these patients the HO-1-positive cell number was well correlated with the microvessel number ($R=0.675$, $p<0.01$). This relationship was also statistically significant in both the DM ($R=0.762$, $p<0.01$) and NDM groups ($R=0.564$, $p<0.01$), but the correlation between the DM and NDM groups was not statistically significant.

3.6. Topographical characteristics of HO-1 expression in atheromatous plaque (lesion type IV) in DM and NDM

Fourteen tissue specimens of AHA-type IV lesion (5 of DM and 9 of NDM) were examined to confirm whether there were any differences in the degree of HO-1 expression among the topographically different areas of atheromatous plaque as described in Section 2. The number of HO-1-positive cells showed a significant variance ($p<0.0001$, Kruskal–Wallis rank test) among the four location areas of AHA-type IV lesions of DM compared with NDM, and the HO-1-positive cell density was significantly higher in the S and Fc regions of DM than in those of NDM ($p<0.01$ and $p<0.05$, respectively), but not in the D and O regions (Supplementary data Fig. II).

4. Discussion

Clinical accumulating evidence indicates that DM increases the incidence of and accelerates atherosclerotic diseases [3]. In the present study, to analyze the pathophysiological role of HO-1 in coronary atherogenesis of diabetic subjects, HO-1 expression was immunohistochemically examined in the coronary arteries of 53 Japanese autopsied patients composed of 19 type 2 diabetics and 34 age- and sex-matched nondiabetic subjects, all of whom had been surveyed in the "Hisayama cohort study" [18,19]. We directly clarified the following key observations: (1) The HO-1 was ubiquitous in human coronary atherosclerotic lesions and was largely expressed by macrophages and ECs of both coronary arteries and intimal newly formed microvessels, and partly by SMCs in atherosclerotic intimas. (2) The extent of HO-1 expression and macrophage infiltration increased as the lesion type and stenotic grade progressed, and was significantly higher not only in early lesions but also in advanced lesions in the DM group compared with the NDM group. (3) Interestingly, the distribution of HO-1-positive cells was accentuated in coronary atherosclerotic lesions with newly formed microvessels in the DM group. These findings indicate that HO-1 expression is intimately associated with human coronary atherogenesis including intimal angiogenesis. Though HO-1 could play an anti-inflammatory role in atherosclerotic lesions, the possible participation of HO-1 in the intimal angiogenesis of atherosclerosis may also be responsible for the progression of atherosclerosis and plaque instability, particularly in diabetic subjects.

Few reports studying the detailed pathological characteristics of HO-1 expression in human atherosclerotic lesions are available [17]. To our best knowledge, this is the first report indicating that HO-1 expression is ubiquitously distributed in human coronary atherosclerotic lesions and is significantly enhanced in diabetic compared with nondiabetic subjects. The inflammatory process evoked via oxidative stress has been thought to intimately participate in the development and progression of atherosclerosis [1], and the oxidative stress induces antioxidatives including HO-1, which has been well established to function antiatherogenically in animal models of atherosclerosis [5,6]. It is, however, unclear whether atherogenic stimuli such as DM, dyslipidemia, hypertension and others can equally promote HO-1 expression in human atherosclerosis. The present study demonstrates that HO-1 expression is significantly enhanced in diabetic compared with nondiabetic subjects, with a greater macrophage content in atherosclerotic lesions. This enhancement of HO-1 expression is additionally upregulated by hypercholesterolemia and smoking, but not by hypertension. These findings support the contention that human atherogenesis is really multifactorial and suggest that DM, hypercholesterolemia and smoking among various types of atherogenic risk factors contribute greatly to HO-1 expression in human coronary atherosclerotic lesions. Recent studies indicate that the stress-responsive elements-Bach1-Nrf2 signal pathway [22] and PPARα and PPARγ/their ligands [23] would transcriptionally regulate HO-1 expression in vascular SMCs and ECs. These transcriptional pathways have been well known to contribute widely to the regulation of the inflammatory-proliferative process in the vasculature, evoked by several atherogenic stimuli including advanced glycation end products overexpressed in DM [24]. In addition, the heterogeneous expression of HO-1 may relate to the severity of human atherosclerosis and the incidence of such atherosclerotic diseases. In fact, a longer GT repeat in human HO-1 promoter has been suggested to result in the decrease of HO-1 transcriptional activity [7,23]. Together with these findings, the cumulative evidence favors therapeutic exploitation using an HO-1 induction strategy [6,25,26].

A few studies comparing the pathologic characteristics of human atherosclerotic lesions in diabetic and nondiabetic patients have been reported [27,28]. A diabetic or prediabetic state as indicated by elevated serum glycohemoglobin levels promotes coronary and aortic atherosclerosis not only in youths but also in adults. Burke et al. [28] reported that macrophage infiltration and necrotic core size play a greater role in the progression of atherosclerosis in diabetic adults who die suddenly. The current study indicates that diabetes is not associated with larger necrotic core size than in nondiabetic subjects, partly due to the small number of subjects examined. In addition, the fact that the major histologic types of more than about three-fourths of the Japanese subjects examined in this study consisted mainly of DIT and early atherosclerotic lesions of types I–III (Supplementary data Fig. I) may be relevant to the larger population of early atherosclerotic lesions and fibrous plaque in Japanese youths [29]. Macrophage infiltration was significantly fre-

quent in diabetic subjects, particularly those with advanced atherosclerotic lesions, in comparison to nondiabetic subjects, and was further accentuated by the co-existence of hypercholesterolemia and smoking. Furthermore, in our study the frequency of MetS was higher in diabetic subjects. The Hisayama cohort study suggested that MetS is a significant risk factor for the development of cardiovascular disease and found a possibility that the increased risk of MetS for cardiovascular disease resulted from the influence of diabetes [30]. These findings suggest that the association of DM with atherosclerosis in the Japanese population is particularly significant for the prevention programs highlighted in the recent Hisayama study [30].

Angiogenesis is an essential process not only physiologically in organ development and tissue regeneration but also pathologically in inflammation and cancer. Newly formed microvessels are ubiquitously distributed in atherosclerotic plaque [11–13], and this angiogenic process has been recently assumed to participate intimately in atherosclerotic progression [14] and in the occurrence of atherothrombosis [15,16]. Recent studies suggest that HO-1 is also involved in physiologic and pathologic angiogenesis [9,10], essentially via the functions of VEGF-A and gas mediators such as NO and CO. Bussolati et al. [9] proposed that HO-1 would play a bifunctional role during an inflammation-repair process, namely, anti-inflammatory action inhibiting leukocytic infiltration and the promotion of VEGF-driven noninflammatory angiogenesis, resulting in the facilitation of a sequential transition from active inflammation to noninflammatory tissue repair. However, the pathological function of HO-1 has not been fully clarified in the chronic inflammatory process including atherogenesis. The current study clearly demonstrated that the extent of newly formed microvessels is well correlated with the degree of HO-1 expression in atherosclerotic plaque, and that hypercholesterolemia in addition to DM accentuates the incidence of intimal angiogenesis. Furthermore, the topographical localization of HO-1 expressed by macrophages and ECs is closely distributed within and around intimal microvessels (Fig. 2). Devesa et al. [9] reported that upregulated HO-1 sustained chronic inflammation in an animal model of chronic arthritis by enhancing inducible nitric oxide synthase expression and VEGF-related angiogenesis. Together with these findings, angiogenesis may play bifunctional and reverse roles in pathological states, partly resulting in the outcome of angiogenic diseases including atherosclerosis and cancer. Though it remains undetermined whether the modulation of HO-1 expression accelerates or suppresses atherogenesis in an animal model of atherosclerosis associated with intimal angiogenesis similar to human lesions, further studies will be necessary to clarify the pathophysiological role of HO-1 in human atherogenesis and to assess the therapeutic effect of HO-1 on atherosclerosis-related disease.

In conclusion, our data demonstrate that HO-1 is ubiquitously upregulated in human coronary atherosclerotic lesions, particularly in diabetics, and that the extent of HO-1 expression is well correlated with the degrees of macrophage infiltration and angiogenesis in atherosclerotic plaque. Thus, HO-1 may participate intimately in the inflammatory-repair process during atherogenesis. Although it has not been determined how HO-1 function activates intraplaque angiogenesis, our findings suggest that HO-1 plays diverse roles in the progression of human coronary atherosclerosis.

Acknowledgments

The authors thank Hiroshi Fujii for his excellent technical assistance. This work was supported in part by a Grant-in-Aid from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, Tokyo, Japan (#16209012 and 13307009).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2008.05.057.

References

[1] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
[2] Qiao Q, Hu G, Tuomilehto J, et al. DECODA Study Group. Age- and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian cohorts. Diabetes Care 2003;26:1770–80.
[3] Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA 2002;287:2570–625.
[4] Ryter SW, Alam J, Choi AMK. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev 2006;86:583–650.
[5] Morita T. Heme oxygenase and atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:1786–95.
[6] Orozco LD, Kapturczak MH, Barajas B, et al. Heme oxygenase-1 expression in macrophages plays a beneficial role in atherosclerosis. Circ Res 2007;100:1703–11.
[7] Kaneda H, Ohno M, Taguchi J, et al. Heme oxygenase-1 gene promoter polymorphism is associated with coronary artery disease in Japanese patients with coronary risk factors. Arterioscler Thromb Vasc Biol 2002;22:1680–5.
[8] Bussolati B, Ahmed A, Pemberton H, et al. Bifunctional role for VEGF-induced heme oxygenas-1 in vivo: induction of angiogenesis and inhibition of leukocytic infiltration. Blood 2004;103:761–6.
[9] Devesa I, Ferárndiz L, Guillén I, et al. Potential role of heme oxygenase-1 in the progression of rat adjuvant arthritis. Lab Invest 2005;85:34–44.
[10] Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. Human Pathol 1995;26:450–6.
[11] Chen Y-X, Nakashima Y, Tanaka K, et al. Immunohistochemical expression of vascular endothelial growth factor/vascular permeability factor in atherosclerotic intimas of human coronary arteries. Arterioscler Thromb Vasc Biol 1999;19:131–9.
[12] Nakano T, Nakashima Y, Yonemitsu Y, et al. Angiogenesis and lymphangiogenesis and expression of lymphangiogenic factors in the

