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Smoking Promotes Subclinical Atherosclerosis in Apparently Healthy Men

– 2-Year Ultrasonographic Follow-up –

Shoji Sanada, MD, PhD; Makoto Nishida, MD, PhD; Kouki Ishii; Toshiki Moriyama, MD, PhD; Issei Komuro, MD, PhD; Keiko Yamauchi-Takihara, MD, PhD

Background: Smoking is a major risk factor for cardiovascular disease. Also, inflammatory activation and metabolic disorder are the mediators of smoking-induced atherosclerotic progression. The aim of the present study was to investigate whether current smoking and smoking cessation alter inflammatory or metabolic status and affect subclinical atherosclerosis in apparently healthy men.

Methods and Results: Classical risk factors and smoking habit were evaluated in 354 men who completed health examinations annually without any current medications. Carotid intima-media thickness (IMT) was followed for 27.1±4.5 months. At baseline, both maximum and mean IMT significantly changed during 2-year follow-up. They tended to increase along with progression of smoking habit, with significantly greater maximum IMT in current smokers compared with never smokers. Both maximum and mean IMT significantly changed during 2-year follow-up, and tended to increase with progression of smoking habit, with maximum IMT being greatest for current smokers. Past smokers tended to have greater IMT increase than never smokers. Among smoking habit and some atherosclerotic risk markers that showed significant correlation with maximum IMT increase, stepwise regression showed that smoking habit and serum low-density lipoprotein-cholesterol (LDL-C) level were the only independent predictors.

Conclusions: Significant 2-year progression of subclinical atherosclerosis was associated with continuous smoking and LDL-C. This was only partly moderated in past smokers despite complete reversal of inflammatory activation, suggesting another crucial factor for inhibiting accelerated progression of subclinical atherosclerosis in men. (*Circ J* 2012; **76:** 2884–2891)

Key Words: Inflammation; Intima-media thickness; Metabolic syndrome; Progression; Smoking cessation

Previous epidemiological studies had proposed numerous risk factors for cardiovascular disease (CVD), such as hypertension, diabetes, and hyperlipidemia,[1] all of which comprise metabolic syndrome (MetS).[2] Also, it has been reported that lower plasma adiponectin[3] is an independent risk factor for CVD.[4] Furthermore, serum high-sensitivity C-reactive protein (hs-CRP) level is recognized as an independent predictor of CVD,[1] and serum interleukin-6 (IL-6) level is associated with increased incidence of CVD,[5] implicating inflammatory responses in the incidence of CVD.

Meanwhile, smoking has also emerged as an important risk factor for CVD,[6] and the inflammatory responses as well as impairment of MetS are thought to be involved in the underlying mechanisms of atherosclerosis development,[7,8] the leading cause of CVD.[9] Therefore, in addition to recovery from MetS through reduction of body weight or salt intake,[3,6] smoking cessation is generally and strongly recommended in current anti-atherosclerotic lifestyle improvement.[6] The impact, however, of smoking cessation on reduction of atherosclerotic changes and, if so, which mechanism confers the improvement, is not fully identified.

Recently, non-invasive measurements of arterial intima-media thickness (IMT) have been widely used for assessment of subclinical arterial alterations, and have demonstrated that this is a predictor of CVD.[9,10] In addition, the association of traditional risk factors with IMT (mainly maximum IMT) has been well examined.[11–13]

In the present study, to elucidate whether smoking cessation reduces or reverses the progression of atherosclerosis, and to explore what underlying mechanisms might be associated with

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Health Care Center, Osaka University, Toyonaka (S.S., M.N., K.I., T.M., K.Y.-T.); Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita (S.S., M.N., I.K., K.Y.-T.), Japan
Mailing address: Keiko Yamauchi-Takihara, MD, PhD, Health Care Center, Osaka University, 1-17 Machikaneyama, Toyonaka 560-0043, Japan. E-mail: takihara@wellness.hss.osaka-u.ac.jp

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Table 1. Baseline Clinical Subject Characteristics

	All	Smoking habit			P-value
		Never	Past	Current	
n	354	195 (55)	84 (24)	75 (21)	
Age (years)	48.5±5.7	47.7±5.6	49.6±5.7	49.4±5.5	0.009
BMI (kg/m^2)	23.2±2.8	23.1±2.7	23.0±2.4	23.8±3.3	0.09
Waist (cm)	82.6±7.7	81.9±7.7	82.1±6.5	85.3±8.6	0.004
SBP (mmHg)	122±14	121±13	122±15	123±15	0.42
DBP (mmHg)	79±11	79±10	80±12	80±12	0.52
UA (mg/dl)	6.0±1.2	6.0±1.1	6.2±1.1	5.9±1.3	0.25
TG (mg/dl)	118±104	110±81	109±62	147±172	0.02
HDL-C (mg/dl)	56±14	57±15	57±12	55±14	0.74
LDL-C (mg/dl)	128±29	126±27	129±33	130±28	0.68
FPG (mg/dl)	92±12	91±12	94±11	90±10	0.18
HbA_{1c}, %	5.0±0.5	5.0±0.5	5.0±0.5	5.0±0.4	0.68
Max. IMT (mm)	0.922±0.502	0.877±0.471	0.958±0.474	1.001±0.597	0.15
Mean IMT (mm)	0.682±0.170	0.662±0.156	0.712±0.198	0.699±0.168	0.05
Presence of plaque	38 (10.7)	16 (8.2)	12 (14.3)	10 (13.3)	0.23

Data given as n (%) or mean±SD.
BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglycerides; UA, uric acid.

this reduction, we evaluated the associations of MetS parameters as well as inflammatory markers with IMT and their relationship with smoking habit in drug-naïve apparently healthy subjects.

