Vascular inflammation evaluated by [^{18}F]-fluorodeoxyglucose-positron emission tomography/computed tomography is associated with endothelial dysfunction

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Running Title: Vascular Inflammation and Endothelial Dysfunction

Key Word: endothelial dysfunction ■ flow-mediated dilation ■ vascular inflammation ■ FDG-PET/CT

Total Word Counts: 4,670 words

Total number of figures and tables: 3 Figures and 4 Tables

Abstract

***Objective*—**Endothelial dysfunction is an initial step in atherosclerotic cardiovascular disease. However, involvement of vascular inflammation in endothelial dysfunction is not fully investigated in humans due to the lack of diagnostic modality to non-invasively evaluate vascular inflammation. We assessed the relationship between endothelial function and vascular inflammation evaluated by [^{18}F]-fluorodeoxyglucose-positron emission tomography (FDG-PET)/computed tomography (CT) imaging.

***Approach and Results*—**We examined endothelial function and vascular inflammation by flow-mediated dilation (FMD) of the brachial artery and FDG-PET/CT imaging of carotid arteries, respectively in 145 subjects (95 males and 50 females; mean age 61.8±9.5 years) who underwent a risk-screening test for cardiovascular disease in Kurume University Hospital. Vascular inflammation was measured by blood-normalized standardized uptake value, known as a target-to-background ratio (TBR). We investigated whether absolute changes from baseline of %FMD after anti-hypertensive treatment for 6 months (Δ%FMD) were correlated with those of TBR in 33 drug-naïve patients with essential hypertension. Multiple logistic regression analysis revealed that age [odds ratio (OR)=1.767 for 10 years increase], male gender (OR=0.434), LDL-cholesterol (OR=1.630 for 26 mg/dL increase) and TBR values (OR=1.759 for 0.2 increase) were independently associated with %FMD in 145 patients. There was an inverse correlation between Δ%FMD and ΔTBR; ΔTBR was a sole independent associate of Δ%FMD in hypertensive patients ($r= -0.558$, $p<0.001$).

***Conclusions*—**The present study showed that vascular inflammation in the

carotid arteries evaluated by FDG-PET/CT was one of the independent correlates of decreased %FMD, thus suggesting the association of vascular inflammation with endothelial dysfunction in humans.

Nonstandard Abbreviations and Acronyms

FMD flow-mediated dilation

FDG [^{18}F]-fluorodeoxyglucose

PET positron emission tomography

SUV standardized uptake value

TBR target-to-background ratio

Endothelial dysfunction is one of the initial steps in atherosclerosis.[1] Indeed, endothelial dysfunction is observed at the early stage of atherosclerosis, which could precede the morphological atherosclerotic changes such as increased intima-media thickness (IMT) of carotid arteries.[2,3] Flow-mediated dilation (FMD) of the brachial artery using a high-resolution ultrasound is a useful screening tool to detect vascular function in humans.[4-6] A number of studies have reported that various cardiovascular risk factors, including male gender,[5] aging,[5-7] smoking,[5,6] obesity,[6,8,9] insulin resistance,[8] dyslipidemia,[10,11] diabetes,[11] and hypertension[6,12] are associated with endothelial dysfunction evaluated by FMD. Furthermore, several reports have shown that the presence of endothelial dysfunction and its severity are of prognostic value.[13,14] Impaired FMD has been shown to predict future cardiovascular events in patients with or without cardiovascular diseases.[14,15]

There is a growing body of evidence, ranging from the results of *in vitro* experiments to pathological analysis to epidemiologic clinical studies, indicating that atherosclerosis is intrinsically an inflammatory disease.[16-18] Inflammatory reactions within the atherosclerotic lesions have been shown to reduce nitric oxide production and/or its bioavailability.[19] Furthermore, inflammatory biomarkers such as C-reactive protein (CRP) are positively associated with endothelial dysfunction in humans.[20,21] These observations suggest that vascular inflammation might contribute to endothelial dysfunction. However, the direct involvement of vascular inflammation in endothelial dysfunction in humans remains unclear due to the lack of diagnostic modality to non-invasively evaluate vascular inflammation non-invasively *in vivo*. We, along with other investigations,

have recently found that [^{18}F]-fluorodeoxyglucose-positron emission tomography (FDG-PET) is capable of identifying and quantifying vascular inflammation within the atherosclerosis in humans.[22-25] In this study, we examined whether vascular inflammation assessed by FDG-PET imaging of carotid arteries was independently associated with endothelial dysfunction evaluated by FMD of the brachial artery in 145 subjects. We further investigated whether absolute changes from baseline of %FMD after anti-hypertensive treatment for 6 months (Δ%FMD) were correlated with those of TBR in 33 drug-naïve essential hypertensive patients among the enrolled subjects.

Materials and Methods

Material and Methods are available in the online-only Supplement.

Results

Clinical characteristics and FDG-PET/CT image of subjects in the study design 1

Table 1 show the clinical characteristics of subjects. Twenty seven of 145 (18.6 %) patients received statins and 26/145 (17.9 %) patients received anti-hypertensive agents without statins. The mean age was 61.8 ± 9.5 years (range: 37-83) and there were 95 males (65.5 %). There were 49 subjects without carotid atherosclerotic plaques (maximum carotid artery IMT < 1.1 mm) and 96 with carotid plaques (maximum carotid artery IMT ≥ 1.1mm). The vessel diameter at baseline and at maximal dilation during reactive hyperemia was 3.98±0.63 mm and 4.20±0.65 mm, respectively. Mean and median (interquartile range) percent change in vasodilation (percent change over the baseline

values; %FMD) was 5.78±2.06 % and 5.52 (4.48-6.91) %, respectively. Nineteen patients had prevalent type 2 diabetes mellitus, in whom 13 patients (68.4 %) received oral hypoglycemic agents. Ninety six patients had essential hypertension. Of these, 36 patients (37.5 %) received anti-hypertensive drugs such as diuretics, β-blockers, calcium-channel blockers, angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers. Twenty six of 145 (17.9 %) patients received statins. Figure 1 shows representative images of FDG-PET of 2 cases; one is the case with low %FMD level (left panel in Fig. 1A and upper panel in Fig. 1B), and the other with high %FMD level (right panel in Fig. 1A and lower panel in Fig. 1B). Note intense FDG uptake in the carotid arteries of low %FMD case.

Association between endothelial function and vascular inflammation

Table 2 shows the results of regression analyses for %FMD [≥ median value 5.52 (interquartile range 4.48-6.91) %=0, < median value=1] in the logistic regression model. As shown in Table 2, there was no significant association between %FMD and carotid artery IMT, adiponectin, ADMA, an endogenous inhibitor of nitric oxide synthase, or hsCRP, an indicator of systemic inflammation. In univariate logistic regression analysis, age (β=0.057, odds ratio; OR=1.765 for 10 years increase [95% confidence interval; CI=1.209-2.575], p=0.003), male gender (β= -0.722, OR=0.486 [0.241-0.978], p=0.043), LDL-cholesterol (β=0.018, OR=1.596 for 26 mg/dL [1.125-2.266], p=0.009), HbA1c (β=0.737, OR=1.458 for 0.5 % increase [1.022-2.081], p=0.038) and TBR values (β=2.808, OR=1.770 for 0.2 increase [1.229-2.550], p=0.002) were significant correlates

of %FMD. Because these significant parameters could be closely correlated with each other, we then performed multivariate logistic regression analysis to determine the independent correlates of %FMD by integrating all these significant parameters. Multiple logistic regression analysis revealed that age (OR=1.767 [1.146-2.723], p=0.010), male gender (OR=0.434 [0.192-0.981], p=0.045), low-density lipoprotein cholesterol (OR=1.630 [1.107-2.398], p=0.013) and TBR values (OR=1.759 [1.168-2.649], p=0.007) were independently associated with %FMD (Table 2).