atherosclerotic intima of human coronary arteries. Human Pathol 2005;36:330–40.
[13] Ohtani K, Egashira K, Hiasa K, et al. Blockade of vascular endothelial growth factor suppresses experimental restenosis after intraluminal injury by inhibiting recruitment of monocyte lineage cells. Circulation 2004;110:2444–52.
[14] Moreno PR, Purushothaman KR, Fuster V, et al. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation 2004;110:2032–8.
[15] Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscl Thromb Vasc Biol 2005;25:2054–61.
[16] Wang L-J, Lee T-S, Lee F-Y, et al. Expression of heme oxygenase-1 in atherosclerotic lesions. Am J Pathol 1998;152:711–20.
[17] Kubo M, Hata J, Ninomiya T, et al. A nonsynonymous SNP in *PRKCH* (protein kinase Cη) increases the risk of cerebral infarction. Nat Genet 2007;39:212–7.
[18] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
[19] World Health Organization/International Association for the Study of Obesity/International Obesity Task Force. The Asia-Pacific perspective: redifining obesity and its treatment. Available at: http://www.diabetes.com.au/pdf/obesity_report.pdf.
[20] Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1995;92:1355–74.
[21] Sumiyoshi S, Nakashima Y, Chen Y-X, et al. Interleukin-10 expression is positively correlated with oxidized LDL deposition and inversely with T-lymphocyte infiltration in atherosclerotic intimas of human coronary arteries. Pathol Res Pract 2006;202:141–50.
[22] Sun J, Brand M, Zenke Y, et al. Heme regulates the dynamic exchange of Bach 1 and NF-E2-related factors in the Maf transcription factor network. Proc Natl Acad Sci USA 2003;101:1461–6.
[23] Kränke G, Kadl A, Ikonomu E, et al. Expression of heme oxygenase-1 in human vascular cells is regulated by peroxisome proliferator-activated receptors. Arterioscl Thromb Vasc Biol 2007;27:1276–82.
[24] Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med 2004;10:355–61.
[25] Wu BJ, Kathir K, Witting PW, et al. Antioxidants protect from atherosclerosis by a heme oxygenase-1 pathway that is independent of free radical scavenging. J Exp Med 2006;203:1117–27.
[26] Ali F, Hamdulay SS, Kinderlerer AR, et al. Statin-mediated cytoprotection of human vascular endothelial cells: a role for Kruppel-like factor 2-dependent induction of heme oxygenase-1. J Thromb Haemost 2007:2537–46.
[27] McGill HC, McMahan CA, Malcom GT, et al. Relation of glycohemoglobin and adiposity to atherosclerosis in youth. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Arterioscler Thromb Vasc Biol 1995;15:431–40.
[28] Burke AP, Kolodgie FD, Zieske A, et al. Morphologic findings of coronary atherosclerotic plaques in diabetics: a postmortem study. Arteriocler Thromb Vasc Biol 2004;24:1266–71.
[29] Kisanuki Y, Asada Y, Sato Y, et al. Coronary atherosclerosis in youths in Kyushu island, Japan: histological findings and stenosis. J Atheroscler Thromb 2000;6:55–9.
[30] Ninomiya T, Kubo M, Doi Y, et al. Impact of metabolic syndrome on the development of cardiovascular disease in Japanese population: the Hisayama study. Stroke 2007;38:2063–9.

Circ J 2009; **73:** 624–625

Uric Acid and Left Ventricular Hypertrophy

Shigeyuki Saitoh, MD

In this issue of the Journal, Mitsuhashi et al report that levels of uric acid (UA) were positively associated with electrocardiographically diagnosed left ventricular hypertrophy (LVH) in healthy Japanese men[1] The result was independent of body mass index, hypertension, diabetes, hyperlipidemia and age; similar results were obtained in both the normal and high blood pressure (BP) subgroups. Theirs was an epidemiological study, but the reported serum concentrations of UA add important information to the assessment of risk factors and preventing cardiovascular disease, especially heart failure.

Article p 667

The relationship between UA and cardiovascular disease has been known since the first half of the 20th century and several studies have identified an association between increased UA and cardiovascular risk in the general population. The positive association between serum UA and hypertension was also observed over a century ago. Although elevated UA levels have been predictive of hypertension in epidemiological studies, the relationship between UA and BP is confounded by numerous factors, including age, diabetes, obesity, alcohol use, and sodium intake or volume status. Recent findings in animal models have helped elucidate possible mechanisms whereby UA may lead to hypertension, and have spurred a renewed interest in discerning a causal role for elevated UA in hypertension.

On the other hand, the presence of hypertensive organ damage signals a condition of increased risk for cardiovascular and renal morbidity and mortality. Thus, the search for LVH, atherosclerosis and microalbuminuria as hypertensive organ damage, which likely reflect both the severity of BP load and other nonhemodynamic risk factors, is currently recommended as part of global risk assessment. Mitsuhashi et al show new findings of a relationship between UA and LVH in Japanese, regardless of the presence of hypertension[1]

There are already reports of the relation between UA and LVH in Japanese with hypertension. For example, Kurata et al reported that serum UA levels correlated positively with left ventricular (LV) mass and indexed LV mass (LVMI) in male hypertensive patients, but not in female hypertensive patients in a cross-sectional study[2] Iwashima et al also demonstrated that UA is independently associated with LVMI and suggested that the combination of hyperuricemia and LVH is an independent and powerful predictor of cardiovascular disease[3]

With the exception of specific genetic defects in purine metabolism, increased UA is generally associated with important risk factors for atherosclerosis, such as hypertension, abdominal obesity, insulin resistance, metabolic syndrome and heart failure. Many studies have also clearly shown an association between increased UA concentrations and oxidative stress, endothelial dysfunction and inflammation. At the very least, an increased UA level is an independent marker of cardiovascular disease and a risk factor in cardiovascular diseases and hypertension. The question is whether UA is the cause of these risk factors or a morbid vascular change.

Because of being an epidemiological study, the results of Mitsuhashi et al's investigation do not suggest whether an elevation of the serum UA level is the cause or result of LVH[1] A consideration of the mechanism of UA production and metabolism offers insight into the relationship between UA level and cardiovascular change. Primarily, the association between UA and LVH might relate to an association of UA with other risk factors, especially renal dysfunction, oxidative stress, BP, and obesity. UA is excreted primarily by the kidney, so decreased renal perfusion could lead to increased serum UA and activation of the renin–angiotensin system; angiotensin II is essential for the development of LVH by myocardial remodeling[4] It is well known that angiotensin II induces hypertrophy and hyperplasia of myocytes and vascular smooth muscle cells, as well as influencing the expression of fibrogenic cytokine, and possibly inducing perivascular and interstitial fibrosis[5]

Secondly, UA levels may reflect xanthine oxidase pathway activity, which has the potential to contribute to the progression of LV dysfunction by interfering with myocardial efficiency[6] and myofilament calcium sensitivity[7] UA is a metabolic byproduct of purine metabolism and its serum level may increase because of increased generation, decreased excretion, or a combination of these mechanisms. UA is produced in the terminal step of purine metabolism catalyzed by xanthine oxidase (XO). XO pathway activity also results in the production of superoxide. XO is inhibited by allopurinol, which inhibited progression of cardiac hypertrophy in an animal model of hypertension without changing BP[8]

Furthermore, there are several possible contributors to increased UA production in cardiac disease, especially heart failure, including increased abundance and activity of XO, increased conversion of xanthine dehydrogenase to XO, or increased XO substrate resulting from enhanced ATP breakdown to adenosine and hypoxanthine under such conditions. XO activity participates in both mechano-energetic uncoupling and vascular dysfunction in the failing circulation. Mechano-energetic uncoupling is the process whereby cardiac energy consumption remains the same or increases

(Received February 2, 2009; accepted February 2, 2009)

Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan
Mailing address: Shigeyuki Saitoh, MD, Second Department of Internal Medicine, Sapporo Medical University, S-1, W-16, Chuo-ku, Sapporo 060-8543, Japan. E-mail: ssaitoh@sapmed.ac.jp

while cardiac work falls dramatically, and is increasingly being perceived as a potential key lesion in the failing heart.

On the other hand, there is the possibility that UA itself may induce LVH. Previous reports have shown that UA impairs NO generation and induces endothelial dysfunction and smooth muscle cell proliferation[9] Moreover, UA is able to induce inflammatory mediators, such as tumor necrosis factor, in vitro and potentially stimulates mitogen-activated protein kinases, which are known to induce cardiac hypertrophy![10] Indeed, accumulating data support the idea that UA possesses specific toxic or other properties that could contribute to cardiac hypertrophy and heart failure pathophysiology. These findings reveal that UA may be the cause of cardiac hypertrophy in part, attributable to an increase in its serum level, via stimulation of endothelial dysfunction, smooth muscle cell proliferation, and inflammation.

So far there is strong evidence that increased UA is associated with atherosclerosis and an increased risk of cardiovascular events. The findings of Mitsuhashi et al. also suggest that the serum level of UA affects cardiac hypertrophy in men! However, whether UA per se is a cause of cardiovascular disease, especially cardiac hypertrophy, remains to be settled. Prospective randomized studies targeting UA reduction are necessary to finish this discussion.

This finding by Mitsuhashi et al[1] is not only potentially of value in preventing cardiac hypertrophy but also raises interesting questions regarding the pathophysiological action of UA on the cardiovascular system.

References

1. Mitsuhashi H, Yatsuya H, Matsushita K, Zhang H, Otsuka R, Muramatsu T, et. al. Uric acid and left ventricular hypertrophy in Japanese men. *Circ J* 2009; **73:** 667–672.
2. Kurata A, Shigematsu Y, Higaki J. Sex-related differences in relations of uric acid to left ventricular hypertrophy and remodeling in Japanese hypertensive patients. *Hypertens Res* 2005; **28:** 133–139.
3. Iwashima Y, Horio T, Kamide K, Rakugi H, Ogihara T, Kawano Y. Uric acid, left ventricular mass index, and risk of cardiovascular disease in essential hypertension. *Hypertension* 2006; **47:** 195–202.
4. Itihara S, Senbonmatsu T, Price E Jr, Ichiki T, Gaffney FA, Inagami T. Angiotensin II type 2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotensin II-induced hypertension. *Circulation* 2001; **104:** 346–351.
5. Nakahara T, Tanaka Y, Hirayama Y, Asano K, Adachi H, Shiokawa G, et al. Left ventricular hypertrophy and geometry in untreated essential hypertension is associated with blood levels of aldosterone and procollagen type III amino-terminal peptide. *Circ J* 2007; **71:** 716–721.
6. Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, et al. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. *Circulation* 2001; **104:** 2407–2411.
7. Pérez NG, Gao WD, Marbán E. Novel myofilament calcium-sensitizing property of xanthine oxidase inhibitors. *Circ Res* 1998; **83:** 423–430.
8. Laakso JT, Teravainen TL, Martelin E, Vaskonen T, Lapatto R. Renal xanthine oxidoreductase activity during development of hypertension in spontaneously hypertensive rats. *J Hypertens* 2004; **22:** 1333–1340.
9. Rao GN, Corson MA, Berk BC. Uric acid stimulates vascular smooth muscle cell proliferation by increasing platelet-derived growth factor A-chain expression. *J Biol Chem* 1991; **266:** 8604–8608.
10. Watanabe S, Kang DH, Feng L, Nakagawa T, Kanellis J, Lan H, et al. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. *Hypertension* 2002; **40:** 355–360.

