min D and the prevalence of MetS[31].

Intake of antioxidants, by contrast, does not appear to have an effect on cardiovascular outcomes[32,33], although some studies have reported conflicting results[34,35]. It is not surprising that a healthy balanced diet with a frequent intake of vegetables, fruits, fish, pasta and rice and a low intake of fried food is protective against MetS[36].

## Management of MetS

The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (ESAD) have published a Joint Statement[37,38], in which it is stressed that the utility of the concept of MetS as the new risk marker for CVD requires further investigation. There is a discussion that clinicians should evaluate and treat all CVD risk factors regardless of whether a patient meets the diagnosis criteria for MetS[38]. Several investigators have questioned whether MetS is really an established clinical entity[39].

Individuals with established dyslipidemia, hypertension, and diabetes are often periodically followed-up at medical institutions in Japan. Even without diagnosis of MetS, patients with these risk factors are already considered to be at high risk for CVD and are often undergoing therapy to ameliorate these disorders in some medical institutes. Thus, an important question would appear to be whether individuals diagnosed with MetS are at substantially higher risk for CVD, especially if the extent of each of their MetS components is mild enough such that on its own it would not seem to require active intervention.

We should certainly continue to pursue whether some drugs, such as peroxisome proliferator-activated receptor (PPAR) agonists, are effective in reducing the incidence of MetS and/or reducing the development of CVD and T2DM in individuals with MetS. On the other hand, lifestyle modification is the cornerstone of MetS treatment and costs less[40]. The clinical importance of diagnosing MetS seems to depend not only on which definition is used, but also on how this syndrome is comprehended and managed by healthcare practitioners.

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# Metabolic Syndrome May Not Associate With Carotid Plaque in Subjects With Optimal, Normal, or High-Normal Blood Pressure

Nobukazu Ishizaka, Yuko Ishizaka, Hideki Hashimoto, Ei-Ichi Toda, Ryozo Nagai, Minoru Yamakado

*Abstract*—Much evidence indicates that metabolic syndrome is a risk factor for the development of cardiovascular disease, but whether metabolic syndrome is an independent risk factor for early atherosclerosis in the individuals with only minor hemodynamic abnormalities, if any, is not well investigated. Here we have investigated the association between metabolic syndrome and carotid atherosclerosis in individuals with blood pressure of <140/90 mm Hg. Between 1994 and 2003, 8143 subjects underwent general health screening including carotid ultrasonography. Of 8143 individuals, 5661 individuals without antihypertensive medications who had blood pressure of <140/90 mm Hg were considered to have optimal, normal, or high-normal blood pressure. After adjustment for age, systolic blood pressure, body mass index, total and high-density lipoprotein cholesterol, triglycerides, fasting glucose, and smoking status, metabolic syndrome was not found to be an independent risk factor for carotid plaque (odds ratio: 1.65; 95% CI; 0.72 to 3.76 in women and odds ratio: 0.95; 95% CI: 0.70 to 1.28 in men) or for carotid intima-media thickening (odds ratio: 0.56; 95% CI: 0.18 to 1.72 in women and odds ratio: 0.93 95% CI: 0.62 to 1.38 in men) in these subjects. Thus, presence of metabolic syndrome may not increase the prevalence of carotid atherosclerosis independent of other cardiovascular risk factors in Japanese individuals with optimal, normal, or high-normal blood pressure. (***Hypertension*. 2006;48:411-417.**)

**Key Words:** metabolism ■ carotid arteries ■ atherosclerosis ■ risk factor ■ hypertension, arterial

Metabolic syndrome (MetS) is a cluster of metabolic and hemodynamic abnormalities linked with insulin resistance.[1–4] Several diagnostic criteria have been advocated for MetS, of which those proposed by the World Health Organization[5] and the National Cholesterol Education Program[1] are used most frequently. Epidemiological studies have shown that MetS is not a rare occurrence[6] and is a risk factor for cardiovascular disease (CVD)[7] and stroke.[8] The coexistence of MetS in individuals with established CVD risk factors, such as diabetes and hypertension, has been shown to increase the risk of mortality and morbidity from CVD,[9–11] and, thus, such individuals should undergo lifestyle advice and/or active treatment to dissolve the clustering of these abnormalities to reduce the risk of future cardiovascular events.

On the other hand, however, subjects with such established risk factors for CVD are already regarded to be at higher risk for CVD irrespective of the presence or absence of MetS. Therefore, it should be tested whether the concept of MetS can be used to identify individuals at higher risk for future CVD events when the extent of their hemodynamic and metabolic abnormalities is only mild, if present at all. Here, by analyzing the cross-sectional data from individuals who underwent general health screening, we have investigated the impact of MetS on carotid atherosclerosis in the subjects with optimal, normal, or high-normal blood pressure (BP). We also performed a similar analysis in the subpopulations that additionally had, if present, only mild abnormalities in glucose metabolism.

## Methods

### Study Subjects

The study was approved by the Ethical Committee of Mitsui Memorial Hospital. Between September 1994 and December 2003, 8143 subjects underwent general health screening, including carotid ultrasonography at the Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital. BP, taken at the center, was classified according to the Sixth Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure[12]: optimal BP: systolic BP (SBP) <120 mm Hg and diastolic BP (DBP) <80 DBP; normal BP: SBP 120 to 129 DBP or DBP 80 to 84 mm Hg; and high-normal BP: SBP 130 to 139 mm Hg or DBP 85 to 89 mm Hg. Fasting glucose levels were classified according to the American Diabetes Association criteria[13]: normal fasting glucose: FPG <100 mg/dL; and impaired fasting glucose: FPG ≥110 and <126 mg/dL. Individuals who were taking antidiabetic medication were not included.

### Definition of MetS

We used a modified version of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII)[1] and MetS was

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From the Departments of Cardiovascular Medicine (N.I., R.N.) and Health Management and Policy (H.H.), University of Tokyo Graduate School of Medicine, and the Center for Multiphasic Health Testing and Services (Y.I., E.-I.T., M.Y.), Mitsui Memorial Hospital, Tokyo, Japan.

Correspondence to Nobukazu Ishizaka, Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Hongo 7-3-1 Bunkyo-ku, Tokyo 113-8655, Japan. E-mail nobuishizka-tky@umin.ac.jp



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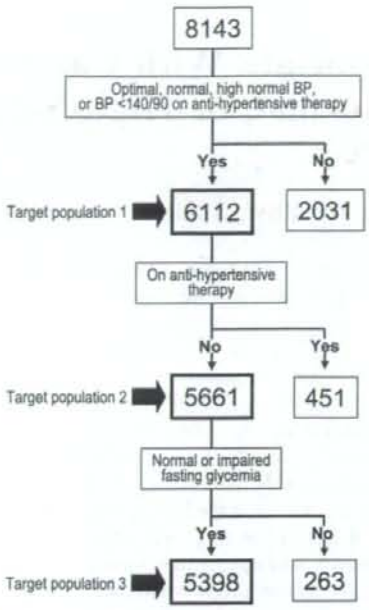


**Figure 1.** Flow chart showing the 3 target populations.

diagnosed when ≥3 of the following components were present: triglycerides ≥150 mg/dL; high-density lipoprotein cholesterol <40 mg/dL in men or <50 mg/dL in women; fasting plasma glucose (FPG) ≥110 mg/dL; SBP ≥130 mm Hg, DBP ≥85 mm Hg, or taking an antihypertensive medication; and body mass index (BMI) ≥25.0 kg/m$^2$ (the waist circumference was not available in this study). This BMI cutoff point was chosen instead of other previously used values[14] because of the discrepancy in BMI between white and Japanese populations in terms of morbidity.[15]

### Selection of Subpopulation

We selected 3 types of target population as follows. Of the 8143 individuals, those with BP of <140/90 mm Hg were selected for target population 1 (n=6112). In addition, of the 6112 individuals in target population 1, those without antihypertensive medications (individuals with optimal, normal, and high-normal BP) were selected for target population 2 (n=5661). Furthermore, of the 5661 individuals in target population 2, those with either normal fasting glucose or impaired fasting glucose were selected for target population 3 (n=5398; Figure 1).