Both endothelial dysfunction and arterial inflammation are well known to associate substantially with atherosclerosis.[18] We further assessed the relationship between %FMD and TBR values in the subgroup of individuals without carotid atherosclerotic plaques. Mean %FMD in individuals without atherosclerosis was significantly higher than that with atherosclerosis (6.26±2.20 % vs. 5.78±2.06 %, p=0.045), while mean TBR was comparable between the 2 groups (1.47±0.23 vs. 1.47±0.20, p=0.995). In individuals without atherosclerosis, univariate logistic regression analysis revealed that age (β=0.081, OR=2.257 for 10 years increase [1.146-4.448], p=0.019), LDL-cholesterol (β=0.035, OR=2.385 for 25 mg/dL increase, 95% CI=1.188-4.788, p=0.015), uric acid (β=0.432, OR=1.908 for 1 mg/dL increase [1.013-3.591], p=0.045) and TBR values (β=4.185, OR=2.649 for 0.2 increase [1.252-5.603], p=0.011) were significant correlates of %FMD (< 6.16 %; median value). Multivariate regression analysis revealed that age (OR=3.011 [1.171-7.742], p=0.022) and TBR values (OR=2.807 [1.079-7.303], p=0.034) were independently associated with %FMD.

Medication for hypertension may affect renal function or vascular inflammation. However, medication for hypertension at baseline was not associated with either renal function (p=0.408) or TBR values (p=0.497).

Anti-hypertensive treatment effects on %FMD

Twenty-seven of 33 (81.8 %) patients achieved the target blood pressure (< 140/90 mm Hg). Four of 33 (12.1 %) patients received statin therapy. Table 3 shows the clinical variables of 33 patients at baseline and after 6-month anti-hypertensive therapy. Co-registration of FDG-PET and CT images revealed that FDG was taken up into the carotid arteries, which was significantly suppressed by anti-hypertensive therapy (Figure 2). As shown in Table 3 and Figure 3, anti-hypertensive treatment significantly reduced systolic, diastolic and mean blood pressure and decreased hsCRP and carotid artery TBR levels, whereas it increased %FMD and adiponectin values. There were no significant differences of anthropometric and metabolic parameters except HDL-cholesterol levels before and after the treatment for hypertension. ΔTBR was inversely and independently correlated with Δ%FMD in 33 patients with essential hypertension (r= -0.558, p<0.001) (Table 4). No treatment-related serious adverse effects were observed in the present study. When we analyzed the class effect of antihypertensive agents, although there was no significant difference of changes in %FMD between 17 olmesartan- and 16 amlodipine-treated patients (1.00±0.21 % vs. 0.58±0.16 % for Δ%FMD, p=0.141), TBR values were more decreased in patients received olmesartan than those with amlodipine (-0.13±0.04 vs. -0.02±0.03 for ΔTBR, p=0.016). Moreover, although only 4 of 33

(12.1 %) patients received statin therapy, it was not associated with either change in TBR (p=0.495) or %FMD values (p=0.061).

Anti-hypertensive therapy for 6 months also did not significantly affect renal function; it was changed from 77.7±15.5 L/min to 79.4±15.5 (p=0.394). Changes in renal function by anti-hypertensive therapy were not correlated with those in hsCRP (p=0.348), adiponectin (p=0.920), %FMD (p=0.187) or TBR values (p=0.354).

Discussion

The major findings of our study are as follows; 1) age, male gender, LDL-cholesterol and vascular inflammation evaluated by TBR in the FDG-PET were independently associated with endothelial dysfunction and 2) reduction in vascular inflammation was a sole independent associate of improvement of endothelial function after anti-hypertensive therapy. The present study suggests that vascular inflammation might be associated with endothelial dysfunction in humans.

Endothelial dysfunction and inflammation

Many clinical studies have reported that endothelial function evaluated by FMD is impaired in patients with classical cardiovascular risk factors such as central obesity, diabetes, hypertension and dyslipidemia.[5-12] Furthermore, subclinical inflammation detected by elevation of hsCRP[20,21] and presence of chronic systemic infection[26] are also associated with endothelial dysfunction. In addition, we have found that vascular inflammation defined by FDG-PET is associated

with the metabolic syndrome,[25] carotid thickness and hsCRP,[25] suggesting the involvement of vascular inflammation in endothelial dysfunction. However, it remains unclear whether vascular inflammation could be independently associated with endothelial dysfunction in humans. We found here that vascular inflammation of carotid arteries defined by FDG-PET was associated with decreased %FMD, which was independent of classical coronary risk factors except age, HbA1c and LDL-cholesterol. Moreover, ΔTBR was inversely correlated with Δ%FMD in essential hypertensive patients after 6-month therapy. These results suggest that vascular inflammation may play a role in endothelial dysfunction, thereby being one of the initial triggers of human atherosclerosis.

Endothelial dysfunction and glycemic state

Although the study design 1 included only 19 type 2 diabetic patients and mean values of HbA1c and FPG were within the normal ranges, HbA1c values were significantly correlated with decreased %FMD (Table 2). We, along with others, have previously reported that serum levels of advanced glycation end products (AGEs), which are formed via non-enzymatic glycation of proteins, are inversely associated with FMD in both diabetic patients and subjects who underwent coronary risk-screening examinations.[27,28] Therefore, even if HbA1c, a ketoamine and one of the intermediates of AGEs, was within a normal range, it may be a biomarker that reflects cumulative glycemic exposure, which may cause endothelial dysfunction.

In the present study, maximum carotid artery IMT, a predictor for future cardiovascular events[29], was not correlated with endothelial dysfunction.

Vascular inflammation could precede the morphological changes in carotid arteries and that FDG-PET might be a more useful tool for detecting early phase of atherosclerosis. In subjects without carotid atherosclerotic plaques, besides carotid artery TBR, age was independently correlated with decreased %FMD. It was contrast to the case of patients with carotid atherosclerotic plaques; in these individuals, TBR was a sole and independent correlate of decreased %FMD (data not shown). These observations suggest that age may exert unfavorable effects on endothelial function, especially in patients without carotid atherosclerotic plaques.

Interventional analysis

In the study design 1, 96 of 145 (66.2 %) subjects had hypertension, which was the most common coronary risk factor observed in our cases. Therefore, we next examined whether ΔTBR was independently correlated with Δ%FMD in 33 essential hypertensive patients when they were treated with blood pressure-lowering agents for 6 months. In the study design 2, anti-hypertensive therapy significantly improved endothelial function and suppressed vascular inflammation, which were accompanied by the increase in adiponectin as well as the decrease in hsCRP levels (Table 3, Figure 3). Multiple stepwise regression analysis demonstrated that reduction of carotid artery TBR, but not blood pressure or hsCRP levels, was a sole independent predictor of improvement of %FMD after 6-month treatment (Table 4). These observations further support the concept that vascular inflammation, *rather than* systemic inflammation or blood pressure, could contribute to endothelial dysfunction in humans. Since

systolic blood pressure was not correlated with decreased %FMD (Table 2), blood pressure-lowering agents may improve endothelial function partly via the direct anti-inflammatory effects on vessels. Further, although several papers have shown the correlation between adiponectin levels and %FMD in high-risk patients or healthy young adults,[30,31] baseline adiponectin levels and ∆adiponectin were not correlated with %FMD and ∆%FMD, respectively (Table 2, Table 4). Anti-hypertensive treatment effects on %FMD may be independent of adiponectin.

