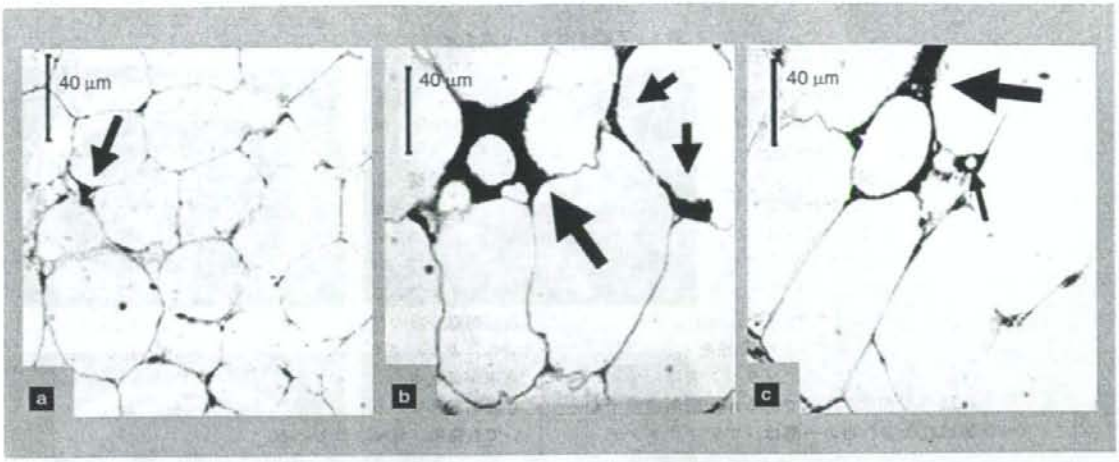


a）Leanマウス：野生型マウス，b）diet-induced肥満マウス：高脂肪食負荷肥満マウス，c）leptin-deficient肥満マウス：レプチン欠損肥満マウス．肥満モデルの脂肪細胞では，F4/80（マクロファージのマーカー）陽性の細胞（矢印）の増加を認める．色矢印は，より集積した状態のものを示す．

図4　肥満による脂肪細胞へのマクロファージの集積［S. P. Weisbergほか，*J. Clin. Invest.*，112，1796（2003）より改変して引用］

また，循環血液中のマクロファージも重要な役割を果たすと考えられている．すなわち，肥満が存在するとマクロファージ誘導作用のある「悪玉」MCP-1が発現し，循環血液中のマクロファージが脂肪組織へ浸潤する（図4）．活性化したマクロファージから分泌されるサイトカインTNF-αはそれ自体がインスリン抵抗性を惹起するうえ，脂肪細胞からのMCP-1の発現を促し，それがまたマクロファージをよび寄せるという悪循環をうながすとされている（表1，文献5）．このようにマクロファージは脂肪細胞肥大による形質転換を誘導する．一方，非肥満の場合，小型脂肪細胞から分泌されるアディポカインは，マクロファージの脂肪細胞への浸潤や活性化を抑制していると考えられている．

脂肪細胞の肥大化が，どのようにしてこのようなアディポサイトカイン分泌パターンの変化につながるについては不明な点が多い．

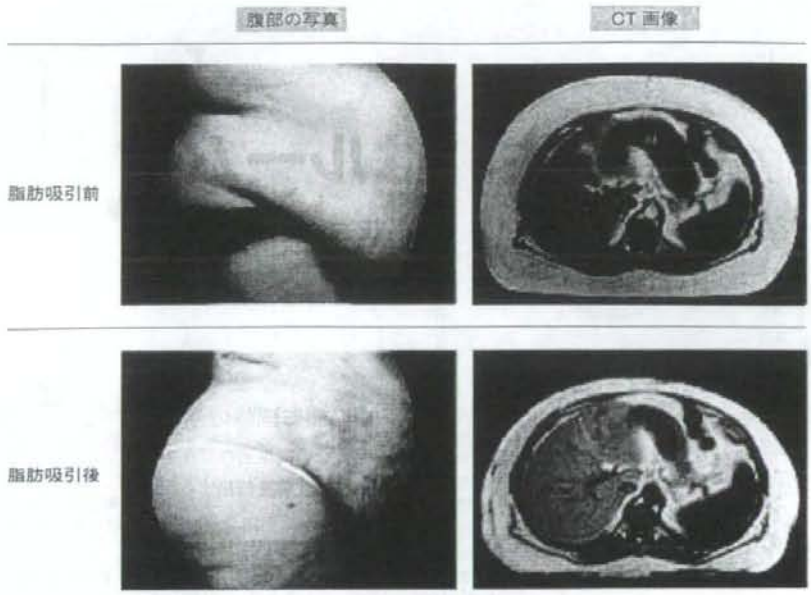
皮下脂肪の影響は？

肥満により，内臓脂肪が沈着し脂肪細胞が大型化すると，アディポサイトカインの分泌様式が変わってメタボリックシンドロームが誘導される，という流れを説明したが，皮下脂肪についてはどうだろう？　運動して皮下脂肪が減ると，インスリン抵抗性は改善するし，糖尿病のコントロールもよくなる．皮下脂肪も内臓脂肪と同様に糖・脂質代謝に相当悪影響があるのではないか，と感じられる方も少なくないだろう．

このことに関連して，興味深いデータがある．2004年に臨床医学の一流紙である*The New England Journal of Medicine*に掲載された論文では，BMI（＝[体重(kg)]/[身長(m)]^2）が平均35 kg/m^2（！）を超える著明な肥満症の症例に対して皮下脂肪吸引を行い，脂肪吸引の前後における代謝異常の程度を比較した結果を報告している（図5）．おどろいたことに，皮下脂肪の吸引は，血圧，糖・脂質代謝異常には有意な影響を与えず，さらに血中のアディポネクチンやTNF-αの濃度にも影響を与えない，という結果だった．皮下脂肪の吸引では，（美容に対する効果はあるだろうが）メタボリックシンドロームの状態を改善することはできなかったことは，やはり，内臓肥満の改善がメタボリックシンドロームのコントロールに重要であることをサポートする結果であるといえる．

おわりに

脂肪組織が単なるエネルギーの貯蔵庫ではなく，生理活性物質の産生・放出により全身の器官にアクティブに働きかける臓器であることが明らかになってきた．これには，わが国の研究者の日夜を分かたぬ研究も大いに貢献している．メタボリックシンドロームの疾患概念は本当に必要であるか，ま



このような皮下脂肪吸引治療では，インスリン抵抗性の改善は得られなかった．

図5 高度肥満者の皮下脂肪吸引［S. Kleinほか，*The New England Journal of Medicine*, **350**, 2549 (2004) より改変して引用］

た，診断には，どのような基準が最適であるか，など未解決な点もある．しかしながらこれらのことは，（内臓）肥満を背景因子として，血圧，糖・脂質代謝異常が「ひとまとめになって発症してくる」ことを認識，理解することの重要性をいささかも減じないだろう．飽食，高齢化の時代にあっては，肥満によるメタボリックシンドロームの発症機序を解明し，それに基づき新たな治療法を提案する，というトレンドは一時のブームに終始せず，ますます加速されていくものと思われる．

あいまいな診断基準に混乱も
——なぜ腹囲の基準値は女性の方が大きいのか？——

メタボリックシンドロームをわざわざ新たな疾患概念として提唱する必要性があるのかとの議論（文献1）がある一方，診断基準に対しても議論がある．メタボリックシンドロームの診断基準は図2のように世界的に統一されていない．こちらの診断基準を使えばメタボリック症候群だが，こちらの基準を使うとそうではないということも起こりうる．必須とする診断項目や，どの項目を重視するかということから，男女共通の値を採用するか男女別の値を採用するかということまでさまざまだ．

たとえば日本の基準値は男女共通の値だが，図2の日本以外は男女別の値を採用している．腹囲は男女別の値だと思われるだろうが，そうではない．腹囲決定の基盤である「病気のリスクが高まる内臓脂肪面積は100 cm^2以上」は，男女混合のCTスキャンデータから割り出した男女共通の値だ．その上で，男女別に内臓脂肪面積vs腹囲のグラフを作成し，そこから内臓脂肪面積100 cm^2以上の腹囲をそれぞれ割り出している．そのため皮下脂肪の多い女性の基準値は男性より5 cm大きい．

内臓脂肪面積の基準値の算出方法への異論や，検討した女性の人数（196人）が男性（559人）と比べて少ないことなどから，国内では腹囲の基準値に対する議論がある．また，国際糖尿病連合（IDF）が日本人向けの基準値を男性90 cm以下，女性80 cm以下と修正したこともあり，混乱も起きている．

参考文献

1. R. Kahn, *Circulation*, **115**, 1806 (2007)；R. S. Beaserほか, *Circulation*, **115**, 1812 (2007).
2. Y. Matsuzawa, *Atheroscler Suppl.*, **6**, 7 (2005).
3. K. Haraほか, *Diabetes Care*, **29**, 1357 (2006).
4. T. Yamauchほか, *Nat. Med.*, **13**, 332 (2007).
5. T. Suganamiほか, *Arterioscler. Thromb. Vasc. Biol.*, **25**, 2062 (2005).
6. N. Kameiほか, *J. Biol. Chem.*, **281**, 26602 (2006).
7. H. Kandaほか, *J. Clin. Invest.*, **116**, 1494 (2006).
8. S. P. Weisbergほか, *J. Clin. Invest.*, **116**, 115 (2006).
9. C. M. Steppanほか, *Nature* (London), **409**, 307 (2001).
10. P. Trayhurnほか, *Br. J. Nutr.*, **92**, 347 (2004).

Critical Role of Bone Marrow Angiotensin II Type 1 Receptor in the Pathogenesis of Atherosclerosis in Apolipoprotein E–Deficient Mice

Daiju Fukuda, Masataka Sata, Nobukazu Ishizaka, Ryozo Nagai

Objective—It is suggested that the angiotensin II (Ang II)–Ang II type 1 receptor (AT1R) pathway plays a pivotal role in the pathogenesis of atherosclerosis. Recently, bone marrow (BM) cells were reported to express AT1R. Here, we investigated the role of AT1R in BM in the pathogenesis of atherosclerosis.

Methods and Results—Genetic ablation or pharmacological blockade of AT1R led to a significant reduction and stabilization of atherosclerotic lesions in ApoE$^{-/-}$ mice. To elucidate the role of AT1R in BM, we generated several BM chimeric mice. Ang II promoted atherosclerosis progression in the BM chimeric mice that had AT1aR in BM, regardless of the absence of AT1aR in the recipient vasculature ($P<0.05$). BM chimeric mice whose BM AT1aR was disrupted showed significantly less atherosclerotic lesions in aorta ($P<0.05$) and more stable plaque with reduced accumulation of BM-derived cells compared with BM chimeric mice that had AT1aR-positive BM. Most of the BM-derived cells in atheroma were positive for a macrophage marker and expressed matrix metalloproteinase (MMP)-9 and monocyte chemoattractant protein-1.

Conclusions—Our findings suggest that AT1R in BM plays an important role in the pathogenesis of atherosclerosis. (***Arterioscler Thromb Vasc Biol.* 2008;28:90-96.**)

Key Words: angiotensin II type I receptor ■ bone marrow ■ atherosclerosis ■ MMP-9 ■ MCP-1

It is a widely accepted view that atherosclerosis is a chronic inflammatory disease.[1] Although multifactorial in etiology, vascular inflammation produces atherosclerosis through the continuous recruitment of circulating leukocytes into the vessel wall and by contributing to an oxidant-rich inflammatory milieu. Recent advances in immunology have dissected several molecular pathways that induce and promote inflammatory responses in atherosclerotic lesions.[1]

The renin–angiotensin system (RAS) has been suggested to play a role in the pathogenesis of atherosclerosis by simulating a series of coordinated cellular and molecular events observed in atherosclerotic lesions.[2] Angiotensin II (Ang II) induces the production of reactive oxygen species and stimulates the expression of adhesion molecules and chemokines, leading to endothelial dysfunction, adhesion and invasion of leukocytes, lipid deposition, and smooth muscle cell proliferation.[2] These observations suggest that local effects of an activated RAS in the vessel wall play a central role in the pathogenesis of chronic vascular inflammation by directly acting on resident vascular cells.

The RAS is reported to be involved in the maintenance of cell proliferation and organ remodeling under physiological or pathophysiological conditions in many tissues other than the cardiovascular system.[2] It was suggested that an activated RAS has local effects in bone marrow (BM), which contributes to the regulation of both normal and malignant hematologic processes.[3] It was demonstrated that Ang II increases hematopoietic progenitor cell proliferation.[4] Recently, Cassis et al reported that Ang II promoted vascular pathology via Ang II type 1a receptor (AT1aR) and that AT1aR expressed on infiltrating cells exerted modest regulation of Ang II–induced atherosclerosis in LDL receptor–deficient mice.[20] It was suggested that the presence of AT1aR in resident tissue was required for the initiation of Ang II–induced atherosclerosis and aneurysm formation.

Although ApoE-deficient and LDL receptor–deficient mice are the most widely used mouse models for atherosclerosis, they differ markedly in lesion type and in their susceptibility to different atherogenic stimuli.[5] Here, we tested the hypothesis that local effects of an activated RAS, especially AT1aR, in BM may play a role in the pathogenesis of atherosclerosis in ApoE-deficient mice. Analyses of BM chimeric mice revealed that AT1aR in BM plays an important role in progression and destabilization of atherosclerotic plaques. We performed a detailed analysis of cellular components of plaque composition and investigated the molecular

Original received April 23, 2007; final version accepted October 12, 2007.