### Carotid Ultrasonography

Carotid artery status was studied using high-resolution B-mode ultrasonography (Sonolayer SSA270A, Toshiba) equipped with a 7.5-MHz transducer as described previously.[16] Plaque was defined to be present when there is ≥1 clearly isolated focal thickening of the intima-media layer with thickness of ≥1.3 mm at the common or internal carotid artery or the carotid bulb. Carotid wall intima-media thickening was said to be present when intima-media thickness, which was measured at the far wall of the distal 10 mm of the common carotid artery, was ≥1.0 mm.[16]

### Statistical Analysis

Comparisons of categorical and continuous variables were made by using $\chi^2$ and Student $t$ tests, respectively. Logistic regression analysis was used to obtain adjusted odds ratios and their 95% CIs to predict the presence of carotid plaque or carotid intima-media thickening. Statistical analyses were carried out by using StatView (version 5.0; SAS Institute Inc). Results are expressed as mean±SD. A value of $P<0.05$ was taken to be statistically significant.

## Results

### Association Between MetS and Carotid Atherosclerosis in Individuals With BP of <140/90 mm Hg (Target Population 1)

The age of the subjects ranged from 21 to 88 years (women, 22 to 87 years; men, 21 to 88 years). In this population, MetS was found in 81 (4%) of 2143 women and 511 (13%) of 3969 men (Table 1). In women, carotid plaque was found in 22 (27%) of the 81 MetS-positive subjects, which was significantly greater than that observed in the MetS-negative subjects (280 of 2062 [14%]; $P<0.001$ by $\chi^2$ test). Similarly, in men, carotid plaque was found in 158 (31%) of the 511 MetS-positive subjects, which was significantly greater than that observed in the MetS-negative subjects (890 of 3458 [26%]; $P=0.013$ by $\chi^2$ test; Figure 2; Table 2). When multivariate logistic regression analysis was performed after adjusting for age, the association between MetS and carotid plaque was statistically significant in both genders (Table 2). After full adjustment for additional CVD risk factors, including total cholesterol, smoking status, and components of the MetS (BMI, SBP, total cholesterol, high-density lipoprotein cholesterol, triglycerides, and FPG), however, this relationship was statistically significant in women, but not in men. After full adjustment, MetS was not significantly associated with carotid intima-media thickening in either gender (Table 3).

### Association Between MetS and Carotid Atherosclerosis in Individuals With Optimal, Normal, or High-Normal BP (Target Population 2)

Of the 6112 individuals in target population 1, 451 individuals were taking antihypertensive medication. To investigate the association between MetS and carotid atherosclerosis in individuals with optimal, normal, and high-normal BP, we omitted these individuals. In this population, the age of the subjects ranged from 21 to 88 years (women, 22 to 87 years; men, 21 to 88 years), and MetS was found in 64 (3%) of 2034 women and 410 (11%) of 3627 men (Table 4). Carotid plaque was found in 11 (17%) of the 64 MetS-positive female subjects and in 103 (25%) of the 410 MetS-positive male subjects. The carotid plaque prevalence was not significantly different from that observed in the MetS-negative subjects (249 of 1970 [13%] women; $P=0.29$; 769 of 3217 [24%] men; $P=0.59$; Figure 2 and Table 2). After full adjustment, MetS was not significantly associated with carotid plaque or carotid intima-media thickening in either gender (Table 3).

### Association Between MetS and Carotid Atherosclerosis in Individuals With Optimal, Normal, or High-Normal BP, Together With Normal or Impaired Fasting Glycemia (Target Population 3)

Of the 5661 individuals in target population 2, 263 individuals whose FPG levels were ≥126 mg/dL were omitted in the target population 3. In this population, the age of the subjects ranged from 21 to 88 years (women, 22 to 87 years; men, 21 to 88 years), and MetS was found in 57 (3%) of 2000 women

**TABLE 1. Baseline Characteristics of the Target Population 1**

| Variables | Women | | | Men | | |
|---|---|---|---|---|---|---|
| | Carotid Plaque (−) (n=1841) | Carotid Plaque (+) (n=302) | P | Carotid Plaque (−) (n=2921) | Carotid Plaque (+) (n=1048) | P |
| Age, y | 54.1±10.0 | 63.5±8.7 | <0.0001 | 53.4±10.0 | 62.8±9.5 | <0.0001 |
| BMI, kg/m² | 21.5±2.9 | 21.6±2.8 | 0.58 | 23.6±2.7 | 23.5±2.5 | 0.20 |
| SBP, mm Hg | 113±13 | 118±14 | <0.0001 | 118±12 | 121±12 | <0.0001 |
| DBP, mm Hg | 70±8 | 71±9 | 0.031 | 74±8 | 74±8 | 0.89 |
| Optimal normal BP, n (%) | 1200 (65) | 141 (47) | <0.0001 | 1475 (50) | 384 (37) | <0.0001 |
| Normal BP, n (%) | 334 (18) | 62 (21) | 0.32 | 684 (23) | 280 (27) | 0.033 |
| High normal BP, n (%) | 240 (13) | 57 (19) | 0.0065 | 596 (20) | 208 (20) | 0.70 |
| On antihypertension medication, n (%) | 67 (4) | 42 (14) | <0.0001 | 166 (6) | 176 (17) | <0.0001 |
| Total cholesterol, mg/dL | 214±35 | 224±33 | <0.0001 | 204±32 | 206±32 | 0.048 |
| High-density lipoprotein cholesterol, mg/dL | 71±17 | 70±17 | 0.51 | 55±5 | 55±15 | 0.79 |
| Triglycerides, mg/dL | 91±58 | 97±53 | 0.095 | 142±106 | 134±100 | 0.043 |
| Uric acid, mg/dL | 4.6±0.9 | 4.8±1.1 | <0.0001 | 6.1±1.2 | 6.1±1.2 | 0.88 |
| Fasting glucose, mg/dL | 90±14 | 91±13 | 0.24 | 99±21 | 101±22 | 0.0020 |
| Hemoglobin A1C, % | 5.1±0.5 | 5.3±0.5 | <0.0001 | 5.3±0.7 | 5.5±0.7 | <0.0001 |
| Metabolic syndrome, n (%) | 59 (3) | 22 (7) | 0.0006 | 353 (12) | 158 (15) | 0.013 |
| Smoking status | | | | | | |
| Never, n (%) | 1534 (83) | 264 (87) | 0.19 | 934 (32) | 281 (27) | <0.0001 |
| Former, n (%) | 97 (5) | 11 (4) | | 841 (29) | 388 (37) | |
| Current, n (%) | 210 (11) | 27 (9) | | 1146 (39) | 379 (36) | |

$\chi^2$ test was used for categorical variables, and $t$ test was used for continuous variables.

and 315 (9%) of 3398 men (Table 5). Carotid plaque was found in 11 (19%) of the 57 MetS-positive female subjects and in 77 (24%) of the 315 MetS-positive male subjects. The carotid plaque prevalence was not significantly different from that observed in the MetS-negative subjects (244 of 1943 [13%] women; $P$=0.13; 723 of 3083 [23%] men; $P$=0.69; Figure 2 and Table 2). After full adjustment, MetS was not found to be significantly associated with carotid plaque or carotid intima-media thickening in either gender (Table 3).

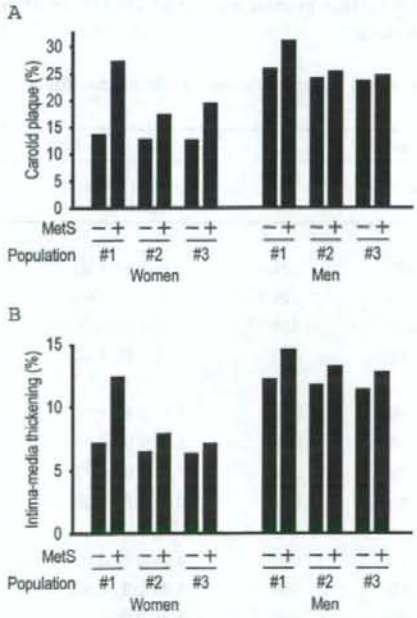


**Figure 2.** Prevalence of carotid plaque and carotid intima-media thickening according to the presence or absence of metabolic syndrome in the 3 target populations.