Methodological considerations

Due to a limited spatial resolution of the PET imaging, FDG activity in the carotid artery may be contaminated by that of blood pool in the target vessel. To address the issue, in this study we obtained the PET imaging 2 hours after the FDG injection because delayed phase image could allow sufficient FDG accumulation in the arterial wall and to permit blood levels of FDG to become more reduced by decay and washout compared with the image obtained 1 hour after FDG administration, which is commonly used for oncology PET.[32,33] Furthermore, TBR calculated as arterial SUV score divided by venous blood SUV is generally used as an index of a quantitative parameter of vascular inflammation.[23,32-36] Indeed, Tawakol et al. reported that TBR values in the carotid arteries were closely correlated with accumulation of CD68-positive macrophages in the corresponding histologic sections of patients with severe carotid stenoses ($r=0.85$, $p<0.001$).[23] Carotid TBR values were higher in patients with high-risk morphological features of atherosclerotic plaques, such as echolucent plaques,

positive remodeling, luminal irregularity, and low attenuation in CT than those without.[34,35] In addition, carotid artery TBR has been shown to strongly predict subsequent cardiovascular disease in patients with no prior history of cardiovascular disease independent of traditional risk factors.[36] Moreover, when we examined which variables including carotid artery SUV, venous blood SUV and TBR value were independently associated with %FMD, TBR value was the strongest correlate of decreased %FMD (β= -2.506, p=0.002). Carotid artery SUV (β=0.295, p<0.001), %FMD (β=0.017, p<0.001) and prevalence of coronary artery disease (β= -0.125, p=0.016) were independently correlated with blood pool SUV. These findings suggest that blood signal was correlated with %FMD, but normalization of arterial wall signal by the blood pool was effective in controlling for the partial volume effects. Therefore, although the partial volume effects might interfere with signal quantification of FDG uptake in the thin carotid arteries because of the limited resolution of the PET imaging, carotid artery TBR values in the FDG-PET imaging could be a reliable marker for vascular inflammation within the carotid atherosclerosis. Higher resolution PET systems and development of novel tracers for the detection of arterial inflammation will overcome the current methodological limitations of FDG-PET imaging.

Limitations

Our study had several limitations. First, we measured endothelial function and vascular inflammation at different vascular regions; the former was evaluated at brachial artery, and the latter at carotid arteries. The subject population enrolled was relatively low-risk, therefore, the generalizability of the study was limited by

the exclusion of individuals with uncontrolled diabetes. Second, we did not know why statin use was not associated with either TBR values or %FMD in the study design 1. It may be due to a lack of statistical power. Another reason why statin therapy was not correlated with either change in TBR values (p=0.495) or %FMD (p=0.061) in the study design 2 may be that statins could not affect the effects of anti-hypertensive agents on endothelial function or vascular inflammation. Third, although univariate regression analysis revealed no significant correlation between various medication and vascular inflammation or %FMD, we can not totally exclude the possibility that other medication could affect the present results because both endothelial function and vascular inflammation may be influenced by numerous therapeutic agents. However, it was unlikely that effects of anti-hypertensive medication on renal function could confound the present findings. Fourth, the treatment sub-study had several limitations; there was a relatively small reduction (5.3 %) in vascular inflammation after 6-month anti-hypertensive treatment, and we had no control group in the study design 2. Nonetheless, anti-hypertensive therapy significantly improved endothelial function evaluated by %FMD (from 5.94±1.70 to 6.73±1.60, p<0.001) and suppressed vascular inflammation measured by carotid artery TBR (from 1.51±0.24 to 1.43±0.20, p=0.007), and reduction of carotid artery TBR was a sole independent predictor of improvement of %FMD after 6-month anti-hypertensive treatment (Table 4). In addition, temporal blinding was used in the analysis. So the present findings suggest the association of vascular inflammation with endothelial dysfunction in humans. Seventh, the association between %FMD and TBR does not necessarily imply biological causation. The

relationship could be in the opposite direction (endothelial dysfunction may predispose to arterial inflammation) or alas the two factors could be separately related to yet another unaccounted factor. Accordingly, further longitudinal studies are needed to clarify if the suppression of arterial inflammation assessed by carotid artery TBR might reduce the risk of future cardiovascular events in humans.

Conclusions

We demonstrated that vascular inflammation in the carotid arteries evaluated by FDG-PET was one of the independent correlates of decreased %FMD and that there was an inverse correlation between ΔTBR and Δ%FMD in hypertensive patients, thus suggesting the association of vascular inflammation with endothelial dysfunction in humans.

Acknowledgments

The authors thank Kouichi Nitta (Hitachi-Medical Co.) and radiation technologists at Kurume University Hospital for their excellent technical assistance. They would also like to thank Mami Nakayama, Yuri Nishino, Miho Nakao-Kogure, Katsue Shiramizu, Miyuki Nishikata, and Makiko Kiyohiro for their efforts.

Sources of Funding

This study was supported in part by research grants from the Kimura Memorial Foundation (to A.H., A.T., and S.I.); the Mitsui Life Social Welfare Foundation (to

N.T.); the FUKUOKA clinical medicine of research prize (NT); and the Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, Tokyo, Japan (to N.T., S-I.Y. and Y.F.).

Disclosures

None.

References

1. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801-809.
2. Juonala M, Viikari JS, Laitinen T, Marniemi J, Helenius H, Rönnemaa T, Raitakari OT. Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. *Circulation* 2004;110:2918-2923.
3. Halcox JP, Donald AE, Ellins E, Witte DR, Shipley MJ, Brunner EJ, Marmot MG, Deanfield JE. Endothelial function predicts progression of carotid intima-media thickness. *Circulation* 2009;119:1005-1012.
4. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111-1115.
5. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol* 1994;24:1468-1474.
6. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, Lehman BT, Fan S, Osypiuk E, Vita JA. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation* 2004;109:613-619.

7. Gerhard M, Roddy MA, Creager SJ, Creager MA. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. *Hypertension* 1996;27:849-853.
8. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest* 1996;97:2601-2610.
9. Tounian P, Aggoun Y, Dubern B, Varille V, Guy-Grand B, Sidi D, Girardet JP, Bonnet D. Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. *Lancet* 2001;358:1400-1404.
10. Sorensen KE, Celermajer DS, Georgakopoulos D, Hatcher G, Betteridge DJ, Deanfield JE. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. *J Clin Invest* 1994;93:50-55.
11. Clarkson P, Celermajer DS, Donald AE, Sampson M, Sorensen KE, Adams M, Yue DK, Betteridge DJ, Deanfield JE. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. *J Am Coll Cardiol* 1996;28:573-579.
12. Gokce N, Holbrook M, Duffy SJ, Demissie S, Cupples LA, Biegelsen E, Keaney JF Jr, Loscalzo J, Vita JA. Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. *Hypertension* 2001;38:1349-1354.

13. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 2001;104:191-196.
14. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* 2002;105:1567-1572.
15. Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. *J Am Coll Cardiol* 2003;42:1037-1043.
16. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2000;20:1262-1275.
17. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868-874.
18. Tahara N, Imaizumi T, Virmani R, Narula J. Clinical feasibility of molecular imaging of plaque inflammation in atherosclerosis. *J Nucl Med* 2009;50:331-334.
19. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. *Annu Rev Immunol* 2009;27:165-197.
20. Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated C-reactive protein levels and impaired endothelial

vasoreactivity in patients with coronary artery disease. *Circulation* 2000;102:1000-1006.

21. Fichtlscherer S, Breuer S, Heeschen C, Dimmeler S, Zeiher AM. Interleukin-10 serum levels and systemic endothelial vasoreactivity in patients with coronary artery disease. *J Am Coll Cardiol* 2004;44:44-49.
22. Rudd JH, Warburton EA, Fryer TD, Jones HA, Clark JC, Antoun N, Johnström P, Davenport AP, Kirkpatrick PJ, Arch BN, Pickard JD, Weissberg PL. Imaging atherosclerotic plaque inflammation with [18F]-fluorodeoxyglucose positron emission tomography. *Circulation* 2002;105:2708-2711.
23. Tawakol A, Migrino RQ, Bashian GG, Bedri S, Vermylen D, Cury RC, Yates D, LaMuraglia GM, Furie K, Houser S, Gewirtz H, Muller JE, Brady TJ, Fischman AJ. In vivo 18F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. *J Am Coll Cardiol* 2006;48:1818-1824.
24. Tahara N, Kai H, Ishibashi M, Nakaura H, Kaida H, Baba K, Hayabuchi N, Imaizumi T. Simvastatin attenuates plaque inflammation: evaluation by fluorodeoxyglucose positron emission tomography. *J Am Coll Cardiol* 2006;48:1825-1831.
25. Tahara N, Kai H, Yamagishi S, Mizoguchi M, Nakaura H, Ishibashi M, Kaida H, Baba K, Hayabuchi N, Imaizumi T. Vascular inflammation evaluated by [18F]-fluorodeoxyglucose positron emission tomography is associated with the metabolic syndrome. *J Am Coll Cardiol* 2007;49:1533-1539.