Methods

Subjects

The subjects were the men who underwent health examinations in the Osaka University Health Care Center during 2005–2007. Apparently healthy Japanese men (n=354), 40–59 years of age, who completed an annual visit for medical checkup in 3 consecutive years, did not take any chronic or frequent medicine from at least 1 year before the first visit to the end of follow-up, did not suffer acute illness within 2 weeks before each visit and successfully underwent carotid ultrasonography in the first and the third visits were consecutively included. Informed consent was obtained from all subjects prior to participation in the study following approval of the study by the Ethics Committee of Osaka University. Because blood tests for hs-CRP, IL-6, and adiponectin concentration were beyond routine annual medical checkup, these tests were also performed in samples from 89 men (42/29/18 in never, past and current smokers, respectively) who participated in this study and who also agreed in writing to additional investigational measurements.

Definition of Past Smoker and Smoking Cessation Period

Smoking habit for each participant was primarily obtained from the mark in the check boxes sorting them into never, current or past smokers, as well as complementary descriptions determining the duration of smoking period in the interview sheet at annual medical checkup. For the past smokers, because the smoking cessation periods were not directly queried on the interview sheet, all the past interview sheet records for each individual were surveyed and the duration of smoking cessation defined as the period starting from the first year after the smoking habit changed from current to past smoker. If it was the case that all the past records indicated past smoking habit or the record was not available, we then referred to a formula of [(Age, years old)−(duration of smoking period, years)−20] year(s), based on the directly acquired data via the interview, to estimate the smoking cessation period.

Table 2. Smoking Status

	Past smoker, n (%)	Current smoker, n (%)
Smoking period (years)		
1–5	20 (23.8)	0 (0)
6–10	24 (28.6)	2 (2.7)
11–20	25 (29.8)	15 (20.3)
21–	15 (17.9)	57 (77.0)
No. cigarettes		
1–10	23 (34.3)	14 (18.7)
11–20	29 (43.3)	31 (41.3)
21–40	13 (19.4)	29 (38.7)
41–	2 (3.0)	1 (1.3)
Smoking cessation period (years)		
2–5	1 (1.2)	
6–10	12 (14.3)	
11–20	33 (39.3)	
21–30	27 (32.1)	
31–	11 (13.1)	

Risk Factor Assessment

Information on medical history, use of medicines and personal smoking habit were obtained via questionnaire, and was reconfirmed in expert interview by trained nurses. Waist circumference at the umbilical level was measured in the late exhalation phase in standing position.

Laboratory Measurements

Serum was collected from subjects after overnight fasting and kept at ≤–20°C until assay. Serum hs-CRP, IL-6 and adipo-

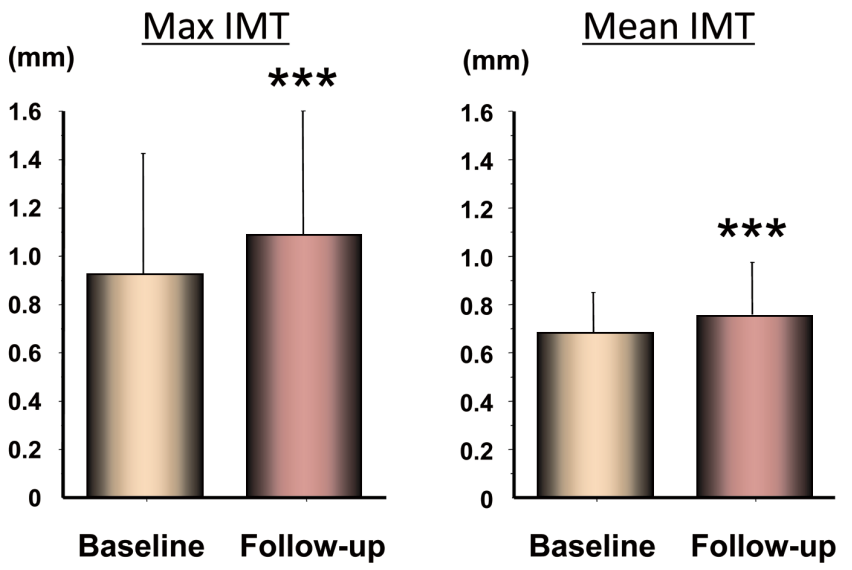

Figure 1. Changes in maximum and mean intima-media thickness (IMT) from baseline to 2 years follow-up in all subjects. Data given as mean±SD. ***P<0.0001 vs. baseline.