## Discussion

Here, we have assessed whether MetS, as diagnosed by modified ATPIII criteria, is an independent risk factor for carotid atherosclerosis in the individuals with optimal, normal, or high-normal BP. Within this population, MetS was not found to be an independent risk factor for carotid atherosclerosis in either gender after the full-adjustment for age and other risk factors for CVD.

In several previous studies, the association between MetS and CVD has been assessed not only in individuals with diabetes[17,18] but also in nondiabetic subjects. For example, in a prospective cohort study of Finnish men who did not have CVD or diabetes at enrollment, MetS was found to be associated with a 2.6- to 3.0-fold higher mortality from CVD.[19] Another study found that MetS was associated with increased risk for CVD in middle-aged adults who did not have CVD or type 2 diabetes mellitus at enrollment.[20] These findings indicate that MetS may increase the future risk for CVD and stroke irrespective of the presence or absence of diabetes. On the other hand, Juutilainen et al[21] have reported that after adjusting for confounding factors, the presence of MetS predicted CVD mortality in nondiabetic women but not in nondiabetic men during the 18-year follow-up.

These studies may seem to substantiate the idea that MetS may be a risk for future CVD mortality, at least in women with low risk profiles. However, hypertension (defined as

**TABLE 2. Logistic Regression Analysis With Metabolic Syndrome as an Independent Variable and the Carotid Plaque as a Dependent Variable**

| | Women | | Men | |
|---|---|---|---|---|
| Variables | Odds Ratio (95% CI) | *P* | Odds Ratio (95% CI) | *P* |
| Target population 1 | | | | |
| Unadjusted | 2.37 (1.43–3.94) | 0.0008 | 1.29 (1.06–1.58) | 0.013 |
| Adjusted for age | 2.11 (1.22–3.64) | 0.0076 | 1.39 (1.12–1.73) | 0.0030 |
| Adjusted for age, TC, smoking status | 2.04 (1.18–3.52) | 0.011 | 1.33 (1.07–1.66) | 0.011 |
| Adjusted for age, TC, smoking status, BMI, SBP, TG, HDL-C, FPG | 2.67 (1.39–5.15) | 0.0033 | 1.14 (0.87–1.49) | 0.34 |
| Target population 2 | | | | |
| Unadjusted | 1.43 (0.74–2.78) | 0.29 | 1.07 (0.84–1.35) | 0.59 |
| Adjusted for age | 1.29 (0.63–1.63) | 0.48 | 1.21 (0.94–1.56) | 0.15 |
| Adjusted for age, TC, smoking status | 1.23 (0.60–2.50) | 0.58 | 1.14 (0.88–1.48) | 0.31 |
| Adjusted for age, TC, smoking status, BMI, SBP, TG, HDL-C, FPG | 1.65 (0.72–3.76) | 0.24 | 0.95 (0.70–1.28) | 0.72 |
| Target population 3 | | | | |
| Unadjusted | 1.67 (0.85–3.26) | 0.14 | 1.06 (0.81–1.38) | 0.69 |
| Adjusted for age | 1.54 (0.75–3.19) | 0.24 | 1.25 (0.94–1.68) | 0.13 |
| Adjusted for age, TC, smoking status | 1.47 (0.71–3.04) | 0.30 | 1.18 (0.88–1.58) | 0.28 |
| Adjusted for age, TC, smoking status, BMI, SBP, TG, HDL-C, FPG | 1.84 (0.79–4.27) | 0.15 | 0.98 (0.69–1.38) | 0.91 |

TC indicates total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol.

≥160/95 mm Hg or on therapy) was prevalent in >65% of the women in the study by Juutilainen et al,[21] but their results were not adjusted for BP. Similarly, Isomaa et al[18] have reported that MetS is a predictor of cardiovascular mortality and morbidity in subjects with normal glucose tolerance. However, their results were adjusted for sex and age but not for BP, although 23% to 24% of their target population included subjects with hypertension (defined as >160/90 mm Hg or on therapy). Furthermore, Wilson et al have reported that in >8 years of follow-up, MetS was found to be associated with increased risk for CVD with odds ratios of 2.3 and 2.9 in middle-aged women and men, respectively, who lacked CVD or type 2 diabetes mellitus at baseline. Again, this result was adjusted only for age, although prevalence of hypertension (defined as ≥130/85 mm Hg or on therapy) was more prevalent in individuals with MetS (89% in women and 91% in men) than in those without MetS (34% in women and 43% in men).

**TABLE 3. Logistic Regression Analysis With Metabolic Syndrome as an Independent Variable and the Carotid Intima-Media Thickening as a Dependent Variable**

| | Women | | Men | |
|---|---|---|---|---|
| Variables | Odds Ratio (95% CI) | *P* | Odds Ratio (95% CI) | *P* |
| Target population 1 | | | | |
| Unadjusted | 1.85 (0.93–3.66) | 0.78 | 1.23 (0.94–1.60) | 0.13 |
| Adjusted for age | 1.53 (0.73–3.21) | 0.26 | 1.37 (1.03–1.81) | 0.030 |
| Adjusted for age, TC, smoking status | 1.54 (0.73–3.24) | 0.26 | 1.28 (0.96–1.70) | 0.094 |
| Adjusted for age, TC, smoking status, BMI, SBP, TG, HDL-C, FPG | 0.87 (0.37–2.05) | 0.75 | 0.98 (0.69–1.39) | 0.89 |
| Target population 2 | | | | |
| Unadjusted | 1.23 (0.49–3.12) | 0.66 | 1.14 (0.84–1.55) | 0.39 |
| Adjusted for age | 1.04 (0.38–2.82) | 0.94 | 1.36 (0.98–1.88) | 0.063 |
| Adjusted for age, TC, smoking status | 1.05 (0.39–2.86) | 0.92 | 1.25 (0.90–1.73) | 0.19 |
| Adjusted for age, TC, smoking status, BMI, SBP, TG, HDL-C, FPG | 0.56 (0.18–1.72) | 0.31 | 0.93 (0.62–1.38) | 0.72 |
| Target population 3 | | | | |
| Unadjusted | 1.13 (0.40–3.16) | 0.82 | 1.14 (0.80–1.62) | 0.46 |
| Adjusted for age | 0.94 (0.31–2.83) | 0.91 | 1.43 (0.99–2.07) | 0.060 |
| Adjusted for age, TC, smoking status | 0.95 (0.31–2.88) | 0.92 | 1.31 (0.90–1.91) | 0.16 |
| Adjusted for age, TC, smoking status, BMI, SBP, TG, HDL-C, FPG | 0.44 (0.13–1.52) | 0.20 | 1.00 (0.64–1.57) | 1.00 |

TC indicates total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol.

**TABLE 4. Baseline Characteristics of the Target Population 2**

| Variables | Women | | | Men | | |
|---|---|---|---|---|---|---|
| | Carotid Plaque (−) (n=1774) | Carotid Plaque (+) (n=260) | P | Carotid Plaque (−) (n=2755) | Carotid Plaque (+) (n=872) | P |
| Age, y | 53.8±9.9 | 63.3±8.9 | <0.0001 | 53.1±10.0 | 62.6±9.8 | <0.0001 |
| BMI, kg/m² | 21.4±2.9 | 21.5±2.7 | 0.52 | 23.6±2.7 | 23.4±2.5 | 0.13 |
| SBP, mm Hg | 112±13112±13 | 116±14 | <0.0001 | 118±12 | 120±12 | <0.0001 |
| DBP, mm Hg | 70±8 | 70±9 | 0.21 | 74±8 | 74±8 | 0.18 |
| Optimal normal BP, n (%) | 1200 (68) | 141 (54) | <0.0001 | 1475 (54) | 384 (44) | <0.0001 |
| Normal BP, n (%) | 334 (19) | 62 (23) | 0.056 | 684 (25) | 280 (32) | <0.0001 |
| High normal BP, n (%) | 240 (13) | 57 (22) | 0.0003 | 596 (22) | 208 (24) | 0.17 |
| Total cholesterol, mg/dL | 214±35 | 225±33 | <0.0001 | 204±32 | 207±33 | 0.061 |
| High-density lipoprotein cholesterol, mg/dL | 71±17 | 71±17 | 0.63 | 55±15 | 55±15 | 0.88 |
| Triglycerides, mg/dL | 90±59 | 94±49 | 0.34 | 142±107 | 134±107 | 0.043 |
| Uric acid, mg/dL | 4.5±0.9 | 4.8±1.1 | <0.0010 | 6.1±1.2 | 6.1±1.2 | 0.83 |
| Fasting glucose, mg/dL | 90±14 | 91±12 | 0.32 | 99±20 | 101±22 | 0.0095 |
| Hemoglobin A1C, % | 5.1±0.5 | 5.3±0.4 | <0.0001 | 5.3±0.8 | 5.5±0.7 | <0.0001 |
| Metabolic syndrome, n (%) | 53 (3) | 11 (4) | 0.28 | 307 (11) | 103 (12) | 0.59 |
| Smoking status | | | | | | |
| Never, n (%) | 1471 (83) | 226 (87) | 0.27 | 871 (32) | 235 (27) | <0.0001 |
| Former, n (%) | 97 (5) | 11 (4) | | 781 (28) | 316 (36) | |
| Current, n (%) | 206 (12) | 23 (9) | | 1103 (40) | 321 (37) | |

