

**FIG. 5.** AMPK plays an important role in Pi-induced calcification. HASMC were treated with or without AICAR (1 mM), a pharmacological activator of AMPK and TNF $\alpha$  (20 ng/ml) in calcification medium for 6 d. A and B, Ca deposition ( $n = 6$ ) (A) was measured, and immunoblotting with antibodies to p-AMPK, AMPK, Gas6, and  $\beta$ -tubulin (B) was performed ( $n = 3$ ). HASMC were cultured with or without compound C (1  $\mu\text{M}$ ), a chemical inhibitor of AMPK, adiponectin (300 ng/ml), and TNF $\alpha$  (20 ng/ml) in calcification medium for 6 d. C and D, Ca deposition (C) was evaluated ( $n = 6$ ), and immunoblotting with antibodies to p-AMPK, AMPK, Gas6, and  $\beta$ -tubulin (D) was performed ( $n = 3$ ). All values are presented as mean  $\pm$  SE. \*,  $P < 0.05$  by Bonferroni test. Each experiment was performed in triplicate for each condition.

phorylation that was inhibited by Pi and TNF $\alpha$  in a calcification-promoting condition.

To clarify the causal relationship between AMPK and calcification, we tried to activate AMPK by treatment with 5-aminimidazole-4-carboxamide ribonucleoside (AICAR) (25). In HASMC, AICAR significantly inhibited Ca deposition in a concentration-dependent manner (Fig. 5A). In addition, TNF $\alpha$ -stimulated Ca deposition was also blunted by AICAR. Interestingly, AICAR restored Gas6 expression down-regulated by Pi and TNF $\alpha$  (Fig. 5B). Next, to investigate whether the effect of adiponectin is dependent on AMPK, we tried to block AMPK using compound C, a chemical inhibitor of AMPK. As shown in Fig. 5C, compound C clearly abrogated the inhibitory effect of adiponectin both on Pi- and TNF $\alpha$ -induced calcification. The increase in Gas6 expression as well as AMPK phosphorylation by adiponectin was also abolished by compound C (Fig. 5D). These results suggest

that AMPK regulates Gas6 expression, followed by regulation of Ca deposition in HASMC.

#### Transcription activity of Gas6 is regulated by adiponectin and TNF $\alpha$ via AMPK

To investigate whether Gas6 expression is transcriptionally regulated by adiponectin, TNF $\alpha$ , and AMPK, a promoter study was undertaken. Reporter assay using the  $-1.9$ -kb Gas6-luciferase DNA construct revealed that adiponectin completely reversed the down-regulation of Gas6 transcription activity by TNF $\alpha$ . Furthermore, compound C abrogated the effect of adiponectin on Gas6 transcription activity, indicating that adiponectin and TNF $\alpha$  regulate Gas6 expression at the transcription level via AMPK activity (Fig. 6).

#### Discussion

The present study showed that adiponectin has a protective effect against Pi-induced calcification and, furthermore,

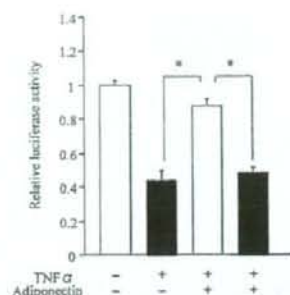


FIG. 6. Effect of adiponectin and TNF $\alpha$  on Gas6 promoter activity. HASMC were transfected with the Gas6 promoter-luciferase construct using lipofectamine 2000. Twenty-four hours after transfection, adiponectin (300 ng/ml), compound C (1  $\mu$ M), and TNF $\alpha$  (20 ng/ml) were added. Cells were incubated for an additional 44 h. Luciferase activity was normalized to that of vehicle-treated cells. All values are presented as mean  $\pm$  SE (n = 4). \*, P < 0.05 by Bonferroni test. Each experiment was performed in triplicate for each condition.

has an antagonistic effect on TNF $\alpha$ -augmented calcification. Based on our previous finding that Pi-induced calcification is dependent on apoptotic cell death in HASMC, we examined the role of adiponectin and TNF $\alpha$  in Pi-induced apoptosis. As expected, we found that adiponectin had an inhibitory effect and TNF $\alpha$  had a stimulatory effect on Pi-induced apoptosis. This study also demonstrated the

regulation of Gas6 expression by TNF $\alpha$  and adiponectin, a suppressive effect and a promoting effect, respectively, at the transcriptional level. Akt, a critical downstream effector of Gas6, was activated by adiponectin, whereas TNF $\alpha$  had an opposite action on its phosphorylation. Given that adiponectin and TNF $\alpha$  did not affect Gas6 expression and Akt phosphorylation in the absence of Pi (data not shown), the effects of adiponectin and TNF $\alpha$  on these molecules may dependent on Pi-induced responses. These results suggest that Gas6 is the target of adiponectin and TNF $\alpha$  in regulating Pi-induced apoptosis, accompanied by modulation of the Akt-dependent survival pathway.

As reported previously (6), Pi-induced VSMC calcification is associated with both phenotypic transition to osteoblastic cells via sodium-dependent phosphate cotransporter and apoptotic cell death. In our preliminary experiments, the expression of osteopontin, an osteoblastic marker, was not affected by TNF $\alpha$  and adiponectin (data not shown). Although this result suggests little influence of TNF $\alpha$  and adiponectin on osteoblastic differentiation of VSMC, extensive and systematic investigation including other markers of osteoblastic differentiation is needed to conclude this issue.

Multiple lines of clinical evidence show that adiponectin has protective actions on the cardiovascular system (26, 27). Circulating levels of adiponectin in humans are as high as 500–30,000  $\mu$ g/ml (28). Therefore, the concentration of adiponectin (300 ng/ml) used in this study are within physiological levels. Especially, consistent with our findings, adiponectin has been implicated in apoptosis of cardiovascular cells (19, 23, 29). Adiponectin inhibits apoptosis in cardiac myocytes and fibroblasts that are exposed to hypoxia-reoxygenation stress (19). In endothelial cells, adiponectin has been reported to inhibit serum starvation-induced apoptosis (23). *In vivo* experiments have also shown that adiponectin-deficient mice develop larger myocardial infarcts due to increased myocardial cell apoptosis and TNF $\alpha$  expression (17). Taking these observations together with our results, the antiapoptotic actions of adiponectin contribute to the inhibition of VSMC calcification.

Most effects of adiponectin have been attributed to the activation of AMPK, which affects many aspects of cellular metabolism including glucose uptake (30, 31), glucose utilization (32), and fatty acid oxidation (33, 34). Recently, AMPK activation in VSMC has been suggested as a target to prevent or treat vascular disease (35, 36). AICAR-induced AMPK activation inhibited angiotensin II-stimulated VSMC proliferation, and administration of AICAR prevented neointimal formation in a rat balloon injury model (35). AMPK activation in VSMC elicited cell cycle arrest at the G1 phase and inhibited cell proliferation via p53 up-regulation (36). Furthermore, in the heart, the inhibitory effects of adiponectin on ischemic injury-induced apoptosis have been shown to be dependent on AMPK activation (19). The results of *in vitro* studies also revealed that AMPK signaling is essential for the antiapoptotic activities of adiponectin on endothelial cells (23). These observations are consistent with the finding of the present study that AMPK activated by adiponectin stimulated Gas6 expression to restore the survival pathway, leading to the suppression of calcification.