Original Article

A Promoter Polymorphism of Lamin A/C Gene is an Independent Genetic Predisposition to Arterial Stiffness in a Japanese General Population (The Tanno and Sobetsu Study)

Hiroshi Akasaka[1], Tomohiro Katsuya[2], Shigeyuki Saitoh[1], Ken Sugimoto[2], Hirofumi Ohnishi[1], Ada Congrains[2], Miyuki Ohnishi[2], Mitsuru Ohishi[2], Hiromi Rakugi[2], Toshio Ogihara[2,3], and Kazuaki Shimamoto[1]

[1]Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Hokkaido, Japan
[2]Department of Geriatric Medicine, Osaka University Graduate School of Medicine, Osaka, Japan
[3]Osaka General Medical Center, Osaka Prefectural Hospital Organization, Osaka, Japan

Aim: We examined the hypothesis that there is a positive, independent association between polymorphisms of lamin A/C gene (*LMNA*) and arterial stiffness in Japanese.
Methods: The subjects were 261 men (mean age, 64.4±0.7 years) selected from inhabitants of the towns of Tanno and Sobetsu in a rural area of Japan who underwent medical check-ups. We conducted clinical examinations, including measurement of bilateral brachial-ankle pulse wave velocity (baPWV) as a marker of arterial stiffness, and genetic analysis. Subjects with atrial fibrillation, subjects with ankle-brachial index <0.9, and subjects taking any medication were excluded. We selected two single nucleotide polymorphisms (SNPs) as markers of *LMNA*, 1908C/T in exon 10 and -1030C/T in the promoter region, which we have recently identified. All genotypes were clearly determined by the TaqMan PCR method.
Results: Genotype frequencies of the two polymorphisms satisfied the Hardy-Weinberg equilibrium. The baPWV of -1030C/T polymorphism was significantly greater in subjects with CC genotype than in subjects with CT+TT genotype (1,652±22.1 cm/s vs. 1,552±43.0 cm/s, $p=0.039$); however, no significant difference was found for 1908C/T polymorphism. The baPWV was found to be significantly associated with age, body height, systolic blood pressure, and smoking habit; therefore, we next performed multiple regression analysis including these parameters, and found an independent, significant association between baPWV and -1030C/T polymorphism.
Conclusion: Promoter -1030C/T polymorphism of *LMNA* is a possible genetic predisposition to arterial stiffness in the Japanese population.

J Atheroscler Thromb, 2009; 16:404-409.

Key words; Arterial stiffness, Nuclear lamina, Genetics, Single nucleotide polymorphism (SNP)

Introduction

Hutchinson-Gilford progeria syndrome (HGPS; Online Mendelian Inheritance in Man #176670) is a rare sporadic disorder with premature aging, and patients with this syndrome are likely to have coronary artery disease, stroke, or other cardiovascular diseases[1-3]. HGPS induces severe systemic arterial stiffness, which leads to fatal myocardial infarction or stroke before an average age of 13 years. Approximately 80% of HGPS cases are caused by a single base change of C to T in position 1824 on exon 11 of a gene encoding nuclear lamins A and C[4,5]. Lamins are structural protein components of nuclear lamina, a protein network underlying the inner nuclear membrane that determines nuclear shape and size, and

Address for correspondence: Hiroshi Akasaka, Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Hokkaido 060-8543, Japan
E-mail: akasaka@sapmed.ac.jp
Received: December 17, 2008
Accepted for publication: January 29, 2009

constitute a class of intermediate filaments. The gene encoding lamins A and C is named lamin A/C gene (*LMNA*; Gene ID 4000), and it spans approximately 24 kb and contains 12 exons on chromosome 1q21. Alternative splicing within exon 10 of *LMNA* gives rise to 2 different mRNAs that code for prelamin A and lamin C.

HGPS is associated with premature arterial stiffness, and it is therefore thought that *LMNA* is involved in the pathophysiology and genesis of arterial stiffness that occurs concurrently with the accelerated aging process. It has been reported that *LMNA* has several single nucleotide polymorphisms (SNPs). The 1908C/T polymorphism (rs #4641), one of the SNPs on *LMNA*, in exon 10 is associated with a risk for developing metabolic traits, including insulin resistance. A positive association between 1908C/T polymorphism and metabolic abnormalities has been reported in Inuit[6], Japanese[7], Pima Indians and Armish[8].

However, there is no data on the association between *LMNA* and arterial stiffness in a general population. Arterial stiffness is mainly determined by measuring pulse wave velocity (PWV)[9]. PWV reflects systemic arteriosclerosis as well as relating to cardiovascular risk factors[10] and ischemic heart disease in type 2 diabetes mellitus[11]. PWV is also a predictor of cardiovascular mortality in patients with end-stage renal disease[12] or hypertension[13] and in elderly individuals[14], independently of age, blood pressure, and cardiac mass.

The purpose of this study was to examine the relationship between polymorphisms of *LMNA* and arterial stiffness in a cross-sectional epidemiological study of a Japanese general population, the Tanno and Sobetsu study.

Materials and Methods

We recruited 586 male inhabitants of Tanno Town and Sobetsu Town who had undergone medical check-ups in 2003. Tanno and Sobetsu are located in Hokkaido, the northernmost island of Japan. The Tanno and Sobetsu study was started in 1977 with a population-based prospective cohort design. Detailed epidemiological findings have already been reported[15-18].

The subjects completed a standard questionnaire regarding their medical history and their smoking and drinking habits. We measured anthropometric parameters, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, plasma glucose, immunoreactive insulin (IRI), highly sensitive C-reactive protein (hs-CRP), and adiponectin in all subjects. Brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI) were measured using Form® PWV/ABI (Omron Colin Co., Ltd., Tokyo, Japan) and the average of right baPWV and left baPWV was adopted[19]. Insulin sensitivity was determined by homeostasis model assessment of the insulin resistance (HOMA-IR) index, which was calculated as plasma glucose (mg/dL) × immunoreactive insulin (μU/L)/405. Blood samples were collected in the early morning after fasting for 8–11 hours. Blood pressure was measured twice after 5 minutes of rest, with the subjects seated.

Exclusion criteria were atrial fibrillation, suspected arteriosclerosis obliterans (ASO) defined as ABI on any side lower than 0.9, and taking any medication, in order to rule out drug effects. After excluding 219 of the 586 male subjects according to the above criteria, we conducted genetic analysis. Finally, 261 male subjects were successfully genotyped. All subjects gave written informed consent to participate in the genetic analysis and in all other procedures associated with the study. The Institutional Review Board (IRB) of Osaka University and the IRB of Sapporo Medical University both approved the study protocol.

Genomic DNA was extracted from 200 μL buffy coat using a QIAamp DNA Blood Kit (QIAGEN K. K., Tokyo, Japan). C-to-T transversion at nucleotide position 1908 in exon 10 of the lamin A/C gene (*LMNA* 1908C/T; rs #4641) and C-to-T transversion at nucleotide position -1030 in the promoter region of *LMNA* (*LMNA* -1030C/T; no rs#) were determined by the TaqMan-polymerase chain reaction (PCR) method. The *LMNA* 1908C/T polymorphism was detected using the following primers and probes: forward, 5'-CGA GGA TGA GGA TGG AGA TGA C-3'; reverse, 5'-CCT CAG CGG CGG CTA C-3'; cytosine base (C)-specific probe, 5'-VIC-CAC TCA CGT GGT GGT G-MGB-3'; and thymine base (T)-specific probe, 5'-FAM-CAC TCA CAT GGT GGT G-MGB-3'. The *LMNA* -1030C/T polymorphism was detected using the following primers and probes: forward, 5'-CCA CTA CCT TCT TTC TGG CTG AA-3'; reverse, 5'-ACT AGG TCC CAG ATT TCT GTG GTT-3'; cytosine base (C)-specific probe, 5'-VIC-CAG CCA ATG TTG GGT C-MGB-3'; and thymine base (T)-specific probe, 5'-FAM-ACA GCC AAT ATT GGG TC-MGB-3'. PCR was carried out using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: initial denaturation at 95℃ for 10 min, followed by 40 cycles of 92℃ for 15 sec and 60℃ for 60 sec. The fluorescence level of PCR

Table 1. Baseline characteristics of study subjects (n=261)

	Male (n=261)
Age (years)	64.6±0.7
BMI (kg/m²)	23.5±0.2
SBP (mmHg)	133±1.3
DBP (mmHg)	75±0.7
Total cholesterol (mg/dL)	193±2.0
Triglyceride (mg/dL)	112±4.6
HDL cholesterol (mg/dL)	52±0.8
Current smoker (%)	33.0
HOMA-IR	1.0±0.07
hsCRP (mg/dL)	0.107±0.008
Adiponectin (ng/mL)	6.1±0.2
baPWV (cm/s)	1,631±19.7

Values are expressed as the mean±SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

Table 2. Comparison of parameters between CC genotype and CT+TT genotype of 1908C/T polymorphism

	CC (n=157)	CT+TT (n=104)	p
Age (years)	65.3±0.9	63.5±1.2	0.24
BMI (kg/m²)	23.5±0.3	23.7±0.3	0.58
SBP (mmHg)	133±1.7	136±2.1	0.30
DBP (mmHg)	75±0.9	77±1.1	0.07
Total cholesterol (mg/dL)	194±2.7	190±3.4	0.09
Triglyceride (mg/dL)	112±5.1	113±10.0	0.91
HDL cholesterol (mg/dL)	52±0.9	52±1.7	0.96
Current smoker (%)	32.4	5.2	0.75
HOMA-IR	1.1±0.1	1.0±0.2	0.56
hsCRP (mg/dL)	0.11±0.008	0.082±0.02	0.10
Adiponectin (ng/mL)	6.1±0.2	6.0±0.4	0.80

Values are expressed as the mean±SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

products measured using an ABI PRISM 7900HT Sequence Detector (Applied Biosystems) differentiated the three genotypes of these two polymorphisms.

Associations between the polymorphisms and clinical variables were analyzed using one-way analysis of variance (ANOVA). Differences in genotype or allele distribution were examined by χ^2 analysis. Multiple regression analysis was used to assess the contribution of confounding factors. All numerical values are expressed as the means±SEM. Significance was defined as $p<0.05$. All statistical analyses were conducted using JMP software version 5.1.2J for Windows (SAS Institute Inc., Cary, NC, USA).