$\chi^2$ test was used for categorical variables, and *t* test was used for continuous variables.

It has been suggested recently by several investigators that clinicians should evaluate and treat all cardiovascular risk factors regardless of whether a patient meets the diagnosis criteria for MetS.[22,23] In fact, individuals with established dyslipidemia, hypertension, and diabetes may be periodically followed-up at medical institutions and undergo medical therapy or lifestyle modification to ameliorate each of these disorders, regardless of the presence or absence of MetS. On

**TABLE 5. Baseline Characteristics of the Target Population 3**

| Variables | Women | | | Men | | |
|---|---|---|---|---|---|---|
| | Carotid Plaque (−) (n=1745) | Carotid Plaque (+) (n=255) | P | Carotid Plaque (−) (n=2598) | Carotid Plaque (+) (n=800) | P |
| Age, y | 53.8±9.9 | 63.3±8.9 | <0.0001 | 52.8±10.0 | 62.7±10.0 | <0.0001 |
| BMI, kg/m² | 21.4±2.9 | 21.6±2.7 | 0.37 | 23.5±2.6 | 23.3±2.5 | 0.21 |
| SBP, mm Hg | 112±13 | 116±14 | <0.0001 | 118±12 | 120±12 | <0.0001 |
| DBP, mm Hg | 70±8 | 70±9 | 0.26 | 74±8 | 74±8 | 0.44 |
| Optimal normal BP, n (%) | 1180 (68) | 139 (55) | <0.0001 | 1409 (54) | 346 (43) | <0.0001 |
| Normal BP, n (%) | 329 (19) | 60 (24) | 0.078 | 637 (25) | 258 (32) | <0.0001 |
| High normal BP, n (%) | 236 (14) | 56 (22) | 0.0004 | 552 (21) | 196 (25) | 0.052 |
| Total cholesterol, mg/dL | 214±36 | 225±33 | <0.0001 | 204±32 | 207±34 | 0.023 |
| High-density lipoprotein cholesterol, mg/dL | 71±17 | 71±17 | 0.69 | 55±15 | 56±16 | 0.49 |
| Triglycerides, mg/dL | 90±58 | 94±50 | 0.26 | 140±105 | 132±108 | 0.067 |
| Uric acid, mg/dL | 4.5±0.9 | 4.8±1.1 | 0.0014 | 6.1±1.2 | 6.1±1.1 | 0.69 |
| Fasting glucose, mg/dL | 89±9 | 90±10 | 0.047 | 95±10 | 96±10 | 0.11 |
| Hemoglobin A1C, % | 5.0±0.4 | 5.2±0.4 | <0.0001 | 5.2±0.4 | 5.3±0.4 | <0.0001 |
| Metabolic syndrome, n (%) | 46 (3) | 11 (4) | 0.13 | 238 (9) | 77 (10) | 0.69 |
| Smoking status | | | | | | |
| Never, n (%) | 1448 (83) | 221 (87) | 0.33 | 829 (32) | 220 (28) | 0.0008 |
| Former, n (%) | 93 (5) | 11 (4) | | 745 (29) | 284 (36) | |
| Current, n (%) | 204 (12) | 23 (9) | | 1024 (39) | 296 (37) | |

$\chi^2$ test was used for categorical variables, and *t* test was used for continuous variables.

the other hand, if individuals who do not have overt hemodynamic or metabolic abnormalities are at higher risk for atherosclerotic diseases when they have MetS, this concept may be useful for isolating individuals at higher risk for atherosclerotic diseases from those with low-risk profiles. For these reasons, we selected individuals with low-risk profiles to assess the usefulness of the MetS concept, and the odds ratio of MetS for carotid atherosclerosis has been calculated after adjusting for other conventional risk factors.

In the current study MetS was not found to be an independent predictor for either carotid plaque or carotid intima-media thickening in the individuals with optimal, normal, or high normal BP; however, we cannot jump to the conclusion that the concept of MetS is insignificant in such population. We may also have to investigate the possible association between MetS and other conditions, such as lacunar infarctions and arterial stiffness. These points should be analyzed in future studies.

The strength of our study is that we could abstract and analyze the data of individuals with optimal, normal, and high-normal BP from a large set of cross-sectional data of those who had undergone general health screening. On the other hand, our study has some limitations. For example, waist circumference data were not available in the study sample; thus, we used BMI as a surrogate of waist circumference, as has been done in several previous studies.[24,25] Although the ability of MetS to predict CVD may differ according to the criteria used[26] and ethnicity,[27] waist circumference may be a more suitable risk factor component for the definition of MetS because of its strong correlation with computed tomography measurements of abdominal fat.[28] In the near future, we are planning to validate the use of other MetS criteria, including waist circumference data, which have now been collected at our institute since 2005, in terms of isolating individuals with higher risk for early atherosclerosis from those with low-metabolic/hemodynamic risk profiles. Because our study was cross-sectional in nature, whether or not MetS, as defined here, would be useful in predicting future cardiovascular events cannot be determined. This point should also be assessed in future studies.

## Perspectives

In the current study, by analyzing the cross-sectional data from individuals who had optimal, normal, or high-normal BP, we showed that MetS defined by modified ATPIII criteria was not an independent predictor for either carotid plaque or carotid intima-media thickening. A body of evidence exists that supports the notion that MetS is a risk factor for atherosclerotic disease. On the other hand, clinicians may have to evaluate and treat all of the atherogenic risk factors regardless of whether a patient meets the diagnosis criteria for MetS. If individuals with only mild hemodynamic and metabolic abnormalities are considered to be at substantially higher risk for CVDs when they have MetS, the concept of MetS would be useful in avoiding the underestimation of the cardiovascular risk in such subjects. Although our data did not support that MetS was an independent risk factor for carotid atherosclerosis in individuals without hypertension, whether the presence of MetS in such subjects would increase the possibility of the future development of CVD should be investigated in longitudinal studies.

## Disclosures

None.