From the Departments of Cardiovascular Medicine (D.F., M.S., N.I., R.N.) and Advanced Clinical Science and Therapeutics (M.S.), University of Tokyo Graduate School of Medicine, Tokyo, Japan.

Correspondence to Dr Masataka Sata, Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail msata-circ@umin.net

Arterioscler Thromb Vasc Biol. **is available at http://atvb.ahajournals.org** **DOI: 10.1161/ATVBAHA.107.152363**

mechanism by which BM AT1aR contributes to progression and destabilization of atherosclerotic plaque.

Methods

Animals

ApoE-deficient ($ApoE^{-/-}$) mice were originally purchased from Jackson Laboratory. Mice deficient in AT1aR, the type 1a receptor of Ang II, ($AT1aR^{-/-}$) were described previously.[6] GFP mice were described previously.[7] Double knockout mice deficient in ApoE and AT1aR were generated by cross-breeding $ApoE^{-/-}$ mice and $AT1aR^{-/-}$ mice. Furthermore, we also generated GFP-positive $ApoE^{-/-}AT1aR^{+/+}$ mice ($ApoE^{-/-}AT1aR^{+/+}GFP^{+/+}$ mice) and GFP-positive $ApoE^{-/-}AT1aR^{-/-}$ mice ($ApoE^{-/-}AT1aR^{-/-}GFP^{+/+}$ mice). We administered 10 mg/kg/d olmesartan, an AT1R blocker, or 30 mg/kg/d hydralazine to 6-week-old male $ApoE^{-/-}$ mice by gavage every day for 24 weeks. An osmotic mini-pump (Alzet) was used to infuse Ang II (5 mg/kg/d).

Bone Marrow Transplantation

Bone marrow transplantation (BMT) was performed as described previously.[7] At 4 weeks after BMT, all animals were started on a Western type diet. We used only BM chimeric mice, in which more than 80% of BM had been replaced by donor BM. All experimental procedures and protocols were approved by the Animal Care and Use Committee of the University of Tokyo.

Preparation of Aortas and Atherosclerotic Lesions

Lipid deposition was quantified by en face analysis of the aorta as previously described.[8] The atherosclerotic lesions in aortic root were analyzed by Oil red O staining, Sirius red staining, and immunohistochemistry as previously described.[9]

Laser Microdissection and Quantitative Real-Time Polymerase Chain Reaction

The atherosclerotic lesions were collected from the aortic root with a Laser Microdissection System (AS LMD, Leica) according to the manufacturer's instructions. Total RNA was isolated with the use of the RNeasy MicroKit (QIAGEN). First strand cDNA was synthesized from the obtained total RNA using a Quantitect Reverse Transcription Kit (QIAGEN) for quantitative real-time polymerase chain reaction (PCR).

Statistical Analysis

Numerical values are expressed as mean±SEM. Comparison of parameters between 2 groups was performed by unpaired Student *t* test. A value of $P<0.05$ was considered significant.

For further details, please refer to the supplemental materials (available online at http://atvb.ahajournals.org).

Results

Effects of Genetic Ablation or Pharmacological Blockade of AT1R on Atherosclerotic Plaque Formation

We generated $ApoE^{-/-}AT1aR^{-/-}$ double knockout mice by cross-breeding $ApoE^{-/-}AT1aR^{+/+}$ mice and $ApoE^{+/+}AT1aR^{-/-}$ mice. We compared atherosclerotic lesion progression between male $ApoE^{-/-}AT1aR^{+/+}$ mice (n=9) and $ApoE^{-/-}AT1aR^{-/-}$ mice (n=7) fed normal chow. As previously reported, systolic blood pressure was significantly lower in $ApoE^{-/-}AT1aR^{-/-}$ mice (90.1±2.9 mm Hg) than in $ApoE^{-/-}AT1aR^{+/+}$ mice (101.1±3.4 mm Hg, $P=0.03$). Plasma total cholesterol level was significantly higher in $ApoE^{-/-}AT1aR^{-/-}$ mice (911±102 mg/dL) than in $ApoE^{-/-}AT1aR^{+/+}$ mice (513±41 mg/dL, $P=0.006$). At 32 weeks of age, en face Sudan IV staining of the aortic arch revealed a

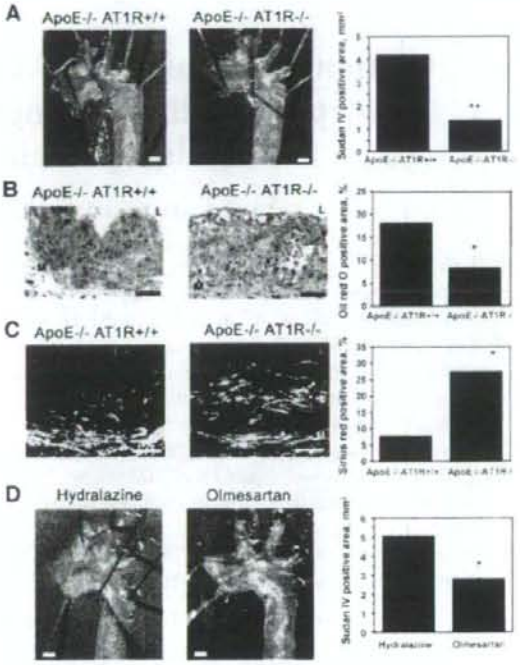


Figure 1. Effects of genetic ablation or pharmacological blockade of AT1R on atherosclerosis. A, En face Sudan IV staining of aortic arch in $ApoE^{-/-}AT1aR^{+/+}$ and $ApoE^{-/-}AT1aR^{-/-}$ mice. Bar, 1 mm. B, Oil red O staining. Bar, 50 μm. C, Sirius red staining. Bar, 50 μm. D, Quantification of lesions in mice treated with olmesartan or hydralazine. Bar, 1 mm. *$P<0.05$, **$P<0.01$. L indicates lumen; M, media.

significant reduction in atherosclerotic lesion formation in $ApoE^{-/-}AT1aR^{-/-}$ mice (1.4±0.3 versus 4.2±0.7 mm^2, $P=0.003$; Figure 1A). Furthermore, Oil red O staining and Sirius red staining of atherosclerotic lesions in the aortic root revealed significantly decreased lipid deposition (8.4±2.4 versus 18.1±2.8%, $P=0.04$) and increased collagen content (27.4±5.3 versus 7.9±4.0%, $P=0.03$) in the plaques of $ApoE^{-/-}AT1aR^{-/-}$ mice compared with those of $ApoE^{-/-}AT1aR^{+/+}$ mice (Figure 1B and 1C). We infused Ang II (5 mg/kg/d) or vehicle into the 20-week-old mice for 2 weeks. In $ApoE^{-/-}AT1aR^{+/+}$ mice, Ang II markedly accelerated atherosclerotic lesion formation in aortic arch (9.3±1.4 versus 2.1±0.4 mm^2, $P=0.0001$) associated with significant elevation in systolic blood pressure (143.0±3.2 versus 95.5±2.6 mm Hg, $P<0.0001$). On the other hand, in $ApoE^{-/-}AT1aR^{-/-}$ mice, there was no significant difference in atherosclerotic lesion area (0.8±0.3 versus 1.5±0.5 mm^2, $P=0.20$) or in blood pressure (92.1±4.2 versus 80.5±4.5 mm Hg, $P=0.09$) between the Ang II–treated and vehicle-treated groups.

Next, we administered 10 mg/kg/d olmesartan (n=6), an AT1R blocker, or 30 mg/kg/d hydralazine (n=6) to 6-week-old male $ApoE^{-/-}AT1aR^{+/+}$ mice fed a Western-type diet every day by gavage for 24 weeks. There was no significant difference between the 2 groups in systolic blood pressure

(hydralazine, 64.7±2.6 versus olmesartan, 61.7±1.4 mm Hg; $P=0.52$) or plasma total cholesterol level (hydralazine, 527±33 versus olmesartan, 523±27 mg/dL, $P=0.93$). Consistent with the effects of genetic ablation of AT1aR, en face Sudan IV staining of the aortic arch revealed significant suppression of atherosclerotic lesion progression by olmesartan (2.8±0.6 versus 5.1±0.5 mm^2, $P=0.01$) (Figure 1D). Furthermore, Oil red O staining of the plaques in the aortic root revealed that olmesartan decreased lipid content (7.3±1.3 versus 14.5±2.9%, $P=0.048$) with increased collagen content (38.7±4.3 versus 23.1±4.5%, $P=0.03$) as detected by Sirius red staining.

Effects of Restoration of BM AT1aR on Atherosclerosis in ApoE$^{-/-}$AT1aR$^{-/-}$ Mice

To evaluate the potential contribution of AT1aR in BM to the pathogenesis of atherosclerosis, we generated several combinations of BM chimeric mice. We performed BMT from ApoE$^{-/-}$AT1aR$^{+/+}$ mice to ApoE$^{-/-}$AT1aR$^{-/-}$ mice at 10 to 14 weeks of age. We also performed BMT from ApoE$^{-/-}$AT1aR$^{+/+}$ mice to ApoE$^{-/-}$AT1aR$^{-/-}$ mice at the same age. These BM chimeric mice had AT1aR in BM, but not in their innate vascular cells. At 12 weeks after BMT, white blood cell count was similar between the AT1aR$^{-/-}$ recipients repopulated with AT1aR$^{+/+}$ BM and AT1aR$^{+/+}$ BM (5.6±0.4 versus 4.9±0.5×10^3/μL, $P=0.28$). From 12 weeks after BMT, we infused 5 mg/kg/d Ang II or vehicle into these BM chimeric mice for 8 weeks using an osmotic mini-pump. Ang II infusion into these BM chimeric mice elevated blood pressure significantly compared with vehicle infusion, though these mice had no AT1aR in their vasculature. There was no significant difference in blood pressure or in plasma cholesterol level between Ang II-treated AT1aR$^{-/-}$ recipient mice repopulated with AT1aR$^{-/-}$ BM and Ang II-treated AT1aR$^{-/-}$ recipient mice repopulated with AT1aR$^{+/+}$ BM (systolic blood pressure, 97.0±6.2 versus 107.4±3.6 mm Hg, $P=0.16$; total cholesterol level, 728±50 versus 650±54 mg/dL, $P=0.32$). After 8 weeks of infusion, en face Sudan IV staining of the aortic arch revealed that atherosclerotic lesions in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM (n=8) were significantly larger than those in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM (n=7; 1.9±0.5 versus 0.6±0.2 mm^2, $P=0.03$; Figure 2). Histological analysis of atherosclerotic lesions in the aortic root revealed that lipid deposition detected by Oil red O staining was accelerated in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM compared with those in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM (12.1±2.2 versus 5.5±1.4%, $P=0.03$). Collagen content demonstrated by Sirius red staining was decreased in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM compared with that in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM (9.7±1.5 versus 18.2±2.6%, $P=0.01$). We measured mRNA expression of MMP-9, MCP-1, and vascular cell adhesion molecule (VCAM)-1 in the plaques by means of a laser microdissection system and quantitative RT-PCR. MMP-9 expression in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM tended to be greater compared with that in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM (3.5±1.5 versus 0.7±0.2 [arbitrary unit], $P=0.11$). There was no statistical difference in MCP-1 (7.6±2.4 versus 3.6±0.8 [arbitrary unit], $P=0.15$) or VCAM-1 (5.6±0.4 versus 6.0±0.3 [arbitrary unit], $P=0.58$) expression. Among the vehicle treated mice (n=7 for each group), collagen content in atheroma in AT1aR$^{-/-}$ recipients with AT1aR$^{+/+}$ BM was significantly decreased compared with that in AT1aR$^{-/-}$ recipients with AT1aR$^{-/-}$ BM, although atherosclerotic lesion area in aorta (Figure 2), lipid content in atheroma and RNA expression in the lesion were similar (supplemental Table I). Taken together, these results suggest that BM transplantation from AT1aR$^{+/+}$ donors to AT1aR$^{-/-}$ recipients could restore Ang II–induced acceleration of atherosclerosis and plaque destabilization.

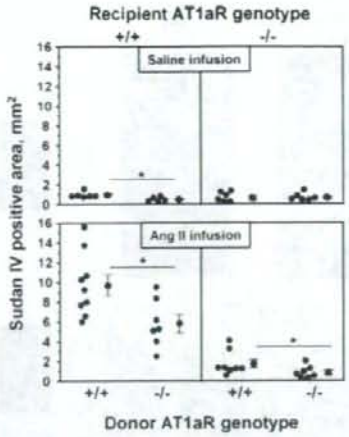


Figure 2. Atherosclerotic lesion development after BMT. BMT was performed between ApoE$^{-/-}$AT1aR$^{+/+}$ and ApoE$^{-/-}$AT1aR$^{-/-}$ mice. Ang II or vehicle was infused for 8 weeks starting at 12 weeks after BMT. Lesions were quantified after en face Sudan IV staining of the aortic arch. Circles represent size in individual mice. The right circles with bars represent means. *$P<0.05$.