In the present study, we further demonstrated that adiponectin significantly augmented the transcriptional activity of Gas6

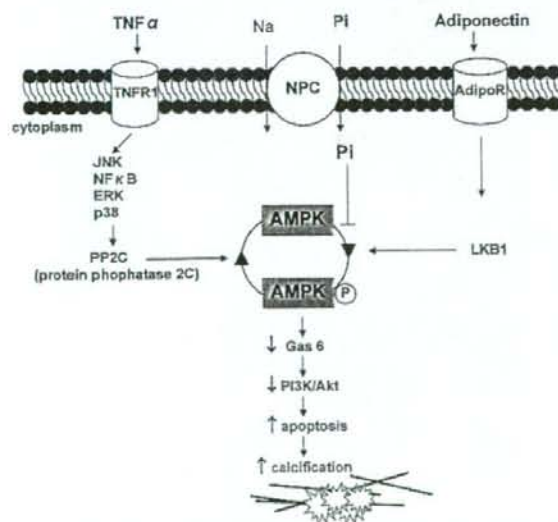


FIG. 7. Scheme of the effect of TNF $\alpha$  and adiponectin on Pi-induced calcification. In HASMC, exogenous Pi is internalized by sodium-dependent phosphate cotransporter (NPC, such as Pit-1) and inhibits AMPK phosphorylation, followed by down-regulation of the Gas6-mediated survival pathway. This pathway stimulates apoptosis, leading to subsequent development of calcification. TNF $\alpha$  directly suppresses AMPK activation by promoting PP2C activation via TNF receptor-1 (TNFR1). On the other hand, adiponectin activates LKB1-AMPK pathway via adiponectin receptors (AdipoR). AMPK activation modulated by TNF $\alpha$  and adiponectin contributes to the regulation of Pi-induced calcification.

that was decreased by TNF $\alpha$ . Indeed, suppression of AMPK by compound C clearly abrogated this beneficial effect of adiponectin. This result suggests that AMPK participates in the transcriptional regulation of Gas6 by adiponectin and TNF $\alpha$ . Several studies support that AMPK regulates the expression of particular genes at the transcriptional level (37–39). For example, AMPK activation by AICAR enhanced activator protein 1-mediated proopiomelanocortin promoter activities, which were completely abolished by compound C (37). AMPK has been shown to mediate the transcription signal that leads to the repression of phosphoenolpyruvate carboxylase expression, a key enzyme of gluconeogenesis, through phosphorylation of a transcription factor, AICAR-responsive element binding protein (38). It has also been observed that AICAR treatment is able to reduce nuclear factor- $\kappa$ B-regulated transcription, which is activated by TNF $\alpha$  (39).

Consistent with our findings, it has been recently reported that TNF $\alpha$  directly suppresses AMPK activation by promoting protein phosphatase 2C (PP2C) activity via TNF receptor-1 (40). PP2C has been proposed as one of modulators of the covalent regulation of AMPK (41). Increased PP2C levels account for the reduced AMPK activity and phosphorylation after TNF $\alpha$  treatment (40). On the other hand, LKB1 [also known as serine/threonine kinase II (STK II)] is the well-known, principal upstream kinase of AMPK (42, 43) that is regulated by adiponectin (44). AMPK activation by adiponectin is considered to be mediated by the cell surface receptors adiponectin receptors 1 and 2 (45). Another adiponectin receptor, T-cadherin, has recently been identified (46). In preliminary experiments, we found that all of the three adiponectin receptors were endogenously expressed in HASMC, and Pi did not affect their expression (data not shown). Taking these observations together, we hypothesized the mechanism of regulation by adiponectin and TNF $\alpha$  on Pi-induced vascular calcification (Fig. 7). However, further intensive investigations are required to elucidate the role of each player in VSMC calcification.

In summary, adiponectin inhibited VSMC calcification and antagonized the stimulatory effect of TNF $\alpha$ . This action was caused by preventing apoptosis via AMPK activation, followed by restoration of the Gas6-mediated survival pathway. AMPK regulated Gas6 expression at the transcriptional level. AMPK activation regulated by adiponectin and TNF $\alpha$  in vascular calcification might be a key to the management of cardiovascular disease.

### Acknowledgments

We thank Yuki Ito for technical assistance.

Received July 25, 2007. Accepted December 26, 2007.

Address all correspondence and requests for reprints to: Masahiro Akishita, M.D., Ph.D., Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: akishita-ky@umin.ac.jp.

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (No. 18590801 and No. 19590854), Novartis Foundation for Gerontological Research, Kanzawa Medical Research Foundation, Ono Medical Research Foundation, and Takeda Research Foundation.

Disclosure Statement: The authors have nothing to disclose.

### References

- Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, Rumberger J, Stanford W, White R, Taubert K 1996 Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association Writing Group. *Circulation* 94:1175–1192
- Johnson RC, Leopold JA, Loscalzo J 2006 Vascular calcification: pathobiological mechanisms and clinical implications. *Circ Res* 99:1044–1059
- van Poele NM, Mattace-Raso FU, Vlietghart R, Grobbee DE, Asmar R, van der Kuip DA, Hofman A, de Feijter PJ, Oudkerk M, Witteman JC 2006 Aortic stiffness is associated with atherosclerosis of the coronary arteries in older adults: the Rotterdam Study. *J Hypertens* 24:2371–2376
- Arad Y, Spadaro LA, Goodman K, Newstein D, Guerci AD 2000 Prediction of coronary events with electron beam computed tomography. *J Am Coll Cardiol* 36:1253–1260
- Thompson GR, Partridge J 2004 Coronary calcification score: the coronary-risk impact factor. *Lancet* 363:557–559
- Son BK, Kozaki K, Iijima K, Eto M, Kojima T, Ota H, Senda Y, Maemura K, Nakano T, Akishita M, Ouchi Y 2006 Statins protect human aortic smooth muscle cells from inorganic phosphate-induced calcification by restoring Gas6-Axl survival pathway. *Circ Res* 98:1024–1031
- Nakamura Y, Shimada K, Fukuda D, Shimada Y, Ehara S, Hirose M, Kataoka T, Kamimori K, Shimodono S, Kobayashi Y, Yoshizawa M, Takeuchi K, Yoshikawa J 2004 Implications of plasma concentrations of adiponectin in patients with coronary artery disease. *Heart* 90:528–533
- Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB 2004 Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 291:1730–1737
- Schulze MB, Shai I, Rimm EB, Li T, Rifai N, Hu FB 2005 Adiponectin and future coronary heart disease events among men with type 2 diabetes. *Diabetes* 54:534–539
- Kojima S, Funahashi T, Sakamoto T, Miyamoto S, Soejima H, Hokamaki J, Kajiwara I, Sugiyama S, Yoshimura M, Fujimoto K, Miyao Y, Suefuji H, Kitagawa A, Ouchi N, Kihara S, Matsuzawa Y, Ogawa H 2003 The variation of plasma concentrations of a novel, adipocyte derived protein, adiponectin, in patients with acute myocardial infarction. *Heart* 89:667
- Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y 1999 Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 100:2473–2476
- Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, Kumada M, Hotta K, Nishida M, Takahashi M, Nakamura T, Shimomura I, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y 2002 Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation* 105:2893–2898
- Maahs DM, Ogden LG, Kinney GL, Wadwa P, Snell-Bergeon JK, Dabelea D, Hokanson JE, Ehrlich J, Eckel RH, Rewers M 2005 Low plasma adiponectin levels predict progression of coronary artery calcification. *Circulation* 111:747–753
- Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y 2001 Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* 103:1057–1063
- Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y 2001 PPAR $\gamma$  ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50:2094–2099
- Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G 2003 Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor- $\alpha$  expression. *Diabetes* 52:1779–1785
- Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y 2002 Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737
- Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y 2000 Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF- $\kappa$ B signaling through a cAMP-dependent pathway. *Circulation* 102:1296–1301
- Shibata R, Sato K, Pimentel DR, Takemura Y, Kihara S, Ohashi K, Funahashi T, Ouchi N, Walsh K 2005 Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med* 11:1096–1103
- Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, Funahashi T, Walsh K 2004 Adiponectin stimulates angiogenesis by promoting cross-talk