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トピックス

# メタボリックシンドロームと喫煙

石坂裕子[*] 石坂信和[**] 山門 実[*]

Keyword
メタボリックシンドローム，喫煙，動脈硬化，炎症，白血球

## はじめに

メタボリックシンドロームの根底にはインスリン抵抗性があると考えられており，喫煙が，インスリン抵抗性を亢進することについては，以前からいくつか報告されている[1]．また，間接喫煙であっても喫煙がメタボリックシンドロームのリスクを増加させる，ということが最近米国から報告されている[2]．喫煙によるインスリン抵抗性亢進，あるいはメタボリックシンドローム合併の機序については不明な点も多いが，アディポサイトカインの分泌に変化が生じている可能性も指摘されている．

喫煙とメタボリックシンドロームに関連があるならば，いくつかの質問が思いつくだろう．喫煙の量や期間はどのように，メタボリックシンドロームの頻度に影響を与えているのだろうか？ 禁煙するとメタボリックシンドロームがよくなるのであろうか？ メタボリックシンドロームは，さまざまな動脈硬化の危険因子の重複した状態ととらえることもできる．喫煙がメタボリックシンドロームを増加させるならば，メタボリックシンドローム発症により，喫煙が動脈硬化を増加させているのであろうか？ さらに，喫煙者のなかにもメタボリックシンドロームを合併しない症例がいるわけだが，喫煙者のなかで，メタボリックシンドロームを合併している症例の特徴はなんなのか？

メタボリックシンドロームが生活習慣病として注目されている現在，わが国の現状を解析し，これらの疑問点に対して答えることは，健康管理，予防医学の観点から重要であるといえる．

## 人間ドック受診者におけるメタボリックシンドロームの頻度

筆者らは，三井記念病院総合健診センターにおいて頸動脈超音波検査を含む健康評価を受けた35～65歳の5,033症例（男性3,300症例，女性1,733症例）のデータを解析し，メタボリックシンドロームの頻度を検討した．平均年齢は，男性53.3歳，女性53.5歳であった．

本検討では，メタボリックシンドロームの診断基準は，以下の5項目のうち3項目以上を満たした場合とした．

(1) 中性脂肪 150 mg/dl 以上
(2) HDL-コレステロール 40 mg/dl 未満（男性），50 mg/dl 未満（女性）
(3) 空腹時血糖 110 mg/dl 以上か糖尿病治療中
(4) 収縮期/拡張期血圧 130/85 mmHg 以上か降圧剤使用中
(5) BMI 25 kg/m$^2$以上

この基準を用いた場合，対象群のメタボリックシンドロームの頻度は，男性で20％，女性で7％と，男性で約3倍近く多かった．

## メタボリックシンドロームと喫煙状況

喫煙状況を確認したところ，男性では，非喫煙群32％，過去喫煙群30％，現喫煙群39％，女性では，非喫煙群84％，過去喫煙群4％，現喫煙群12％であった．喫煙状況別メタボリックシンドロームの頻度を図1に示す．男女とも喫煙によりメタボリックシンドロームの頻度が増加している．

つぎに喫煙本数別に検討した．その結果，喫煙数が増すほど，メタボリックシンドロームに対するオッズ比が高くなること，過去喫煙群，喫煙群いずれも，1日10本

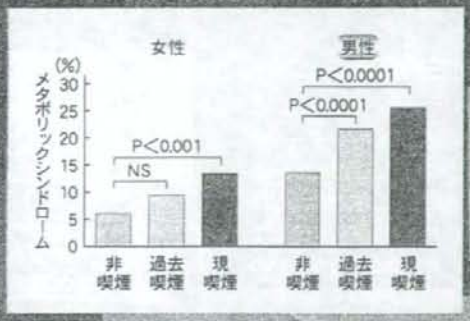


図1　喫煙状況とメタボリックシンドロームの頻度
（文献*より改変して引用）

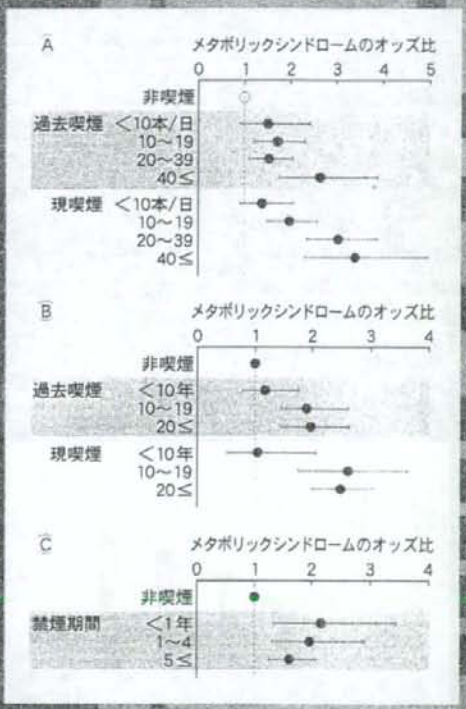


図2　喫煙本数，年数，禁煙期間とメタボリックシンドローム（文献*より改変して引用）

未満の喫煙では，メタボリックシンドロームの頻度が上昇しないことなどがわかった（図2A）．喫煙年数別で同様の検討を行うと，過去喫煙群，喫煙群いずれも，10年以上の喫煙歴でメタボリックシンドロームの頻度が上昇していた（図2B）．さらに，過去喫煙群で禁煙期間別に検討すると，5年以上の禁煙期間があっても，非喫煙群より依然としてメタボリックシンドロームの頻度が有意に高かった（オッズ比1.46，95% CI 1.25～2.05，P=0.002）．

## 喫煙，メタボリックシンドローム，頸動脈硬化の関連

メタボリックシンドロームは，動脈硬化のリスクを加算させると考えられているが，では，メタボリックシンドロームが喫煙と動脈硬化の間を介在している可能性はあるだろうか．そうであれば，メタボリックシンドローム非合併例では，喫煙は動脈硬化の危険因子となっていないはずである．

そこで上記の症例から，メタボリックシンドロームの非合併例のみを抽出し，喫煙が頸動脈硬化（頸動脈プラーク）の危険因子となっているかどうかについて検討した．メタボリックシンドロームを合併していない症例に限定した場合，多変量解析を行った結果，過去喫煙群は，オッズ比1.45（95% CI 1.14～1.84，P=0.0023），現喫煙群は，オッズ比1.47（95% CI 1.17～1.84，P=0.001）と，非喫

煙群に比較して，有意に頸動脈硬化の頻度が高かった．すなわち，メタボリックシンドロームのない症例においても，喫煙群では動脈硬化の頻度が高いということである．このことは，メタボリックシンドロームが喫煙と動脈硬化の間の介在因子である，とは必ずしも結論づけられないことを意味している．

## 喫煙群でメタボリックシンドロームを合併している症例の特徴とは

喫煙者のうち，メタボリックシンドローム合併例には，検査データ上なにか特徴があるだろうか．最近，メタボリックシンドロームの症例でinterleukin 18の活性化[4]，白血球数の増加，CRP亢進[5]などが認められることから，メタボリックシンドロームと炎症との関連が注目されている．ただし，慢性炎症が存在するとメタボリックシンドロームが生じやすくなるのか，メタボリックシンドロームが存在すると炎症が増悪しやすいのか，ということは結論づけられない．

白血球も炎症の一つのマーカーであるが，喫煙は血中の白血球数を増加させることが報告されている．では，喫煙者でメタボリックシンドロームを合併している症例では白血球数が増加しているのだろうか？

## 喫煙と末梢血白血球数

この点について，筆者らは，三井記念病院総合健診センターを受診した男性27,972症例のデータを解析し検討した．前述の検討に比較して，対象を男性に限定していながら，症例数がかなり多いのは，頸動脈超音波検査を施行していない症例も含んでいるためである．対象症例のうち，非喫煙群は9,729例（35％），過去喫煙群7,242例（29％），現喫煙群11,001例（39％）であった．白血球数（$\times 10^3/\mu l$）は，過去喫煙群で5.4±1.3，現喫煙群で6.4±1.8と，いずれも非喫煙群の5.2±1.3に比較して有意に多かった．各四分位の，白血球数（$\times 10^3/\mu l$）は表1のとおりである．白血球数が，第3，第4の四分位に入っている症例は，非喫煙群では36％，過去喫煙群では43％，現喫煙では69％であった．

表1　各四分位の白血球数（$\times 10^3/\mu l$）

| | |
|---|---|
| 第1 | 2.1～4.6 |
| 第2 | 4.7～5.4 |
| 第3 | 5.5～6.5 |
| 第4 | 6.6～22.0 |

## メタボリックシンドロームと白血球数

メタボリックシンドロームの診断基準は前述の検討と同じものを用いた．白血球数の四分位をとると，白血球数の低いほうから，メタボリックシンドロームの頻度は5.7％，11.4％，15.8％，22％と白血球数に応じて明らかに増加していた．では，喫煙状況を考慮した場合，白血球とメタボリックシンドロームの関連はどのようになるのだろうか．