Effects of Targeted Disruption of BM AT1aR on Atherosclerosis in ApoE$^{-/-}$AT1aR$^{+/+}$ Mice

Next, to keep track of BM-derived cells in the process of atherosclerotic lesion progression, we replaced BM of ApoE$^{-/-}$AT1aR$^{+/+}$ mice with that of ApoE$^{-/-}$AT1aR$^{-/-}$GFP$^{+/+}$ mice or ApoE$^{-/-}$AT1aR$^{+/+}$GFP$^{+/+}$ mice at 10 weeks of age. The former BM chimeric mice lacked AT1aR only in BM, and the latter chimeric mice had AT1aR in both BM and the vasculature. At 12 weeks after BMT, white blood cell count was similar between the AT1aR$^{+/+}$ recipient mice repopulated with AT1aR$^{+/+}$ BM and the AT1aR$^{-/-}$ recipient mice repopulated with AT1aR$^{-/-}$ BM (6.9±0.6 versus 6.3±0.8×10^3/μL, $P=0.52$). In these BM chimeric mice, we compared the effects of Ang II on atherosclerotic lesion formation. We infused Ang II from 12 weeks after BMT. After 8 weeks infusion, en face Sudan IV staining of the aortic arch revealed that acceleration of atherosclerotic lesion was significantly attenuated in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{-/-}$ BM (n=7) compared with that in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{+/+}$ BM (n=9) (5.8±0.9 versus 9.8±1.1 mm^2, $P=0.02$; Figures 2 and 3A), with reduced accumulation of GFP-positive cells (5.8±0.3 versus 9.3±1.3 mm^2, $P=0.03$; Figure 3A). In these

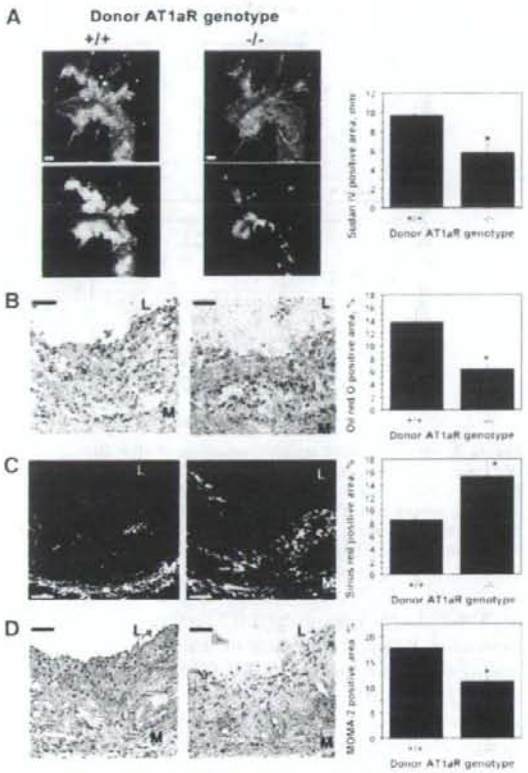


Figure 3. Targeted disruption of AT1aR in BM-ameliorated atherosclerotic lesion progression and destabilization in ApoE $^{-/-}$ AT1aR$^{+/-}$ mice. A, En face Sudan IV staining of aorta. GFP signal indicates accumulation of BM-derived cells. Bar, 1 mm. B, Oil red O staining of the lesions in aortic root. Bar, 50 μm. C, Sirius red staining. Bar, 50 μm. D, Anti–MOMA-2 immunohistochemistry. Bar, 50 μm. L indicates lumen; M, media. *$P<0.05$.

2 types of BM chimeric mice, there was no significant difference in blood pressure (136.9±4.7 versus 141.7±3.1 mm Hg, $P=0.40$) or in total cholesterol level (1019±87 versus 912±76 mg/dL, $P=0.37$). In atherosclerotic plaques in the aortic root in the AT1aR$^{+/-}$ recipients with AT1aR$^{-/-}$ BM showed significantly reduced lipid deposition (6.5±0.8 versus 13.8±2.7%, $P=0.04$) and increased collagen content (15.3±2.3 versus 8.6±1.9%, $P=0.01$) compared with that in AT1aR$^{+/+}$ recipients with AT1aR$^{+/+}$ BM (Figure 3B and 3C). These results suggest that BM-derived cells may play a role in the pathogenesis of accelerated atherosclerosis induced by Ang II. Infiltration of macrophages into the lesions was significantly reduced in the AT1aR$^{+/+}$ recipients with AT1aR$^{-/-}$ BM compared with that in the AT1aR$^{+/+}$ recipient with AT1aR$^{+/+}$ BM as demonstrated by immunostaining for MOMA-2 (11.2±1.2 versus 17.8±2.1%, $P=0.02$; Figure 3D). MMP-9 RNA expression in the AT1aR$^{+/+}$ recipient with AT1aR$^{-/-}$ BM in atheroma was significantly reduced compared with that in the AT1aR$^{+/-}$ recipient with AT1aR$^{+/+}$ BM (0.9±0.7 versus 8.9±3.1 [arbitrary unit], $P=0.03$). In these BM chimeric mice, there was no significant difference in MCP-1 (4.6±0.4 versus 4.7±0.6 [arbitrary unit], $P=0.87$) nor VCAM-1 (5.4±0.3 versus 5.3±0.4 [arbitrary unit], $P=0.87$) expression.

Next, we infused vehicle into these BM chimeric mice for 8 weeks. There was no significant difference in blood pressure between the AT1aR$^{+/+}$ recipients with AT1aR$^{+/+}$ BM and those with AT1aR$^{-/-}$ BM. Similar to Ang II–treated mice, atherosclerotic lesion of aorta was significantly attenuated in the AT1aR$^{+/+}$ recipients with AT1aR$^{-/-}$ BM (n=5) compared with that in the AT1aR$^{+/+}$ recipients with AT1aR$^{+/+}$ BM (n=6; Figure 2). However, lipid deposition, collagen content, and RNA expression in atheroma were similar in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{+/+}$ BM or AT1aR$^{-/-}$ BM (supplemental Table I).

To investigate how AT1aR positive BM cells contribute to the pathogenesis of atherosclerosis, we examined gene expression in the plaques by means of a laser microdissection system and quantitative RT-PCR (n=4 in each group) at 4 weeks after Ang II infusion (Figure 4A). Expressions of MMP-9 (3.0-fold, $P=0.04$) and MCP-1 (7.1-fold, $P=0.02$) in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{+/+}$ BM was significantly greater than those in the AT1aR$^{+/-}$ recipients repopulated with AT1aR$^{-/-}$ BM. On the other hand, there was no significant difference in VCAM-1 expression between the 2 BM chimeric mice. Accumulation of BM-derived GFP-positive cells was significantly accelerated in AT1aR$^{+/+}$ recipients with AT1aR$^{+/+}$ BM (n=8) compared with that in AT1aR$^{+/-}$ recipients with AT1aR$^{-/-}$ BM (n=7; 60.3±3.8 versus 38.4±1.9%, $P=0.0003$). Most of the BM-derived cells were positive for a macrophage marker (Figure 4B). Furthermore, the percentage of BM-derived GFP-positive cells among the MMP-9–positive cells (72.7±6.3 versus 38.2±1.9%, $P=0.0003$) or the MCP-1–positive cells (55.1±2.8 versus 42.5±4.8%, $P=0.10$) was greater in the AT1aR$^{+/+}$ recipient with AT1aR$^{+/+}$ BM than in the AT1aR$^{+/+}$ recipient with AT1aR$^{-/-}$ BM (Figure 4C and 4D).

Discussion

In this study, we demonstrated that genetic ablation or pharmacological blockade of AT1R effectively suppressed atherosclerotic lesion formation with more stabilized morphological characteristics of the plaque. We found that AT1aR-positive BM cells accelerated atherosclerotic lesion progression and plaque destabilization, even if the recipient vasculature cells did not express AT1aR. On the other hand, lack of AT1aR in BM cells decreased atherosclerotic lesion progression and stabilized plaques with or without Ang II infusion despite the existence of AT1aR in vascular cells. Histological studies revealed that accumulation of BM-derived cells in atherosclerotic lesions was enhanced when AT1aR was expressed in BM cells. Moreover, the existence of AT1aR in BM significantly increased the expression of MMP-9 and MCP-1 in atherosclerotic plaques. The percentage of BM-derived cells among the MCP-1– or MMP-9–expressing cells in the lesions was decreased by the disruption of AT1aR in BM. Most of the BM-derived cells accumulated in the lesions were positive for a macrophage marker. Taken together, our present study demonstrated

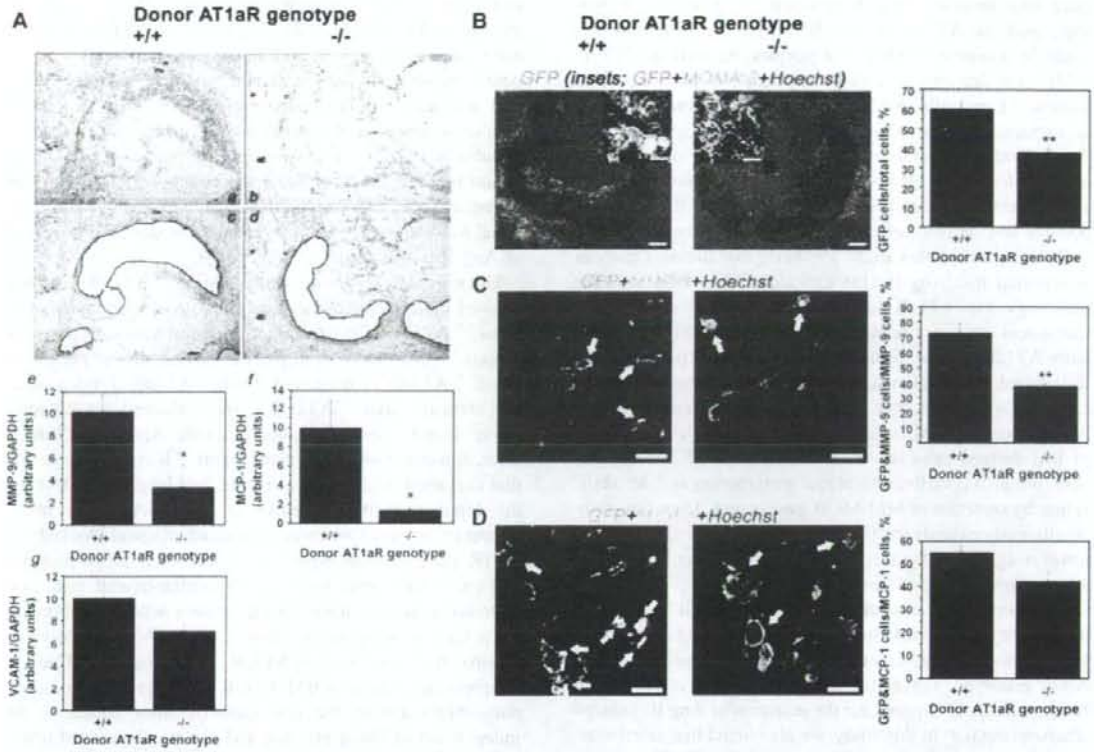


Figure 4. Targeted disruption of AT1aR in BM cells altered characteristics of atherosclerotic lesions. Characteristics of the plaque at 4 weeks after Ang II infusion in AT1aR$^{-/+}$ recipients. A, Total RNA was isolated from the lesions collected with the use of a laser microdissection system. B through D, Double immunofluorescent studies. B, Bar, 100 μm. Bar, 10 μm (insets). C and D, Bar, 10 μm. $^{*}P<0.05$, $^{**}P<0.001$.

functional contribution of the AT1aR in BM to the pathogenesis of atherosclerosis in vivo.

The RAS has been considered to be a circulating hormonal system that regulates blood pressure and flow. Recent studies have provided evidence for local effects of an activated RAS, particularly in the cardiac, vascular, and renal systems.[10] It is now well established that Ang II has significant proinflammatory actions on the vessel wall, leading to progression of atherosclerosis.[11] It is well known that there are 2 different types of Ang II receptors, AT1R and AT2R, in mammals. Both AT1R and AT2R have been identified in the vessel wall. In rodents, 2 AT1R subtypes, AT1aR and AT1bR, have been identified. In the vasculature, AT1aR is predominant and mediates most of the physiological and pathophysiological responses to Ang II in mice.[6] There is increasing evidence of cross-talk between RAS and dyslipidemia in atherogenesis.[12] It was demonstrated that hypercholesterolemia stimulates angiotensin peptide synthesis[12] and increased the density of AT1R,[13] suggesting that Ang II–AT1R pathway may meditate, at least in part, the atherogenic effects of hypercholesterolemia. Consistently, previous reports demonstrated that inhibition of AT1R-signaling reduces atherosclerosis.[14] The greatest AT1R density has been found on vascular smooth muscle cells and endothelial cells. Thus, the antiatherogenic effects of AT1R blockade are thought to result from inhibition of AT1R-mediated signaling in resident vascular cells.

Recent reports suggest that local effects of an activated RAS exist in BM and functions to promote differentiation and proliferation of BM cells.[4] Our preliminary study revealed that AT1aR was abundantly expressed in BM, whereas other receptors were hardly detected (supplemental Figure IA). We also found that AT1aR could be detected in atherosclerotic lesions in the AT1aR$^{-/-}$ recipients repopulated with AT1aR$^{+/+}$ BM (supplemental Figure IB). Thus, we here focused on AT1aR in BM, although it is plausible that other receptors of Ang II may participate in the atherogenic effects of Ang II.