26. Prasad A, Zhu J, Halcox JP, Waclawiw MA, Epstein SE, Quyyumi AA. Predisposition to atherosclerosis by infections: role of endothelial dysfunction. *Circulation* 2002;106:184-190.
27. Tan KC, Chow WS, Ai VH, Metz C, Bucala R, Lam KS. Advanced glycation end products and endothelial dysfunction in type 2 diabetes. *Diabetes Care* 2002;25:1055-1059.
28. Kajikawa M, Nakashima A, Fujimura N, Maruhashi T, Iwamoto Y, Iwamoto A, Matsumoto T, Oda N, Hidaka T, Kihara Y, Chayama K, Goto C, Aibara Y, Noma K, Takeuchi M, Matsui T, Yamagishi S, Higashi Y. Ratio of serum levels of AGEs to soluble form of RAGE is a predictor of endothelial function. *Diabetes Care* 2015;38:119-125.
29. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 1999;340:14-22.
30. Li M, Xu A, Lam KS, Cheung BM, Tse HF. Impact of combination therapy with amlodipine and atorvastatin on plasma adiponectin levels in hypertensive patients with coronary artery disease: combination therapy and adiponectin. *Postgrad Med* 2011;123:66-71.
31. Saarikoski LA, Huupponen RK, Viikari JS, Marniemi J, Juonala M, Kähönen M, Raitakari OT. Adiponectin is related with carotid artery intima-media thickness and brachial flow-mediated dilatation in young adults--the Cardiovascular Risk in Young Finns Study. *Ann Med* 2010;42:603-611

32. Bucerius J, Mani V, Moncrieff C, Machac J, Fuster V, Farkouh ME, Tawakol A, Rudd JH, Fayad ZA. Optimizing 18F-FDG PET/CT imaging of vessel wall inflammation: the impact of 18F-FDG circulation time, injected dose, uptake parameters, and fasting blood glucose levels. *Eur J Nucl Med Mol Imaging* 2014;41:369-383.
33. Bucerius J, Hyafil F, Verberne HJ, Slart RH, Lindner O, Sciagra R, Agostini D, Übleis C, Gimelli A, Hacker M; Cardiovascular Committee of the European Association of Nuclear Medicine (EANM). Position paper of the Cardiovascular Committee of the European Association of Nuclear Medicine (EANM) on PET imaging of atherosclerosis. *Eur J Nucl Med Mol Imaging.* 2016;43:780-792.
34. Choi YS, Youn HJ, Chung WB, Hwang HJ, Lee DH, Park CS, Lee JB, Kim PJ, Chung WS, Lee MY, Seung KB, Chung YA. Uptake of F-18 FDG and ultrasound analysis of carotid plaque. *J Nucl Cardiol* 2011;18:267-272.
35. Figueroa AL, Subramanian SS, Cury RC, Truong QA, Gardecki JA, Tearney GJ, Hoffmann U, Brady TJ, Tawakol A. Distribution of inflammation within carotid atherosclerotic plaques with high-risk morphological features: a comparison between positron emission tomography activity, plaque morphology, and histopathology. *Circ Cardiovasc Imaging* 2012;5:69-77.
36. Moon SH, Cho YS, Noh TS, Choi JY, Kim BT, Lee KH. Carotid FDG Uptake Improves Prediction of Future Cardiovascular Events in Asymptomatic Individuals. *JACC Cardiovasc Imaging* 2015;8:949-956.

Highlights section

1) Vascular inflammation was associated with endothelial dysfunction.

2) Reduction in vascular inflammation was correlated with improvement of endothelial dysfunction.

3) Vascular inflammation may play a role in atherosclerosis.

Figure legends

Figure 1. Representative coronal images of FDG-PET in 2 cases. One is the case with low %FMD level at 3.0 % (left panel in Fig. 1A and upper panel in Fig. 1B) and the other with high %FMD level at 11.6 % (right panel in Fig. 1A and lower panel in Fig. 1B). (B) CT (left panels), FDG-PET (middle panels) and PET/CT images (right panels) in cases with low and high %FMD. White arrows indicate carotid artery, while red arrows its FDG uptake. Circle shows the region of interest on cross sectional FDG-PET/CT images. Note intense FDG uptake in the carotid arteries of low %FMD case.

Figure 2. Representative cases before and after 6-month anti-hypertensive therapy. Representative CT (left panels), FDG-PET (middle panels) and PET/CT (right panels) images at baseline (top) and after 6-month anti-hypertensive therapy (bottom). Note reduction in FDG uptake in the left carotid artery (red arrows) after 6-month anti-hypertensive therapy. White arrows indicate carotid artery, while red arrows its FDG uptake. Circle shows the region of interest on cross sectional FDG-PET/CT images.

Figure 3. Changes from baseline of %FMD and TBR values after 6-month anti-hypertensive therapy. %FMD and TBR at baseline and after anti-hypertensive therapy were measured in each patient. Square and bar show means and standard deviation, respectively.

Materials and Methods

[Protocol 1]

Patients and study design 1

The study involved 145 consecutive subjects who underwent a risk-screening test for cardiovascular disease in Kurume University Hospital. We excluded any patients with inflammatory, neoplastic disorders, uncontrolled diabetes (fasting plasma glucose [FPG] ≥200 mg/dL), and any acute infection. Patients who received insulin injections for the treatment of diabetes mellitus were also excluded. All participants gave informed consent to participate in this study. The Ethical Committee for the Clinical Research of Kurume University approved this study.

Data collection

The medical history and use of smoking were ascertained by a questionnaire. Smoking was classified as current habitual use or not. Waist circumference was measured as an index of the presence or absence of central obesity. Blood pressure (BP) was measured in the sitting position using an upright standard sphygmomanometer. Vigorous physical activity and smoking were avoided for at least 30 minutes before BP and resting heart rate measurements. Blood samples for laboratory assays were obtained following overnight fasting from the antecubital vein in the morning for determinations of lipids {total cholesterol, low-density lipoprotein cholesterol (LDL-cholesterol), triglycerides, and high-density lipoprotein cholesterol (HDL-cholesterol)}, plasma glucose, insulin, glycated hemoglobin (HbA1c), uric acid, estimated glomerular filtration rate

(eGFR), high-sensitivity CRP (hsCRP), adiponectin, and asymmetric dimethylarginine (ADMA). These blood chemistry variables were measured by standard methods at a commercial laboratory (The Kyodo Igaku Laboratory, Fukuoka, Japan and SRL Inc., Tokyo, Japan) as described previously.[1-3] Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR index was calculated from the values of FPG (mg/dL) and fasting insulin (μU/mL) using the following formula [(FPG × fasting insulin)/405]. The value for HbA1c (%) is estimated as a National Glycohemoglobin Standardization Program equivalent value (%) calculated by the formula HbA1c (National Glycohemoglobin Standardization Program) (%) = 1.02 × HbA1c (Japan Diabetes Society) (%) + 0.25%.[4] eGFR was calculated using the Modification of Diet in Renal Disease study equation modified with a Japanese coefficient.[5] Hypertension was defined as BP≧ 140/90 mmHg or current treatment with antihypertensive medication. Diabetes was defined as FPG ≥ 126 mg/dL and/or current treatment with oral hypoglycemic agents.

Carotid ultrasonography

The carotid wall thickness of the bilateral carotid arteries was measured by duplex ultrasonography (SSA-380A, Toshiba) with a 10-MHz transducer as described previously.[1-3] Longitudinal B-mode images at the diastolic phase of the cardiac cycle were recorded by a single trained technician, who was blinded to the subjects' background. The images were magnified and printed using a high-resolution line recorder (LSR-100A, Toshiba, Japan). The maximum intima-media thickness was measured at the thickest wall of internal and

common carotid arteries. The intra-observer or inter-observer variability of IMT measurements was less than 5% as described previously.[1-3]

Measurement of endothelial function by flow-mediated vasodilation

Endothelial function was assessed in the brachial artery using the FMD technique by 1 trained ultrasonographer in a blinded manner as previously described.[6,7] The patients were instructed to fast and to abstain from exercise, caffeine/alcohol intake, taking medications or smoking for at least 12 hours before the examination. All patients rested for at least 15min in the supine position before the FMD measurements. The procedure was performed in a quiet, dark, temperature-controlled examination room according to the guidelines of the International Brachial Artery Reactivity Task Force.[8] A longitudinal image of the right brachial artery was obtained using a 10-MHz linear array transducer probe (UNEXEF18G). Using the FMD mode of the tracking system, the artery diameter was semi-automatically tracked, and the waveform of diameter changes over the cardiac cycle was recorded. Then, a forearm-cuff was inflated for 5 minutes at 50mmHg above the ordinary systolic blood pressure.[6-8] An end diastolic image of the right brachial artery was recorded continuously until 5 minutes after cuff deflation and diameters were measured with R-wave synchronized automated edge-detection software (UNEX Corporation, Nagoya, Japan).[6,7] FMD was estimated as the percent change in the vessel diameter over the baseline value at maximal dilation during reactive hyperemia.