- between AMP-activated protein kinase and Akt signaling in endothelial cells. *J Biol Chem* 279:1304–1309
21. Kudo N, Barr AJ, Barr RL, Desai S, Lopaschuk GD 1995 High rates of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA levels due to an increase in 5'-AMP-activated protein kinase inhibition of acetyl-CoA carboxylase. *J Biol Chem* 270:17513–17520
  22. Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K 2003 Obesity, adiponectin and vascular inflammatory disease. *Curr Opin Lipidol* 14:561–566
  23. Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y 2004 Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res* 94:e27–e31
  24. Son BK, Kozaki K, Iijima K, Eto M, Nakano T, Akishita M, Ouchi Y 2007 Gas6/Axl-PI3K/Akt pathway plays a central role in the effect of statins on inorganic phosphate-induced calcification of vascular smooth muscle cells. *Eur J Pharmacol* 556:1–8
  25. Corton JM, Gillespie JG, Hawley SA, Hardie DG 1995 5-Aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells? *Eur J Biochem* 229:558–565
  26. Gualillo O, González-Juanatey JR, Lago F 2007 The emerging role of adipokines as mediators of cardiovascular function: physiologic and clinical perspectives. *Trends Cardiovasc Med* 17:275–283
  27. Inoue T, Kotooka N, Morooka T, Komoda H, Uchida T, Aso Y, Inukai T, Okuno T, Nade K 2007 High molecular weight adiponectin as a predictor of long-term clinical outcome in patients with coronary artery disease. *Am J Cardiol* 100:569–574
  28. Berg AH, Combs TP, Scherer PE 2002 ACRP 30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab* 13:84–89
  29. Lin LY, Lin CY, Su TC, Liao CS 2004 Angiotensin II-induced apoptosis in human endothelial cells is inhibited by adiponectin through restoration of the association between endothelial nitric oxide synthase and heat shock protein 90. *FEBS Lett* 574:106–110
  30. Russell 3rd RR, Li J, Coven DL, Pypaert M, Zechner C, Palmeri M, Giordano FJ, Mu J, Birnbaum MJ, Young LH 2004 AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. *J Clin Invest* 114:495–503
  31. Li J, Miller EJ, Ninomiya-Tsuji J, Russell 3rd RR, Young LH 2005 AMP-activated protein kinase activates p38 mitogen-activated protein kinase by increasing recruitment of p38 MAPK to TAB1 in the ischemic heart. *Circ Res* 97:672–679
  32. Marsin AS, Bertrand L, Rider MH, Deprez J, Beauloye C, Vincent MF, Van den Berghe G, Carling D, Hue L 2000 Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. *Curr Biol* 10:1247–1255
  33. Kudo N, Gillespie JG, Kung L, Witters LA, Schulz R, Clanchan AS, Lopaschuk GD 1996 Characterization of 5'-AMP-activated protein kinase activity in the heart and its role in inhibiting acetyl-CoA carboxylase during reperfusion following ischemia. *Biochim Biophys Acta* 1301:67–75
  34. Makinde AO, Gamble J, Lopaschuk GD 1997 Upregulation of 5'-AMP-activated protein kinase is responsible for the increase in myocardial fatty acid oxidation rates following birth in the newborn rabbit. *Circ Res* 80:482–489
  35. Nagata D, Takeda R, Sata M, Satonaka H, Suzuki E, Nagano T, Hirata Y 2004 AMP-activated protein kinase inhibits angiotensin II-stimulated vascular smooth muscle proliferation. *Circulation* 110:444–451
  36. Igata M, Motoshima H, Tsuruzoe K, Kojima K, Matsumura T, Kondo T, Taguchi T, Nakamaru K, Yano M, Kukidome D, Matsumoto K, Toyonaga T, Asano T, Nishikawa T, Araki E 2005 Adenosine monophosphate-activated protein kinase suppresses vascular smooth muscle cell proliferation through the inhibition of cell cycle progression. *Circ Res* 97:837–844
  37. Iwasaki Y, Nishiyama M, Taguchi T, Kambayashi M, Asai M, Yoshida M, Nigawara T, Hashimoto K 2007 Activation of AMP-activated protein kinase stimulates proopiomelanocortin gene transcription in AIT20 corticotroph cells. *Am J Physiol Endocrinol Metab* 292:E1899–E1905
  38. Inoue E, Yamauchi J 2006 AMP-activated protein kinase regulates PEPCK gene expression by direct phosphorylation of a novel zinc finger transcription factor. *Biochem Biophys Res Commun* 351:793–799
  39. Solaz-Fuster MC, Gimeno-Alcainz JV, Casado M, Sanz P 2006 TRIP6 transcriptional co-activator is a novel substrate of AMP-activated protein kinase. *Cell Signal* 18:1702–1712
  40. Steinberg GR, Michell BJ, van Denderen BJ, Watt MJ, Carey AL, Fam BC, Andrikopoulos S, Proietto J, Görgün CZ, Carling D, Hotamisligil GS, Febbraio MA, Kay TW, Kemp BE 2006 Tumor necrosis factor  $\alpha$ -induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. *Cell Metab* 4:465–474
  41. Davies SP, Helps NR, Cohen PT, Hardie DG 1995 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C $\alpha$  and native bovine protein phosphatase-2A/C. *FEBS Lett* 377:421–425
  42. Hawley SA, Boudeau J, Reid JL, Mustard KJ, Udd L, McKelvie TP, Alessi DR, Hardie DG 2003 Complexes between the LKB1 tumor suppressor, STRAD  $\alpha/\beta$  and MO25  $\alpha/\beta$  are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* 2:28
  43. Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LG, Neumann D, Schlatterer U, Wallimann T, Carlson M, Carling D 2003 LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol* 13:2004–2008
  44. Imai K, Inukai K, Ikegami Y, Awata T, Katayama S 2006 LKB1, an upstream AMPK kinase, regulates glucose and lipid metabolism in cultured liver and muscle cells. *Biochem Biophys Res Commun* 351:595–601
  45. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T 2003 Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423:762–769
  46. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF 2004 T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. *Proc Natl Acad Sci USA* 101:10308–10313

*Endocrinology* is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

## White matter lesions as a feature of cognitive impairment, low vitality and other symptoms of geriatric syndrome in the elderly

Kazuki Sonohara,<sup>1</sup> Koichi Kozaki,<sup>1</sup> Masahiro Akishita,<sup>2</sup> Kumiko Nagai,<sup>1</sup> Hiroshi Hasegawa,<sup>1</sup> Masafumi Kuzuya,<sup>3</sup> Koutaro Yokote<sup>4</sup> and Kenji Toba<sup>1</sup>

<sup>1</sup>Department of Geriatric Medicine, Kyorin University School of Medicine, Mitaka, <sup>2</sup>Department of Geriatric Medicine, University of Tokyo Graduate School of Medicine, Tokyo, <sup>3</sup>Department of Geriatrics, Nagoya University Graduate School of Medicine, Nagoya, and <sup>4</sup>Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan

**Aim:** White matter lesions (WML) are common findings on magnetic resonance imaging (MRI) in elderly persons. In this study, we analyzed the relation of WML with global cognitive function, depression, vitality/volition, and 19 symptoms of geriatric syndrome in Japanese elderly patients who attended three university geriatric outpatient clinics.

**Methods:** Two hundred and eighty-six subjects (103 men and 183 women; mean  $\pm$  standard deviation age, 74.5  $\pm$  7.8 years) were included in this study. MRI scans were performed for the diagnosis of WML, and the severity of periventricular and deep white matter hyperintensities (PVH and DWMH) was rated semiquantitatively. Concurrently, all subjects underwent tests of cognitive function, depressive state and vitality, and were examined for 19 symptoms of geriatric syndrome.

**Results:** The study subjects showed cognitive decline, depression and low vitality, all to a mild extent. Univariate linear regression analysis showed a negative correlation between the severity of WML and cognitive function or vitality. Multiple logistic analysis revealed that the severity of WML was a significant determinant of cognitive impairment and low vitality, after adjustment for confounding factors such as age, sex and concomitant diseases. PVH and/or DWMH score was significantly greater in subjects who exhibited 13 out of 19 symptoms of geriatric syndrome. Logistic regression analysis indicated that WML were associated with psychological disorders, gait disturbance, urinary problems and parkinsonism.

**Conclusion:** WML were associated with various symptoms of functional decline in older persons. Evaluating WML in relation to functional decline would be important for preventing disability in elderly people.