Previous reports have demonstrated that AT1R in the vasculature mediates upregulation of adhesion molecules and chemokines, thus promoting infiltration of inflammatory cells into the vessel wall.[15] MCP-1 in vascular cells is one of the essential inflammatory mediators in Ang II–induced progression of atherosclerosis.[16] Several reports have demonstrated that monocytes/macrophages release MCP-1 through activation of the Ang II–AT1R pathway in vitro.[17] Our results showed the expression of MCP-1 from BM-derived cells in plaques. Selective disruption of AT1aR in BM significantly decreased MCP-1 expression in plaques in ApoE$^{-/-}$ AT1aR$^{-/+}$

mice that received Ang II infusion for 4 weeks. It was suggested that AT1aR-positive BM-derived cells themselves could be a source of MCP-1 in plaques. As well as MCP-1, MMPs are demonstrated to be expressed in atherosclerotic lesions.[18] Especially, MMP-9 is important for the resorption of extracellular matrix and contributes to progression and destabilization of atherosclerosis. An AT1R antagonist is reported to inhibit MMP-9 expression in a mouse model of atherosclerosis.[19] Our present results showed that AT1aR-positive BM-derived cells are an important source of MMP-9.

Recently, when this study was being conducted, Cassis et al reported that Ang II (1.0 μg/kg/min) promotes vascular pathology via AT1aR in LDL receptor–deficient mice.[20] Consistent with our findings, repopulation of AT1aR$^{+/+}$ mice with AT1aR$^{-/-}$ BM resulted in modest reductions in Ang II–induced atherosclerosis.[20] Here, we confirm the importance of AT1aR in BM. In addition, we demonstrate that AT1aR-positive BM cells not only accelerate accumulation of BM-derived cells in the lesions through MCP-1 expression, but also contribute to plaque progression and destabilization by secretion of MMP-9, at least in part. Thus, our study significantly extends the findings of Cassis et al and provides novel insights into the mechanism by which Ang II promotes atherosclerosis progression and instability.

Unexpectedly, Cassis et al found that AT1aR$^{-/-}$ recipient mice were dramatically protected from Ang II (1.44 mg/kg/d for 4 weeks)-induced vascular pathologies irrespective of BM donor genotype, suggesting that the presence of AT1aR in resident tissue is required for the initiation of Ang II–induced atherosclerosis.[20] In this study, we also found that atherosclerosis development was notably retarded in AT1aR$^{-/-}$ recipients with vehicle infusion regardless of the existence of AT1aR in BM. However, Ang II infusion at a higher dose for a longer period (5.0 mg/kg/d for 8 weeks) could promote atherosclerosis significantly even in AT1aR$^{-/-}$ recipients when the BM cells were repopulated with AT1aR$^{+/+}$ BM. Others reported that there are several differences in the pathogenesis of dyslipidemia and atherosclerosis between ApoE$^{-/-}$ and LDL-R$^{-/-}$ mice.[5] Moreover, we infused Ang II into BM chimeric mice at 12 weeks after BMT, whereas Cassis et al started Ang II treatment at 7 weeks after BMT. In our study, blood cell count at 12 weeks after BMT was similar among 4 BMT groups. At 40 weeks after BMT, the blood cell count and the BM cell composition were also similar in the AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{+/+}$ BM and AT1aR$^{-/-}$ BM (supplemental Table II). However, many reports documented that only the limited number of engrafted cells are matured with skewed population frequencies at 4 to 6 weeks after BMT, although high numbers of donor cells can be observed in peripheral blood at 4 to 6 weeks after BMT.[21] Assays of immune function indicate deficient functions at earlier time points after BMT. Thus, the differences in the type of hypercholesterolemic mice, the protocol of Ang II infusion, and the timing of Ang II infusion relative to the BMT might lead to the different results by us and by Cassis et al.

The dose of Ang II used in our study might be high compared with those used in previous studies. However, 5 mg/kg/d Ang II did not cause apparent toxic effects, such as increased mortality (supplemental Table III), changes in plasma lipid profile, or body weight loss, in the BM chimeric mice. Moreover, in our preliminary study, 3.0 mg/kg/d of Ang II infusion was not sufficient for blood pressure elevation and acceleration of atherosclerosis even in AT1aR$^{+/+}$ recipients repopulated with AT1aR$^{+/+}$ BM (Fukuda and Sata, unpublished data), suggesting that higher dose of Ang II would be required to evaluate the effects of Ang II in BM chimeric ApoE$^{-/-}$ mice repopulated with exogenous BM after lethal irradiation. Therefore, we chose the dose of 5 mg/kg/d of Ang II in this study.

Unexpectedly, in our study, ApoE$^{-/-}$AT1aR$^{-/-}$ mice showed significantly higher total cholesterol level than that in ApoE$^{-/-}$AT1aR$^{+/+}$ mice. This was inconsistent with previous reports.[22] Analysis of lipid profile showed similar pattern in ApoE$^{-/-}$AT1aR$^{+/+}$ mice and ApoE$^{-/-}$AT1aR$^{-/-}$ mice (data not shown). ApoE$^{-/-}$AT1aR$^{-/-}$ mice showed significantly lower blood pressure compared with ApoE$^{-/-}$AT1aR$^{+/+}$ mice, consistent with a previous report.[22] It could be possible that alteration of blood pressure and lipid level may affect the development of atherosclerosis in ApoE$^{-/-}$AT1aR$^{-/-}$ mice. However, in our experiment on pharmacological blockade of AT1R, there were no significant difference in blood pressure and cholesterol level between olmesartan-treated mice and hydralazine-treated mice. In our studies with BM chimeric mice, there was no significant difference in blood pressure or plasma cholesterol level in AT1aR$^{-/-}$ recipients or AT1aR$^{+/+}$ recipients regardless of BM AT1aR genotype. Therefore, our data obtained with the BM chimeric mice appear to be independent of blood pressure and plasma cholesterol level.

In summary, our results suggest that AT1aR expressed not only on vascular cells but also on BM-derived cells plays a role in the pathogenesis of atherosclerosis, at least in part. Therefore, blockade of AT1R not only in vascular cells but also in BM could be an important strategy to prevent the progression and destabilization of atherosclerotic plaques.

Sources of Funding

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology and the Ministry of Health, Labor, and Welfare of Japan. Dr Fukuda is supported by a Research Fellowship from the Japan Society for the Promotion of Science for Young Scientists.

Disclosures

None.

References

1. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med*. 1999; 340:115–126.
2. Daugherty A, Cassis L. Angiotensin II-mediated development of vascular diseases. *Trends Cardiovasc Med*. 2004;14:117–120.
3. Haznedaroglu IC, Ozturk MA. Towards the understanding of the local hematopoietic bone marrow renin-angiotensin system. *Int J Biochem Cell Biol*. 2003;35:867–880.
4. Rodgers KE, Xiong S, Steer R, diZerega GS. Effect of angiotensin II on hematopoietic progenitor cell proliferation. *Stem Cells*. 2000;18:287–294.
5. Zadelaar S, Kleemann R, Verschuren L, de Vries-Van der Weij J, van der Hoorn J, Princen HM, Kooistra T. Mouse models for atherosclerosis and pharmaceutical modifiers. *Arterioscler Thromb Vasc Biol*. 2007;27: 1706–1721.
6. Chen R, Iwai M, Wu L, Suzuki J, Min LJ, Shiuchi T, Sugaya T, Liu HW, Cui TX, Horiuchi M. Important role of nitric oxide in the effect of

angiotensin-converting enzyme inhibitor imidapril on vascular injury. *Hypertension*. 2003;42:542–547.
7. Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, Hirai H, Makuuchi M, Hirata Y, Nagai R. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat Med*. 2002;8:403–409.
8. Sata M, Nishimatsu H, Osuga J, Tanaka K, Ishizaka N, Ishibashi S, Hirata Y, Nagai R. Statins augment collateral growth in response to ischemia but they do not promote cancer and atherosclerosis. *Hypertension*. 2004;43:1214–1220.
9. Fukumoto Y, Libby P, Rabkin E, Hill CC, Enomoto M, Hirouchi Y, Shiomi M, Aikawa M. Statins alter smooth muscle cell accumulation and collagen content in established atheroma of watanabe heritable hyperlipidemic rabbits. *Circulation*. 2001;103:993–999.
10. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev*. 2006;86:747–803.
11. Brasier AR, Recinos A 3rd, Eledrisi MS. Vascular inflammation and the renin-angiotensin system. *Arterioscler Thromb Vasc Biol*. 2002;22:1257–1266.
12. Daugherty A, Rateri DL, Lu H, Inagami T, Cassis LA. Hypercholesterolemia stimulates angiotensin peptide synthesis and contributes to atherosclerosis through the AT1A receptor. *Circulation*. 2004;110:3849–3857.
13. Nickenig G, Sachinidis A, Michaelsen F, Bohm M, Seewald S, Vetter H. Upregulation of vascular angiotensin II receptor gene expression by low-density lipoprotein in vascular smooth muscle cells. *Circulation*. 1997;95:473–478.
14. Li Z, Iwai M, Wu L, Liu HW, Chen R, Jinno T, Suzuki J, Tsuda M, Gao XY, Okumura M, Cui TX, Horiuchi M. Fluvastatin enhances the inhibitory effects of a selective AT1 receptor blocker, valsartan, on atherosclerosis. *Hypertension*. 2004;44:758–763.
15. Petnehazy T, Stokes KY, Wood KC, Russell J, Granger DN. Role of blood cell-associated AT1 receptors in the microvascular responses to hypercholesterolemia. *Arterioscler Thromb Vasc Biol*. 2006;26:313–318.
16. Ni W, Kitamoto S, Ishibashi M, Usui M, Inoue S, Hiasa K, Zhao Q, Nishida K, Takeshita A, Egashira K. Monocyte chemoattractant protein-1 is an essential inflammatory mediator in angiotensin II-induced progression of established atherosclerosis in hypercholesterolemic mice. *Arterioscler Thromb Vasc Biol*. 2004;24:534–539.
17. Schmeisser A, Soehnlein O, Illmer T, Lorenz HM, Eskafi S, Roerick O, Gabler C, Strasser R, Daniel WG, Garlichs CD. ACE inhibition lowers angiotensin II-induced chemokine expression by reduction of NF-kappaB activity and AT1 receptor expression. *Biochem Biophys Res Commun*. 2004;325:532–540.
18. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res*. 2002;90:251–262.
19. Chen J, Li D, Schaefer RF, Mehta JL. Inhibitory effect of candesartan and rosuvastatin on CD40 and MMPs expression in apo-E knockout mice: novel insights into the role of RAS and dyslipidemia in atherogenesis. *J Cardiovasc Pharmacol*. 2004;44:446–452.
20. Cassis LA, Rateri DL, Lu H, Daugherty A. Bone marrow transplantation reveals that recipient AT1a receptors are required to initiate angiotensin II-induced atherosclerosis and aneurysms. *Arterioscler Thromb Vasc Biol*. 2007;27:380–386.
21. Kennedy DW, Abkowitz JL. Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. *Blood*. 1997;90:986–993.
22. Wassmann S, Czech T, van Eickels M, Fleming I, Bohm M, Nickenig G. Inhibition of diet-induced atherosclerosis and endothelial dysfunction in apolipoprotein E/angiotensin II type 1A receptor double-knockout mice. *Circulation*. 2004;110:3062–3067.

Are Serum Carcinoembryonic Antigen Levels Associated With Carotid Atherosclerosis in Japanese Men?

Nobukazu Ishizaka, Yuko Ishizaka, Ei-Ichi Toda, Kazuhiko Koike, Minoru Yamakado, Ryozo Nagai

Objective—Carcinoembryonic antigen (CEA), a serological marker of malignant tumors, may show a modest increase under some nonmalignant conditions, such as ageing and cigarette smoking. We have investigated whether serum CEA levels are associated with early carotid atherosclerosis.

Methods and Results—Cross-sectional data from 4181 male individuals who underwent general health screening were analyzed. The interquartile of cutoff values of serum CEA levels were 1.0, 1.6, and 2.5 ng/mL. Cigarette smoking was associated with increased serum CEA levels in a dose- and duration-dependent manner, and this association was more prominent in current than former smokers. Logistic regression analysis adjusted for age, body mass index, serum lipid and glucose profiles, white blood cell count, C-reactive protein, and smoking habits showed that the first, second, third, and fourth CEA quartiles were associated with carotid plaque with an odds ratio of 1 (reference), 1.25 (95% CI 1.03 to 1.52, $P=0.023$), 1.49 (95% CI 1.23 to 1.82 $P<0.001$), and 1.34 (95% CI 1.08 to 1.65, $P=0.007$), respectively. Although serum CEA levels were associated with metabolic syndrome, association between serum CEA and carotid plaque was significant in individuals without metabolic syndrome.

Conclusions—Serum CEA was associated with carotid atherosclerosis independently of atherogenic risk factors and markers of inflammation. Our data suggest that a slight elevation of CEA in current smokers, as well as in never smokers, may not be an innocuous observation from the viewpoint of atherosclerosis. (***Arterioscler Thromb Vasc Biol.* 2008;28:160-165.**)

Key Words: tumor marker ■ carotid atherosclerosis ■ health screening ■ cigarette smoking

Carcinoembryonic antigen (CEA) is a glycoprotein with a molecular weight of 180 to 200 kDa.[1] CEA is overexpressed in adenocarcinomas in the colon and other organs including pancreas, lung, prostate, urinary bladder, ovary, and breast; therefore, it is used as a serological marker of malignant tumors worldwide. On the other hand, serum CEA levels may increase under some nonmalignant conditions, for example, ageing, chronic renal failure, hypothyroidism, cigarette smoking, and some chronic inflammatory diseases,[2–5] although the extent of CEA elevation in such nonmalignant conditions, when present, is usually only modest. Stimulation of monocytes and macrophages with CEA may result in an increase in the production of proinflammatory cytokines,[6,7] which may subsequently upregulate adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin, on the surface of vascular endothelial cells.[7] These processes are thought to play a role in facilitating the metastasis of cancer cells. Interestingly, the early stage of atherosclerosis involves recruitment of inflammatory cells and their transendothelial migration, which is mediated by such cellular adhesion molecules on the surface of vascular endothelial cells.[8] Of note, some epidemiological studies have demonstrated a possible association between neoplastic diseases that would potently increase serum CEA and coronary artery disease.[9–11] Although modest elevation of serum CEA can be observed in apparently healthy individuals, especially in cigarette smokers,[3,12] little information is available on the possible association between serum CEA and atherosclerosis in the general population. In the current study, by analyzing the data of Japanese men who underwent general health screening, we have investigated whether there is an association between serum CEA levels and early carotid atherosclerosis.