FDG-PET and CT

Hybrid FDG-PET and computed tomography (CT) were performed as described previously.[2,3] In brief, after at least 12 hour-fasting prior to PET scanning, patients received an intravenous injection administration of FDG {4.2 MBq (0.12 mCi)/kg body weight} via the antecubital vein. Two hours after the FDG injection, 3-dimensional whole-body PET imaging and CT were carried out using an integrated full-ring PET/CT scanner (Gemini-GXL 16; Philips Medical Systems, Inc., Cleveland, Ohio, USA). The subjects rested for 2 hours in a comfortable position in a quiet room and were then conveyed to the scanning suite. The CT data were used for attenuation correction and lesion localization. After both the transmission and emission images were obtained, the images were reconstructed using the 3D line-of-response row-action maximum likelihood algorithm (3D-LOR-RAMLA; Philips, Eindhoven, The Netherlands). The co-registration of PET and CT imaging was performed for review on a workstation (Sun Microsystems, Inc., Santa Clara, California). The intensity of FDG uptake was quantified by measuring the standardized uptake value (SUV) corrected for body weight. The SUV was calculated by using the maximum pixel activity value within the region of interest placed on the vascular wall of the transaxial PET/CT image. The arterial SUV score was determined as the average of the SUVs of both the common carotid arteries obtained from 10 consecutive PET/CT images, each separated by 4 mm in length with the most cranial site starting at the carotid bifurcation. Afterward, target-to-background ratio (TBR) was calculated as arterial SUV score divided by venous blood SUV as described previously.[2,3] As to the reproducibility of analysis, TBR values in all

145 patients were re-analyzed by two blinded investigators 12 weeks after the initial evaluation. The intra-observer and inter-observer variability of measurements of carotid TBR values were very small; intra-class correlation coefficients between readers of 0.98 and 0.96, respectively.

[Protocol 2]

Patients and study design 2

Among total 145 subjects, 33 drug-naïve essential hypertensive patients (13 males and 20 females, mean age 59.1 ± 11.7 years) were enrolled in the study design 2. Although none of them had diabetes or coronary artery disease, 7 patients were active smokers, 4 received statin therapy, and one aspirin and bezafibrate. Sixteen patients received a calcium-channel blocker, amlodipine (5 to 10 mg daily) and 17 an angiotensin II type 1 receptor blocker, olmesartan (20 to 40 mg daily) for 6 months, and then %FMD and TBR were re-evaluated. BP was checked at least every 2 weeks, with assigned medication titrated to reach a target BP < 140/90 mm Hg. During the study period, subjects were instructed not to change their life styles and to continue taking the same dose of any concomitant drugs, including statins. We examined which Δclinical variables were independently associated with Δ%FMD after anti-hypertensive therapy. The study protocol was also approved by the Ethics Committee of Kurume University. All subjects provided written informed consent.

Statistics

Data were presented as mean values ± standard deviation or medians with the interquartile range. We performed the Shapiro-Wilk test to evaluate the assumption of normality. Statistical analysis was performed by means of appropriate parametric and nonparametric methods. In the study design 1, correlates between %FMD ($\geq$ median value=0, < median value=1) and other clinical variables were determined by univariate logistic regression analysis. Then we performed multivariate logistic regression analysis to determine the independent correlates of %FMD by integrating all the significant parameters in univariate analysis. We estimated odds ratios and their 95% confidence intervals per 1-unit (approximately 1 SD) increase in the variable. In the study design 2, Pearson's product-moment correlation test was performed to determine the association between Δ%FMD and Δclinical variables. Values of less than 0.05 were considered to be statistical significant. All statistical analyses were performed with the use of the SAS software (Release 9.3, SAS Institute, Cary, NC, USA) and SPSS system (SPSS Inc., Chicago, IL, USA) .

References

1.Tahara N, Kai H, Nakaura H, Mizoguchi M, Ishibashi M, Kaida H, Baba K, Hayabuchi N, Imaizumi T. The prevalence of inflammation in carotid atherosclerosis: analysis with fluorodeoxyglucose-positron emission tomography. *Eur Heart J* 2007;28:2243-2248.

2. Nitta Y, Tahara N, Tahara A, Honda A, Kodama N, Mizoguchi M, Kaida H, Ishibashi M, Hayabuchi N, Ikeda H, Yamagishi S, Imaizumi T. Pioglitazone decreases coronary artery inflammation in impaired glucose tolerance and diabetes mellitus: evaluation by FDG-PET/CT imaging. *JACC Cardiovasc Imaging* 2013;6:1172-1182.

3. Mizoguchi M, Tahara N, Tahara A, Nitta Y, Kodama N, Oba T, Mawatari K, Yasukawa H, Kaida H, Ishibashi M, Hayabuchi N, Harada H, Ikeda H, Yamagishi S, Imaizumi T. Pioglitazone attenuates atherosclerotic plaque inflammation in patients with impaired glucose tolerance or diabetes a prospective, randomized, comparator-controlled study using serial FDG PET/CT imaging study of carotid artery and ascending aorta. *JACC Cardiovasc Imaging* 2011;4:1110-1118.

4. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM; IFCC Working Group on HbA1c Standardization. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem* 2004;50:166-174.

5. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino

Y, Yokoyama H, Hishida A; Collaborators developing the Japanese equation for estimated GFR. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53:982-992.

6. Watanabe K, Oba K, Suzuki T, Ouchi M, Suzuki K, Futami-Suda S, Sekimizu K, Yamamoto N, Nakano H. Oral glucose loading attenuates endothelial function in normal individual. *Eur J Clin Invest* 2011;41:465-473.

7. Tomiyama H, Matsumoto C, Yamada J, Teramoto T, Abe K, Ohta H, Kiso Y, Kawauchi T, Yamashina A. The relationships of cardiovascular disease risk factors to flow-mediated dilatation in Japanese subjects free of cardiovascular disease. *Hypertens Res* 2008;31:2019-2025.

8. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;39:257-265.