**Keywords:** deep white matter hyperintensity, geriatric syndrome, periventricular hyperintensity, white matter lesion.

Accepted for publication 10 December 2007.

Correspondence: Assistant Professor Koichi Kozaki MD, Department of Geriatric Medicine, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan. Email: kozaki-tky@umin.ac.jp

## Introduction

Brain magnetic resonance imaging (MRI) has markedly enhanced the chance of detecting characteristic hyperintense signals in the periventricular and subcortical areas on T2-weighted images, even in asymptomatic older persons.<sup>1</sup> These lesions are known as white matter lesions (WML), leukoaraiosis or white matter (periventricular and subcortical) hyperintensities.<sup>2-4</sup> WML, which accompany symptoms of gait abnormalities,<sup>5-7</sup> urinary symptoms<sup>8,9</sup> and cognitive impairment,<sup>4,10,11</sup> are reported to be associated with aging,<sup>12-14</sup> hypertension,<sup>14</sup> diabetes<sup>15</sup> and atherosclerosis.<sup>5</sup> There is poor understanding of the pathogenesis of the lesions, and it remains unknown whether WML are mere innocuous radiological changes that appear as a result of the aging process,<sup>2,3,10</sup> or whether they are one of the causal factors of the functional decline in elderly people.

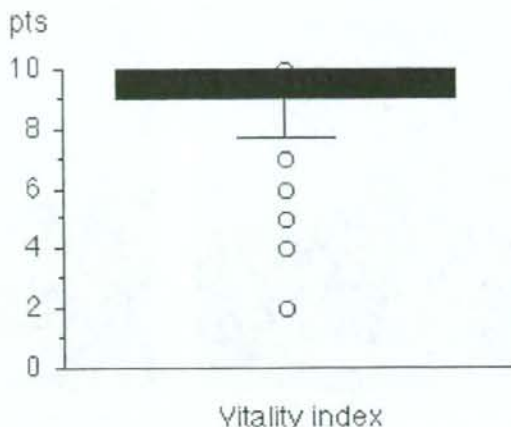
Geriatric syndrome is a group of symptoms that are related to daily life, and the comorbidity triggers the loss of independence of elderly persons. Hence, evaluation of geriatric syndrome is important for the physical and mental care of the elderly. To address the pathological significance of WML in the global cognitive and psychological functions, and in geriatric syndrome in representative Japanese elderly subjects, we organized a group of geriatric outpatient clinics, and investigated the clinical manifestations of WML in those patients. Especially, we analyzed the relation of WML with global cognitive function, depressive state, vitality/volition and 19 symptoms of geriatric syndrome.

## Methods

### Subjects

This was a multicenter study performed at three different university geriatric outpatient clinics in Japan under the organization of a Longevity Science Research Grant from the Ministry of Health, Labor and Welfare of Japan (H15-Choju-013). Two hundred and eighty-six consecutive subjects (103 men and 183 women; mean  $\pm$  standard deviation [SD] age,  $74.5 \pm 7.8$  years) were included in this study: 187 at Kyorin University Hospital, 74 at Chiba University Hospital, and 25 at Nagoya University Hospital, from January 2004 to January 2005.

The diagnosis of dementia was made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The definition of hypertension was systolic blood pressure (BP) of more than 140 mmHg or diastolic BP of more than 90 mmHg, or receiving antihypertensive drugs. The definition of diabetes was glycosylated hemoglobin A1c of more than 6.5%, or receiving antidiabetic drugs. The definition of hyperlipidemia was total cholesterol of more than

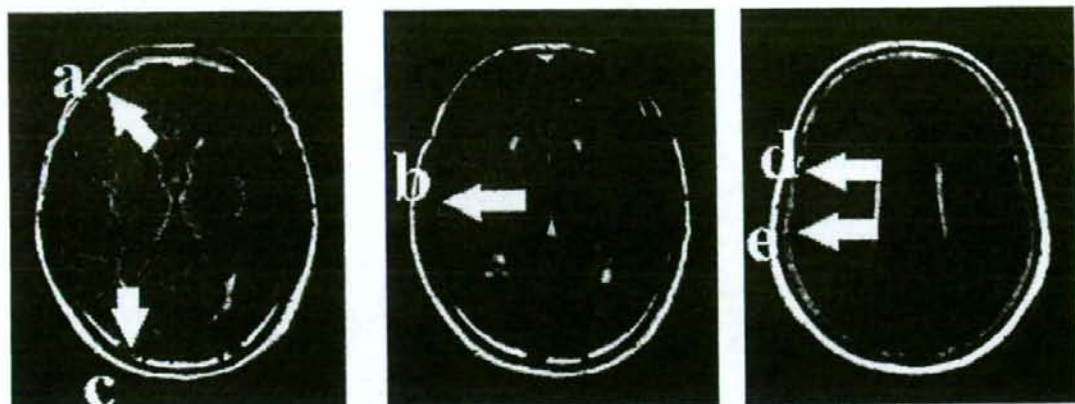


**Figure 1** Distribution of vitality index. All subjects underwent assessment of vitality index as a measure of vitality related to activities of daily living (waking pattern, communication, feeding, getting on and off the toilet, rehabilitation and other activities; 2 points each; range, 0-10).

5.72 mmol/L, triglyceride of more than 1.70 mmol/L, or receiving antihyperlipidemic drugs.

All subjects underwent the following assessment of global cognitive and psychological function. Cognitive function was evaluated by Mini-Mental State Examination (MMSE).<sup>16</sup> In this examination, we focused on calculation (serial subtraction of 7 from 100) to evaluate attention and working memory (part of the frontal lobe function). We also performed verbal fluency or word recollection test by asking the subjects to name as many vegetables as possible, which is also indicative of the frontal lobe function. Depression was evaluated by the 15-item Geriatric Depression Scale (GDS-15), which consists of 15 dichotomous questions for screening depressive symptoms in elderly subjects (range, 0-15).<sup>17</sup> Vitality index was used to measure vitality or volition in daily life (waking pattern, communication, feeding, getting on and off the toilet, rehabilitation and other activities; 2 points each; range, 0-10).<sup>18</sup> A full score can be maintained until one is severely disabled in cognition or function. The distribution of vitality index in the subjects of this study is shown in Figure 1.

We examined symptoms of geriatric syndrome: 19 dichotomous questions about hallucinations, delusions, insomnia, vertigo, paralysis, numbness, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, constipation, decreased appetite, weight loss, apathy, speech impairment, swallowing difficulty, tremor and muscle stiffness.



**Figure 2** Evaluation of periventricular hyperintensity (PVH). PVH were evaluated in six regions in three slices: (a) adjacent to the frontal horns, (b) lateral ventricular body, (c) occipital horns, (d) frontal central semiovale in the parietal region and (e) occipital centrum semiovale in the parietal region in both hemispheres. Each area was rated as five grades according to the method of Junque *et al.*: (0) no hyperintensities; (1) <25% of the brain area; (2) 25–50%; (3) 50–75%; and (4) >75%.<sup>11</sup> The sum of all grades in the six regions was defined as the PVH score (range, 0–24).

### Magnetic resonance imaging

Magnetic resonance imaging scans were performed for the diagnosis of WML and cerebral infarction on 1.5-T scanners (Toshiba, Nasu, Japan). T1-weighted images (repetition time [TR], 496 ms; echo time [TE], 12 ms), T2-weighted images (TR, 4280 ms; TE, 105 ms), and fluid-attenuated inversion-recovery (FLAIR)-weighted images (TR, 8000 ms; TE, 105 ms; 5-mm slice thickness) were obtained in the axial plane. MRI images were examined to differentiate between WML, characterized by isointense signals on T1-weighted images and hyperintense signals on T2-weighted and FLAIR images, and cerebral infarction, characterized by hypointense signals on T1-weighted images and hyperintense signals on T2-weighted and FLAIR images.

White matter lesions were classified as periventricular hyperintensities (PVH), which adjoined the lateral ventricle, and deep white matter hyperintensities (DWMH), located in the deep white matter apart from the lateral ventricles.