Table 3. Risk Factors and 2-Year Increase of IMT

	Delta-maximum IMT		Delta-mean IMT	
	r	P-value	r	P-value
Age	0.041	0.44	0.063	0.24
BMI	0.035	0.51	0.023	0.67
Waist	0.042	0.43	0.013	0.80
SBP	0.114	0.031	0.097	0.068
DBP	0.025	0.64	0.073	0.17
UA	0.079	0.14	0.110	0.039
TG	0.108	0.042	0.019	0.72
HDL-C	–0.029	0.58	0.052	0.33
LDL-C	0.142	0.009	0.155	0.004
FPG	0.087	0.10	0.092	0.082
HbA_{1c}	0.108	0.042	0.118	0.026
Smoking habit	0.130	0.015	0.096	0.071

Abbreviations as in Table 1.

nectin concentration were measured as described previously.[14,15] Briefly, they were measured using an immunoenzyme assay, a chemiluminescent enzyme immunoassay (CLEIA) and a sandwich enzyme-linked immunosorbent assay (ELISA) system, respectively.

The mean interclass coefficient of variation (CV) of hs-CRP, IL-6, and adiponectin measurements (n=40) in the assays before this study were 1.1%, 4.5%, and 1.2%, respectively. Kits from the same lots were used in this study to maintain reliability of measurement.

Evaluation of Carotid Atherosclerosis

All ultrasound examinations were performed by a single well-trained sonographer (K.I.) who regularly participated in quality control measurement sessions and was totally blinded to all clinical information, using LOGIQ 5 (GE Yokogawa Medical Systems, Tokyo, Japan) with an 8.8-MHz linear transducer. Three different longitudinal images (anterior oblique, lateral, and posterior oblique) of the left common carotid artery (CAA) of a 1.0–1.5-cm section at the distal end of the CCA proximal to the carotid bulb were obtained as described previously,[14,15] complying with validated protocols. In addition, transverse images were then obtained to confirm the accuracy of longitudinal images. After examination, the best longitudinal images were analyzed for each individual. Maximum and mean IMT was obtained using computer software that automatically traces the intima-media edge of the far wall. The presence of plaque was defined as detection of a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT or having a thickness of ≥1.5 mm, in concordance with a previous report.[16]

Statistical Analysis

Data were analyzed using SPSS 14.0 (Chicago, IL, USA). Pearson's correlation coefficients were calculated for variables with skewed distribution after logarithmic transformation. Stepwise multiple regression analysis was conducted using the enter method. ANOVA with modified Bonferroni's post-hoc test was used to assess differences between groups based on category. In order to analyze correlation of smoking with the progression of IMT, current, past and never smokers were scored as 1, 0.5 and 0, respectively, and the sum of this score was used, together with IMT progression within 2 years in each individual. P<0.05 was considered statistically significant.

Results

Baseline Demographics

Clinical characteristics of the study subjects are summarized in Table 1. With regard to risk factors, age was significantly older, and waist circumference and serum triglyceride (TG) level significantly higher as smoking habit progressed in men, whereas no significant differences were seen in body mass index (BMI), blood pressure (BP), uric acid (UA), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG) and HbA_{1c}. As shown in Table 1, both maximum and mean IMT tended to increase as smoking habit progressed, reaching significance in mean IMT. Plaques as defined in a previous report[16] were

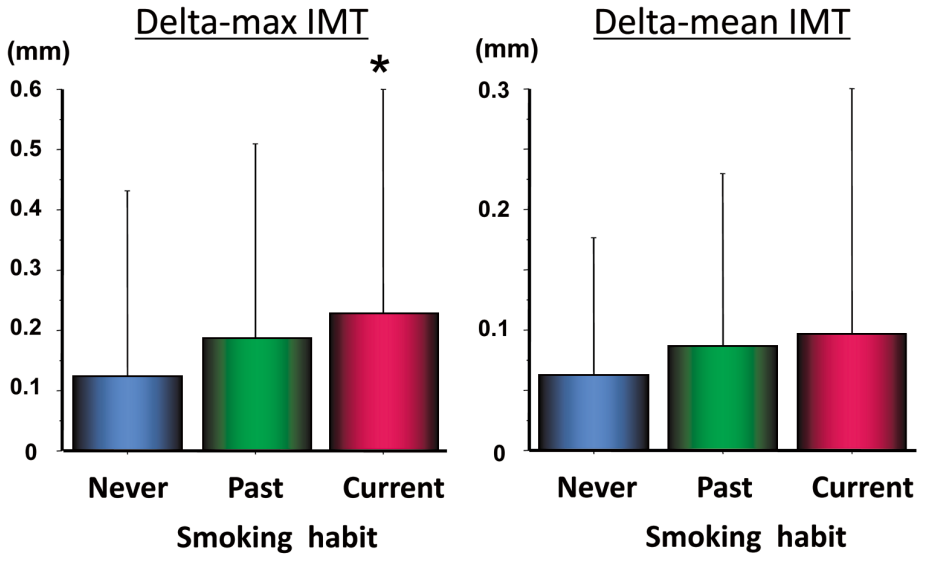

Figure 2. Difference in (**A**) maximum and (**B**) mean intima-media thickness (IMT) from baseline to 2 years follow-up vs. smoking habit. Data given as mean±SD. *P<0.05 vs. never smokers.

found in only 38 out of 355 individuals in the cohort (10.7%), without significant association with smoking habit.