Figure 1A

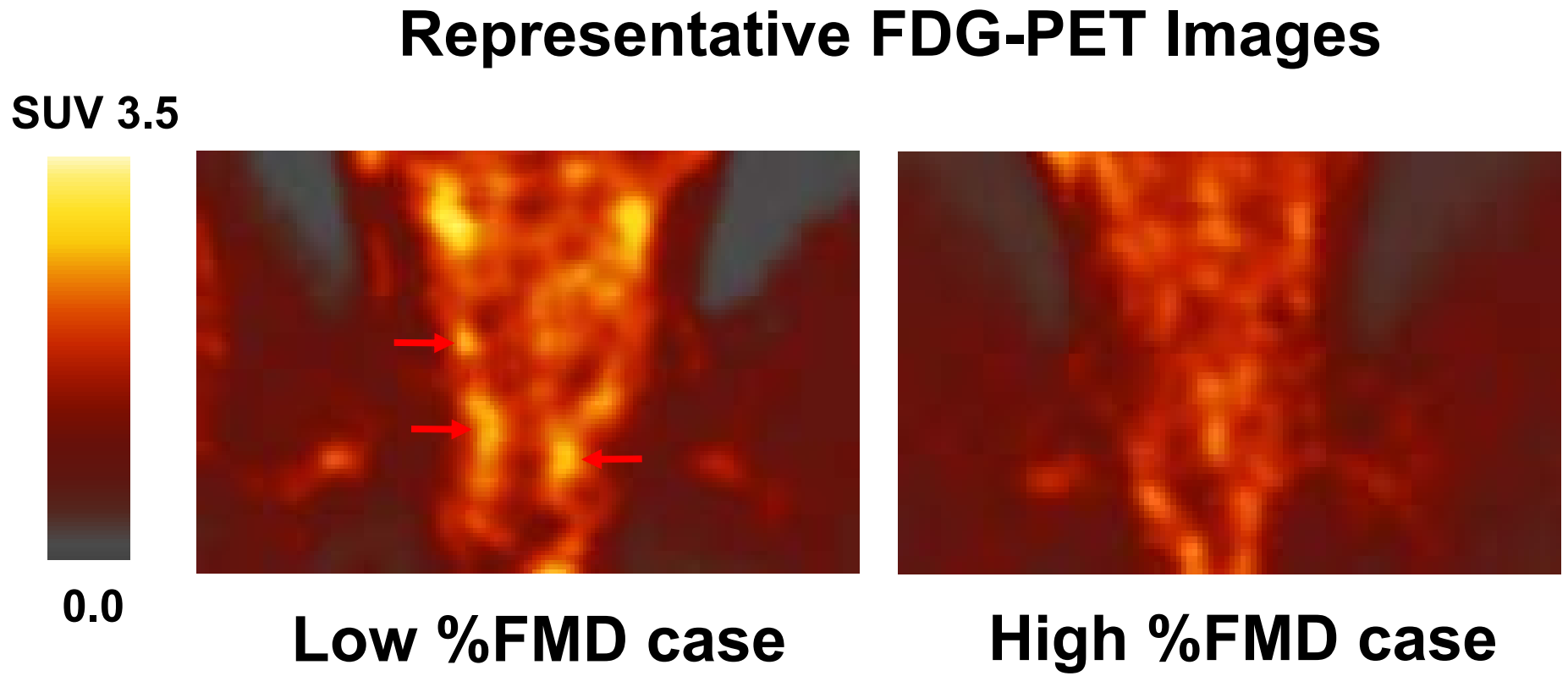
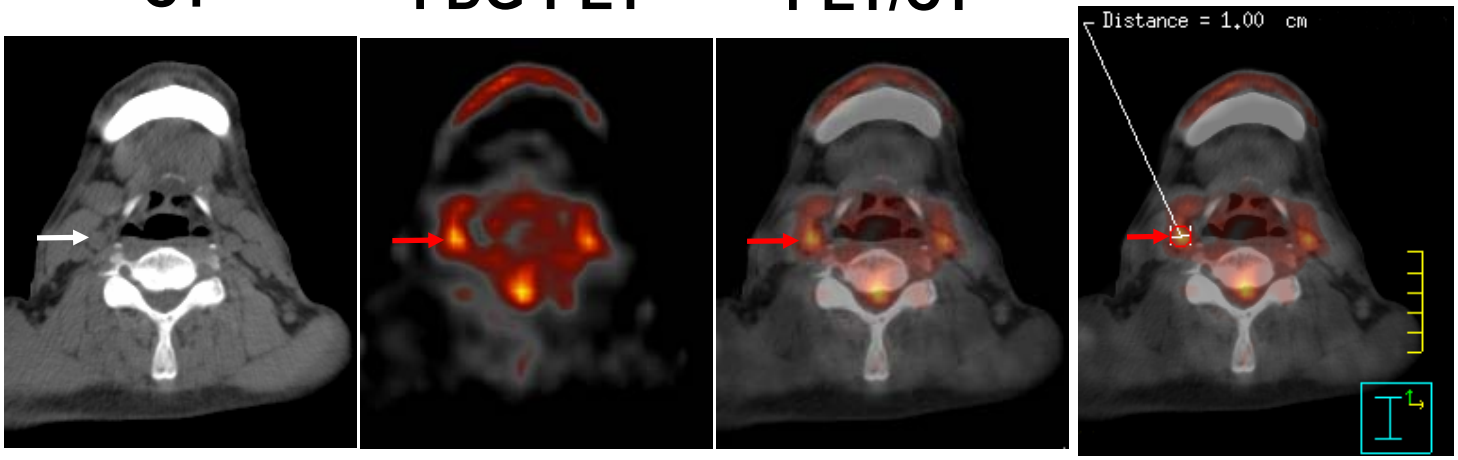