### Periventricular and deep white matter hyperintensity scores

Periventricular hyperintensities were evaluated in six regions in three slices: adjacent to the frontal horns, lateral ventricular body, occipital horns, frontal central semiovale in the parietal region, and occipital centrum semiovale in the parietal region in both hemispheres (Fig. 2). Each area was rated as five grades according to the systematic quantification method developed by Junque *et al.*: (0) no hyperintensities; (1) less than 25%

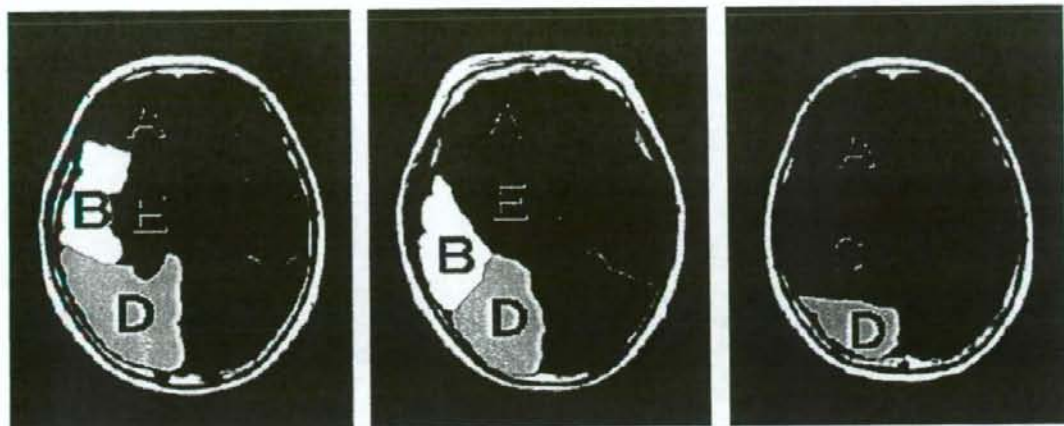
of the brain area; (2) 25–50%; (3) 50–75%; and (4) more than 75%.<sup>11</sup> The sum of all grades in the six regions was defined as the PVH score (range, 0–24).

Deep white matter hyperintensities were evaluated in the frontal, temporal, parietal and occipital lobes, and in the basal ganglia in both hemispheres (Fig. 3). Each lesion was rated as three grades according to the diameter by the study of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) more than 10 mm. The sum of all grades in five regions in both hemispheres was defined as the DWMH score.<sup>4</sup> Analysis was performed assuming that the white matter scores of PVH and DWMH were quantitative interval scales.

### Statistical analysis

The relationship between two continuous variables such as MMSE, GDS-15 or vitality index, and WML (PVH or DWMH) score was analyzed by univariate linear regression analysis, and the correlation was analyzed by means of Pearson's simple correlation coefficients. Statistical significance was set at  $P < 0.05$ .

The relation of cognitive impairment or low vitality with PVH score or DWMH score was assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. Cognitive impairment and low vitality were defined as an MMSE score of 23 or less<sup>19</sup> and a vitality index of 9 or less, respectively. Odds ratios and 95% confidence interval were calculated from the coefficients and their standard errors.



**Figure 3** Evaluation of deep white matter hyperintensities (DWMH). DWMH were evaluated in the (A) frontal, (B) temporal, (C) parietal and (D) occipital lobes, and (E) in the basal ganglia in both hemispheres. Each lesion was rated as three grades according to diameter by the method of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) >10 mm.<sup>4</sup> The sum of all grades in five regions in both hemispheres was defined as the DWMH score.

Periventricular hyperintensity score or DWMH score was compared between subjects who did or did not exhibit each symptom of geriatric syndrome and analyzed by Student's *t*-test. When the difference was considered to be significant ( $P < 0.05$ ), the difference was further assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease.

#### Ethical considerations

This study was approved by the ethical committees of the institutes involved in this project. We explained this study clearly, and obtained written consent from all participants and their guardians (mainly family members). All the data were stored and analyzed carefully to preserve the subjects' anonymity and protect their privacy.

## Results

#### Clinical data

The clinical characteristics of the study subjects are shown in Table 1. The mean age of subjects was  $74.5 \pm 7.8$  years (mean  $\pm$  SD), and subjects aged 65 or older comprised 88.1%. The mean body mass index was  $21.8 \pm 3.3$  kg/m<sup>2</sup> and none of the subjects were obese. Of the subjects, 10.1% had experienced stroke or other cerebrovascular disease and 22.7% were smokers.

Hypertension, diabetes and hyperlipidemia were present in 50.7%, 27.3% and 50.0% of the subjects, respectively.

#### White matter lesions

Periventricular hyperintensities and DWMH were observed in 77.7% and 96.7% of the total subjects, respectively. The mean score of PVH and DWMH was  $5.5 \pm 4.8$  and  $35.5 \pm 39.8$ , respectively (Table 1). Pearson's correlation analysis showed a strong positive correlation between PVH score and DWMH score ( $r = 0.56$ ,  $P < 0.0001$ ). In relation to aging, a positive correlation was found between PVH score and age ( $r = 0.34$ ,  $P < 0.0001$ ), and between DWMH score and age ( $r = 0.28$ ,  $P < 0.0001$ ).

#### Cognitive and psychological assessment

The mean score of MMSE, GDS-15 and vitality index was  $23.1 \pm 5.3$ ,  $5.0 \pm 3.5$  and  $9.4 \pm 1.2$  points, respectively, indicating that the subjects showed cognitive decline, depression and decreased vitality, all to a mild extent. Given that a score of 23 or below on MMSE is regarded as the presence of cognitive impairment,<sup>19</sup> 47.5% of the subjects fell into this category. The causes of cognitive impairment were Alzheimer disease (AD; 53.3%), vascular dementia (VaD; 16.4%), combined dementia of AD and VaD (9.0%) and other types of dementia (21.3%). Pearson's correlation analysis revealed a negative correlation between PVH score and MMSE, PVH score and vitality index, DWMH score and MMSE, and DWMH score and vitality index,



**Table 1** Clinical characteristics of study subjects

	Prevalence (n = 286)	Mean ± standard deviation
Clinical characteristics		
Age (years)		74.5 ± 7.8
Women (%)	74.0	
Height (m)		1.55 ± 0.08
Bodyweight (kg)		52.4 ± 10.6
Body mass index (kg/m <sup>2</sup> )		21.8 ± 3.3
Systolic blood pressure (mmHg)		135.3 ± 20.2
Diastolic blood pressure (mmHg)		76.3 ± 11.8
Prevalence of complications		
Hypertension (%)	50.7	
Diabetes (%)	27.3	
Hyperlipidemia (%)	50.0	
Past history of cerebrovascular disease (%)	10.1	
Smoking (%)	22.7	
Cognitive and psychological assessment		
Mini-Mental State Examination (0–30 points)		23.1 ± 5.3
Geriatric depression scale (0–15 points)		5.0 ± 3.5
Vitality index (0–10 points)		9.4 ± 1.2
White matter lesions		
Periventricular hyperintensities (points)	5.5 ± 4.8	
Deep white matter hyperintensities (points)	35.5 ± 39.8	

**Table 2** Relationship between white matter lesions and global cognition (MMSE), depressive state (GDS-15) and vitality (vitality index)

	Linear regression	
	PVH score	DWMH score
MMSE	-0.380**	-0.272**
GDS-15	0.022	-0.066
Vitality index	-0.432**	-0.184*

Univariate linear regression analysis: \* $P < 0.01$ , \*\* $P < 0.0001$ . DWMH, deep white matter hyperintensity; GDS-15, 15-item Geriatric Depression Scale; MMSE, Mini-Mental State Examination; PVH, periventricular hyperintensity.

respectively (Table 2). It was also found that calculation (serial subtraction of 7 from 100) was negatively correlated with PVH score ( $r = -0.156$ ,  $P = 0.04$ , data not shown), and verbal fluency (naming as many vegetables as possible) was negatively correlated with PVH score ( $r = -0.216$ ,  $P < 0.01$ , data not shown). On the other hand, no significant correlation was found between PVH score and GDS-15, or between DWMH score and

GDS-15. Multiple logistic analysis revealed that PVH score and DWMH score remained significant determinants of cognitive impairment (MMSE,  $\leq 23$ ) and low vitality (vitality index,  $\leq 9$ ) after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 3).