Factors of smoking habit such as duration of smoking or number of cigarettes per day in current and past smokers are listed in Table 2. Current smokers were liable to have a longer smoking history, but there was no significant change in the number of cigarettes, and there were very few heavy smokers consuming >40 cigarettes per day in each group.

Progression of IMT During Follow-up and Association With MetS Components

After 2 years of follow-up, both maximum and mean IMT in all subjects significantly increased (P<0.0001) compared with baseline (Figure 1). Single regression analysis between the traditional risk factors and the change in IMT (delta-IMT) from before to after 2 years follow-up is given in Table 3. Among them, systolic BP, serum TG, LDL-C and HbA_{1c} as well as smoking habit were significantly correlated with delta-maximum IMT, whereas serum UA correlated only with delta-mean IMT, and serum LDL-C as well as HbA_{1c} were associated with both parameters. In contrast, age, BMI, waist circumference, diastolic BP, HDL-C and FPG were not correlated with delta-IMT.

Change in IMT During Follow-up and Association With Smoking Habit

Figure 2 charts delta-IMT during 2 years follow-up along with smoking habit (never, past and current smoker). Current smoking was associated with a tendency for increase in both delta-maximum and delta-mean IMT, which was significant in delta-maximum IMT. Past smokers tended to have less of an increase, and this did not reach significance. Among the aforedescribed MetS parameters as well as smoking habit, stepwise regression analysis (Table 4) showed that only smoking habit and serum LDL-C were significantly correlated with delta-maximum IMT, suggesting that these 2 parameters would be independent contributors for increased progression of atherosclerosis. Furthermore, to clarify whether MetS components had changed during the 2-year observation period, we additionally evaluated the relationship between IMT and the mean of the

Table 4. Independent Factors for IMT Increase in 2 Years

Independent variables	Delta-max. IMT		Delta-mean IMT	
	r	P-value	r	P-value
SBP	0.089	0.11		
UA			0.091	0.10
TG	–0.032	0.62		
LDL-C	0.119	0.036	0.115	0.040
HbA_{1c}	0.056	0.31	0.077	0.16
Smoking habit	0.111	0.041	0.099	0.082

Abbreviations as in Table 1.

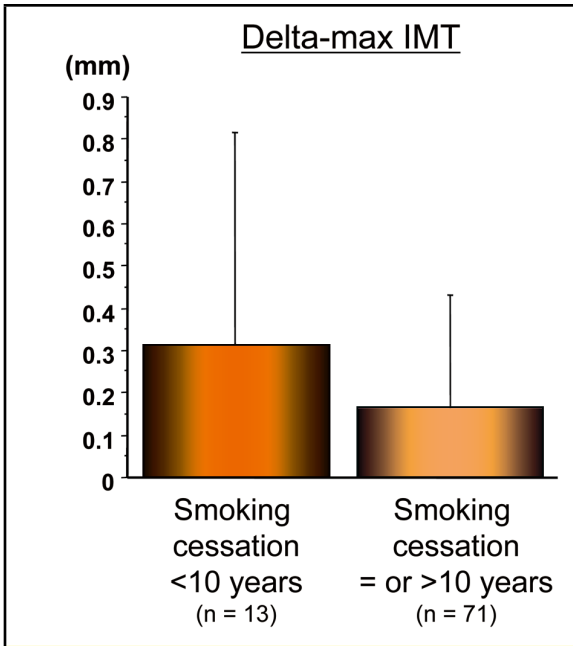

Figure 3. Smoking cessation period and increase of intima-media thickness (IMT).