Methods

Study Subjects

The study was approved by The Ethical Committee of Mitsui Memorial Hospital and that of University of Tokyo, Graduate School of Medicine. In Japan, regular health check-ups for employees are legally mandated. Therefore, the majority of the subjects enrolled did not have serious health problems. Between January 2003 and April 2007, 7292 subjects (2471 women, 4821 men) underwent general health screening for whom data on carotid ultrasonography and fasting insulin were available. Data on cigarette smoking habit data were collected in a self reported questionnaire, and among 4821 male

Original received July 17, 2007; final version accepted October 2, 2007.

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

Correspondence to Dr 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

Arterioscler Thromb Vasc Biol. is available at http://atvb.ahajournals.org DOI: 10.1161/ATVBAHA.107.155465

Table 1. Baseline Characteristics of the Study Subjects

	Never Smoker (n=1556)	Former Smoker (n=1427)	Current Smoker (n=1198)	*P* Value
Age, years	56.5±11.5	58.8±10.2	54.4±10.2	<0.001
Body mass index, kg/m^2	23.9±3.0	24.2±2.7	24.1±2.9	0.092
Systolic blood pressure, mm Hg	128±19	131±19	125±19	<0.001
Diastolic blood pressure, mm Hg	81±12	82±11	79±12	<0.001
Laboratory data				
WBC count, ×10^3/μL	5.2±1.2	5.3±1.2	6.3±1.7	<0.001
Hemoglobin, g/dL	15.1±1.1	15.1±1.0	15.5±1.1	<0.001
Platelet count, ×10^4/μL	21.8±4.6	22.0±4.7	23.2±5.8	<0.001
LDL-cholesterol, mg/dL	128±29	129±30	127±32	0.26
HDL-cholesterol, mg/dL	55±13	57±13	53±14	<0.001
Triglycerides, mg/dL	121±72	135±97	167±139	<0.001
Uric acid, mg/dL	6.1±1.2	6.2±1.1	6.2±1.2	0.023
hsCRP, mg/dL	0.14±0.40	0.16±0.64	0.18±0.38	0.086
Fasting glucose, mg/dL	100±19	103±19	104±25	<0.001
Haemoglobin A1C, %	5.3±0.7	5.4±0.7	5.6±0.8	<0.001
Fasting insulin, μU/mL	6.8±4.7	7.0±4.4	7.2±9.0	0.36
CEA, ng/mL	1.2±0.7	1.4±1.3	2.0±1.4	<0.001
Carotid ultrasonography				
Max intima-media thickness, mm	1.27±0.65	1.36±0.72	1.32±0.71	<0.001

subjects, 4181 answered the questionnaire in full concerning the amount and the duration of smoking, and concerning the duration since they had stopped smoking at the time of the general health check if when they were former smokers. In the current study, subjects who had quit smoking for 1 month or less and those who had quit for more than 1 month before the time of the health screening were considered to be, respectively, current and former smokers, and those without a smoking history were considered to be never smokers. We were unable to identify any specific reasons for why the remaining 640 subjects failed to complete the questionnaire about their smoking status. We found that these the 640 individuals excluded were slightly but significantly older than those enrolled in the study (60±10 and 57±11 years old, respectively, $P<0.001$).

Laboratory Analysis

Blood samples were obtained from the subjects in the morning after an overnight fast, and the assays for variables analyzed in the current study were performed on the same day of blood draw without freezing the samples. Serum levels of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, and hemoglobin A_{1C} was determined using the latex agglutination immunoassay. Plasma glucose was measured by the hexokinase method and serum insulin was measured by enzyme immunoassay. Serum CEA was measured using a commercially available immunometric chemiluminescent assay kit (Bayer Medical Co) with an interassay coefficient of variation ranging between 2.0 and 3.5%. High sensitivity C-reactive protein (hsCRP) concentration was measured by a turbidimetric immunoassay. Metabolic syndrome was diagnosed by National Cholesterol Education Program Adult Treatment Panel III[13] with a modification and it was said to be present when 3 or more of following conditions were present: (1) fasting plasma glucose (FPG) ≥110 mg/dL; (2) systolic blood pressure (SBP)/diastolic blood pressure ≥130/85 mm Hg; (3) TG ≥150 mg/dL; (4) HDL-C <40 mg/dL; and (5) BMI ≥25 kg/m^2.

Carotid Ultrasonography

Carotid artery status was assessed by high resolution B-mode ultrasonography, using a machine (Sonolayer SSA270A, Toshiba, Japan) equipped with a 7.5-MHz transducer (PLF-703ST, Toshiba). The carotid arteries were examined bilaterally at the levels of the common carotid, the bifurcation, and the internal carotid arteries from transverse and longitudinal orientations by trained sonographers. The intima-media thickness (IMT) was measured using a computer-assisted method by experienced sonographers who were unaware of the subjects' clinical and laboratory findings. Carotid intima-media wall thickening was said to occur when the IMT which was measured at the far wall of the distal 10 mm of the common carotid artery was ≥1.0 mm. Max IMT was defined as the thickest IMT in the scanned regions, and carotid plaque was defined when there was one or more focally thickened region(s) with the IMT of ≥1.1 mm.[14] The difference in the prevalence of carotid plaque in the individuals undergoing general health screening in the current study and that reported in some previous studies[15] would be explained by the difference in diagnostic criteria for carotid plaque.

Statistical Analysis

The data in this study were analyzed by the χ^2 test, ANOVA with Bonferroni post-hoc analysis, and univariate and multivariate logistic regression analysis using computer software, StatView ver. 5.0 (SAS Institute). A value of $P<0.05$ was taken to be statistically significant. Results are expressed as the mean±SD unless stated otherwise.

Results

Baseline Characteristics

The age of the subjects enrolled ranged from 21 to 89 years with a median of 57 years. Former smokers were significantly older ($P<0.001$) whereas current smokers were significantly younger ($P<0.001$) than never smokers (Table 1). Carotid plaque was found in 632/1556 (41%) of never smokers, 678/1427 (48%) of former smokers, and 539/1198 (45%) of current smokers. Carotid intima-media thickening was found in 125/1556 (8%) of never smokers, 161/1427 (11%) of former smokers, and 114/1198 (10%) of current smokers. Pearson's correlation coefficients between CEA and various

Table 2. Pearson's Correlation Coefficients Between Serum CEA Levels and Various Parameters

Variable	Coefficient	*P* Value
Age, years	0.16	<0.001
Body mass index, kg/m^2	−0.06	<0.001
Systolic blood pressure, mm Hg	0.04	0.044
Diastolic blood pressure, mm Hg	0.01	0.89
Laboratory data		
WBC count, ×10^3/mL	0.16	<0.001
Hemoglobin, g/dL	0.02	0.64
Platelet count, ×10^4/mL	−0.03	0.11
LDL-cholesterol, mg/dL	−0.01	0.64
HDL-cholesterol, mg/dL	0.02	0.50
Triglycerides, mg/dL	0.00	0.99
Uric acid, mg/dL	0.01	0.66
hsCRP, mg/dL	0.03	0.22
Fasting glucose, mg/dL	0.14	<0.001
Haemoglobin A1C, %	0.19	<0.001
Fasting insulin, μU/mL	0.03	0.26
Carotid ultrasonography		
Max intima-media thickness, mm	0.10	<0.001

variables are described in Table 2. Age, WBC count, FPG, hemoglobin A_{1c} showed only a weak correlation with CEA with the correlation coefficients ranging between 0.1 and 0.2; however, the correlation between serum CEA and hsCRP was not statistically significant (Table 2). The median (range) of the first to fourth quartiles of CEA values was 0.5 (0.5 to 0.7), 1.0 (0.8 to 1.2), 1.6 (1.3 to 1.9), and 2.5 (2.0 to 37.2) ng/mL, respectively. Of the 4181 subjects enrolled, 489 (12%) and 36 (0.9%) had CEA levels greater than 2.5 ng/mL (upper normal limit) and 5.0 ng/mL, respectively.

Smoking Habits and Serum CEA Levels

In current smokers, CEA levels increased according to the amount and duration of smoking, whereas this tendency was less apparent in former smokers (Figure). In smokers, the prevalence of the highest CEA quartile was found to increase with the daily number of cigarettes smoked as well as with smoking duration (Table 3). This trend was more prominent in current smokers than in former smokers. The odds ratio for the highest CEA quartile tended to get smaller with the length of smoking cessation in former smokers; however, after adjusting for age, former smokers who had last smoked ≥5 years ago at the time of assessment were found to still have a greater prevalence of the highest serum CEA quartile as compared with never smokers (Table 3), in agreement with a previous observation.[16]

Association Between CEA and Carotid Atherosclerosis

Of the individuals in the first to fourth CEA quartiles, carotid plaque was found in 343/1051 (33%), 613/1071 (43%), 532/1074 (50%), and 516/985 (52%), respectively, and carotid intima-media thickening was found in 73/1051 (7%), 92/1071 (9%), 101/1074 (9%), and 134/985 (14%), respectively. When the lowest serum CEA quartile was used as a reference, logistic regression analysis showed that the higher serum CEA quartiles were positively associated with carotid plaque even after adjusting for age, SBP, lipid and glucose data, smoking status, and the inflammatory markers, WBC and hsCRP (Model 4 in Table 4). In this model, hsCRP was also significantly associated with carotid plaque with an odds ratio of 1.26 (95% CI 1.04 to 1.54, per 1 mg/dL increase, P=0.019). In contrast, after adjusting for the same variables, the association between the second, third, and fourth serum CEA quartiles with carotid intima-media thickening was not significant with an odds ratio of 0.92 (95% CI 0.66 to 1.29), 0.84 (95% CI 0.60 to 1.18), and 1.10 (0.79 to 1.55), respectively. Of 4173 study subjects, 3890 (93%) had a

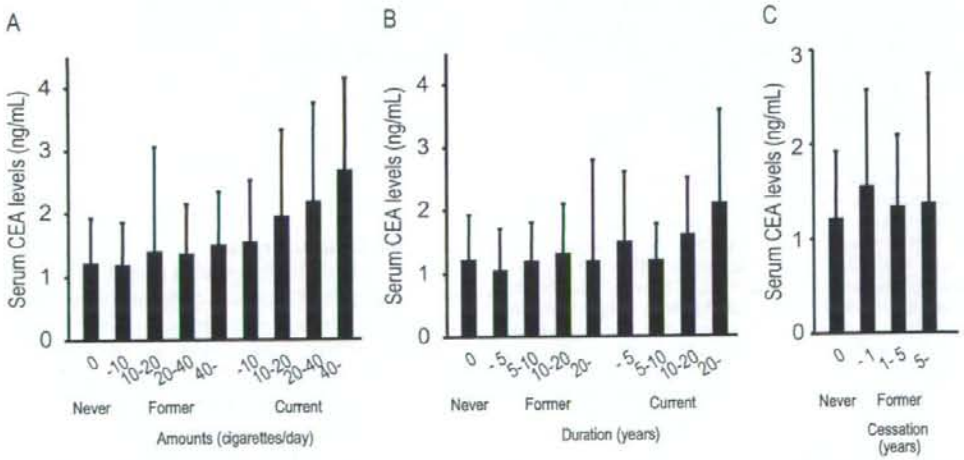


Figure. Serum CEA levels according to smoking habits. A, According to daily number of cigarettes smoked. B, According to duration of cigarette smoking. C, According to cessation period.