Results

The 261 male subjects had a mean age of 64.6± 0.7 years, mean body mass index (BMI) of 23.5±0.2 kg/m², and mean brachial-ankle pulse wave velocity (baPWV) of 1,631±19.7 cm/sec. **Table 1** shows the baseline characteristics of all study subjects. The genotype frequencies of the two polymorphisms of *LMNA* examined did not significantly differ from the values predicted by the Hardy-Weinberg equilibrium. The frequencies of CC, CT and TT genotypes of exon 10 1908C/T polymorphism were 60%, 32% and 8%, respectively, and the frequencies of CC, CT and TT genotypes of promoter -1030C/T polymorphism were 79%, 16% and 5%, respectively. Since the number of subjects with TT genotype of these two polymorphisms was small, we adopted a recessive model of the C allele (CC vs. CT+TT) for the two polymorphisms. **Tables 2** and **3** show the clinical parameters of each genotype of the 1908C/T polymorphism and -1030C/T polymorphism of *LMNA*, respectively. Despite previous findings[6-8], there was no significant relationship between the T allele of 1908C/T polymorphism and metabolic traits in our study cohort. BaPWV of subjects with CC genotype of -1030C/T polymorphism was significantly greater than that of

Table 3. Comparison of parameters between CC genotype and CT+TT genotype of -1030C/T polymorphism

	CC (n=207)	CT+TT (n=54)	p
Age (years)	64.8±0.8	63.9±1.5	0.61
BMI (kg/m²)	23.6±0.2	23.2±0.4	0.37
SBP (mmHg)	134±1.4	131±2.8	0.27
DBP (mmHg)	76±0.8	74±1.6	0.24
Total cholesterol (mg/dL)	191±2.2	199±4.4	0.38
Triglyceride (mg/dL)	112±5.9	106±7.3	0.56
HDL cholesterol (mg/dL)	53±1.0	52±1.3	0.88
Current smoker (%)	35.4	28.1	0.26
HOMA-IR	1.1±0.1	1.0±0.1	0.27
hsCRP (mg/dL)	0.11±0.01	0.10±0.01	0.43
Adiponectin (ng/mL)	6.0±0.3	6.2±0.3	0.52

Values are expressed as the mean±SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

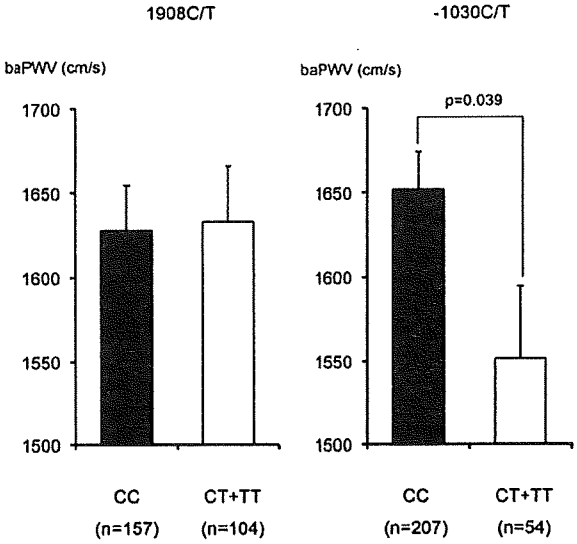

Fig. 1. Comparison of brachial-ankle pulse wave velocity (baPWV) according to genotypes of *LMNA* 1908C/T and -1030C/T polymorphisms.

subjects with CT+TT genotype of -1030C/T polymorphism (1,652±22.1 cm/s vs. 1,552±43.0 cm/s, $p=0.039$), while there was no significant difference in the genotype of 1908C/T polymorphism (**Fig. 1**). BaPWV showed significant positive correlations with age, systolic blood pressure and smoking habit and a significant negative correlation with body height. We therefore selected these factors as covariates of multiple regression analysis for baPWV and *LMNA* -1030C/T polymorphism (**Table 4**). There was an independent relationship between the genotype of -1030C/T polymorphism and baPWV after adjusting covariates. Mean baPWVs of -1030/CC and -1030/CT +TT genotypes were 1,650±17.1 cm/s and 1,571± 32.0 cm/s, respectively, after adjusting covariates.

Since there was a strong linear correlation between age and baPWV, we analyzed the genotypic difference of -1030C/T in this correlation. The gradient of the regression line of subjects with CC genotype was significantly high compared to that of subjects with CT+TT genotype (data not shown), indicating that subjects with CC genotype of -1030C/T polymorphism might be susceptible to the progression of arterial stiffness by aging.

Discussion

We examined the hypothesis that there is a positive, independent association between polymorphisms

Table 4. Multiple regression analysis for brachial-ankle pulse wave velocity (baPWV)

Term	Estimate	SE	t	p
Age	12.5	1.58	7.9	<0.0001
Body height	0.684	2.55	0.3	0.79
Systolic blood pressure	7.69	1.15	6.7	<0.0001
Smoking habit	−6.34	15.9	−0.40	0.69
-1030C/T CC vs. CT+TT	−39.4	17.8	−2.2	0.028

$R^2=0.48$ ($n=261$)

of *LMNA* and arterial stiffness in a cross-sectional study of a Japanese population. Genotype frequencies of the two polymorphisms satisfied the Hardy-Weinberg equilibrium. The mean baPWV of -1030C/T polymorphism was significantly greater in subjects with CC genotype than in subjects with CT+TT genotype; however, no significant difference was found for 1908C/T polymorphism. On the other hand, age, body height, systolic blood pressure, and smoking habit were significantly associated with baPWV. Multiple regression analysis including covariates revealed that subjects with the -1030T allele had a significantly lower level of baPWV.

The 1908C/T polymorphism of *LMNA* has been reported to be related to metabolic abnormalities or insulin resistance in Japanese[7] and Armish[8], but a relationship between this polymorphism and arterial stiffness was not found in the present study. In addition, no relationship was found between 1908C/T polymorphism and metabolic traits in our population. This may due to the characteristics of our subjects. Subjects taking any medication were excluded from this study, and the number of subjects with type 2 diabetes mellitus or dyslipidemia was therefore small; however, the promoter -1030C/T polymorphism of *LMNA*, which we have recently identified by direct sequencing in the subjects, was independently associated with baPWV as a marker of arterial stiffness. To our knowledge, this is the first report of a relationship between *LMNA* polymorphism and arterial stiffness.

In addition, we investigated the relation between the prevalence of cardiovascular diseases and SNPs in *LMNA* using a cross-sectional method; however, there was no significant relation between cardiovascular diseases and SNPs. Because the subjects of our study were relatively healthy and had a low prevalence of cardiovascular diseases, our investigation might lack statistical power. A prospective study to elucidate this relation is now ongoing. Because of the shortness of the follow-up period, we do not have valuable results

at present, but we will report the obtained results in the future.

Mutations in *LMNA* have been discovered in a staggering variety of inherited diseases called "laminopathies[20]". To date, several laminopathies are more familiar than Hutchinson-Gilford progeria syndrome (HGPS), such as Dunnigan-type familial partial lipodystrophy (FPLD), Emery-Dreifuss muscular dystrophy, Charcot-Marie-Tooth disease, limb-girdle muscular dystrophy, mandibuloacral dysplasia, dilated cardiomyopathy with conduction abnormality and early onset of atrial fibrillation. Laminopathies are caused by a mutation of *LMNA*, and are likely to include cardiovascular diseases[21, 22]. The precise mechanisms of this relationship are unknown, but it is speculated that *LMNA* regulates metabolic traits as well as arterial stiffness and thus results in the aging process. HGPS is a laminopathy that is mainly caused by C-to-T mutation in position 1824 of *LMNA* and results in systemic arteriosclerosis. Recent studies have shown that this mutation causes nuclear blebbing induced by anchoring the mutant lamin A (called "progerin", which lacks 50 amino acids near the carboxy terminus) to the inner nuclear membrane, resulting in dysregulated gene transcription, heterochromatin disorganization[23, 24], and increased vulnerability of the nuclear membrane. Numerous abnormalities present in HGPS are common phenomena that occur in cells not only of HGPS patients but also aged individuals in the general population, such as nuclear blebbing, epigenetic changes and increased levels of DNA damage[25]; therefore, *LMNA* seems to be one of the key genes regulating aging as well as arterial stiffness.

In order to elucidate the function of -1030C/T promoter polymorphism of *LMNA*, we searched for transcription factors likely to bind around -1030C/T, using online databases of TRANSFAC, JASPAR, IMD, and CBIL/GibbsMat (http://www.cbil.upenn.edu/cgi-bin/tess/tess/). Motif analysis revealed one transcription factor, CREB-binding protein/CCAAT recognition factor (CBP/CRF), which has homology with the sequence including the -1030T allele. This may result in a difference in *LMNA* expression. Subjects with the -1030T allele are likely to have a strong expression of lamin A and C matrix and might have a stable nuclear membrane against environmental insult, represented by reactive oxygen species.

Our study has several limitations. First, our study was conducted using a small number of subjects in a Japanese population. Second, this study was is designed as a cross-sectional method. Although arteriosclerosis occurs mostly in aged individuals, the genotype-phenotype relationship showd also be analyzed in a time-considered, longitudinal study. Third, the mechanisms by which transcriptional activities of *LMNA* are regulated by promoter -1030C/T polymorphism are unclear. Further study is required to clarify the function of promoter -1030C/T polymorphism.

In conclusion, promoter -1030C/T polymorphism of *LMNA* might be associated with arterial stiffness in Japanese independent of metabolic traits. The mechanism of the influence of *LMNA* on arterial stiffness may reveal part of the mechanism of a common condition susceptible to arteriosclerosis, aging.

Acknowledgements

We would like to express our gratitude to Ms. Kazuko Iwasa and Ms. Eriko Nagata for their continued support of our investigations. The present study was supported by a Grant-in-Aid for Scientific Research (H17-pharmaco-common-003) from the Japanese Ministry of Health, Labor, and Welfare, by Grants-in-Aid for Scientific Research (18590265, 18590811 and 19650188) from the Ministry of Education, Science, Sports and Culture of Japan, and by research grants from Takeda Science Foundation and the Japan Research Foundation for Clinical Pharmacology.