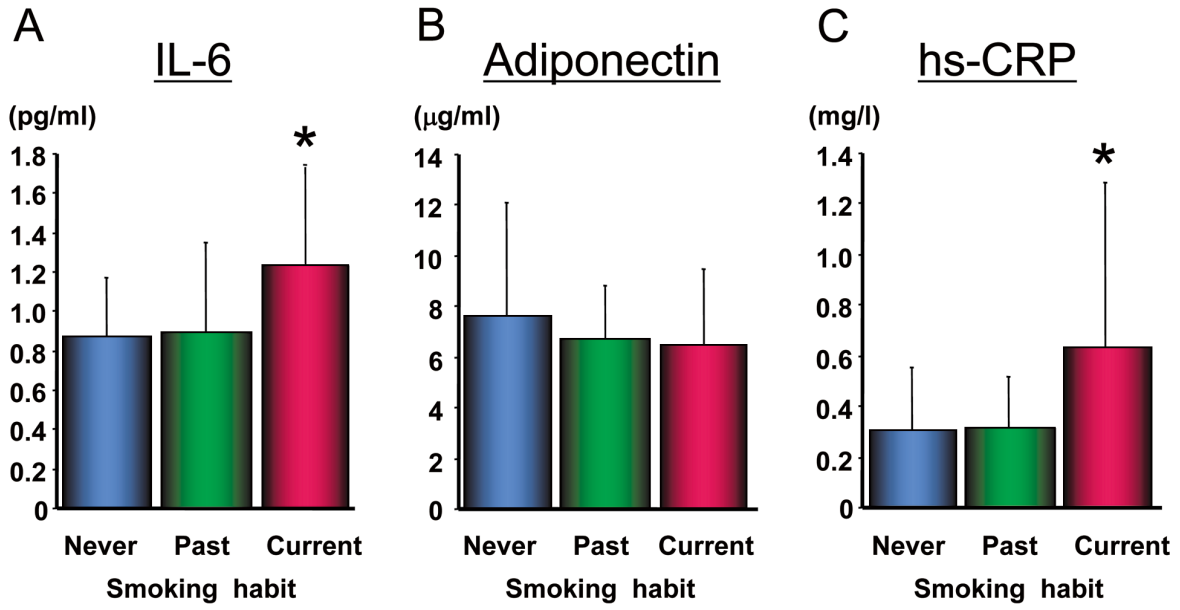

Figure 4. Comparison of (**A**) serum interleukin (IL)-6, (**B**) adiponectin and (**C**) high-sensitivity C-reactive protein (hs-CRP) levels at baseline vs. smoking habit. Data given as mean±SD. *P<0.05 vs. never smokers.

2-year blood test results (Table S1). We observed that only mean LDL-C during 2 years of follow-up had a significant association with progression of both maximum and mean IMT, further suggesting the important contribution of LDL-C to the progression of atherosclerosis. As to period of smoking cessation, longer estimated cessation >10 years tended to provide further moderation of progression of delta-maximum IMT (Figure 3), but was not significant (P=0.1139 and 0.534 in maximum and mean IMT, respectively). We then further investigated the association of atherosclerosis progression with either smoking or smoking cessation period, and found that positive correlation with smoking period might be stronger than the negative correlation with smoking cessation period among past smokers, although not significant (Table S2).

Smoking Habit, Serum IL-6, Adiponectin and hs-CRP

To further elucidate the underlying mechanisms, serum IL-6, adiponectin, and hs-CRP at baseline were evaluated. As shown in Figure 4, serum IL-6 and hs-CRP at baseline were significantly higher in current smokers (1.22±0.53 pg/ml and 0.63±0.65 mg/L, respectively), compared with never and past smokers. Serum adiponectin tended to be higher in never smokers than past and current smokers, but no significant difference was observed. Furthermore, we found that IL-6 and hs-CRP had a significant positive correlation with the duration of smoking, and hs-CRP also had a significant negative correlation with duration of smoking cessation among past smokers (Table S3). Taken together, moderated progression of subclinical atherosclerosis was achieved in past smokers compared to current smokers, associated with complete reversal of inflammatory activation. This implies that smoking cessation and associated inflammatory deactivation might be another critical factor for inhibiting accelerated progression of subclinical atherosclerosis in addition to LDL-C lowering in men.

Discussion

In the present group of apparently healthy men we found that atherosclerosis, as evaluated via carotid IMT, significantly progressed, and was independently and significantly accelerated as serum LDL-C increased and as smoking habit progressed, as shown on multiple regression analysis. Serum hs-CRP and IL-6 were significantly higher in only current smokers, but in past smokers were completely the same as in never smokers.

Many previous studies have reported that smoking is a major promoter of atherosclerotic change,[17] and that cessation of smoking is strongly recommended, and is associated with possible reduction of risk for CVD.[18,19] Direct and quantitative evaluation of the impact of smoking cessation on long-term atherosclerotic change, however, was still to be documented. Here, in the present study, we have carried out a prospective 2-year follow-up of IMT changes in apparently healthy men. This had been done only in 1 previous report in healthy men/women,[19] which primarily supported the present findings of incomplete recovery from accelerated mean IMT increase after 2 years. The direct mechanistic linkage underlying smoking cessation and atherosclerotic progression, however, had not been investigated.

For the first time, we analyzed IMT change over time along with smoking habit, MetS status and multiple inflammatory parameters. We evaluated 89 samples from participants who agreed to additional blood sampling in writing, and on analysis of direct correlation between IL-6, hs-CRP and IMT, IL-6 had P=0.054 for positive correlation, whereas CRP did not have any association with IMT. Accordingly, our previous report evaluated this issue in a similar cohort of 153 apparently healthy men, and successfully observed the identical tendency.[15] Briefly, IL-6 was significantly correlated with either delta-maximum (P=0.02) or delta-mean (P=0.008) IMT, whereas the hs-CRP correlation was not significant (P=0.24 in delta-maximum