Table 3. Unadjusted and Age-adjusted Association of Smoking Habits With the Highest Serum CEA Quartile

Smoking Status	Odds Ratio (95% CI) Unadjusted	P	Odds Ratio (95% CI) Adjusted for Age	P
Amount of smoking				
Never smoking	1.00	...	1.00	...
Former smoking§				
<10 (cigarettes/d)	0.83 (0.52–1.33)	0.45	0.85 (0.53–1.37)	0.51
10–19	1.53 (1.18–1.98)	0.001	1.43 (1.10–1.86)	0.008
20–39	1.43 (1.09–1.87)	0.010	1.31 (1.00–1.72)	0.054
40≤	2.02 (1.46–2.81)	<0.001	1.76 (1.26–2.47)	<0.001
Current smoking§				
<10	1.84 (1.28–2.65)	0.001	2.02 (1.40–2.93)	<0.001
10–19	3.94 (3.11–4.98)	<0.001	4.52 (3.54–5.76)	<0.001
20–39	5.35 (4.23–6.76)	<0.001	6.40 (5.02–8.17)	<0.001
40≤	9.93 (6.33–15.59)	<0.001	11.32 (7.14–17.94)	<0.001
Duration of smoking				
Never smoking	1.00	...	1.00	...
Former smoking§				
<5y	0.75 (0.40–1.43)	0.38	0.87 (0.45–1.66)	0.67
5–9	0.59 (0.34–1.00)	0.048	0.66 (0.39–1.13)	0.13
10–19	1.40 (1.06–1.85)	0.019	1.39 (1.05–1.85)	0.023
20≤	1.92 (1.53–2.40)	<0.001	1.59 (1.27–2.01)	<0.001
Current smoking§				
<5y	1.64 (0.54–4.98)	0.39	1.83 (0.59–5.67)	0.30
5–9	0.66 (0.23–1.88)	0.44	0.93 (0.32–2.67)	0.89
10–19	2.20 (1.48–3.27)	<0.001	3.28 (2.16–5.00)	<0.001
20≤	4.98 (4.11–6.02)	<0.001	5.37 (4.42–6.53)	<0.001
Years of cessation				
Never smoking	1.00	...	1.00	...
Former smoking§				
Last smoked <1y ago	2.75 (1.75–4.33)	<0.001	3.07 (1.92–4.90)	<0.001
Last smoked 1–4y ago	1.30 (0.91–1.84)	0.15	1.42 (0.99–2.02)	0.055
Last smoked ≥5y ago	1.42 (1.15–1.75)	0.001	1.24 (1.00–1.54)	0.046

§Never smoking was used as a reference.

fasting glucose level of less than 140 mg/dL and were not taking antidiabetic medication. In these subjects, the second, third, and fourth serum CEA quartiles were associated with carotid plaque with an odds ratio of 1.28 (95% CI 1.05 to 1.57, $P=0.013$), 1.49 (95% CI 1.22 to 1.82, $P=0.0001$), and 1.32 (1.06 to 1.64, $P=0.013$), respectively, after adjusting for HOMA-IR and the covariates used in Model 4 of Table 4. The odds ratio of each serum CEA quartile for the carotid plaque in never, former, and current smokers is described in Table 5. The association between the highest serum CEA quartile and carotid plaque did not reach statistical significance after this subdivision.

Metabolic syndrome was found in 783 (19%) individuals. After adjusting for age, logistic regression analysis showed that the first to fourth CEA quartiles were associated with metabolic syndrome with an odds ratio of 1 (reference), 1.04 (95% CI 0.83 to 1.31, $P=0.72$), 1.15 (95%CI 0.92 to 1.44, $P=0.21$), and 1.40 (95% CI 1.12 to 1.75, $P=0.004$), respectively. Among the 3383 individuals who did not have metabolic syndrome, the first to fourth serum CEA quartiles were associated with carotid plaque with an odds ratio of 1 (reference), 1.04 (95% CI 0.83 to 1.31, $P=0.72$), 1.48 (95% CI 1.20 to 1.84, $P<0.001$), and 1.30 (95% CI 1.02 to 1.64, $P=0.031$), respectively. We then investigated the association between serum CEA and increased insulin resistance, defined here as the highest HOMA-IR quartile (HOMA-IR >2.15), in 3890 individuals who had a fasting glucose level of less than 140 mg/dL and were not taking antidiabetic medication. We found that the first to fourth CEA quartiles were associated with the highest HOMA-IR quartile with an odds ratio of 1 (reference), 1.26 (95% CI 1.02 to 1.56, $P=0.032$), 1.30 (95% CI 1.05 to 1.64, $P=0.015$), and 1.32 (95% CI 1.05 to 1.65, $P=0.015$), respectively.

Discussion

In the current study, we have investigated the possible association between serum CEA levels and carotid atherosclerosis by analyzing the data of 4181 male individuals who

Table 4. Logistic Regression Analysis of the CEA Quartiles as Independent Variables and Carotid Plaque as a Dependent Variable

CEA quartiles	Odds Ratio for Carotid Plaque	P Value
Model 1		
Q1	1.00	...
Q2	1.54 (1.29–1.84)	<0.001
Q3	2.03 (1.70–2.42)	<0.001
Q4	2.27 (1.90–2.72)	<0.001
Model 2		
Q1	1.00	...
Q2	1.29 (1.07–1.56)	0.009
Q3	1.62 (1.34–1.95)	<0.001
Q4	1.59 (1.31–1.93)	<0.001
Model 3		
Q1	1.00	...
Q2	1.26 (1.04–1.53)	0.017
Q3	1.50 (1.24–1.82)	<0.001
Q4	1.38 (1.13–1.70)	0.002
Model 4		
Q1	1.00	...
Q2	1.25 (1.03–1.52)	0.023
Q3	1.49 (1.23–1.82)	<0.001
Q4	1.34 (1.08–1.65)	0.007

Model 1: Unadjusted.
Model 2: Adjusted for age.
Model 3: Adjusted for age, SBP, and smoking status.
Model 4: Adjusted for age, SBP, FPG, BMI, LDL-C, HDL-C, TG, smoking status, WBC, and hsCRP.

underwent general health screening. As compared with the lowest serum CEA quartile, individuals in the 3 higher serum CEA quartiles had significantly increased prevalence of carotid plaque (Table 3). This association remained statistically significant even after adjusting for age, SBP, FPG, BMI, LDL-C, HDL-C, TG, smoking status, WBC, and hsCRP. In the smokers, especially the current smokers, serum CEA levels were increased according to the daily number of cigarettes smoked and duration of smoking (Figure, Table 3). We found previously that circulating WBC count, a marker for systemic inflammation, was also increased according to the amount and duration of smoking,[17,18] and increased WBC count was a risk factor for carotid atherosclerosis independent of other conventional risk factors.[19] Therefore, the association between serum CEA levels and carotid atherosclerosis might be confounded by that between circulating WBC count and atherosclerosis. However, the association between CEA and carotid plaque remained statistically significant after adjustment for WBC count and hsCRP (Model 4, Table 4).

What is the possible underlying mechanism, if present, which would explain the observed link between serum CEA and carotid plaque? First, serum CEA was associated with metabolic syndrome and increased insulin resistance. Because both of these conditions can increase the risk for carotid atherosclerosis, increased insulin resistance or metabolic syndrome may explain the observed link between serum CEA and carotid plaque. On the other hand, the association

Table 5. Logistic Regression Analysis of the CEA Quartiles as Independent Variables and Carotid Plaque or Carotid Intima-Media Thickening as a Dependent Variable According to Smoking Status

CEA Quartiles	Odds Ratio for Carotid Plaque	P Value
Never smoker		
Q1	1.00	...
Q2	1.17 (0.87–1.56)	0.30
Q3	1.51 (1.11–2.04)	0.008
Q4	1.42 (0.98–2.04)	0.063
Former smoker		
Q1	1.00	...
Q2	1.36 (1.00–1.86)	0.049
Q3	1.41 (1.02–1.94)	0.039
Q4	1.15 (0.81–1.64)	0.43
Current smoker		
Q1	1.00	...
Q2	1.26 (0.77–2.08)	0.36
Q3	1.74 (1.10–2.75)	0.018
Q4	1.46 (0.94–2.28)	0.096

Adjusted for age, SBP, FPG, BMI, LDL-C, HDL-C, TG, WBC, and hsCRP.

between serum CEA and carotid plaque remained statistically significant after adjustment for HOMA-IR, and this association was found to be statistically significant among individuals who did not have metabolic syndrome, suggesting that the association was, at least in part, independent of increased insulin resistance or metabolic syndrome. Second, several previous studies have suggested that CEA may stimulate the monocytes/macrophages to release proinflammatory cytokines, which eventually lead to the induction of adhesion molecules on the surface of vascular endothelial cells, which may facilitate the metastasis of malignant cells.[6,7,20] Interestingly, it has been found that ICAM-1 and VCAM-1 levels correlate with serum CEA levels in colorectal cancer patients.[21] Recruitment of inflammatory cells from the circulation after the induction of adhesion molecules on the surface of vascular endothelial cells is postulated to be an early phase of atherosclerosis[8]; therefore, enhanced expression of certain adhesion molecules on vascular cells may explain the observed link between CEA and carotid plaque. Third, serum CEA may be increased in patients with chronic inflammatory disorders[22] such as inflammatory bowel disease,[23,24] collagen disease, and chronic viral hepatitis,[25] although this idea remains controversial.[26] Patients with such chronic inflammatory diseases may have an increased risk of carotid atherosclerosis.[27–29] It is possible that a similar immune-inflammatory reaction, such as activation of the CD40/CD40 ligand system, might play a crucial role in both atherosclerosis and inflammatory diseases.[30,31] Whether serum CEA levels are associated with blood levels or membrane-bound levels of adhesion molecules and CD40 ligand should be investigated in future studies. Although cigarette smoking increases serum CEA levels without evidence of malignant diseases,[2] elevated serum CEA levels may be associated with a more accelerated decline in the percent forced expiratory volume in one second (FEV1%) value among smokers.[32]

Thus, our data may provide additional evidence that an increase in serum CEA levels among cigarette smokers may not be an innocuous observation also from the viewpoint of atherosclerosis.

This study has several potential limitations. First, we analyzed only men in the current study, because the number of female subjects who had a smoking history was much smaller during the study period. Second, because of the cross-sectional nature of the study, we cannot determine whether there is a causal or resultant relationship between the elevation of serum CEA and carotid plaque. Third, in addition to cigarette smoking, serum CEA is known to be elevated in other nonmalignant conditions, such as hypothyroidism,[4] and end-stage lung diseases.[33] We did not include these disorders as confounding variables, because their prevalence is considered to be very low among the study population.

In conclusion, we have shown that cigarette smoking increases serum levels of CEA in a dose- and duration-dependent manner in Japanese men who underwent general health screening. The increase in serum CEA was found to be associated with an increased prevalence of carotid plaque independent of blood pressure, fasting glucose, serum lipids, and inflammatory markers. Our data suggested that an elevation of CEA in smokers may not be an innocuous observation from the viewpoint of atherosclerosis.

Acknowledgments

The work was supported in part by a Grant from the Smoking Research Foundation.

Disclosures

None.

References

1. Krupey J, Wilson T, Freedman SO, Gold P. The preparation of purified carcinoembryonic antigen of the human digestive system from large quantities of tumor tissue. *Immunochemistry*. 1972;9:617–622.
2. Stevens DP, Mackay IR. Increased carcinoembryonic antigen in heavy cigarette smokers. *Lancet*. 1973;2:1238–1239.
3. Fukuda I, Yamakado M, Kiyose H. Influence of smoking on serum carcinoembryonic antigen levels in subjects who underwent multiphasic health testing and services. *J Med Syst*. 1998;22:89–93.
4. Amino N, Kuro R, Yabu Y, Takai SI, Kawashima M, Morimoto S, Ichihara K, Miyai K, Kumahara Y. Elevated levels of circulating carcinoembryonic antigen in hypothyroidism. *J Clin Endocrinol Metab*. 1981; 52:457–462.
5. Witherspoon LR, Shuler SE, Alyea K, Husserl FE. Carcinoembryonic antigen: assay following heat compared with perchloric acid extraction in patients with colon cancer, non-neoplastic gastrointestinal diseases, or chronic renal failure. *J Nucl Med*. 1983;24:916–921.
6. Ganguly A, Yeltsin E, Robbins J. Identification of a carcinoembryonic antigen binding protein on monocytes. *Biochem Biophys Res Commun*. 2003;311:319–323.
7. Aarons CB, Bajenova O, Andrews C, Heydrick S, Bushell KN, Reed KL, Thomas P, Becker JM, Stucchi AF. Carcinoembryonic antigen-stimulated THP-1 macrophages activate endothelial cells and increase cell-cell adhesion of colorectal cancer cells. *Clin Exp Metastasis*. 2007;24:201–209.
8. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med*. 1999; 340:115–126.
9. Henderson BE, Bogdanoff E, Gerkins VR, SooHoo J, Arthur M. Evaluation of cancer risk factors in a retirement community. *Cancer Res*. 1974;34:1045–1048.
10. Reicher-Reiss H, Jonas M, Goldbourt U, Boyko V, Modan B. Selectively increased risk of cancer in men with coronary heart disease. *Am J Cardiol*. 2001;87:459–462, A456.
11. Neugut AI, Rosenberg DJ, Ahsan H, Jacobson JS, Wahid N, Hagan M, Rahman MI, Khan ZR, Chen L, Pablos-Mendez A, Shea S. Association between coronary heart disease and cancers of the breast, prostate, and colon. *Cancer Epidemiol Biomarkers Prev*. 1998;7:869–873.
12. Alexander JC, Silverman NA, Chretien PB. Effect of age and cigarette smoking on carcinoembryonic antigen levels. *JAMA*. 1976;235:1975–1979.
13. 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;285:2486–2497.
14. Handa N, Matsumoto M, Maeda H, Hougaku H, Kamada T. Ischemic stroke events and carotid atherosclerosis. Results of the Osaka Follow-up Study for Ultrasonographic Assessment of Carotid Atherosclerosis (the OSACA Study). *Stroke*. 1995;26:1781–1786.
15. Ishizaka N, Ishizaka Y, Toda E, Nagai R, Yamakado M. Association between serum uric acid, metabolic syndrome, and carotid atherosclerosis in Japanese individuals. *Arterioscler Thromb Vasc Biol*. 2005;25: 1038–1044.
16. Kashiwabara K, Nakamura H, Yokoi T. Chronological change of serum carcinoembryonic antigen (CEA) concentrations and pulmonary function data after cessation of smoking in subjects with smoking-associated CEA abnormality. *Clin Chim Acta*. 2001;303:25–32.
17. Ishizaka N, Ishizaka Y, Toda E, Nagai R, Yamakado M. Association between smoking, hematological parameters, and metabolic syndrome in Japanese men. *Diabetes Care*. 2006;29:741.
18. Ishizaka N, Ishizaka Y, Toda EI, Nagai R, Koike K, Hashimoto H, Yamakado M. Relationship between smoking, white blood cell count and metabolic syndrome in Japanese women. *Diabetes Res Clin Pract*. 2007.
19. Ishizaka N, Ishizaka Y, Toda E, Hashimoto H, Nagai R, Yamakado M. Association between white blood cell count and carotid arteriosclerosis in Japanese smokers. *Atherosclerosis*. 2004;175:95–100.
20. Minami S, Furui J, Kanematsu T. Role of carcinoembryonic antigen in the progression of colon cancer cells that express carbohydrate antigen. *Cancer Res*. 2001;61:2732–2735.
21. Alexiou D, Karayiannakis AJ, Syrigos KN, Zbar A, Kremmyda A, Bramis I, Tsigris C. Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. *Eur J Cancer*. 2001;37:2392–2397.
22. Ruibal Morell A. CEA serum levels in non-neoplastic disease. *Int J Biol Markers*. 1992;7:160–166.
23. Moore TL, Kantrowitz PA, Zamcheck N. Carcinoembryonic antigen (CEA) in inflammatory bowel disease. *JAMA*. 1972;222:944–947.
24. Rule AH, Goleski-Reilly C, Sachar DB, Vandevoorde J, Janowitz HD. Circulating carcinoembryonic antigen (CEA): relationship to clinical status of patients with inflammatory bowel disease. *Gut*. 1973;14:880–884.
25. Booth SN, King JP, Leonard JC, Dykes PW. Serum carcinoembryonic antigen in clinical disorders. *Gut*. 1973;14:794–799.
26. Maharshak N, Arbel Y, Bornstein NM, Gal-Oz A, Gur AY, Shapira I, Rogowski O, Berliner S, Halpern Z, Dotan I. Inflammatory bowel disease is not associated with increased intimal media thickening. *Am J Gastroenterol*. 2007;102:1050–1055.
27. Papa A, Santoliquido A, Danese S, Covino M, Di Campli C, Urgesi R, Grillo A, Guglielmo S, Tondi P, Guidi L, De Vitis I, Fedeli G, Gasbarrini G, Gasbarrini A. Increased carotid intima-media thickness in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2005;22:839–846.
28. Ishizaka N, Ishizaka Y, Takahashi E, Tooda E, Hashimoto H, Nagai R, Yamakado M. Association between hepatitis C virus seropositivity, carotid-artery plaque, and intima-media thickening. *Lancet*. 2002;359:133–135.
29. Roman MJ, Moeller E, Davis A, Paget SA, Crow MK, Lockshin MD, Sammaritano L, Devereux RB, Schwartz JE, Levine DM, Salmon JE. Preclinical carotid atherosclerosis in patients with rheumatoid arthritis. *Ann Intern Med*. 2006;144:249–256.
30. Urbich C, Dernbach E, Aicher A, Zeiher AM, Dimmeler S. CD40 ligand inhibits endothelial cell migration by increasing production of endothelial reactive oxygen species. *Circulation*. 2002;106:981–986.
31. Vowinkel T, Anthoni C, Wood KC, Stokes KY, Russell J, Gray L, Bharwani S, Senninger N, Alexander JS, Kriegstein CF, Grisham MB, Granger DN. CD40-CD40 ligand mediates the recruitment of leukocytes and platelets in the inflamed murine colon. *Gastroenterology*. 2007;132:955–965.
32. Kashiwabara K, Nakamura H, Kiguchi T, Yagyu H, Yokoi T, Matsuoka T. Chronological change of respiratory function in smokers with elevated serum carcinoembryonic antigen levels. *Clin Chim Acta*. 1998;276:179–186.
33. Hadjiliadis D, Tapson VF, Davis RD, Palmer SM. Prognostic value of serum carcinoembryonic antigen levels in patients who undergo lung transplantation. *J Heart Lung Transplant*. 2001;20:1305–1309.