References

1) DeBusk FL: The Hutchinson-Gilford progeria syndrome. Report of 4 cases and review of the literature. J Pediatr, 1972; 80: 697-724
2) Hennekam RC: Hutchinson-Gilford progeria syndrome: review of the phenotype. Am J Med Genet A, 2006; 140: 2603-2624
3) Ogihara T, Hata T, Tanaka K, Fukuchi K, Tabuchi Y, Kumahara Y: Hutchinson-Gilford progeria syndrome in a 45-year-old man. Am J Med, 1986; 81: 135-138
4) Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glover TW, Collins FS: Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature, 2003; 423: 293-298
5) Fukuchi K, Katsuya T, Sugimoto K, Kuremura M, Kim HD, Li L, Ogihara T: LMNA mutation in a 45 year old Japanese subject with Hutchinson-Gilford progeria syndrome. J Med Genet, 2004; 41: e67
6) Hegele RA, Huff MW, Young TK: Common genomic variation in LMNA modulates indexes of obesity in Inuit. J Clin Endocrinol Metab, 2001; 86: 2747-2751
7) Murase Y, Yagi K, Katsuda Y, Asano A, Koizumi J, Mabuchi H: An LMNA variant is associated with dyslipidemia and insulin resistance in the Japanese. Metabolism, 2002; 51: 1017-1021
8) Steinle NI, Kazlauskaite R, Imumorin IG, Hsueh WC,

Pollin TI, O'Connell JR, Mitchell BD, Shuldiner AR: Variation in the lamin A/C gene: associations with metabolic syndrome. Arterioscler Thromb Vasc Biol, 2004; 24: 1708-1713
9) Weber MA: The measurement of arterial properties in hypertension. Am J Hypertens, 2001; 14: 183-185
10) Sato H, Hayashi J, Harashima K, Shimazu H, Kitamoto K: A population-based study of arterial stiffness index in relation to cardiovascular risk factors. J Atheroscler Thromb, 2005; 12: 175-180
11) Hatsuda S, Shoji T, Shinohara K, Kimoto E, Mori K, Fukumoto S, Koyama H, Emoto M, Nishizawa Y: Regional arterial stiffness associated with ischemic heart disease in type 2 diabetes mellitus. J Atheroscler Thromb, 2006; 13: 114-121
12) Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM: Aortic pulse wave velocity index and mortality in end-stage renal disease. Kidney Int, 2003; 63: 1852-1860
13) Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S: Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension, 2002; 39: 10-15
14) Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME: Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. Arterioscler Thromb Vasc Biol, 2001; 21: 2046-2050
15) Ohnishi H, Saitoh S, Ura N, Takagi S, Obara F, Akasaka H, Oimatsu H, Shimamoto K: Relationship between insulin resistance and accumulation of coronary risk factors. Diabetes Obes Metab, 2002; 4: 388-393
16) Ohnishi H, Saitoh S, Takagi S, Ohata J, Isobe T, Kikuchi Y, Takeuchi H, Shimamoto K: Pulse wave velocity as an indicator of atherosclerosis in impaired fasting glucose: the Tanno and Sobetsu study. Diabetes Care, 2003; 26: 437-440
17) Fujiwara T, Saitoh S, Takagi S, Ohnishi H, Ohata J, Takeuchi H, Isobe T, Chiba Y, Katoh N, Akasaka H, Shimamoto K: Prevalence of asymptomatic arteriosclerosis obliterans and its relationship with risk factors in inhabitants of rural communities in Japan: Tanno-Sobetsu study. Atherosclerosis, 2004; 177: 83-88
18) Takeuchi H, Saitoh S, Takagi S, Ohnishi H, Ohhata J, Isobe T, Shimamoto K: Metabolic syndrome and cardiac disease in Japanese men: applicability of the concept of metabolic syndrome defined by the National Cholesterol Education Program-Adult Treatment Panel III to Japanese men-the Tanno and Sobetsu Study. Hypertens Res, 2005; 28: 203-208
19) Munakata M, Ito N, Nunokawa T, Yoshinaga K: Utility of automated brachial ankle pulse wave velocity measurements in hypertensive patients. Am J Hypertens, 2003; 16: 653-657
20) Al-Shali KZ, Hegele RA: Laminopathies and atherosclerosis. Arterioscler Thromb Vasc Biol, 2004; 24: 1591-1595
21) Hegele RA: Monogenic forms of insulin resistance: apertures that expose the common metabolic syndrome. Trends Endocrinol Metab, 2003; 14: 371-377
22) Hegele RA, Kraw ME, Ban MR, Miskie BA, Huff MW, Cao H: Elevated serum C-reactive protein and free fatty acids among nondiabetic carriers of missense mutations in the gene encoding lamin A/C (LMNA) with partial lipodystrophy. Arterioscler Thromb Vasc Biol, 2003; 23: 111-116
23) Capell BC, Collins FS, Nabel EG: Mechanisms of cardiovascular disease in accelerated aging syndromes. Circ Res, 2007; 101: 13-26
24) Merideth MA, Gordon LB, Clauss S, Sachdev V, Smith AC, Perry MB, Brewer CC, Zalewski C, Kim HJ, Solomon B, Brooks BP, Gerber LH, Turner ML, Domingo DL, Hart TC, Graf J, Reynolds JC, Gropman A, Yanovski JA, Gerhard-Herman M, Collins FS, Nabel EG, Cannon RO 3rd, Gahl WA, Introne WJ: Phenotype and course of Hutchinson-Gilford progeria syndrome. N Engl J Med, 2008; 358: 592-604
25) Scaffidi P, Misteli T: Lamin A-dependent nuclear defects in human aging. Science, 2006; 312: 1059-1063

Original Article

Increased Serum Apolipoprotein B48 Concentration in Patients with Metabolic Syndrome

Makoto Kinoshita[1], Hirofumi Ohnishi[2], Tomomi Maeda[1], Nakayuki Yoshimura[1], Yoko Takeoka[1], Daijiro Yasuda[1], Jun Kusano[1], Yamato Mashimo[1], Shigeyuki Saito[2], Kazuaki Shimamoto[2], and Tamio Teramoto[1]

[1]Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan
[2]Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan

Aim: Postprandial hyperlipidemia is characterized by an increase of chylomicron remnants (CM-R), and is a risk factor for atherosclerosis. Apolipoprotein (apo) B48 exists exclusively in chylomicroms and CM-R, and fasting plasma levels of apo B48 may reflect high postprandial levels of chylomicrons and/or CM-R. We hypothesized that fasting apo B48 levels may be increased in metabolic syndrome.
Methods: We investigated 1,349 inhabitants (528 men and 821 women aged 62.4 ± 12.8 y; mean ± S.D.) of two towns in rural Hokkaido, who underwent health checks in 2005.
Results: The fasting apo B48 level was significantly higher in males than females (geometric mean 1.92; 95% CI 1.80–2.04 μg/mL, vs. 1.69; 95% CI 1.61–1.76 μg/mL; $p < 0.001$). Ln (apo B48) showed a significant positive correlation with total cholesterol and ln (triglycerides), and a negative correlation with HDL-cholesterol. The correlation between ln (apo B48) and ln (triglycerides) was strong. Apo B48 was significantly higher in men and women with than without metabolic syndrome. Regression analysis revealed that ln (apo B48) was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and ln (triglyceride).
Conclusion: Fasting apo B48 levels are raised in individuals with metabolic syndrome.

J Atheroscler Thromb, 2009; 16:517-522.

Key words; Chylomicrons, Hypertriglyceridemia, Apolipoprotein B48, Metabolic syndrome

Introduction

Postprandial hyperlipidemia, which is characterized by increased levels of chylomicron remnants (CM-R), is considered to be a risk factor for atherosclerosis[1, 2]. Chylomicrons are assembled in the small intestine and undergo lipolysis by lipoprotein lipase in the plasma to generate CM-R. Because CM-R are then rapidly taken up by the liver, it has been assumed that fasting plasma levels of these particles are very low[3].

Postprandial hyperlipidemia is related to metabolic syndrome[4-6]. This syndrome is characterized by insulin resistance, hypertension, dyslipidemia, and hyperglycemia, and is an important risk factor for atherosclerosis. In metabolic syndrome, dyslipidemia is characterized by hypertriglyceridemia and a low high density lipoprotein (HDL)-cholesterol level. Recent studies have shown that postprandial hyperlipidemia is a major cause of hypertriglyceridemia associated with metabolic syndrome[6, 7].

Though CM-R are thought to play an important role in dyslipidemia associated with metabolic syndrome, CM-R concentrations in patients with this syndrome have not yet been reported. Apo B48 exists exclusively in chylomicroms and CM-R. Several methods of measuring the apo B48 concentration in plasma or in triglyceride-rich lipoproteins have been reported[8-15]. These methods include sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[8-10], SDS-PAGE coupled with Western blotting[11, 12], and competitive enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies[13, 14].

Address for correspondence: Makoto Kinoshita, Department of Internal Medicine, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo, 173-8605, Japan
E-mail: makkino@med.teikyo-u.ac.jp
Received: July 8, 2008
Accepted for publication: June 4, 2009

Sakai *et al.* reported an ELISA for measuring apo B48 in fasting serum that employed a monoclonal antibody against apo B48[15]. We also recently developed an ELISA to measure the serum level of apo B48 using another monoclonal antibody[16].

We hypothesized that the fasting plasma level of apo B48 may be increased in patients with metabolic syndrome; therefore, we measured fasting plasma levels of apo B48 in order to evaluate the relationship between CM-R and metabolic syndrome. We also investigated the factors regulating apo B48 levels in fasting plasma.

Subjects and Methods

The subjects were 1,349 inhabitants (528 men and 821 women) of two towns in a rural area of Hokkaido, Japan, who underwent routine health checks in 2005. Blood samples were collected from all subjects after an overnight fast. Systolic and diastolic blood pressures were measured at rest in the sitting position. The fasting plasma glucose (FPG) level and plasma levels of total cholesterol, triglycerides, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol were measured by enzymatic methods. Immunoreactive insulin (IRI) was measured by an enzyme immunoassay (EIA). Waist circumference was measured at the level of the umbilicus in the standing position. The HOMA-IR (homeostasis model assessment insulin resistance index) was calculated as FPGxIRI/405, after excluding individuals who had an FPG above 126 mg/dL and/or were on treatment for diabetes[17].