Representative FDG-PET Images
SUV 3.5
0.0
Low %FMD case
High %FMD case

Figure 1B

Low %FMD case

CT | FDG-PET | PET/CT | PET/CT



High %FMD case

CT | FDG-PET | PET/CT | PET/CT

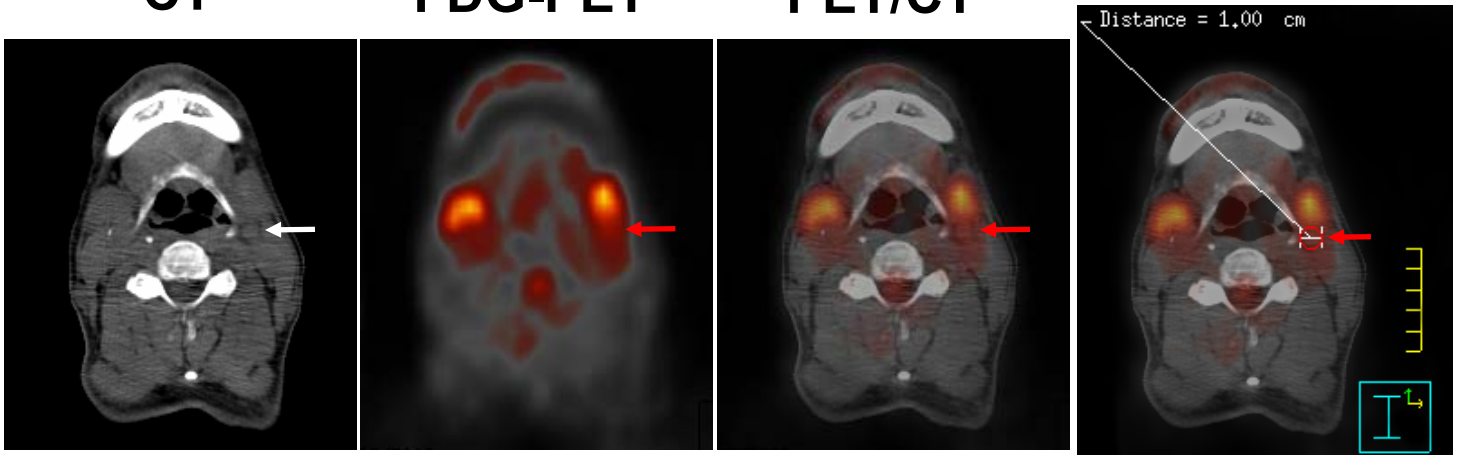


Figure 2

Representative FDG-PET and PET/CT Images After 6-Month Anti-hypertensive therapy

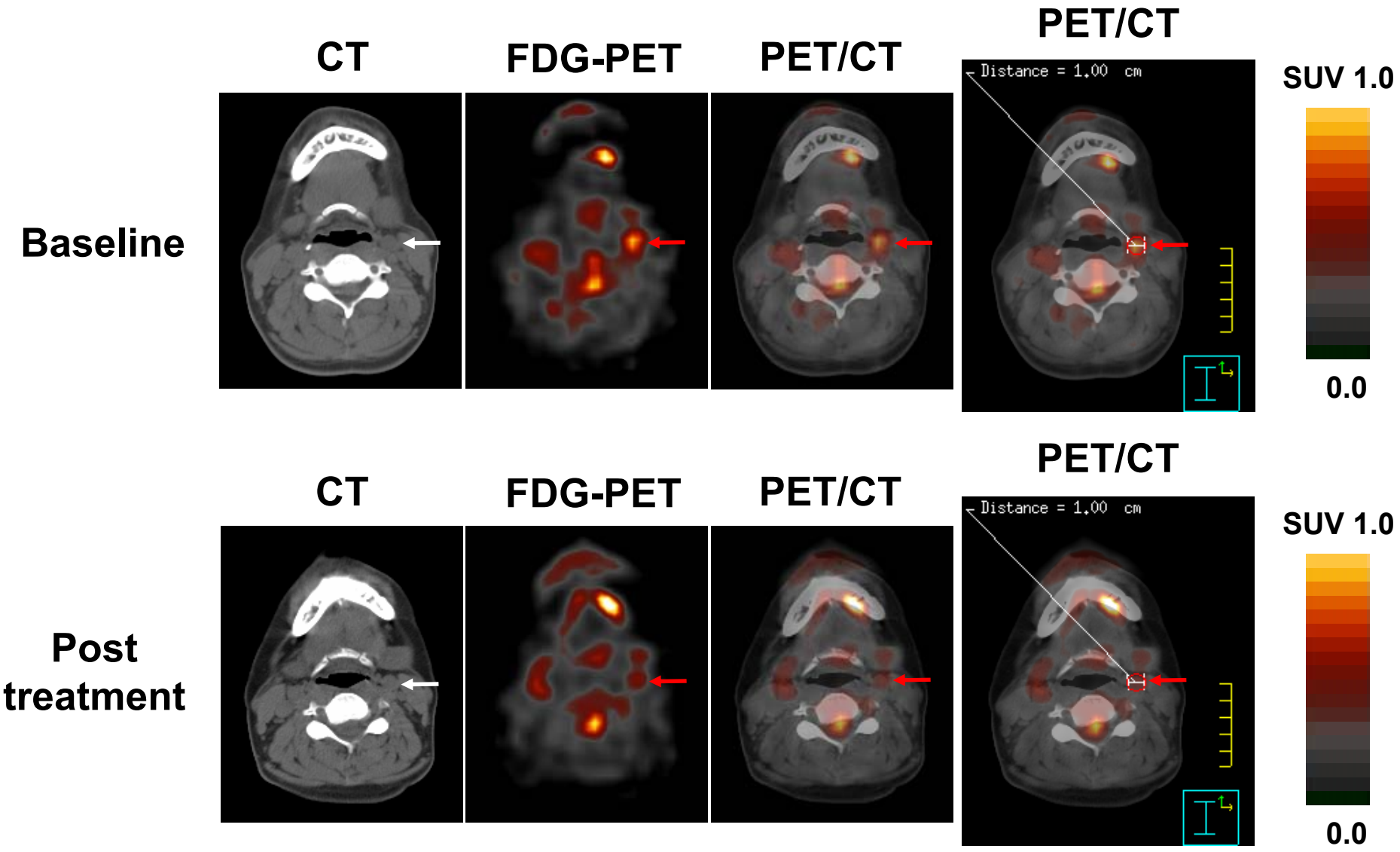


Figure 3

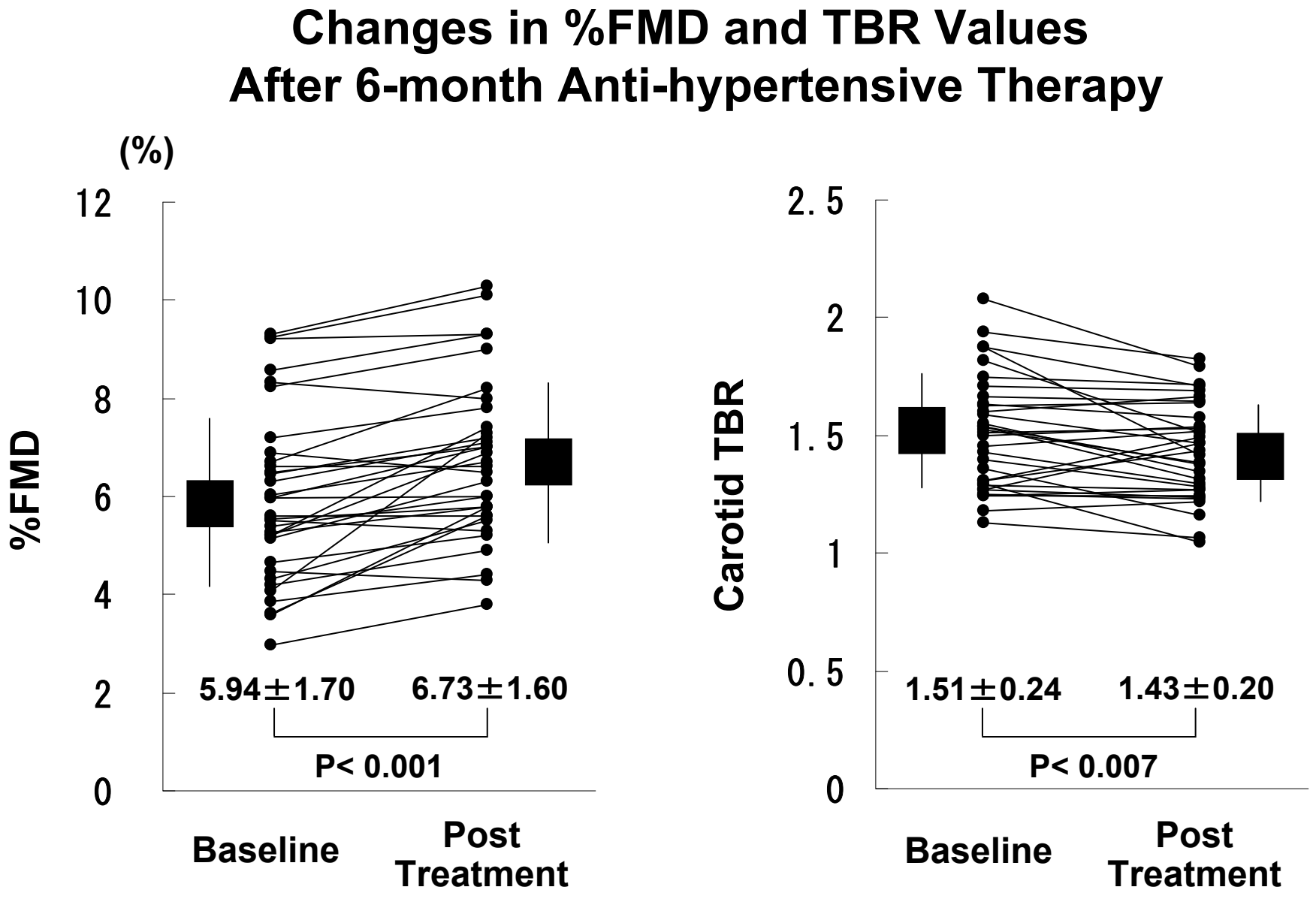

Changes in %FMD and TBR Values
After 6-month Anti-hypertensive Therapy
(%)
%FMD
12
10
8
6
4
2
0
5.94±1.70
6.73±1.60
P< 0.001
Baseline
Post Treatment
Carotid TBR
2.5
2
1.5
1
0.5
0
1.51±0.24
1.43±0.20
P< 0.007
Baseline
Post Treatment

Table 1. Clinical variables of subjects

Clinical variables	
Number	145
Age, years	61.8±9.5
Age, years (range)	37-83
Male, n (%)	95 (65.5)
Body mass index, kg/m^2	23.7±3.1
Waist circumference, cm	86.7±9.6
Active smoker, n (%)	28 (19.3)
Hypertension, n (%)	96 (66.2)
Diabetes, n (%)	19 (13.1)
Heart rate, beats/minute	64.7±11.7
Blood pressure, mmHg	
Systolic	140.4±19.8
Diastolic	84.3±11.6
Mean	103.0±13.4
Maximum carotid artery IMT*, mm	1.40 (0.91-1.89)
Carotid artery TBR	1.47±0.20
Flow-mediated dilation	
Baseline diameter, mm	3.98±0.63
Maximum diameter, mm	4.20±0.65
%flow-mediated dilation, %	5.78±2.06
Lipid profile	
LDL-cholesterol, mg/dL	124.9±26.3
HDL-cholesterol, mg/dL	58.2±14.0
Triglycerides*, mg/dL	101.0 (72.0-151.0)
Glycemic state	
Fasting plasma glucose*, mg/dL	96.0 (90.0-101.0)
Fasting plasma insulin*, mU/mL	4.80 (3.30-7.85)
HOMA-IR*	1.15 (0.75-2.00)
HbA1c, %	5.83±0.51
Estimated glomerular filtration rate, mL/min	76.8±16.0
Uric acid, mg/dL	5.67±1.33
Adiponectin*, μg/mL	6.19 (3.77-9.70)

ADMA, nmoL/mL	0.45±0.06
hsCRP*, mg/L	0.49 (0.24-0.87)
Coronary artery disease, n (%)	5 (3.4)
Drugs, n (%)	
Aspirin	7 (4.8)
Statins	26 (17.9)
For hypertension	36 (24.8)
For diabetes	13 (9.0)

Values are mean ± SD, n (%), or *median (interquartile range).