Original Article

Relationship between Blood Pressure and Chronic Kidney Disease in the Japanese Population: The Lower the Better Even in Individuals without Hypertension?

Yasutomi HIGASHIKUNI[1], Nobukazu ISHIZAKA[1], Yuko ISHIZAKA[2], Ei-ichi TODA[2], Ryozo NAGAI[1], and Minoru YAMAKADO[2]

In hypertensive subjects, it has been demonstrated that the lower the blood pressure, the lower the incidence of chronic kidney disease (CKD). However, whether this relationship holds true in individuals without hypertension—that is, in individuals with a blood pressure <140/90 mmHg—remains unknown. This study was performed to assess the relationship between blood pressure and CKD in a Japanese population without hypertension. Among 13,007 Japanese participants in a general health screening, 9,596 (5,691 men and 3,905 women) were found to have either normal blood pressure or prehypertension, and were enrolled in this study. We categorized these individuals' blood pressure into six classes: BP-C1, <90/<65 mmHg; BP-C2, 90–100/65–70 mmHg; BP-C3, 100–110/70–75 mmHg; BP-C4, 110–120/75–80 mmHg; BP-C5, 120–130/80–85 mmHg; and BP-C6, 130–140/85–90 mmHg. Albuminuria was defined as a urinary albumin excretion ratio of ≥30 mg/g. Low estimated glomerular filtration rate (eGFR) was defined as eGFR <60 mL/min/1.73 m^2. In men, when BP-C3 was used as a reference, multivariate logistic regression analysis adjusted for age, body mass index, serum lipid profiles, fasting plasma glucose and smoking status showed that BP-C1, BP-C2, BP-C4, BP-C5 and BP-C6 were associated with albuminuria with an adjusted odds ratio of 1.85 (0.53–6.46), 1.22 (0.59–2.51), 1.62 (1.01–2.59), 2.57 (1.64–4.02), and 3.81 (2.44–5.96). In women, the adjusted odds ratios of the risk for albuminuria in BP-C2, BP-C3, BP-C4, BP-C5 and BP-C6, as compared with BP-C1 as a reference, were 1.83 (0.70–4.79), 2.13 (0.84–5.42), 2.80 (1.10–7.14), 2.59 (0.99–6.78), and 3.99 (1.50–10.64). Blood pressure was not significantly associated with low eGFR in either gender. The risk for albuminuria was significantly greater when blood pressure exceeded 110/75 mmHg in both genders. (*Hypertens Res* 2008; 31: 213–219)

Key Words: **blood pressure, chronic kidney disease, Japanese population**

Introduction

The increasing prevalence of end-stage renal disease (ESRD) that may require hemodialysis is now a worldwide public health problem because of poor outcomes and high costs. To prevent an increase in the number of ESRD patients, intensive intervention at an early stage of kidney impairment has recently been emphasized. This enthusiasm has led to the establishment of the concept of chronic kidney disease (CKD). According to the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (K/DOQI) crite-

From the [1]Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Tokyo, Japan; and [2]Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital, Tokyo, Japan.

Address for Reprints: Nobukazu Ishizaka, M.D., Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan. E-mail: nobuishizka-tky@umin.ac.jp

Received May 22, 2007; Accepted in revised form August 24, 2007.

Table 1. Characteristics of the Enrolled Male Subjects According to Blood Pressure Levels

	Total (n=5,691)	BP-C1 (n=67)	BP-C2 (n=401)	BP-C3 (n=1,130)	BP-C4 (n=1,565)	BP-C5 (n=1,140)	BP-C6 (n=1,088)	p value
Age, years old	52.2±10.5	53.4±11.9	51.0±10.8	50.8±10.6	51.3±10.5	53.2±10.4	54.2±11.9	<0.0001
BMI, kg/m^2	23.3±2.7	20.9±2.1	21.8±2.2	22.5±2.3	23.1±2.5	23.8±2.7	24.3±2.9	<0.0001
Systolic BP, mmHg	116.3±11.9	85.5±3.5	95.6±3.0	104.9±3.0	114.0±3.2	123.0±3.9	132.2±4.7	<0.0001
Diastolic BP, mmHg	74.3±7.9	56.4±4.0	62.5±4.3	67.6±4.1	72.8±4.3	78.3±4.3	83.6±4.5	<0.0001
TC, mg/dL	206.7±32.2	208.2±32.9	200.0±31.3	202.8±32.6	206.9±32.1	208.4±31.9	210.3±31.9	<0.0001
HDL-C, mg/dL	55.0±13.4	60.4±16.5	55.0±13.4	55.2±12.9	55.2±13.9	54.8±13.0	54.7±13.6	0.032
LDL-C, mg/dL	127.2±30.6	125.3±38.2	123.8±30.5	125.4±30.6	127.6±30.3	127.5±30.9	129.2±29.9	0.017
TG, mg/dL	128.0±88.7	108.9±96.1	106.9±58.2	113.8±73.6	127.5±97.0	136.7±96.9	140.6±84.4	<0.0001
Hyperglycemia, n (%)	698 (12.3%)	6 (9.0%)	30 (7.5%)	78 (6.9%)	175 (11.2%)	212 (14.7%)	197 (18.1%)	<0.0001
Fasting plasma glucose, mg/dL	98.1±19.3	96.6±24.2	94.2±16.9	94.9±15.5	97.6±19.2	99.4±17.7	102.2±24.0	<0.0001
HbA1c, %	5.34±0.72	5.43±0.91	5.28±0.68	5.26±0.62	5.33±0.69	5.36±0.68	5.44±0.86	<0.0001
Smoking status								<0.0001
Former smoking, n (%)	1,849 (32.5%)	17 (25.4%)	95 (23.7%)	316 (28.0%)	496 (31.7%)	496 (34.4%)	429 (39.4%)	
Current smoking, n (%)	1,893 (33.3%)	32 (47.8%)	183 (45.6%)	402 (35.6%)	522 (33.4%)	440 (30.6%)	314 (28.9%)	
UAER, mg/g	14.1±73.7	9.1±22.0	11.1±72.4	8.0±33.5	11.5±50.3	19.8±117.8	18.0±56.3	0.0005
Albuminuria, n (%)	331 (5.8%)	3 (4.5%)	11 (2.7%)	26 (2.3%)	65 (4.2%)	102 (7.1%)	124 (11.4%)	<0.0001
eGFR, mL/min/1.73 m^2	70.7±9.9	71.5±11.3	71.3±9.9	71.3±9.8	70.8±9.6	70.5±10.0	70.1±9.9	0.055
Low eGFR, n (%)	735 (12.9%)	9 (13.4%)	42 (10.5%)	131 (11.6%)	189 (12.1%)	206 (14.3%)	158 (14.5%)	0.078

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; UAER, urinary albumin excretion ratio.

ria, CKD is defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2 and/or kidney damage identified mainly by microalbuminuria (*1*).

Hypertension is a well-known risk factor for the progression of renal and cardiovascular diseases (*2–5*). Previous studies have demonstrated that lower blood pressure leads to better clinical outcomes in regard to renal and cardiovascular diseases (*2*, *6–10*), thereby providing the academic basis of "the lower the better" strategy in daily practice. Only a few studies, however, have evaluated the relationship between blood pressure and CKD in the non-hypertensive range of blood pressure. Haroun *et al.* (*10*) demonstrated that a blood pressure of 130–140/85–90 mmHg was associated with an increase in serum creatinine levels in a predominantly Caucasian population. Ramirez *et al.* (*4*) showed that the risk for proteinuria was significantly increased at a systolic blood pressure of 130–140 mmHg in a Chinese population, whereas a significant increase in the risk for proteinuria was not found at a blood pressure level below 140/90 mmHg in a Malaysian population.

In the current study, we sought to assess the relationship between blood pressure level and CKD in Japanese individuals without hypertension. We used albuminuria and low eGFR as indicators of CKD, and the two indicators were evaluated separately so that the pathophysiology of kidney damage could be clearly assessed.

Methods

Study Subjects

The study was approved by the Ethical Committee of Mitsui Memorial Hospital. Between April 2005 and August 2006, 13,007 subjects (8,298 men, 4,709 women) underwent a general health screening. Among these subjects, 9,596 subjects (5,691 men, 3,905 women) who had blood pressure <140/90 mmHg and were not taking any antihypertensive medication were enrolled in this study. In Japan, regular health check-ups for employees are legally mandated, and thus, most of these subjects did not have serious health problems.

Body mass index (BMI) was calculated as body weight (kg) divided by the square of the height (m^2). Smoking habits were self-reported by means of a questionnaire, and subjects were classified as non-smokers, former smokers or current smokers. Subjects were judged to be former smokers if they had stopped smoking at least 1 month before the health evaluation.