Apolipoprotein B48 was measured by EIA[16]. Briefly, a 96-well microtiter plate (Nalge Nunc International, Japan) was coated with an anti-apoB-48 monoclonal antibody (4C8) by overnight incubation at 4℃. After washing the microtiter plate with phosphate-buffered saline, 50-μL aliquots of 100-fold-diluted serum or plasma (diluted with 0.05 mol/L Tris-HCl buffer, pH 7.5, 0.15 mol/L NaCl, and 0.1% Triton X-100) were added in duplicate to the wells and the plate was incubated at room temperature (20–25℃) for 1 hr. Aliquots (50 μL) of the apoB-48 standard (2.5 ng/mL to 160 ng/mL: 7-point calibration curve) were incubated in the same way. After the plate was washed three times, 50 μL biotin-conjugated anti-apoB-48/B-100 (ICN Pharmaceuticals Inc., USA) diluted in 0.01 mol/L phosphate buffer (pH 7.2) with 0.15 mol/L NaCl and 0.1% bovine serum albumin was added to each well and incubated with gentle shaking at room temperature for 1 hr. After the plate was washed, 50 μL horseradish peroxidase-conjugated avidin solution was added followed by incubation at room temperature for 30 min. After the plate was washed, 50 μL chromogenic substrate solution was added to each well and incubated with shaking at room temperature for 20 min until the color developed. Then 50 μL of stop solution was added to each well and plate was read at 450 nm using a Spectra-Fluor-Plus plate reader (Tecan, USA).

Metabolic syndrome was defined according to Japanese criteria[18]. Briefly, a waist circumference of more than 85 cm in men and 90 cm in women combined with more than one of the following factors led to a diagnosis of metabolic syndrome: plasma triglycerides >150 mg/dL and/or HDL cholesterol <40 mg/dL, systolic blood pressure >130 mmHg and/or diastolic blood pressure >85 mmHg, and FPG >110 mg/dL. Some subjects were taking medications, but subjects with or without medications were grouped together for this study.

The mean ± SD or median with interquartile range is shown to summarize the characteristics of the study subjects by sex. Between-group comparisons of the means and median were performed by unpaired t-test and the Wilcoxon rank-sum test, respectively. The relationship of serum lipids and lipoproteins with apo B48 was examined by correlation and multiple regression analysis. Pearson's correlation coefficients were calculated for the correlation. Stepwise multiple regression analysis was used to determine independent predictors of apo B48, with p-to-enter and p-to-retain set at 0.10 each. Statistical significance was declared if the two-sided p value was less than 0.05. Statistical analyses were performed using JMP software (SAS Institute, Cary, NC).

Results

The age of all subjects, men and women was 62.4 ± 12.8 years, 64.2 ± 12.8 years and 61.2 ± 12.7 years (mean ± S.D.), respectively. The mean body mass index (BMI) did not differ significantly between men and women (23.9 ± 3.1 vs. 23.5 ± 3.6, respectively). Plasma levels of total cholesterol, HDL-cholesterol and LDL-cholesterol were significantly lower ($p<0.001$) in men than in women, whereas the values of apo B48, triglycerides, and FPG were significantly higher in men ($p<0.001$) (**Table 1**). **Fig. 1** shows the distribution of apo B48 in men and women. The mean apo B48 level was 1.92 μg/mL in men and 1.69 μg/mL in women.

As shown in **Fig. 1**, the distribution of apo B48 was skewed to the left. Data were therefore normalized by logarithmic transformation for further statistical analysis. The triglyceride and HOMA-IR data were

Table 1. Characteristics of the subjects

	Men (n=524)	Women (n=819)	p-value
Age (years)[a]	64.2±12.8	61.2±12.7	<0.0001
BMI (kg/m²)[a]	23.9±3.1	23.5±3.6	0.01
Waist (cm)[a]	85.9±8.7	83.1±10.7	0.04
MS with/without	397/125	741/78	<0.0001
T. chol (mg/dL)[a]	192.4±31.1	204.4±30.9	<0.0001
TG (mg/dL)[b]	99 (74-140)	83 (63-117)	<0.0001
HDL-C (mg/dL)[a]	53.9±13.2	62.1±14.3	<0.0001
LDL-C (mg/dL)[a]	106.2±27.6	115.8±27.3	<0.0001
SBP (mmHg)[b]	138 (124-153)	135 (117-151)	<0.001
DBP (mmHg)[b]	78 (70-86)	74.5 (66-83)	<0.0001
FPG (mg/dL)[a]	102.9±23.4	95.0±18.9	<0.0001
IRI (μU/mL)[b]	3.9 (2.6-5.8)	4.1 (2.8-5.8)	0.38
HOMA-IR (mg/dL×μU/mL)[b]	0.924 (0.602-1.387)	0.909 (0.636-1.341)	0.02
ApoB 48 (μg/mL)[b]	1.80 (1.20-3.10)	1.61 (1.14-2.45)	<0.001

[a]Mean±SD
[b]Median (25th and 75th interquartile range)
p values were based on paired t-test and Wilcoxon rank-sum test for mean and median, respectively.

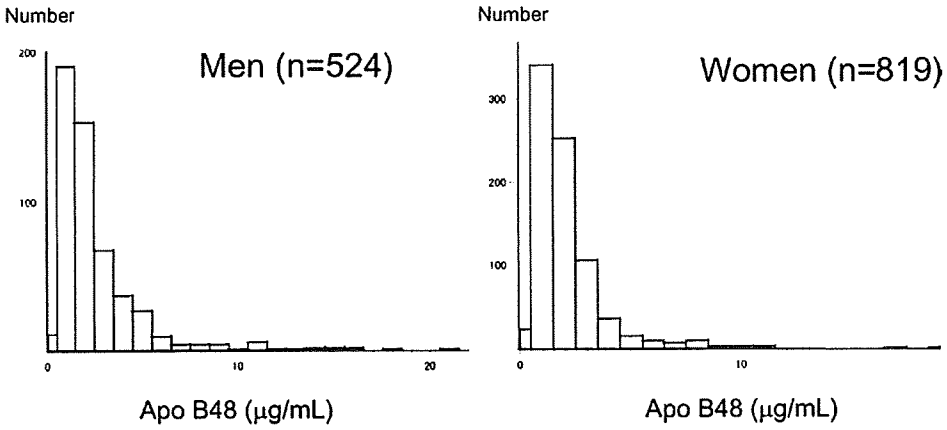

Fig. 1. Distribution of ln (apo B48) in men and women.
Geometric means and 95% central limits are 1.92 μg/mL (1.80-2.04) in men and 1.69 μg/mL (1.61-1.76) in women.

also normalized because of their skewed distribution (data not shown).

The ln (apo B48) showed a weak positive correlation with total cholesterol, and a weak negative correlation with HDL-cholesterol (**Fig. 2**). In addition, ln (apo B48) and ln (triglycerides) showed a strong positive correlation (r=0.53 in men and r=0.48 in women).

Ln (apo B48) also showed a strong positive correlation with ln (HOMA-IR) (**Fig. 2**). The fasting apo B48 level was significantly higher in both men and women with metabolic syndrome than without (**Table 2**).

Ln (apo B48) was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and ln (triglyceride) by multiple regression analysis (**Table 3**).

Discussion

In this study, we measured plasma apo B48 levels with a novel ELISA. According to previous reports, the fasting plasma apo B48 concentration ranges between

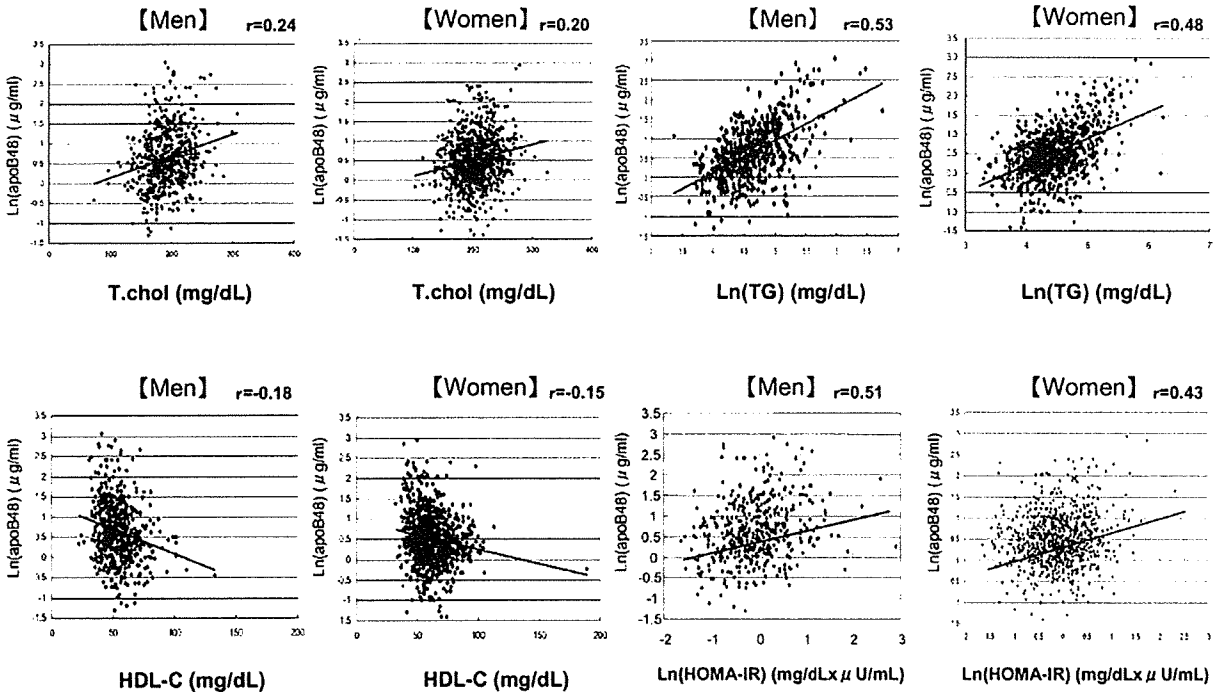

Fig. 2. Correlation of ln (apo B48) with total cholesterol, ln (triglycerides), HDL-cholesterol, and ln (HOMA-IR) in men and women.

Table 2. Apo B48 levels according to metabolic syndrome in men and women

Sex	Metabolic syndrome	N	Geometric mean (95% CI)
Men	(−)	397	1.76 (1.65–1.89)
	(+)	125	2.50 (2.17–2.88)
			$p<0.0001$
Women	(−)	741	1.64 (1.57–1.72)
	(+)	78	2.19 (1.86–2.59)
			$p<0.0001$

CI, confidence interval
p-value was based on unpaired *t*-test.

0.08 μg/mL and 60 μg/mL[8-15]. Our data (men: 1.92 μg/mL; women: 1.69 μg/mL (mean value)) were also in this range, and were similar to the level reported by Sakai *et al.* for normolipidemic subjects (5.2 ± 3.8 mg/mL) using a similar ELISA with another monoclonal antibody against apo B48[15]. Among the methods available to measure apo B48, ELISA systems based on monoclonal antibodies are valuable because they are simple and quantitative methods.