図3に喫煙状況，白血球数の四分位とメタボリックシンドロームの頻度をグラフにした．一見すると，喫煙状況のいかんにかかわらず，白血球数の増加に応じて，メ

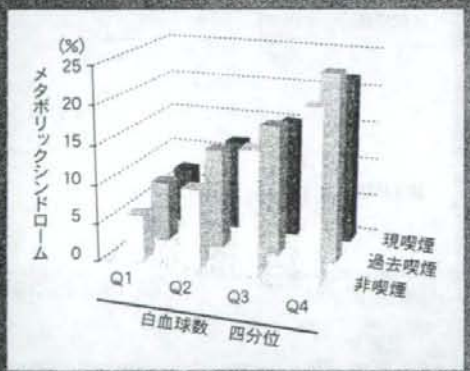


図3　喫煙状況，白血球数とメタボリックシンドロームの頻度

タボリックシンドロームの頻度が増加しているようにみえる．ここではしかし，喫煙群では非喫煙群よりも白血球数の四分位が高いほうにシフトしている点に留意する必要がある．

年齢，TC で補正した多変量解析で検討すると，第1または，第2の白血球の四分位に入る症例では過去喫煙，現喫煙はいずれもオッズ比 1.3〜1.4 のメタボリックシンドロームの独立した危険因子であった．しかし，図3からも理解されるように，第1，第2の白血球の四分位における非喫煙群のメタボリックシンドロームの頻度は比較的低く，それゆえ，喫煙による増加分もあまり目立たないといえる．他方で，第3，第4の白血球の四分位に入る症例では，現喫煙も過去喫煙もメタボリックシンドロームの頻度を有意に上昇させない[6]．

今回の検討では，喫煙が白血球増加に働くこと，喫煙の有無にかかわらず白血球数が比較的多い症例においてメタボリックシンドロームの頻度が増加していることがみてとれる．本研究がクロスセクショナルな検討であることから，喫煙，白血球数，メタボリックシンドロームの因果関係については言及できない．しかし，白血球数をみれば，喫煙者のなかから，メタボリックシンドロームの合併しやすい群を抽出できる可能性がある，と考えることもできるだろう．

## まとめと今後の展望

本研究では人間ドック受診者のデータを解析し，喫煙とメタボリックシンドロームの関連を探った．その結果，喫煙が容量依存性，喫煙期間依存性にメタボリックシンドロームの頻度増加に関連していること，禁煙後5年以上経過しても，非喫煙群に比較して，メタボリックシンドロームの頻度が依然として高いことが示された．また，喫煙が白血球増加と関連していること，白血球数が多い症例では，喫煙状況のいかんにかかわらずメタボリックシンドロームの頻度が増加していることから，喫煙群のなかから，白血球数の増加の有無によって，メタボリックシンドロームのハイリスク群を抽出できる可能性があることが示された．

これらの点をふまえ，今後解決すべきいくつかの課題が残る．

①禁煙により，はたして白血球数は減少するのか，また，禁煙後，白血球数の減少した群では，上昇し続けている群に比較して，メタボリックシンドロームの頻度が低下するのか．

②メタボリックシンドロームが合併していない症例に限定した場合，喫煙群で白血球が多い群は，白血球が少ない群に比較して，「将来的なメタボリックシンドロームのリスク」が増加するのか．

③非喫煙群で白血球が多い症例は，なにか特徴があるのか，たとえば，間接喫煙とは関連があるのか．

これらの点を解決するためには，プロスペクティブな検討も必要になると考えられる．喫煙，白血球増加，メタボリックシンドロームの関係を明らかにすることは，メタボリックシンドロームの発症機序の解明，ハイリスク群の抽定，メタボリックシンドロームの予防などに役立つものと考えられ，今後の検討が期待される．


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VI. 予防・治療・管理

ライフスタイルへの介入によるメタボリックシンドロームの予防と治療
禁煙指導

# メタボリックシンドローム診療における禁煙指導の重要性

Importance of smoking cessation intervention for subjects with metabolic syndrome

石坂裕子[1]　石坂信和[2]　山門　實[1]

Key words：喫煙，禁煙，メタボリックシンドローム，インスリン抵抗性

## はじめに

厚生労働省の平成16年国民健康・栄養調査によると，中高年の男性の2人に1人，女性では5人に1人がメタボリックシンドローム，もしくは，その"予備軍"である．メタボリックシンドロームは虚血性心疾患，動脈硬化性疾患の危険因子の一つであり，その予防，治療は，国民の健康維持および，医療費の削減の両方の観点から，重要な課題であるといえる．

喫煙の有害性は，発癌，動脈硬化性疾患，慢性閉塞性肺疾患などに対しては周知のとおりであり，'タバコを吸わないこと'は，重要な生活習慣上のポイントであることは，一般にも広く認識されつつある．日本における嫌煙権運動の始まりは30年ほど前にさかのぼるが，最近10年間は殊に'職場における喫煙対策のためのガイドライン'の策定，健康増進法の施行，各学会からの禁煙宣言，禁煙関連9学会合同禁煙ガイドラインなどの発表などがあり，禁煙への意識がますます高まっている．しかしながら，我が国の成人男性の喫煙率は43％と欧米諸国に比べ高く，女性においても若い年齢層の喫煙率は上昇傾向にある．

喫煙は糖代謝に悪影響を与えることが知られており，糖尿病の発症率を増加させることも報告されている．本稿では，喫煙がどの程度メタボリックシンドロームの頻度を増加させているのか，その機序としてどのようなことが考えられているのか，また，禁煙はどのような効果があるのかについて，現在までに報告されていることについて概説する．

## 1. 喫煙・禁煙とインスリン抵抗性

メタボリックシンドロームの発症および進展の基盤には，インスリン抵抗性が存在すると考えられている．Facchini ら[2]は，喫煙者では非喫煙者に比較して，インスリン抵抗性が上昇していることから，喫煙がインスリン抵抗性亢進を介して動脈硬化を促進する可能性を示唆している．また彼らは，喫煙群では，非喫煙群より中性脂肪が高く，HDLコレステロールが低いことも示しており，喫煙は脂質代謝にも好ましくない影響を与える，と報告している．Eliasson ら[3]は，1日10本以上を20年以上喫煙していた症例を対象に8週間禁煙させた群と，喫煙継続

[1]Yuko Ishizaka, Minoru Yamakado: Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital 三井記念病院 総合健診センター　[2]Nobukazu Ishizaka: Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine 東京大学医学部附属病院 循環器内科

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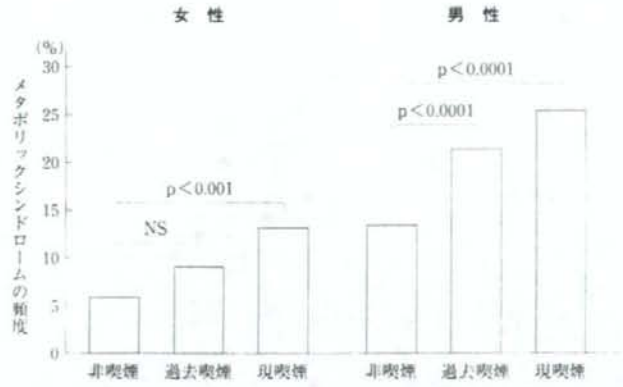


**図1 喫煙状況別のメタボリックシンドロームの頻度**（文献 より改変）

群に分けることにより，禁煙がインスリン感受性に与える影響を比較している．その結果，禁煙群でのみ，インスリン感受性が改善していた．彼らはまた，禁煙は，BMI，体脂肪の増加に働くものの，LDLコレステロール，HDLコレステロールなどの脂質プロファイルは改善することを報告している．

## 2. 喫煙・禁煙とメタボリックシンドローム

### a. 喫煙とメタボリックシンドローム

喫煙とメタボリックシンドロームの関連について，受動喫煙の事例を含めて幾つかの報告がなされている[4]．Ohら[5]は，The Korea National Health and Nutrition Examination Surveyの結果から，喫煙が用量依存性にメタボリックシンドロームの頻度を増加させることを示している．彼らはまた，喫煙が用量依存性に中性脂肪上昇とHDLコレステロール低下の頻度を増加させていることも見いだしており，前述のFacchini，Eliassonらの報告と同様の知見であるといえる．彼らの検討では，現喫煙者では，用量依存性に腹部肥満が増大していた．日常臨床においては，禁煙すると太る，というケースを少なからず経験する．しかし，Ohらの知見や，Brinkman indexが554以上の喫煙者において，内臓脂肪が増加していた，という我が国の知見[6]を合わせて考えると，内臓脂肪という観点からは，喫煙は必ずしも有利に働かないと考えられる．