One hundred and ninety subjects reported symptoms of geriatric syndrome. The frequency is shown in Table 4. Frequent symptoms ( $>20\%$ ) were tripping (32.1%), constipation (26.3%), gait disturbance (23.2%) and pollakiuria (22.1%). Student's *t*-test showed that PVH score was significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, weight loss, apathy, swallowing difficulty, tremor and muscle stiffness. Multiple logistic analysis revealed that PVH score remained a significant determinant of hallucinations, tripping, pollakiuria, urinary incontinence, weight loss, apathy and swallowing difficulty after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 5). By the same method, DWMH score was

**Table 3** Periventricular hyperintensity and deep white matter hyperintensity scores as determinants of cognitive impairment and low vitality

	PVH score		P-value	DWMH score		P-value
	OR	95% CI		OR	95% CI	
Cognitive impairment	1.185	1.084–1.295	<0.001	1.010	1.001–1.021	<0.05
Low vitality	1.260	1.133–1.401	<0.0001	1.025	1.012–1.039	<0.001

Cognitive impairment and low vitality were defined as MMSE  $\leq 23$  and vitality index  $\leq 9$ , respectively. Multiple logistic analysis was performed after adjustment for age, sex, hypertension, diabetes, hyperlipidemia, and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; DWMH, deep white matter hyperintensity; OR, odds ratio; PVH, periventricular hyperintensity.

significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence and constipation. Multiple logistic analysis revealed that DWMH score remained a significant determinant of hallucinations, delusions, tripping, urinary incontinence and constipation after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 6).

## Discussion

Elderly persons are affected by multiple chronic diseases. Once they are affected by serious illness, full recovery cannot be expected with medical treatment, because elderly patients are often trapped in a vicious circle of illness and poor quality of life (QOL). This is the reason why care and welfare contribute to the total well-being of the elderly. Physicians need to pay great attention to improving QOL as well as treating illness. Thus, it is important to comprehend the whole picture of their life by means of comprehensive geriatric assessment, which evaluates multiple aspects of an elderly person's life, such as activities of daily living, cognition, mood, vitality, communication and social environment.

The present study confirmed a negative correlation between the severity of WML and MMSE score. Multivariate analysis showed that the presence of WML was a significant risk factor for cognitive impairment, even after adjustment for confounding factors of age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease. The mechanism and the size and location of WML that impair cognitive function are not yet clear. However, from previous studies, it seems convincing that a reduction of blood flow in the frontal lobe plays an important role in cognitive impairment in elderly people who exhibit WML.<sup>20,21</sup> Clinical manifestations of WML include attention deficit and a decline in information-processing ability.<sup>4,13,22</sup> Junque *et al.* reported the reappearance of primitive reflexes, one of the symptoms of frontal lobe dysfunction, in patients with WML.<sup>11</sup> In this study, patients with PVH showed

attention deficit (incapability of calculation) and verbal inarticulacy (naming less vegetables), implying the impairment of frontal lobe function. WML, as reported previously,<sup>6,23</sup> were negatively correlated with vitality. Multiple logistic regression analysis, using potential risk factors including advanced age as confounding variables, found that the presence of WML was an independent risk factor for low vitality. Additionally, a relation between PVH score and apathy, a significant symptom of geriatric syndrome, was also found. From previous studies showing the importance of frontal lobe function in vitality,<sup>24–26</sup> we assume that blood flow reduction in the frontal lobe may account for the apathy and low vitality in patients with WML. More precisely, WML disrupting the frontal-subcortical circuit may result in dysfunction in the anterior cingulate and dorsolateral prefrontal circuits, thereby leading to apathy and decreased vitality.<sup>5,6,20</sup> Increase in PVH score or DWMH score was not apparently correlated with depression, probably because depression is associated with many factors such as aging, female sex, hyperlipidemia and medication.<sup>27–29</sup> The subjects in this study were mostly elderly (88.1%) and female (74.0%). We assume that these confounding conditions made it difficult to prove a true relation between WML and depression. From analysis of the association of WML with geriatric syndrome, it appears that WML have a relation to psychiatric symptoms (hallucinations and delusions), gait abnormalities (gait disturbance, tripping and falls), urinary symptoms (pollakiuria and urinary incontinence) and possibly with parkinsonism (swallowing difficulty, tremor and muscle stiffness). It was reported that WML were related to gait abnormalities,<sup>5–7</sup> presumably caused by disruption of the frontal-subcortical circuit.<sup>30</sup> Some other studies suggested that parkinsonism is also a contributing factor to gait disturbance in patients with WML.<sup>6,31</sup> Interestingly, we found that both gait abnormalities and symptoms of parkinsonism were associated with WML.

The present study confirmed an association between WML and voiding dysfunction (pollakiuria and incontinence). It was reported that urinary dysfunction was derived from damage to the frontal-subcortical

**Table 4** Comparison of periventricular hyperintensity and deep white matter hyperintensity scores between subjects who did or did not exhibit each symptom of geriatric syndrome

Geriatric syndrome	Prevalence (%)	PVH score		P-value	DWMH score		P-value
		Symptom Present	Absent		Symptom Present	Absent	
Hallucination	6.8	8.5 ± 5.9	4.4 ± 4.7	<0.01	59.8 ± 43.9	28.6 ± 35.4	<0.01
Delusion	9.5	7.6 ± 5.2	4.4 ± 4.8	0.01	56.1 ± 37.6	28.2 ± 35.9	<0.01
Insomnia	18.9	4.2 ± 3.6	4.7 ± 4.9	0.56	31.4 ± 36.0	31.3 ± 37.6	0.98
Vertigo	18.9	6.1 ± 6.5	4.4 ± 4.4	0.06	33.4 ± 38.1	30.7 ± 37.0	0.70
Paralysis	2.1	8.5 ± 4.8	4.6 ± 4.9	0.12	59.5 ± 47.2	30.1 ± 36.3	0.11
Numbness	16.6	5.1 ± 4.6	4.6 ± 4.8	0.62	34.6 ± 40.0	29.9 ± 36.0	0.52
Gait disturbance	23.2	6.7 ± 5.1	4.2 ± 4.7	<0.01	43.3 ± 41.7	27.5 ± 34.9	0.01
Tripping	32.1	6.4 ± 4.5	3.9 ± 4.9	<0.01	42.1 ± 43.7	25.9 ± 32.4	<0.01
Falls	17.9	6.6 ± 4.9	4.3 ± 4.8	0.01	45.8 ± 43.1	28.0 ± 35.0	0.01
Pollakiuria	22.1	8.0 ± 5.8	3.8 ± 4.2	<0.01	41.5 ± 41.0	41.5 ± 41.0	0.04
Urinary incontinence	13.8	7.5 ± 5.1	4.3 ± 4.8	<0.01	52.4 ± 44.9	52.4 ± 44.9	<0.01
Constipation	26.3	5.8 ± 4.3	4.4 ± 5.1	0.08	44.5 ± 45.1	44.5 ± 45.1	<0.01
Decreased appetite	14.7	6.1 ± 4.4	4.5 ± 5.0	0.12	42.1 ± 42.6	42.1 ± 42.6	0.11
Weight loss	14.2	6.9 ± 4.1	4.4 ± 5.0	0.01	40.7 ± 41.3	40.7 ± 41.3	0.15
Apathy	7.6	7.4 ± 3.6	4.4 ± 5.0	0.03	30.7 ± 28.1	30.7 ± 28.1	0.97
Speech impairment	2.7	5.6 ± 5.2	4.5 ± 4.7	0.62	35.3 ± 48.0	35.3 ± 48.0	0.80
Swallowing difficulty	14.7	12.2 ± 4.4	4.5 ± 4.8	<0.01	44.6 ± 34.6	44.6 ± 34.6	0.40
Tremor	5.3	9.1 ± 6.5	4.4 ± 4.7	<0.01	45.0 ± 38.1	45.0 ± 38.1	0.24
Muscle stiffness	3.2	9.2 ± 4.8	4.5 ± 4.9	0.02	48.7 ± 43.4	48.7 ± 43.4	0.23

PVH and DWMH score are shown as mean ± SD. Boldface values are statistically significant ( $P < 0.05$  by Student's *t*-test). DWMH, deep white matter hyperintensity; PVH, periventricular hyperintensity.