In this study, we measured fasting plasma levels of apo B48. It has been suggested that a high fasting apo B48 level reflects high postprandial concentrations of chylomicrons and/or CM-R[12]; therefore, we assumed that a high fasting plasma level of apo B48 indicated the existence of postprandial hyperlipidemia.

The B48 concentration was higher among men than women (**Fig. 1**). Sakai *et al.* previously found that men also had higher apo B48 levels than women among normolipidemic subjects[15]. These results may indicate that women show more rapid catabolism of chylomicrons and/or CM-R, or less intestinal fat absorption, or both.

Apo B48 showed a significant and strong correlation with triglycerides (**Fig. 2**). Cortner *et al.* reported that delayed catabolism of CM-R leads to hypertriglyceridemia[3]. Since very low density lipoprotein (VLDL) and VLDL remnants are considered the main contributors to plasma triglyceride concentration, the close relationship between apo B48 and triglyceride levels indicates that delayed catabolism of CM-R leads to the accumulation of VLDL or VLDL remnants. It is interesting that the plasma concentration of apo B48, which is far lower than that of apo B100 (0.15–0.2 vs.

Table 3. Stepwise multiple regression analysis of ln (apoB48) in relation to serum lipids, lipoproteins, and glucose-related parameters (n = 1,089)

	Regression coefficient	S.E.	t value	p value
Age	−0.0069	0.0013	−5.12	<0.0001
BMI	−0.0213	0.0599	−3.55	0.0004
Total cholesterol	0.0066	0.0021	3.13	0.0018
HDL cholesterol	−0.0068	0.0022	−3.15	0.0017
LDL cholesterol	−0.0047	0.0020	−2.28	0.0227
ln (Triglycerides)	0.5520	0.0715	7.72	<0.0001
ln (HOMA-IR)	0.0583	0.0337	1.73	0.0939

Sex, SBP, DBP, total cholesterol, HDL cholesterol, LDL cholesterol, apo E, ln (triglyceride), and ln (IRI) were also included as explanatory variables in the model, but they did not remain in the final model.

100–120 mg/dL), has such a significant relationship with the VLDL or VLDL remnant level. This may because the triglyceride content of CM-R is very high when compared to VLDL or VLDL remnants.

Apo B48 was also positively correlated with HOMA-IR (**Fig. 2**), which is a marker of insulin resistance, and the apo B48 level was significantly higher in subjects with metabolic syndrome than without (**Table 2**). These results indicate that apo B48 increases in the presence of insulin resistance and/or metabolic syndrome. Since insulin resistance is considered to be involved in the development of metabolic syndrome[19, 20], insulin sensitivity might influence the level of apo B48. It has been reported that insulin resistance shows a negative correlation with lipoprotein lipase mRNA expression and activity in adipose tissue[21]. Thus, defects of lipoprotein lipase may cause the accumulation of apo B48 particles. In fact, it has been reported that insulin resistance might lead to postprandial hyperlipidemia[22, 23].

Because our subjects with metabolic syndrome showed higher fasting plasma concentrations of apo B48, there is a possibility that CM-R may play a role in the increased risk of atherosclerosis related to this syndrome. Vine *et al.* reported that impaired postprandial metabolism of apo B48 led to atherosclerosis in rats with metabolic syndrome[24]. On the other hand, Velero *et al.* reported that the fasting apo B48 level does not predict the risk of coronary heart disease[25]. Thus, whether the fasting apo B48 level influences the risk of atherosclerosis remains to be determined.

Multiple regression analysis revealed that apo B48 was significantly associated with age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride. Gender does not affect apo B48 by this method, which may be due to an other factor related to gender (i.e., LDL cholesterol or HDL cholesterol) having a strong association with apo B48.

In conclusion, the fasting plasma level of apo B48 was correlated with the serum triglyceride concentration, and apo B48 levels were higher in rural Japanese subjects with metabolic syndrome than those without; however, further studies of other populations are needed to confirm these results.

References

1) Boquist S, Ruotolo G, Tang R, Björkegren J, Bond MG, de Faire U, Karpe F, Hamsten A: Alimentary lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima-media thickness in healthy, middle-aged men. Circulation, 1999; 100: 723-728
2) Groot PH, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L: Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb, 1991; 11: 653-662
3) Cortner JA, Coates PM, Le NA, Cryer DR, Ragni MC, Faulkner A, Langer T: Kinetics of chylomicron remnant clearance in normal and inhyperlipoproteinemic subjects. J Lipid Res, 1987; 28: 195-206
4) Kolovou GD, Anagnostopoulou KK, Salpea KD, Pilatis ND, Iraklianou S, Grapsa G, Pantelakis A, Tsarpalis K, Kapnia E, Cokkinos DV: Postprandial lipemia in postmenopausal women with high fasting high-density lipoproteincholesterol. Am J Med Sci, 2006; 331: 10-16
5) Rana JS, Nieuwdorp M, Jukema JW, Kastelein JJ: Cardiovascular metabolic syndrome -an interplay, of obesity, inflammation, diabetes and coronary heart disease. Diabetes Obes Metab, 2007; 9: 218-232
6) Kolovou GD, Anagnostopoulou KK, Cokkinos DV: Pathophysiology of dyslipidaemia in the metabolic syndrome. Postgrad Med J, 2005; 81: 358-366
7) Couillard C, Bergeron N, Pascot A, Alméras N, Bergeron J, Tremblay A, Prud'homme D, Després JP: Evidence for impaired lipolysis in abdominally obese men: postprandial study of apolipoprotein B-48-and B-100-containing lipoproteins. Am J Clin Nutr, 2002; 76: 311-318
8) Karpe F, Hamsten A: Determination of apolipoproteins

B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. J Lipid Res, 1994; 35: 1311-1317
9) Lemieux S, Fontani R, Uffelman KD, Lewis GF, Steiner G: Apolipoprotein B-48 and retinyl palmitate are not equivalent markers of postprandial intestinal lipoproteins. J Lipid Res, 1998; 39: 1964-1971
10) Schneeman BO, Kotite L, Todd KM, Havel RJ: Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. Proc Natl Acad Sci USA, 1993; 90: 2069-2073
11) Smith D, Proctor SD, Mamo JC: A highly sensitive assay for quantitation of apolipoprotein B48 using an antibody to human apolipoprotein B and enhanced chemiluminescence. Ann Clin Biochem, 1997; 34: 185-189
12) Smith D, Watts GF, Dane-Stewart C, Mamo JC: Postprandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. Eur J Clin Invest, 1999; 29: 204-209
13) Lorec AM, Juhel C, Pafumi Y, Portugal H, Pauli AM, Lairon D, Defoort C: Determination of apolipoprotein B-48 in plasma by a competitive ELISA. Clin Chem, 2000; 46: 1638-1642
14) Lovegrove JA, Isherwood SG, Jackson KG, Williams CM, Gould BJ: Quantitation of apolipoprotein B-48 in triacylglycerol-rich lipoproteins by a specific enzyme-linked immunosorbent assay. Biochim Biophys Acta, 1996; 1301: 221-229
15) Sakai N, Uchida Y, Ohashi K, Hibuse T, Saika Y, Tomari Y, Kihara S, Hiraoka H, Nakamura T, Ito S, Yamashita S, Matsuzawa Y: Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. J Lipid Res, 2003; 44: 1256-1262
16) Kinoshita M, Kojima M, Matsushima T, Teramoto T: Determination of apolipoprotein B-48 in serum by a sandwich ELISA. Clin Chim Acta, 2005; 351: 115-120
17) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function fromfasting plasma glucose and insulin concentrations in man. Diabetologia, 1985; 28: 412-419
18) Matsuzawa Y: Metabolic syndrome: Definition and diagnostic criteria in Japan. J Atheroscler Thromb, 2005; 12: 301-330
19) Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, Cline GW, Befroy D, Zemany L, Kahn BB, Papademetris X, Rothman DL, Shulman GI: The role of skeletal muscle insulin resistance in the pathogenesis of themetabolic syndrome. Proc Natl Acad Sci USA, 2007; 104: 12587-12594
20) Bigazzi R, Bianchi S: Insulin resistance, metabolic syndrome and endothelial dysfunction. J Nephrol, 2007; 20: 10-14
21) Panarotto D, Rémillard P, Bouffard L, Maheux P: Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. Eur J Clin Invest, 2002; 32: 84-92
22) Annuzzi G, De Natale C, Iovine C, Patti L, Di Marino L, Coppola S, Del Prato S, Riccardi G, Rivellese AA: Insulin resistance is independently associated with postprandial alterations of triglyceride-rich lipoproteins in type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol, 2004; 24: 2397-2402
23) Eriksson JW, Burén J, Svensson M, Olivecrona T, Olivecrona G: Postprandial regulation of blood lipids and adipose tissue lipoprotein lipase in type 2 diabetes patients and healthy control subjects. Atherosclerosis, 2003; 166: 359-367
24) Vine DF, Takechi R, Russell JC, Proctor SD: Impaired postprandial apolipoprotein-B48 metabolism in the obese, insulin-resistant JCR: LA-cp rat:increased atherogenicity for the metabolic syndrome. Atherosclerosis, 2007; 190: 282-290
25) Valero R, Lorec AM, Paganelli F, Beliard S, Atlan C, Lairon D, Vialettes B, Portugal H: Fasting apoprotein B-48 level and coronary artery disease in a population without frank fasting hypertriglyceridemia. Metabolism, 2005; 54: 1442-1447

Geriat. Med. 47(7)：859～863, 2009

各論

高齢者
—ガイドラインと今後の

高血圧 ●メタボリックシンドロー
定健診 ●TONE

7．高齢者の メタボリックシンドローム における血圧管理

斎藤 重幸

SUMMARY

■メタボリックシンドローム(MetS)は腹部肥満を基盤とした高血圧を含む危険因子の集積で，動脈硬化性疾患の易発症状態である．わが国でのMetSの頻度は高齢者男性で50%，女性で30%程度に及ぶ．高齢者でのMetSのCVD発症リスクは非高齢者より低いものであるが，リスク集積は認知症，CKD，糖尿病発症などとも関連し，特に高血圧の治療が重要になる．MetSを合併した高齢者では肥満の是正が重要であり，減量により高齢者でも一定程度の降圧効果が期待されるが，生活習慣の改善に当たってはQOL低下，認知症進行，低血圧による転倒事故などに注意を払う必要がある．降圧薬療法でMetSの背景にある肥満，インスリン抵抗性を踏まえた治療が必須でACE阻害薬，ARBが中心となる．