IMT and P=0.35 in delta-mean IMT). Therefore, we could assume that the inflammatory status represented by serum IL-6 potentially affects IMT progression. Although the NHANES III study (15,489 individuals) showed that blood CRP returned to baseline 5 years after smoking cessation,[20] which is consistent with the time frame associated with cardiovascular risk reduction observed in both the MONICA and Northwick Park Heart studies,[20–22] we intriguingly obtained the novel finding that the past smokers do not achieve complete recovery from accelerated increase in both maximum and mean IMT, despite complete reversal of inflammatory status after the shorter period of 2 years. This time frame-dependent dissociation might be explained in some way by the different characteristics and size of the subject group, but it may also be due to another long-lasting producer of IMT progression other than inflammatory status, that is, smoking. In this way, the underlying mechanisms of preclinical IMT progression and clinically relevant CVD incidence, as well as the markers representing the respective risks, should be somewhat different. Accordingly, regression analysis of the relationship between atherosclerosis progression and the duration of either smoking or smoking cessation period (**Tables S2,3**) showed that maximum-IMT had a stronger positive correlation with the duration of smoking period than negative correlation with the duration of smoking cessation period in past smokers, further supporting this idea. This might ultimately lead to the idea that interventions to avoid smoking from the beginning might be as important as those to stop smoking.

Because the inflammatory response is widely recognized as an independent risk factor for CVD,[1,5] and is reported to be closely associated with vulnerable plaque,[23] we can say that smoking cessation rapidly reduces the vulnerability of atherosclerotic regions via inflammatory inactivation, whereas more time, or even years, would be needed to reverse the acceleration of primary atherosclerotic progress represented by increased IMT due to other mechanisms. Accordingly, the Heinz Nixdorf Recall Study of 4,814 individuals without overt CHD also showed that the growth of the coronary atherosclerotic region is accelerated by smoking and slows down after smoking cessation, but advanced atherosclerotic change is present for a long period.[24] The question then arises as to what other mechanism than inflammatory response may be responsible for the prolonged IMT progression.

In this study the annual increase of mean IMT was around 0.030–0.050 mm, which might be greater than that reported in the many previous studies on the progression of IMT in healthy subjects. There are some reports, however, indicating that the progression of mean IMT in asymptomatic young adults varies from 0.015 to 0.029 mm/year.[25] Furthermore, we collected the present data from untreated and middle-aged individuals, who might be substantially more susceptible to IMT progression.[26] Taken together, we could say that the current data regarding the annual increase of mean IMT of around 0.030–0.050 mm might be within reasonable range.

As a possible mechanism, Oyama et al showed that green tea catechins have anti-atherosclerotic properties among smokers by increasing the level of nitric oxide and reducing oxidative stress.[27] In contrast, it is widely accepted that the impairment of MetS status substantially promotes the progression of atherosclerosis[28] and increases the risk of CVD.[1] Also, another study on a cohort of 5,033 individuals with the same characteristics as the present subjects suggested that the exposure to MetS would explain at least in part the increasing risk of excessive carotid plaque in past smokers.[29] The present result from stepwise regression analysis also showed that serum LDL-C level and smoking habit were the only independent predictors of IMT progression. This result is primarily supported by a previous study in a cohort of 2,421individuals who have similar characteristics with the present subjects followed up for 14 years.[30] According to **Table 1**, however, past smokers have normal MetS parameters including BMI, waist circumference, BP, UA, TG, LDL-C and HDL-C and HbA_{1c}, which are equal to those of never smokers. This is possibly in part due to the relatively small size of the data set, because the data in **Table 2** differ from those in previous reports that suggested waist circumference[31] or HDL-C[32] as independent risk factors for accelerated atherosclerosis. Accordingly, the JART study in the same ethnic population showed that intensive lipid-lowering treatment with rosuvastatin effectively eliminated the progression of IMT compared with pravastatin treatment, and was associated with a much higher rate of achieving lower LDL-C/HDL-C ratio <1.5.[33] The present cohort had a mean LDL-C level within normal limits but a relatively higher LDL-C/HDL-C ratio (2.373±0.056 for never, 2.390±0.089 for past and 2.496±0.109 for current smokers, respectively, P=0.55), suggesting that more intensive lipid-lowering strategy beyond normalizing LDL-C level might facilitate the reduction of atherosclerosis progression. Otherwise, the present results might downgrade the relative importance of impaired MetS status including hypercholesterolemia as a putative promoter of smoking-induced prolonged atherosclerotic progression. The only difference we observed in past smokers compared with never smokers was a trend toward lower blood adiponectin level, which was almost identical to that of current smokers. Adiponectin is an adipocytokine mainly secreted from visceral fat tissues,[34] and the reduction of its blood level is reported to be an independent risk factor for atherosclerotic progression.[35] Although the duration for recovery of blood adiponectin level after smoking cessation is currently elusive, varying from 2 months to up to 20 years in men,[36,37] complete recovery from accelerated peripheral arterial atherosclerosis due to smoking represented by impaired ankle-brachial index will take up to 20 years after smoking cessation.[38] Experimental studies show that adiponectin has a direct cardioprotective property,[35] therefore adiponectin could be a potential contributor to smoking-induced prolonged atherosclerotic progression. Accordingly, **Table S3** suggests a potential inverse correlation of adiponectin level with smoking habit: decreasing with intensity and length of smoking ands increasing with the duration of smoking cessation, but it is possible that the 2-year follow-up period was too short to evaluate the long-term effect of adiponectin. This issue should be directly addressed by further study with an increased number of participants.