**Table 5** Periventricular hyperintensity score as determinant of geriatric syndrome

	OR	P-value	95% CI
Hallucination	1.12	0.043	1.004–1.248
Tripping	1.11	0.005	1.032–1.194
Pollakiuria	1.17	0.001	1.067–1.278
Urinary incontinence	1.11	0.022	1.015–1.207
Weight loss	1.14	0.007	1.036–1.246
Apathy	1.14	0.027	1.015–1.276
Swallowing difficulty	1.35	0.019	1.050–1.741

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

circuit.<sup>5,20</sup> In relation to the symptoms of parkinsonism (swallowing difficulty, tremor and muscle stiffness), this association was previously explained by dysfunction of the frontal-subcortical circuit.<sup>6,31</sup> The importance of this lesion was also suggested by a study showing that swallowing difficulty occurs with dysfunction of inter-nuclear neurons that link the brainstem to the cerebral cortex.<sup>32</sup>

**Table 6** Deep white matter hyperintensity score as determinant of geriatric syndrome

	OR	P-value	95% CI
Hallucination	1.017	0.020	1.003–1.032
Delusion	1.016	0.024	1.002–1.030
Tripping	1.011	0.020	1.002–1.020
Urinary incontinence	1.016	0.008	1.004–1.028
Constipation	1.011	0.025	1.001–1.021

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

Considering the cause of manifestation of geriatric syndrome in patients with WML, it appears that damage to associative pathways in the frontal and subcortical regions due to ischemic hypoperfusion is an important mechanism.<sup>5,20,21</sup> It is necessary to localize the responsible connecting pathway for each symptom by a sophisticated approach in the future.

In conclusion, we showed that WML were associated with cognitive impairment, low vitality and geriatric syndrome of psychological disorders, gait disturbance,

urinary problems and parkinsonism. Evaluating WML in relation to geriatric syndrome and building a preventive measure against WML is an important future task for maintaining the independence of elderly people.

## Acknowledgments

This study was supported by a Longevity Science Research Grant from the Ministry of Health, Labor and Welfare of Japan (H15-choju-013) and by Mitsui Sumitomo Insurance Welfare Foundation (2004, 2006), and by the Japan Health Foundation. We thank Yukiko Yamada and Ayako Machida for their technical assistance.

## References

- Breteler MM, van Swieten JC, Bots ML et al. Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. *Neurology* 1994; **44**: 1246-1252.
- Hachinski VC, Potter P, Merskey H. Leuko-araiosis. *Arch Neurol* 1987; **44**: 21-23.
- Hunt AL, Orrison WW, Yeo RA et al. Clinical significance of MRI white matter lesions in the elderly. *Neurology* 1989; **39**: 1470-1474.
- de Groot JC, de Leeuw FE, Oudkerk M et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann Neurol* 2000; **47**: 145-151.
- Kuo HK, Lipsitz LA. Cerebral white matter changes and geriatric syndromes: is there a link? *J Gerontol A Biol Sci Med Sci* 2004; **59**: 818-826.
- Starkstein SE, Sabe L, Vazquez S et al. Neuropsychological, psychiatric, and cerebral perfusion correlates of leuko-araiosis in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 1997; **63**: 66-73.
- Baloh RW, Ying SH, Jacobson KM. A longitudinal study of gait and balance dysfunction in normal older people. *Arch Neurol* 2003; **60**: 835-839.
- Sakakibara R, Hattori T, Uchiyama T, Yamanishi T. Urinary function in elderly people with and without leuko-araiosis: relation to cognitive and gait function. *J Neurol Neurosurg Psychiatry* 1999; **67**: 658-660.
- Tarvonen-Schroder S, Roytta M, Raiha I, Kurki T, Rajala T, Sourander L. Clinical features of leuko-araiosis. *J Neurol Neurosurg Psychiatry* 1996; **60**: 431-436.
- Pantoni L, Garcia JH. The significance of cerebral white matter abnormalities 100 years after Binswanger's report. *Stroke* 1995; **26**: 1293-1301.
- Junque C, Pujol J, Vendrell P et al. Leuko-araiosis on magnetic resonance imaging and speed of mental processing. *Arch Neurol* 1990; **47**: 151-156.
- Fazekas F. Magnetic resonance signal abnormalities in asymptomatic individuals: their incidence and functional correlates. *Eur Neurol* 1989; **29**: 164-168.
- Ylikoski R, Ylikoski A, Erkinjuntti T, Sulkava R, Raininko R, Tilvis R. White matter changes in healthy elderly persons correlate with attention and speed of mental processing. *Arch Neurol* 1993; **50**: 818-824.
- Fu JH, Lu CZ, Hong Z, Dong Q, Luo Y, Wong KS. Extent of white matter lesions is related to acute subcortical infarcts and predicts further stroke risk in patients with first ever ischaemic stroke. *J Neurol Neurosurg Psychiatry* 2005; **76**: 793-796.
- Taylor WD, MacFall JR, Provenzale JM et al. Serial MR imaging of volumes of hyperintense white matter lesions in elderly patients: correlation with vascular risk factors. *Am J Roentgenol* 2003; **181**: 571-576.
- Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State": A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189-198.
- Sheikh JJ, Yesavage JA. Geriatric Depression Scale (GDS): recent evidence and development of a short version. *Clin Gerontol* 1986; **56**: 165-173.
- Toba K, Nakai R, Akishita M et al. Vitality index as a useful tool to assess elderly with dementia. *Geriatr Gerontol Int* 2002; **2**: 23-29.
- Cullen B, Fahy S, Cunningham CJ et al. Screening for dementia in an Irish community sample using MMSE: a comparison of norm-adjusted versus fixed cut-points. *Int J Geriatr Psychiatry* 2005; **20**: 371-376.
- Pugh KG, Lipsitz LA. The microvascular frontal-subcortical syndrome of aging. *Neurobiol Aging* 2002; **23**: 421-431.
- Yao H, Sadoshima S, Kuwabara Y, Ichiya Y, Fujishima M. Cerebral blood flow and oxygen metabolism in patients with vascular dementia of the Binswanger type. *Stroke* 1990; **21**: 1694-1699.
- Burton EJ, Kenny RA, O'Brien J et al. White matter hyperintensities are associated with impairment of memory, attention, and global cognitive performance in older stroke patients. *Stroke* 2004; **35**: 1270-1275.
- Thomas P, Hazif-Thomas C, Saccardy F, Vandermarq P. Loss of motivation and frontal dysfunction. Role of the white matter change. *Encephale* 2004; **30**: 52-59.
- Okada K, Kobayashi S, Yamagata S, Takahashi K, Yamaguchi S. Poststroke apathy and regional cerebral blood flow. *Stroke* 1997; **28**: 2437-2441.
- Craig AH, Cummings JL, Fairbanks L et al. Cerebral blood flow correlates of apathy in Alzheimer disease. *Arch Neurol* 1996; **53**: 1116-1120.
- Benoit M, Koulibaly PM, Migneco O, Darcourt J, Pringuey DJ, Robert PH. Brain perfusion in Alzheimer's disease with and without apathy: a SPECT study with statistical parametric mapping analysis. *Psychiatry Res* 2002; **15**: 103-111.
- Stordal E, Mykletun A, Dahl AA. The association between age and depression in the general population: a multivariate examination. *Acta Psychiatr Scand* 2003; **107**: 132-141.
- Terao T, Iwata N, Kanazawa K et al. Low serum cholesterol levels and depressive state in human dock visitors. *Acta Psychiatr Scand* 2000; **101**: 231-234.
- Noble RE. Depression in women. *Metabolism* 2005; **54**: 49-52.
- Hennerici MG, Oster M, Cohen S, Schwartz A, Motsch L, Daffertshofer M. Are gait disturbances and white matter degeneration early indicators of vascular dementia? *Dementia* 1994; **5**: 197-202.
- Piccini P, Pavese N, Canapicchi R et al. White matter hyperintensities in Parkinson's disease. Clinical correlations. *Arch Neurol* 1995; **52**: 191-194.
- Daniels SK, Foundas AL. Lesion localization in acute stroke patients with risk of aspiration. *J Neuroimaging* 1999; **9**: 91-98.