人間ドックを受診した5,033症例のデータを解析することにより，著者らも喫煙とメタボリックシンドロームの関連について検討した[7]ところ，男女とも喫煙によりメタボリックシンドロームの頻度は増加していた（**図1**）．多変量解析においても，現喫煙，過去喫煙は，用量依存性にメタボリックシンドロームの頻度が上昇しており，1日10本超の喫煙はメタボリックシンドロームの頻度を有意に増加した（**図2-a，b**）．

### b. 禁煙とメタボリックシンドローム

では，禁煙により，メタボリックシンドロームの頻度は減少するのであろうか．著者らの検討[7]では，禁煙期間が長いほど，メタボリックシンドロームのオッズ比は低下するという結果であった（**図2-c**）．これは，禁煙がメタボリックシンドロームの頻度を減少させる可能性を示唆しているが，cross-sectionalスタディなのではっきりとは結論づけられない．

前述のEliassonらの論文[3]でも述べられているとおり，禁煙で中性脂肪は減少，HDLコレステロールは上昇する[8]．中性脂肪，HDLコレステロールがメタボリックシンドロームのコンポーネントであることを考えると，禁煙はやはりメタボリックシンドロームの頻度を減少させる可能性がある．

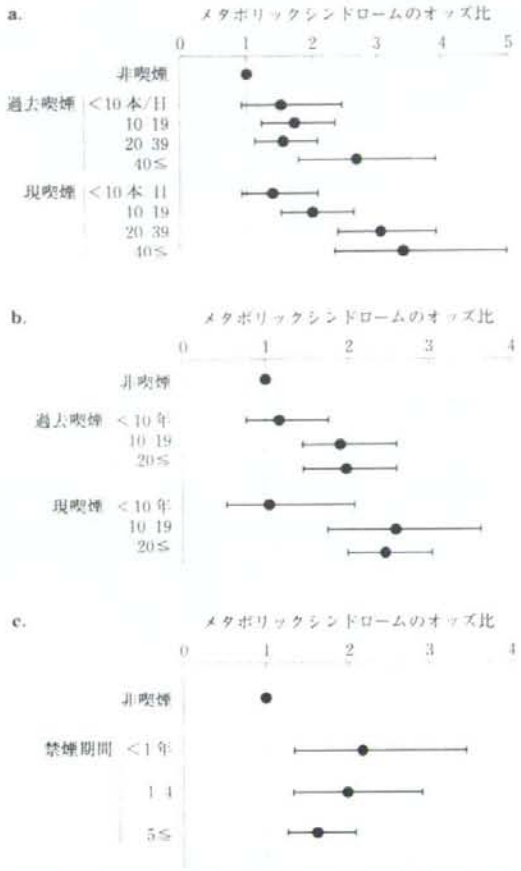


**図2 喫煙本数，年数，禁煙期間とメタボリックシンドロームの頻度**
（文献[7]より改変）
年齢，性別，総コレステロール値で補正したロジスティック回帰分析の結果．
a：喫煙本数別，b：喫煙年数別，c：禁煙期間別．

一方，著者らのデータでは，年齢，総コレステロール値で補正したロジスティック回帰分析では，男性においてメタボリックシンドロームに対するオッズ比は現喫煙群では2.3(95%CI 1.4-2.2)であるが，5年以上禁煙している症例でも，依然として非喫煙者に比較してメタボリックシンドロームの頻度が高い(**図2** c)．Tonstadら[7]の報告でも，メタボリックシンドロームのオッズ比は，非喫煙群と比較して現喫煙群，過去喫煙群において，いずれも2.2-2.3と大差ない．過去喫煙者においてもメタボリックシンドロームの頻度が非喫煙者より高いということは，"はじめから吸わないこと"の重要性を示唆しているといえるだろう．

### c．喫煙とメタボリックシンドロームを結びつけるもの

喫煙やニコチンの薬理学的作用については多くの研究がなされているが，喫煙がメタボリッ

クシンドロームをどのような機序で増加させるのかについては不明な点も多い．喫煙がインスリン抵抗性を亢進させる可能性[23]については前述した．Eliassonら[10]は，ニコチンガムの使用でもインスリン抵抗性が増加することから，ニコチンがタバコ成分の中でインスリン抵抗性増加に強く関与している因子の一つであろう，と結論している．一方，イタリアのMasulliら[11]のグループは，喫煙がメタボリックシンドロームの頻度を増加させることや，用量依存性に中性脂肪上昇・HDLコレステロール低下と関連があることを確認する一方，喫煙が(インスリン抵抗性亢進によって生じてくるであろう)高血圧や高血糖の頻度は増加させないことから，必ずしも喫煙とメタボリックシンドロームの関連をインスリン抵抗性の亢進ということで説明できない可能性を指摘している．喫煙が血圧を上昇させるか，また，禁煙が血圧を下げるか，という議論は，禁煙による肥満の問題と相まって単純ではないかもしれない[12]．

Iwashimaら[13]は，喫煙がメタボリックシンドロームの発症に密接に関連しているアディポネクチンの血中濃度を低下させることを報告している．そのほか，喫煙とメタボリックシンドロームを結ぶものとして，ニコチンの遊離脂肪酸増加作用，抗エストロゲン作用によるHDLコレステロールの低下，慢性炎症状態などの関与の可能性がある．

## 3. メタボリックシンドローム診療における禁煙指導

メタボリックシンドロームという診断がなされても，動脈硬化性疾患や糖代謝疾患への危険性が一層増加している，と感じている人は少なく，禁煙に対する本人の動機付けが十分でない場合も多い．メタボリックシンドローム診療では，無関心期や関心期にある人を準備期，実行期へ導くことが重要である．そのためには，禁煙を含めた食事，運動などの生活習慣の改善が必須である．また，診療のたびに，喫煙状況，禁煙への意思，禁煙への決断の障害などを尋ね，定期的に助言していかなくてはならない．

著者らの検討では，過去喫煙群でも，メタボリックシンドロームの頻度が非喫煙群より高かった[1]．しかし，喫煙本数が1日10本以下，喫煙年数が10年以下の場合は，現喫煙群，過去喫煙群ともメタボリックシンドロームの頻度の有意な増加を認めていない(**図2 a, b**)ことから，早期に喫煙習慣を解消することは有意義であると考えられる．禁煙後もメタボリックシンドロームのリスク上昇が依然として残存していることに関しては，禁煙後の肥満が悪影響を及ぼしている可能性もある[9]．我が国においても，Nakanishiら[14]は，禁煙した症例では7年の経過観察中に，21%の症例において5kg以上の体重増加を認め，同じ期間で，非喫煙者，喫煙継続者では5kg以上体重が増加したのは9-11%であったことと比較してより高率であったと報告している．これらの知見は，メタボリックシンドロームの予防・改善のために，禁煙後の体重増加に対する指導も重要であることを意味している．

## おわりに

喫煙はメタボリックシンドロームの危険因子である．その影響は用量依存性であり，また，禁煙後もメタボリックシンドロームのリスクは依然高い．それゆえ"やめること"と同時に，"最初から吸わない"ことが重要である．喫煙は飲酒と並んで個人の嗜好ととらえられがちである．しかし，受動喫煙もメタボリックシンドロームの危険因子となる可能性があり，喫煙が本人のみならず周囲の人にとってどのような問題であるかをきちんと説明し禁煙に導く必要がある．また，禁煙後の体重増加を抑制することも禁煙指導上重要なポイントである．

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*Original Article*

# Angiotensin II–Induced Regulation of the Expression and Localization of Iron Metabolism–Related Genes in the Rat Kidney

Nobukazu ISHIZAKA[1], Kan SAITO[1], Kyoko FURUTA[1], Gen Matsuzaki[1], Kazuhiko KOIKE[2], Eisei NOIRI[3], and Ryozo NAGAI[1]