As a limitation, because smoking habit was confirmed only by questionnaire and interview, we cannot exclude the possibility that some of the past smokers were occasionally exposed to temporary smoking episodes during follow-up. Also, we did not follow the subjects for a longer period because of the study design. Furthermore, we did not measure luminal diameter routinely in this study. The primary aim of IMT measurement was to study the surrogate marker of atherosclerotic change in normal or preclinical stages. The core requirement to achieve this goal was to measure the initial and small changes of maximum and mean IMT precisely in a large cohort. To achieve this, we ensured that a well-trained and established sonographer performed the entire IMT test himself, to avoid inter-individual variance. This meant that it was necessary to limit the list of measurements to scoring of maximum and mean IMT, accompanied by informal observation of visually advanced narrowing, because IMT measurements were

performed on approximately 5,000 candidates and applicants every year, along with the institutional annual general medical checkup.

In addition, we could not measure the inflammatory markers (IL-6, adiponectin and hs-CRP) repeatedly because written informed consent limited the measurements to once only, at the time of entry.

Finally, we can conclude that subclinical atherosclerosis is independently accelerated via continuous smoking or LDL-C, and that the smoking-induced promotion of atherosclerotic change is closely associated with inflammatory reactions. Furthermore, the entire inhibition of activated inflammatory responses by smoking cessation is still insufficient to abrogate accelerated progression of subclinical atherosclerosis after 2 years in men, and reduced adiponectin level can be potentially proposed as an underlying mediator. Further investigation into the role of adiponectin in relation to smoking cessation in a larger cohort for a longer period is therefore warranted, to enable investigation of a direct mechanistic link between them, and to verify strategies to increase adiponectin as a therapeutic intervention against atherosclerosis and subsequent CVD events.

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Disclosures

Conflict of Interest: None.

References

1. Fruchart JC, Nierman MC, Stroes ES, Kastelein JJ, Duriez P. New risk factors for atherosclerosis and patient risk assessment. *Circulation* 2004; **109:** III15–III19.
2. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; **109:** 433–438.
3. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2004; **24:** 29–33.
4. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 2003; **23:** 85–89.
5. Luc G, Bard JM, Juhan-Vague I, Ferrieres J, Evans A, Amouyel P, et al. C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: The PRIME Study. *Arterioscler Thromb Vasc Biol* 2003; **23:** 1255–1261.
6. Erhardt L. Cigarette smoking: An undertreated risk factor for cardiovascular disease. *Atherosclerosis* 2009; **205:** 23–32.
7. Nguyen AB, Rohatgi A, Garcia CK, Ayers CR, Das SR, Lakoski SG, et al. Interactions between smoking, pulmonary surfactant protein B, and atherosclerosis in the general population: The Dallas Heart Study. *Arterioscler Thromb Vasc Biol* 2011; **31:** 2136–2143.
8. Ishizaka N, Ishizaka Y, Toda E, Hashimoto H, Nagai R, Yamakado M. Association between cigarette smoking, metabolic syndrome, and carotid arteriosclerosis in Japanese individuals. *Atherosclerosis* 2005; **181:** 381–388.
9. Peters SA, Grobbee DE, Bots ML. Carotid intima-media thickness: A suitable alternative for cardiovascular risk as outcome? *Eur J Cardiovasc Prev Rehabil* 2011; **18:** 167–174.
10. Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M. Carotid intima-media thickening indicates a higher vascular risk across a wide age range: Prospective data from the Carotid Atherosclerosis Progression Study (CAPS). *Stroke* 2006; **37:** 87–92.
11. Blackburn R, Giral P, Bruckert E, André JM, Gonbert S, Bernard M, et al. Elevated C-reactive protein constitutes an independent predictor of advanced carotid plaques in dyslipidemic subjects. *Arterioscler Thromb Vasc Biol* 2001; **21:** 1962–1968.
12. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol* 2004; **24:** 969–974.
13. Mahmud A, Feely J. Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension* 2005; **46:** 1118–1122.
14. Nishida M, Moriyama T, Sugita Y, Yamauchi-Takihara K. Interleukin-10 associates with adiponectin predominantly in subjects with metabolic syndrome. *Circ J* 2007; **71:** 1234–1238.
15. Nishida M, Moriyama T, Ishii K, Takashima S, Yoshizaki K, Sugita Y, et al. Effects of IL-6, adiponectin, CRP and metabolic syndrome on subclinical atherosclerosis. *Clin Chim Acta* 2007; **384:** 99–104.
16. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Desvarieux M, et al; Advisory Board of the 3rd Watching the Risk Symposium 2004, 13th European Stroke Conference. Mannheim intima-media thickness consensus. *Cerebrovasc Dis* 2004; **18:** 346–349.
17. Erhardt L. Cigarette smoking: An undertreated risk factor for cardiovascular disease. *Atherosclerosis* 2009; **205:** 23–32.
18. Pipe AL, Papadakis S, Reid RD. The role of smoking cessation in the prevention of coronary artery disease. *Curr Atheroscler Rep* 2010; **12:** 145–150.
19. van den Berkmortel FW, Wollersheim H, van Langen H, Smilde TJ, den Arend J, Thien T. Two years of smoking cessation does not reduce arterial wall thickness and stiffness. *Neth J Med* 2004; **62:** 235–241.
20. Bakhru A, Erlinger TP. Smoking cessation and cardiovascular disease risk factors: Results from the Third National Health and Nutrition Examination Survey. *PLoS Med* 2005; **2:** e160.
21. Koenig W, Sund M, Fröhlich M, Fischer HG, Löwel H, Döring A, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: Results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 1999; **99:** 237–242.
22. Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, et al. Haemostatic function and ischaemic heart disease: Principal results of the Northwick Park Heart Study. *Lancet* 1986; **2:** 533–537.
23. Shah PK. Inflammation and plaque vulnerability. *Cardiovasc Drugs Ther* 2009; **23:** 31–40.
24. Jöckel KH, Lehmann N, Jaeger BR, Moebus S, Möhlenkamp S, Schmermund A, et al. Smoking cessation and subclinical atherosclerosis: Results from the Heinz Nixdorf Recall Study. *Atherosclerosis* 2009; **203:** 221–227.
25. Toprak A, Kandavar R, Toprak D, Chen W, Srinivasan S, Xu JH, et al. C-reactive protein is an independent predictor for carotid artery intima-media thickness progression in asymptomatic younger adults (from the Bogalusa Heart Study). *BMC Cardiovasc Disord* 2011; **11:** 78.
26. Liviakis L, Pogue B, Paramsothy P, Bourne A, Gill EA. Carotid intima-media thickness for the practicing lipidologist. *J Clin Lipidol* 2010; **4:** 24–35.
27. Oyama J, Maeda T, Kouzuma K, Ochiai R, Tokimitsu I, Higuchi Y, et al. Green tea catechins improve human forearm endothelial dysfunction and have antiatherosclerotic effects in smokers. *Circ J* 2010; **74:** 578–588.
28. Scuteri A, Najjar SS, Muller DC, Andres R, Hougaku H, Metter EJ, et al. Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. *J Am Coll Cardiol* 2004; **43:** 1388–1395.
29. Ishizaka N, Ishizaka Y, Toda E, Hashimoto H, Nagai R, Yamakado M. Association between cigarette smoking, metabolic syndrome, and carotid arteriosclerosis in Japanese individuals. *Atherosclerosis* 2005; **181:** 381–388.
30. Hata J, Doi Y, Ninomiya T, Fukuhara M, Ikeda F, Mukai N, et al. Combined effects of smoking and hypercholesterolemia on the risk of stroke and coronary heart disease in Japanese: The Hisayama study. *Cerebrovasc Dis* 2011; **31:** 477–484.
31. Liu KH, Chan YL, Chan WB, Chan JC, Chu CW. Mesenteric fat thickness is an independent determinant of metabolic syndrome and identifies subjects with increased carotid intima-media thickness. *Diabetes Care* 2006; **29:** 379–384.