Laboratory Analysis

Blood samples were taken from the subjects after an overnight fast. Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were determined

Table 2. Characteristics of the Enrolled Female Subjects According to Blood Pressure Levels

	Total (n=3,905)	BP-C1 (n=182)	BP-C2 (n=645)	BP-C3 (n=1,095)	BP-C4 (n=962)	BP-C5 (n=636)	BP-C6 (n=385)	p value
Age, years old	50.7±10.5	46.9±10.3	47.2±10.5	49.2±9.8	51.2±10.3	53.9±10.1	56.3±10.3	<0.0001
BMI, kg/m^2	20.8±2.7	19.2±2.2	19.7±2.1	20.4±2.3	21.1±2.6	21.8±2.8	22.3±2.9	<0.0001
Systolic BP, mmHg	110.3±13.0	85.6±3.7	95.1±2.8	104.5±2.9	113.9±3.1	123.5±3.6	133.1±4.1	<0.0001
Diastolic BP, mmHg	68.9±8.8	55.0±4.8	60.3±4.8	65.5±4.9	71.1±5.0	76.7±5.2	81.7±5.9	<0.0001
TC, mg/dL	212.1±35.5	201.6±31.8	205.3±35.4	209.3±35.8	212.8±35.3	219.1±34.0	223.5±34.5	<0.0001
HDL-C, mg/dL	68.0±14.4	69.1±13.4	69.0±14.0	68.6±14.4	67.9±14.5	66.9±14.1	66.4±15.3	0.014
LDL-C, mg/dL	123.4±32.7	113.5±27.4	117.0±32.0	120.4±32.0	124.2±32.7	130.1±32.4	134.1±33.2	<0.0001
TG, mg/dL	80.5±43.2	65.3±31.4	69.4±32.9	77.1±41.2	82.3±45.0	90.1±43.6	96.0±53.6	<0.0001
Hyperglycemia, n (%)	129 (3.3%)	0 (0%)	5 (0.8%)	25 (2.3%)	31 (3.2%)	38 (6.0%)	30 (7.8%)	<0.0001
Fasting plasma glucose, mg/dL	89.4±12.5	85.2±7.7	86.6±9.2	88.4±10.5	89.6±12.8	92.6±14.7	92.9±16.8	<0.0001
HbA1c, %	5.11±0.50	5.02±0.41	5.03±0.41	5.07±0.47	5.13±0.49	5.20±0.54	5.20±0.61	<0.0001
Smoking status								<0.0001
Former smoking, n (%)	230 (5.9%)	10 (5.5%)	40 (6.2%)	71 (6.5%)	53 (5.5%)	36 (5.7%)	20 (5.2%)	
Current smoking, n (%)	369 (9.4%)	33 (18.1%)	81 (12.6%)	110 (10.0%)	90 (9.4%)	39 (6.1%)	16 (4.2%)	
UAER, mg/g	13.9±46.1	8.1±7.1	10.7±19.5	14.0±58.6	14.2±39.9	13.0±20.2	22.1±80.0	0.0027
Albuminuria, n (%)	234 (6.0%)	5 (2.7%)	30 (4.7%)	58 (5.3%)	65 (6.8%)	40 (6.3%)	36 (9.4%)	0.0095
eGFR, mL/min/1.73 m^2	69.5±9.8	70.3±9.7	70.3±9.7	69.7±10.0	69.5±9.8	68.9±9.7	67.9±9.7	0.0025
Low eGFR, n (%)	592 (15.2%)	22 (12.1%)	85 (13.2%)	150 (13.7%)	143 (14.9%)	111 (17.5%)	81 (21.0%)	0.0028

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; UAER, urinary albumin excretion ratio.

enzymatically. Plasma glucose was measured by the hexokinase method, and hemoglobin A1c was determined using the latex agglutination immunoassay. The serum creatinine level was determined by the enzymatic method.

Serum creatinine was calibrated by the following formula: calibrated serum creatinine = 0.2 + serum creatinine (enzyme method). Glomerular filtration rate (GFR) was estimated by the abbreviated Modification of Diet in Renal Disease (MDRD) equation as follows (*11*):

$$\text{eGFR (mL/min/1.73 m}^2) = 186.3 \times (\text{calibrated serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times 0.881 \ (\times 0.742, \text{ if female}).$$

In the MDRD formula, 0.881 is the coefficient of Japanese for an eGFR specific to the Japanese population, as recommended by the Japanese Society of Nephrology (*11*). The urinary albumin excretion ratio (UAER) was calculated as urinary albumin (mg) per urinary creatinine (g).

Hyperglycemia was defined as fasting plasma glucose (FPG) ≥110 mg/dL and/or treatment with hypoglycemic agents. Albuminuria was defined as UAER ≥30 mg/g, and low eGFR was defined as eGFR <60 mL/min/1.73 m^2.

Classification of Blood Pressure Levels

Blood pressure level was classified into six categories as follows: BP-C1, systolic blood pressure (SBP) <90 mmHg and diastolic blood pressure (DBP) <65 mmHg; BP-C2, SBP 90–100 mmHg and/or DBP 65–70 mmHg; BP-C3, SBP 100–110 mmHg and/or DBP 70–75 mmHg; BP-C4, SBP 110–120 mmHg and/or DBP 75–80 mmHg; BP-C5, SBP 120–130 mmHg and/or DBP 80–85 mmHg; BP-C6, SBP 130–140 mmHg and/or DBP 85–90 mmHg.

Statistical Analysis

Continuous variables are expressed as the means±SD. Discrete variables are presented as numbers and percentages. Statistical differences in the means among groups were analyzed by one-way analysis of variance (ANOVA). Frequencies were compared by using the χ^2 test. A multivariate logistic regression model was used to examine the relationship between blood pressure level and the prevalence of albuminuria and low eGFR after adjustment for age, BMI, LDL-C, HDL-C, TG, FPG level and smoking status. A p value of less than 0.05 was taken to indicate statistical significance. Statistical analyses were performed with Stat View software version 5.0 (SAS Institute, Cary, USA).

Results

Characteristics of the Enrolled Subjects

Characteristics of the enrolled male and female subjects are shown in Tables 1 and 2, respectively. Albuminuria was found in 331 (5.8%) men and in 234 (6.0%) women. Low

Table 3. Logistic Regression Analysis of the Association between Blood Pressure and Prevalence of Albuminuria in Individuals without Hypertension

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Male						
BP-C1	1.99	0.59–6.75	0.27	1.85	0.53–6.46	0.33
BP-C2	1.20	0.59–2.45	0.62	1.22	0.59–2.51	0.60
BP-C3	1.00	—	—	1.00	—	—
BP-C4	1.84	1.16–2.92	0.0096	1.62	1.01–2.59	0.044
BP-C5	3.24	2.09–5.02	<0.0001	2.57	1.64–4.02	<0.0001
BP-C6	5.46	3.55–8.41	<0.0001	3.81	2.44–5.96	<0.0001
Female						
BP-C1	1.00	—	—	1.00	—	—
BP-C2	1.72	0.66–4.52	0.27	1.83	0.70–4.79	0.22
BP-C3	1.98	0.78–5.01	0.15	2.13	0.84–5.42	0.11
BP-C4	2.57	1.02–6.46	0.046	2.80	1.10–7.14	0.03
BP-C5	2.38	0.92–6.11	0.073	2.59	0.99–6.78	0.053
BP-C6	3.65	1.41–9.47	0.0077	3.99	1.50–10.64	0.0056

CI, confidence interval; OR, odds ratio. BP-C1, <90/<65 mmHg; BP-C2, 90–100/65–70 mmHg; BP-C3, 100–110/70–75 mmHg; BP-C4, 110–120/75–80 mmHg; BP-C5, 120–130/80–85 mmHg; BP-C6, 130–140/85–90 mmHg.

eGFR was observed in 735 (12.9%) men and in 592 (15.2%) women. In men, about two-thirds of subjects were either current or former smokers, whereas in women, only about 15% of subjects were either current or former smokers. BMI and the prevalence of hyperglycemia were higher in men than in women.

In men, BMI was larger and HDL-C level was lower when blood pressure level was high. TC, LDL-C, TG and fasting plasma glucose levels were the lowest in the BP-C2 group, and hemoglobin A1c level and the prevalence of hyperglycemia were the lowest in the BP-C3 group. There was a tendency for these parameters to increase as the blood pressure increased above these blood pressure levels. In women, BMI, glucose and lipid profile got worse when the blood pressure level increased.

Impact of Blood Pressure Level on the Prevalence of Albuminuria

The prevalence of albuminuria according to blood pressure levels in both genders is shown in Tables 1 and 2. In men, the prevalence of albuminuria showed a J-shaped relationship, whereas in women the prevalence of albuminuria showed an almost graded increase according to blood pressure level.

Next, multivariate logistic regression analysis adjusted for age, BMI, LDL-C, HDL-C, TG, FPG level and smoking status was performed and the results are shown in Table 3. In men, when BP-C3, the category with the lowest prevalence of albuminuria, was used as a reference, a significant graded increase in the risk for albuminuria was found in BP-C4, BP-C5, and BP-C6. In women, when BP-C1 was used as a reference, the increase in the risk for albuminuria was significant in both BP-C4 and BP-C6, and borderline significant in BP-C5.

Impact of Blood Pressure Level on the Prevalence of Low eGFR

The eGFR value according to blood pressure levels is shown in Tables 1 and 2. Female subjects with low blood pressure had a slightly higher eGFR than those with high blood pressure. The same tendency was found in men, although the differences among each category were not found to be statistically significant.

The prevalence of low eGFR according to blood pressure levels is also shown in Tables 1 and 2. In men, a J-shaped relationship was found between blood pressure and the prevalence of low eGFR, whereas in women a positive graded association was observed.

The results of multivariate logistic regression analysis adjusted for age, BMI, LDL-C, HDL-C, TG, FPG level and smoking status are shown in Table 4. The reference categories were BP-C2 in men and BP-C1 in women; these were the categories of the lowest prevalence of low eGFR in men and women. The risk for low eGFR did not significantly differ in non-hypertensive individuals of either gender.

Discussion

In the present study, we demonstrated that, in both men and women, the risk for albuminuria was significantly greater when blood pressure exceeded 110/75 mmHg in a Japanese population without hypertension. On the other hand, the risk for albuminuria did not significantly differ in individuals with

Table 4. Logistic Regression Analysis of the Association between Blood Pressure and Prevalence of Low eGFR in Individuals without Hypertension

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Male						
BP-C1	1.33	0.61–2.87	0.47	1.19	0.53–2.69	0.68
BP-C2	1.00	—	—	1.00	—	—
BP-C3	1.12	0.78–1.62	0.54	1.07	0.73–1.58	0.72
BP-C4	1.17	0.82–1.67	0.37	1.04	0.71–1.51	0.84
BP-C5	1.43	1.00–2.03	0.048	1.10	0.75–1.60	0.64
BP-C6	1.45	1.01–2.08	0.043	1.04	0.70–1.54	0.84
Female						
BP-C1	1.00	—	—	1.00	—	—
BP-C2	1.10	0.67–1.82	0.7	1.05	0.62–1.79	0.86
BP-C3	1.15	0.72–1.86	0.56	0.94	0.57–1.56	0.81
BP-C4	1.27	0.79–2.05	0.33	0.84	0.50–1.40	0.50
BP-C5	1.54	0.94–2.51	0.086	0.82	0.48–1.39	0.45
BP-C6	1.94	1.17–3.22	0.011	0.83	0.48–1.46	0.52

eGFR, estimated glomerular filtration rate; CI, confidence interval; OR, odds ratio. BP-C1, <90/<65 mmHg; BP-C2, 90–100/65–70 mmHg; BP-C3, 100–110/70–75 mmHg; BP-C4, 110–120/75–80 mmHg; BP-C5, 120–130/80–85 mmHg; BP-C6, 130–140/85–90 mmHg.

blood pressure lower than 100–110/70–75 mmHg. On the other hand, such type of association was not present between blood pressure and the risk for low eGFR in our study population.

Hypertension has been shown to be a risk factor for kidney dysfunction as well as cardiovascular diseases (*2–5*). Previous studies have established that, in hypertensives, the lower the blood pressure level, the better the clinical outcomes in regard to cardiovascular diseases and kidney dysfunction (*2, 6–10*). However, the relationship between blood pressure and renal function in apparently healthy Japanese with normal blood pressure or prehypertension has not been fully evaluated. Accordingly, this study was performed to determine whether any intervention is needed to regulate blood pressure in this population.

In the present study, a blood pressure level of ≥110/75 mmHg increased the prevalence of albuminuria in both men and women. Because albuminuria has been shown to be associated with the development of cardiovascular events and deaths (*12, 13*), and because albuminuria is in itself a risk factor for the progression of kidney dysfunction (*14*), the current study suggests that reduction of blood pressure to below 110/75 mmHg might help to prevent atherosclerotic diseases in the Japanese population. This threshold is nearly the same as that shown in the meta-analysis by Lewington *et al.*, in which a "the lower the better" relationship was found between blood pressure and cardiovascular mortality in individuals with blood pressure above 115/75 mmHg (*6*). In the Japanese population, Tozawa *et al.* (*15*) demonstrated that the incidence of ESRD was the lowest in the group with blood pressure below 120/80 mmHg, and it increased along with increases in blood pressure, which may support our present findings.

Furthermore, in both genders in the present study, there was no significant difference in the risk for albuminuria among the different blood pressure categories below 110/75 mmHg after multivariate adjustment. Therefore, when individuals have blood pressure less than 110/75 mmHg, they may not be considered to be at either higher or lower risk for albuminuria or CKD.

We found that the pattern of the relationship between BMI, glucose or lipid profile and blood pressure level showed a similar tendency to that between the prevalence of albuminuria and blood pressure level in both genders. This finding suggests that lifestyle intervention may help in realizing an optimal blood pressure level in apparently healthy Japanese subjects without hypertension.

Interestingly, the pattern of the relationship between blood pressure classes and prevalence of albuminuria seemed to differ slightly between the genders. The risk for albuminuria showed a more abrupt increase in proportion to a blood pressure level above 110/75 mmHg in men than in women. This variation might result from differences in the concentration of sex hormones between men and women. Androgen has been shown to stimulate the renin-angiotensin-aldosterone system (RAAS) and to increase the sensitivity to angiotensin-II through modulation of angiotensin-II receptors (*16*). Therefore, baseline RAAS activity may be higher in men than in women. Because angiotensin-II increases the resistance of mainly efferent arterioles, intraglomerular pressure may be higher in men than in women, even in individuals with the same blood pressure level, and may be more susceptible in men than in women to changes in systemic blood pressure *via*