はじめに

メタボリックシンドローム(MetS)は内臓脂肪蓄積型肥満を背景として，血圧高値，耐糖能異常，脂質異常が合併した易動脈硬化性発症状態として定義される．わが国では，2005年に関連8学会より合同で診断基準が発表され，これを基に2008年からは40～74歳までの特定健診・特定保健指導制度が開始されている．わが国のMetSの特徴は肥満を腹囲径でスクリーニングし，腹囲径で男性85 cm以上，女性90 cm以上を腹部肥満としてこれを必須項目とすることで，正常高値血圧以上の血圧高値，空腹時血糖値110 mg/dL以上(特定健診では100 mg/dL以上)の高血糖，中性脂肪150 mg/dL以上あるいはHDLコレステロール40 mg/dL未満の脂質異常の2項目以上が集積するとMetSと診断される．

年齢にかかわらずMetSの診断基準は同一であるが，特定保健指導では，前期高齢者では動機付け支援までであり，年齢により特定健診後の対処方法は異なる．本稿では，高齢者のMetSの臨床意義と高血圧治療に関して概説する．

高齢者のメタボリックシンドロームの病態

わが国は世界で有数の長寿国であるが，全年齢層での死因の第2位，3位が脳血管障害，心疾患であり，現在この多くを，脳梗塞，虚血性心疾患などの動脈硬化性疾患が占める．そして，高齢者になるに従い心疾患・脳血管疾患の死亡割合が増加する．特に女性ではこの傾向が顕著で，80歳以上の死因の37%が心疾患と脳血管疾患である(図1)[1]．さらに，心疾患・脳血管疾患(CVD)は，認知症や転倒，骨折の背景要因でもあり，CVD予防は予後の延長，ADLの確保から要介護者の増加防止に至る社会負担の軽減のために重要となる．MetSは，内臓脂肪蓄積型肥満に着目しこれに合併するCVD多危険因子を肥満の是正により，一元的に管理しようとする概念である．進行した高血圧，糖尿病，動脈硬化には，それぞれについての厳格な管理が必要なことは明白であるが，それによって予防できない動脈硬化性疾患への対策にはMetSの予防と管理が重要であると考えられる．動脈硬化の進展と動脈硬化性疾患の発症は，複合的な危険因子の長期間にわたる曝露の後に起こる

■さいとう しげゆき(札幌医科大学医学部第二内科)

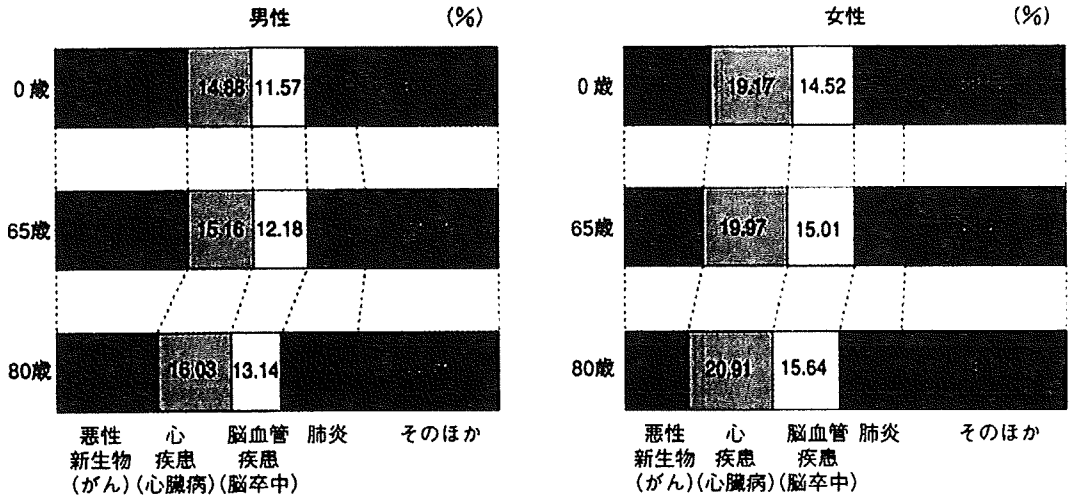

図1　0歳，65歳，80歳の死亡原因(平成17年)

ことは明らかである．高齢者での心血管疾患の予防のために，若年時よりのMetSへの介入が必要である．

高齢者のメタボリックシンドロームの頻度

生理的加齢，病的加齢いずれにおいても，加齢に従い血圧値，血糖値は上昇し，体脂肪分布も変化する．したがって高齢者でのMetSの頻度は上昇する．図2[2]は，国民栄養調査時に評価された日本人のMetSの頻度である．20歳以上全体では男性の45.6%，女性の16.7%，70歳以上では男性の55.3%，女性の30.4%が腹部肥満診断基準を満たし，かつMetS診断基準を1つ以上合併しており，これがわが国の動脈硬化性疾患の高リスク者であると考えられる．しかしながら，この中には既に高血圧，糖尿病，脂質異常症などの治療者が含まれ，管理の状況や罹病期間もまちまちであり，MetSとして一定のリスクを持つものではない．

高齢者のメタボリックシンドロームの予後

教室で継続している前向き疫学研究(端野・壮瞥町研究)では，NCEP-ATPⅢ基準でのMetSは非MetSに比して心疾患発症リスクが1.8倍に上昇することを報告している[3]．65歳以上の高齢者で同様な解析を行うと，心血管疾患発症には非MetS群と比べてMetS群で有意の差違がなかった．このとき，予後規定因子として高血圧リスクが最大である．

Mozaffarianらは高齢者を対象に，NCEP-ATPⅢ基準のMetSと死亡リスクをCardiovascular Health Studyの対象より評価した(図3)[4]．Cardiovascular Health Studyは米国の65歳以上を対象としたコホート研究であるが，CVD既往のない4,258人を追跡した．平均年齢は73歳．男性の31%，女性の38%がMetSであり，15年間の追跡で2,116人が死亡している．多変量解析でMetSの死亡率は，非MetSに比し相対リスク1.22(95%信頼区間：1.11～1.34)であった．しかしながら死亡リスク上昇は，空腹時高血糖があるMetSで相対リスク1.41(1.27～1.57)，高血圧があるMetSで相対リスク1.26(1.15～1.39)だが，空腹時高血糖がないMetSでは相対リスク0.97(0.85～1.11)，高血圧がないMetSでは0.92(0.71～1.19)で，リスク上昇はみられなかった．高血圧や空腹時高血糖のないMetSの死亡における集団寄与リスクは認められなかった．CVD既往者を加えた検討でも同様であ

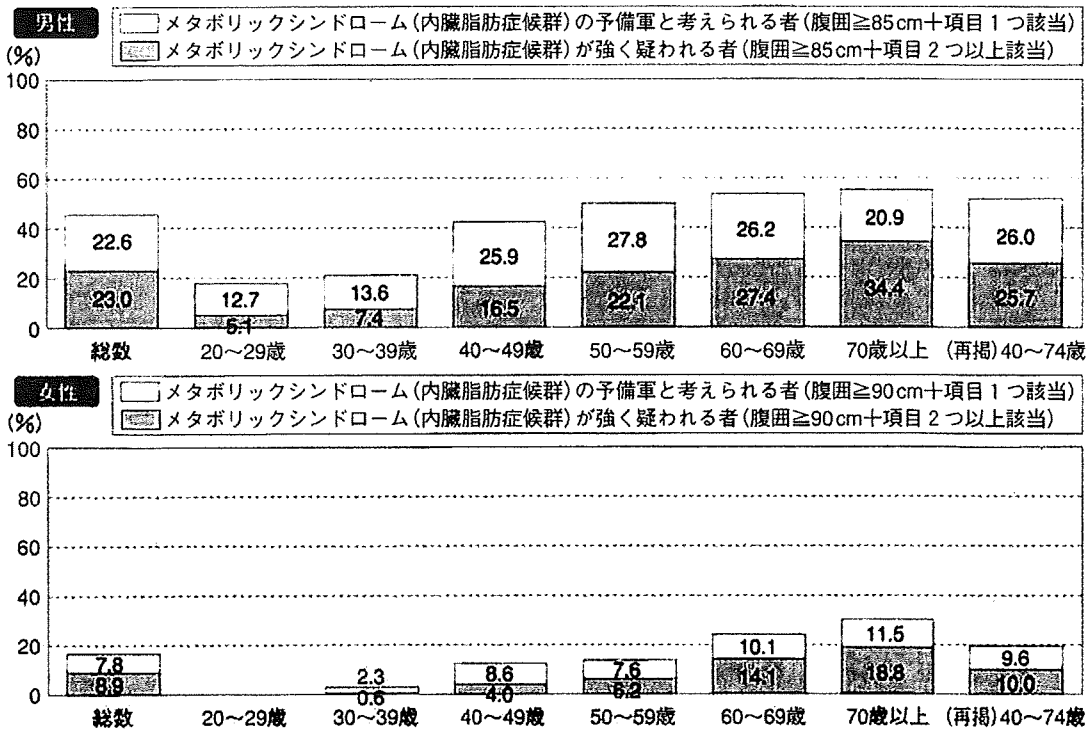

図2　メタボリックシンドローム(内臓脂肪症候群)の状況(20歳以上)
—平成16年　国民健康・栄養調査結果—

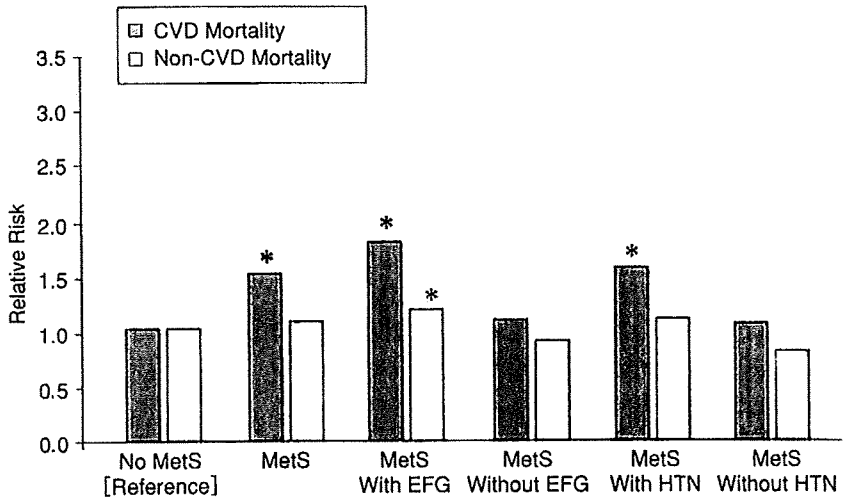

図3　65歳以上高齢者でのメタボリックシンドローム(MetS)の有無による心血管疾患死亡(CVD)と非CVD死亡の相対危険
EFG：空腹時高血糖，HTN：高血圧，文献4より改変引用．