32. Johnsen SH, Mathiesen EB, Fosse E, Joakimsen O, Stensland-Bugge E, Njølstad I, et al. Elevated high-density lipoprotein cholesterol levels are protective against plaque progression: A follow-up study of 1952 persons with carotid atherosclerosis the Tromsø study. *Circulation* 2005; **112:** 498–504.
33. Nohara R, Daida H, Hata M, Kaku K, Kawamori R, Kishimoto J, et al. The Justification for Atherosclerosis Regression Treatment (JART) Investigators. Effect of intensive lipid-lowering therapy with rosuvastatin on progression of carotid intima-media thickness in Japanese patients. *Circ J* 2011; **76:** 221–229.
34. Matsuzawa Y. Therapy insight: Adipocytokines in metabolic syndrome and related cardiovascular disease. *Nat Clin Pract Cardiovasc Med* 2006; **3:** 35–42.
35. Ouchi N, Shibata R, Walsh K. Cardioprotection by adiponectin. *Trends Cardiovasc Med* 2006; **16:** 141–146.
36. Efstathiou SP, Skeva II, Dimas C, Panagiotou A, Parisi K, Tzanoumis L, et al. Smoking cessation increases serum adiponectin levels in an apparently healthy Greek population. *Atherosclerosis* 2009; **205:** 632–636.
37. Takefuji S, Yatsuya H, Tamakoshi K, Otsuka R, Wada K, Matsushita K, et al. Smoking status and adiponectin in healthy Japanese men and women. *Prev Med* 2007; **45:** 471–475.
38. Cui R, Iso H, Yamagishi K, Tanigawa T, Imano H, Ohira T, et al. Relationship of smoking and smoking cessation with ankle-to-arm blood pressure index in elderly Japanese men. *Eur J Cardiovasc Prev Rehabil* 2006; **13:** 243–248.

Supplementary Files

Supplementary File 1

Table S1. Correlation Between Mean Risk Factors During Study Period of 2 Years and Progression of IMT

Table S2. Correlation Between Smoking Habit and Progression of IMT in 2 Years

Table S3. Correlation Between Smoking Habit and Inflammatory Markers

Please find supplementary file(s);
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