IMT, intima-media thickness; TBR, target-to-background ratio; LDL, low-density lipoprotein; HDL, high-density protein; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycosylated hemoglobin; ADMA, asymmetric dimethyl arginine; hsCRP, high-sensitivity C-reactive protein.

TABLE 2. Univariate and multivariate logistic regression analyses for correlates of %FMD

Dependent variable (increment)	Univariable logistic regression					Multivariable logistic regression				
	β	SE	OR	95% CI	p Value	β	SE	OR	95% CI	p Value
Age (10 years)	0.057	0.019	1.765	1.209, 2.575	**0.003**	0.057	0.022	1.767	1.146, 2.723	**0.010**
Sex*	-0.722	0.357	0.486	0.241, 0.978	**0.043**	-0.835	0.416	0.434	0.192, 0.981	**0.045**
Active smoker*	0.194	0.422	1.214	0.531, 2.775	0.645					
Body mass index (3 kg/m^2)	0.011	0.053	1.034	0.746, 1.433	0.842					
Waist circumference (10 cm)	0.012	0.017	1.121	0.807, 1.556	0.496					
Heart rate (12 beats/minute)	0.003	0.014	1.033	0.745, 1.433	0.844					
Systolic blood pressure (20 mmHg)	0.008	0.008	1.183	0.850, 1.646	0.318					
Diastolic blood pressure (12 mmHg)	-0.001	0.014	0.987	0.712, 1.369	0.939					
Maximum carotid artery IMT† (0.5 times)	-0.002	0.340	0.999	0.721, 1.385	0.995					
Carotid artery TBR (0.2)	2.808	0.915	1.770	1.229,	**0.002**	2.777	1.028	1.759	1.168,	**0.007**

				2.550					2.649	
LDL-cholesterol (26 mg/dL)	0.018	0.007	1.596	1.125, 2.266	**0.009**	0.019	0.007	1.630	1.107, 2.398	**0.013**
HDL-cholesterol (14 mg/dL)	-0.010	0.012	0.865	0.622, 1.203	0.389					
Triglycerides† (0.5 times)	-0.028	0.325	0.986	0.711, 1.367	0.932					
Fasting plasma glucose† (0.1 times)	2.188	1.222	1.378	0.970, 1.958	0.073					
Fasting plasma insulin† (0.6 times)	-0.206	0.266	0.878	0.632, 1.220	0.440					
HOMA-IR† (0.7 times)	-0.081	0.246	0.946	0.683, 1.313	0.742					
HbA1c (0.5 %)	0.737	0.355	1.458	1.022, 2.081	**0.038**	0.644	0.390	1.390	0.940, 2.056	0.099
Estimated glomerular filtration rate (16 mL/min)	-0.006	0.010	0.903	0.650, 1.256	0.546					
Uric acid (1 mg/dL)	0.206	0.128	1.317	0.942, 1.839	0.107					
hsCRP† (1.1 imes)	0.090	0.150	1.105	0.796, 1.536	0.550					
Adiponectin† (0.7 times)	-0.132	0.277	0.910	0.618, 1.340	0.633					

ADMA (0.06 nmoL/mL)	4.019	2.658	1.294	0.926, 1.807	0.131
Coronary artery disease*	14.259	700.8	>999.9	<0.001, >999.9	0.984
Aspirin use*	0.317	0.783	1.373	0.296, 6.362	0.686
Statin use*	0.291	0.429	1.338	0.577, 3.100	0.498
Medication for hypertension*	0.465	0.389	1.592	0.743, 3.411	0.232
Medication for diabetes*	0.531	0.596	1.700	0.529, 5.468	0.373

%FMD: median value=0, <median value=1.

* Men=0, Women=1 or No=0, Yes=1.

†Log-transformed value was used.

β = regression coefficient; OR = odds ratio; CI = confidence interval; FMD = flow-mediated dilation.

Other abbreviations as in Table 1.

Table 3. Clinical variables at baseline and after anti-hypertensive treatment

Parameters	Baseline	Follow-up	P value
Age, years	59.1±11.7		
Body mass index, kg/m^2	24.0±3.6	24.1±3.7	0.350
Waist circumference, cm	84.7±9.9	84.2±9.0	0.416
Heart rate, bpm	67.7±13.1	63.2±9.3	0.058
Blood pressure, mmHg			
Systolic	157.1±13.8	130.9±12.4	**<0.001**
Diastolic	90.0±10.5	80.0±9.2	**<0.001**
Mean	112.9±11.0	96.9±9.7	**<0.001**
Flow-mediated dilation			
Baseline diameter, mm	3.81±0.76	3.78±0.65	0.582
Maximum diameter, mm	4.04±0.79	4.02±0.66	0.760
%FMD, %	5.94±1.70	6.73±1.60	**<0.001**
Carotid artery TBR	1.51±0.24	1.43±0.20	**0.007**
Lipid profile			
LDL-cholesterol, mg/dL	121.0±21.1	119.3±24.9	0.666
HDL-cholesterol, mg/dL	62.7±15.7	59.4±16.5	**0.021**
Triglycerides*, mg/dL	85.0 (68.5-159.0)	85.0 (68.0-148.5)	0.391
Glycemic state			
Fasting plasma glucose*, mg/dL	102.0 (94.0-111.5)	102.0 (95.5-114.0)	0.689
Fasting plasma insulin*, mU/mL	5.40 (3.60-8.90)	5.60 (3.95-9.45)	0.206
HOMA-IR*	1.38 (1.05-2.29)	1.49 (1.07-2.39)	0.184
HbA1c, %	5.75±0.37	5.68±0.37	0.197
Estimated glomerular filtration rate, mL/min	77.7±15.5	79.4±18.5	0.394
Uric acid, mg/dL	5.27±1.37	5.25±1.40	0.926
Adiponectin*, µg/mL	5.96 (4.31-8.18)	6.50 (4.87-13.69)	**0.011**
ADMA, nmoL/mL	0.46±0.06	0.46±0.06	1.000
hsCRP*, mg/L	0.86	0.54	**0.049**

	(0.37-1.83)	(0.23-1.32)

Values are mean ± SD or *median (interquartile range).

Abbreviations as in Table 1.

Table 4. Univariate and multivariate analyses for associates of absolute change from baseline of %FMD (∆%FMD)

Parameters	Univariate analysis		Multivariate analysis	
	β	p Value	β	p Value
ΔHeart rate	0.019	0.079		
ΔSystolic blood pressure	-0.011	0.257		
ΔDiatolic blood pressure	-0.013	0.413		
ΔLDL-cholesterol	0.004	0.520		
ΔHDL-cholesterol	-0.033	0.064		
Δtriglycerides†	-0.175	0.744		
ΔEstimated glomerular filtration rate	-0.017	0.187		
ΔUric acid	0.117	0.432		
ΔFasting plasma glucose†	2.486	0.151		
ΔFasting plasma insulin†	-0.349	0.456		
ΔHOMA-IR†	-0.162	0.722		
ΔhsCRP†	-0.063	0.582		
ΔADMA	-0.176	0.912		
ΔAdiponectin†	-0.113	0.791		
ΔCarotid artery TBR	-3.128	**0.001**	-3.128	**0.001**
R^2			R^2=0.311	

*****Log-transformed value was used.

Abbreviations as in Tables 1 and 2.