**Due to recent discoveries of novel genes involved in iron metabolism, our understanding of the molecular mechanisms underlying iron metabolism has dramatically increased. We have previously shown that the administration of angiotensin II alters iron homeostasis in the rat kidney, which may in turn aggravate angiotensin II–induced renal damage. Here we have investigated the effect of angiotensin II administration on the localization and expression of transferrin receptor (TfR), divalent metal transporter 1 (DMT1), ferroportin 1 (FPN), and hepcidin mRNA in the rat kidney. Weak expression of TfR, DMT1, FPN, and hepcidin mRNA was observed in the kidneys of control rats. In contrast, after 7 days of angiotensin II infusion by osmotic minipump, the expression of these mRNAs was more widely distributed. Staining of serial sections revealed that some, but not all, of the renal tubular cells positive for these genes contained iron deposits in the kidney of angiotensin II–infused animals. Real-time polymerase chain reaction (PCR) showed that the mRNA expression of TfR, iron-responsive element–negative DMT1, FPN, and hepcidin mRNA increased ~1.9-fold, ~1.7-fold, ~2.3-fold, and ~4.7-fold, respectively, after angiotensin II infusion as compared with that of untreated controls, and that these increases could be suppressed by the concomitant administration of losartan. Our data demonstrate that these genes were unequivocally expressed in the kidney and could be regulated by angiotensin II infusion. The relative contribution, if any, of these genes to renal and/or whole-body iron homeostasis in various disorders in which the renin angiotensin system is activated should be investigated in future studies. (*Hypertens Res* 2007; 30: 195–202)**

***Key Words*: angiotensin II, iron metabolism, hypertension, gene regulation**

## Introduction

Iron is an essential element that is required for fundamental cell functions in all living organisms. On the other hand, excess body iron is potentially harmful because of its ability to catalyze the conversion of hydrogen peroxide to toxic free radicals. Thus, maintaining an appropriate balance of iron in the body is important. Recently, our understanding of the mechanisms underlying iron metabolism has dramatically increased due to discoveries of novel genes related to iron metabolism. Divalent metal transporter 1 (DMT1), also referred to as natural resistance–associated macrophage protein 2 (Nramp2) or divalent cation transporter (DCT1), is a

From the [1]Departments of Cardiovascular Medicine, [2]Infectious Diseases, and [3]Nephrology and Endocrinology, University of Tokyo Graduate School of Medicine, Tokyo, Japan.

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Address for Reprints: Nobukazu Ishizaka, M.D., Ph.D., Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Hongo 7-3-1, Bunkyo-ku, Tokyo 113–8655, Japan. E-mail: nobuishizka-tky@umin.ac.jp

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12-segment transmembrane-spanning integral membrane protein (*1*) that is expressed in the duodenum, where it transports divalent metals across the apical membrane of enterocytes. A G185R point mutation of this gene causes microcytic hypochromic anemia in rodents (*2*, *3*), thus indicating the fundamental role played by this gene in iron homeostasis. Ferroportin 1 (FPN), also referred to as metal transporter protein (MTP1) or iron-regulated protein 1 (Ireg1), is a multiple transmembrane-spanning protein that transports iron out of cells. FPN is expressed strongly in the basolateral region of absorptive duodenal enterocytes and in tissue macrophages in the liver, *i.e.*, Kupffer cells (*4*, *5*). Hepcidin (hepc) is a recently discovered cysteine-rich 25-amino acid peptide that has antimicrobial properties and also acts as a negative regulator of intestinal iron absorption and macrophage iron release, and its overproduction may contribute to the anemia associated with inflammation (*6*). Hepc was initially isolated from human urine (*6*); however, it is mainly produced in the liver, from where it is released into the systemic circulation. Binding of hepc to the iron exporter FPN leads to the internalization and degradation of FPN (*7*, *8*); it is presumably by this mechanism that hepc functions as a negative regulator of intestinal iron absorption.

In general, the kidney is not considered to be the major expression site of these newly discovered iron metabolism–related genes. Although expression of the transferrin receptor (TfR) (*9*, *10*), DMT1 (*11*, *12*), FPN (*13*), and hepc (*14*) has been demonstrated in the kidney, information about the physiological importance and the regulation of these genes has been limited to date. We previously reported that the administration of angiotensin II to rats causes prominent iron deposition in the kidney, which occurs primarily in the proximal tubular epithelial cells; this deposition is thought to be associated with increased proteinuria and the upregulation of fibrosis-related genes (*15*, *16*). Here, we have characterized the renal expression patterns of these iron metabolism–related genes and investigated how their expression might be regulated by angiotensin II in the kidney.

## Methods

### Generation of Animal Models

The experiments were performed in accordance with the guidelines for animal experimentation approved by the Animal Center for Biomedical Research, Faculty of Medicine, University of Tokyo. Angiotensin II was continuously infused into male Sprague-Dawley rats by subcutaneous implantation of an osmotic minipump (Alzet model 2001; Alza Pharmaceutical, Mountain View, USA) as described previously. In brief, Val5-angiotensin II was infused at doses of 0.7 mg/kg/day for 7 days by subcutaneously implanted osmotic minipumps (Alza Pharmaceutical) which exerted hypertensive effects (192±5 mmHg [$n$=12], $p$<0.01 *vs.* control rats, 131±3 mmHg [$n$=6]). In some experiments, the selective angiotensin type 1 ($AT_1$) receptor antagonist, losartan (25 mg/kg/day) or the nonspecific vasodilator, hydralazine (15 mg/kg/day) (Sigma Chemical, St. Louis, USA) was given in the drinking water, beginning 2 days before pump implantation and throughout angiotensin II infusion (angiotensin II+losartan, 126±5 mmHg [$n$=7]; angiotensin II+hydralazine 126±3 mmHg [$n$=7]). In some experiments, norepinephrine was infused at a dose (2.8 mg/kg/day) which exerted hypertensive effects (192±4 mmHg) comparable to those of angiotensin II. Systolic blood pressure was measured in conscious rats by tail-cuff plethysmography (Ueda Seisakusho, Tokyo, Japan).

### *In Situ* Hybridization, Histological Analyses

Rat cDNAs corresponding to rat sequences of TfR, DMT1, FPN1, and hepc were obtained by subcloning the reverse-transcription (RT)–polymerase chain reaction (PCR) product using rat kidney mRNA. Sense and antisense primers were as follows: 5′-TACCTGTGCAGACGATCTCAAGAG-3′ and 5′-AGGACGACTTTATCCAGATTAAT-3′, respectively, for TfR; 5′-CTACCTGGATCCAGGAAACATT-3′ and 5′-AAGTACTTATTGGCTTCTCGAA-3′, respectively, for DMT1; 5′-AGACCCCTGCTCTGGCT GTA-3′ and 5′-AGA CACATTAGCATAAGCAT-3′, respectively, for FPN; and 5′-GGCAGGACAGAAGGCAAGAT-3′ and 5′-GGTAGG ACAGGAATAAATAAT-3′, respectively, for hepc. The sequence targeted for the amplification of DMT1 was a common region of DMT1 with or without iron-responsive element (IRE), which was designated here as IRE(+)DMT1 and IRE(−)DMT1, respectively. Rat cDNA corresponding to these iron metabolism–related genes were subcloned into a pGEM-T vector, and then *in situ* hybridization was performed as described previously (*17*). After digestion with a restriction enzyme and linearization of the plasmid, antisense and sense cRNA riboprobes were transcribed *in vitro* using the DIG RNA labeling Kit SP6/T7 (Roche Diagnostics, Basel, Switzerland). Hybridization was performed using *In Situ* Hybridization Reagents (Nippon Gene, Tokyo, Japan). *In situ* hybridization was performed on either formalin-fixed specimens or un-fixed frozen specimens. Prussian blue staining was used for iron staining, and Oil red O staining was used to detect the accumulation of lipid in unfixed frozen tissue sections.

### RNA Extraction, Northern Blot Analysis, and Real Time RT-PCR

Total RNA was isolated from homogenized aorta by the acid guanidinium thiocyanate–phenol chloroform method as described previously (*18*). To investigate the mRNA expression by quantitative PCR with gene-specific HybriProbes was performed by LightCycler (Roche Diagnostics). The following respective sense and antisense primers were used: 5′-AAGTCCTGCTGAGCGAAGAT-3′ and 5′-TGGTCCCTA