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

## Stress-induced blood pressure elevation in subjects with mild cognitive impairment: Effects of the dual-type calcium channel blocker, cilnidipine

Yumiko Kawashima,<sup>1</sup> Masahiro Akishita,<sup>2</sup> Hiroshi Hasegawa,<sup>1</sup> Koichi Kozaki<sup>1</sup> and Kenji Toba<sup>1</sup>

<sup>1</sup>Department of Geriatric Medicine, Kyorin University School of Medicine, Mitaka, and <sup>2</sup>Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

**Aim:** We investigated whether mental stress-induced blood pressure elevation was related to cognitive function in the elderly, and further examined the effects of the dual-type calcium channel blocker, cilnidipine, on stress induced hypertension in subjects with mild cognitive impairment.

**Methods:** In study I, 39 consecutive outpatients (mean age  $\pm$  standard deviation,  $77 \pm 8$  years), who were referred to our memory clinic and were not taking any medications, were studied. They were divided into three groups according to cognitive function on the Hasegawa Dementia Scale-Revised (HDSR): group 1 ( $n = 8$ ), 28 points or more; group 2 ( $n = 18$ ), 21–27 points; and group 3 ( $n = 13$ ), 20 points or less. In study II, 14 outpatients with hypertension and mild cognitive impairment (aged  $79 \pm 8$  years; HDSR score,  $24 \pm 4$ ) were assigned to receive cilnidipine (10–20 mg/day). The control group ( $n = 10$ ) matched for age, HDSR and blood pressure was followed without cilnidipine.

**Results:** In study I, although age and basal blood pressure were similar among the three groups, the blood pressure response to a mental arithmetic test was twice as large in group 2 ( $26 \pm 12$  mmHg in systolic pressure and  $11 \pm 8$  mmHg in diastolic pressure) as those in groups 1 and 3. In study II, after 4 weeks, cilnidipine treatment significantly decreased the blood pressure responses to the mental arithmetic test compared to the baseline as well as to those of the control group.

**Conclusions:** Stress-induced blood pressure elevations are exaggerated in subjects with mild cognitive impairment. Cilnidipine may have inhibitory effects on stress-induced hypertension.

**Keywords:** calcium antagonists, dementia, hypertension, mental stress.

Accepted for publication 1 July 2008.

Correspondence: Dr Masahiro Akishita MD PhD, Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.  
Email: akishita-ky@umin.ac.jp

### Introduction

Mental stress-induced increases in blood pressure (BP) and heart rate are often experienced during daily living. Psychophysiological cardiovascular reactivity is caused by autonomic nervous system activation via the hypothalamus–pituitary–adrenal axis,<sup>1–3</sup> and is

modulated by individual characteristics, environmental exposures, interpersonal and social contexts and genetic factors.<sup>6-7</sup> It has been reported that the subjects with exaggerated cardiovascular reactivity to mental stress are predisposed to hypertension,<sup>8,9</sup> atherosclerosis<sup>10-12</sup> and cerebrovascular disease such as stroke and silent cerebral infarction.<sup>13,14</sup>

Although the central nervous system plays an important role in mental stress-induced cardiovascular reactivity,<sup>1-3</sup> little is known about the relationship between the reactivity and cognitive function or dementia. Association of increased short-time BP variability with cognitive impairment<sup>15</sup> suggests but does not directly demonstrate the link between BP responses to mental stress and cognitive function. Because the patients with cognitive impairment cannot easily perform cognitive tasks, we hypothesized that exaggerated mental stress responses would result in BP elevation in such patients.

To test this hypothesis, we conducted a cross-sectional study examining the relationship between cognitive function and BP responses to a mental arithmetic test using the subjects who were referred to our memory clinic and not taking any medications. Furthermore, we examined the effects of cilnidipine, an N- and L-type calcium channel blocker, on BP responses to a mental arithmetic test in hypertensive patients with mild cognitive impairment.

## Methods

### Subjects

The subjects who were referred to our memory clinic and were suspected to have hypertension on the first visit were enrolled. Depressive patients (15-item Geriatric Depression Scale score of  $\geq 10$  points) and post-stroke patients were excluded from the study. Each subject gave written informed consent before enrollment in this study. The study protocol was approved by the ethics committee of Kyorin University School of Medicine.

In study I, 39 consecutive patients (20 men and 19 women, aged  $77 \pm 8$  years), who showed high-normal or higher BP ( $>130$  mmHg in systolic or  $>85$  mmHg in diastolic) and were not taking any medications, were enrolled. They underwent mental stress tests and were divided into three groups according to cognitive function on the Hasegawa Dementia Scale-Revised (HDSR): group 1 ( $n = 8$ ), 28 points or more; group 2 ( $n = 18$ ), 21–27 points; and group 3 ( $n = 13$ ), 20 points or less. All the patients in group 3 and five patients in group 2 were clinically diagnosed to have Alzheimer's disease, but none were so in group 1.

In study II, 14 patients with hypertension ( $>140$  mmHg in systolic or 90 mmHg in diastolic BP,

or taking antihypertensive agents) and mild cognitive impairment (aged  $79 \pm 8$  years; HDSR score,  $24 \pm 4$  points; HDSR range, 21–27) were assigned to receive cilnidipine. Nine patients of group 2 in study I, who showed more than 140 mmHg in systolic or 90 mmHg in diastolic BP, were included in study II. Fifteen treated ( $n = 11$ ) or untreated ( $n = 4$ ) hypertensive patients were additionally included in study II. The dose of cilnidipine was initiated at 10 mg/day, and was increased to 20 mg/day if systolic BP was more than 150 mmHg or diastolic BP was more than 90 mmHg 2 weeks later. The patients were followed for an additional 2 weeks. Separately, the control group ( $n = 10$ ) matched for age, HDSR and baseline BP were followed for 4 weeks. Mental stress tests were performed before and after the 4-week study period. Any medications except for cilnidipine were not changed throughout the study period.

### Mental arithmetic test

After resting for 5 min in a quiet room, baseline BP and pulse rate (PR) were measured using an automated, digital electro sphygmomanometer (HEM-7271C; Omron Healthcare, Kyoto, Japan) on the non-dominant arm in the sitting position. Then, each subject was instructed to continuously subtract 7 from 213 as accurately as possible. BP and PR were measured again after 1 min of subtraction to evaluate the response to mental arithmetic. Measurements of BP and PR were repeated twice at each step, and the average values were used in the analyses. This test was modified for patients with cognitive impairment from the original version.<sup>16</sup> The correlation coefficients between the two repeated measurements of a 4-week interval were 0.971 for systolic BP and 0.850 for diastolic BP ( $n = 15$ ,  $P < 0.01$ ) after mental arithmetic.

### Data analysis

The values are expressed as mean  $\pm$  standard deviation in the text, tables and figures unless otherwise specified. Differences between the groups were analyzed using one-factor ANOVA, followed by a Newman-Keuls test. Changes in BP and PR during the study period were analyzed using a paired Student's *t*-test.  $P < 0.05$  was considered statistically significant.

## Results

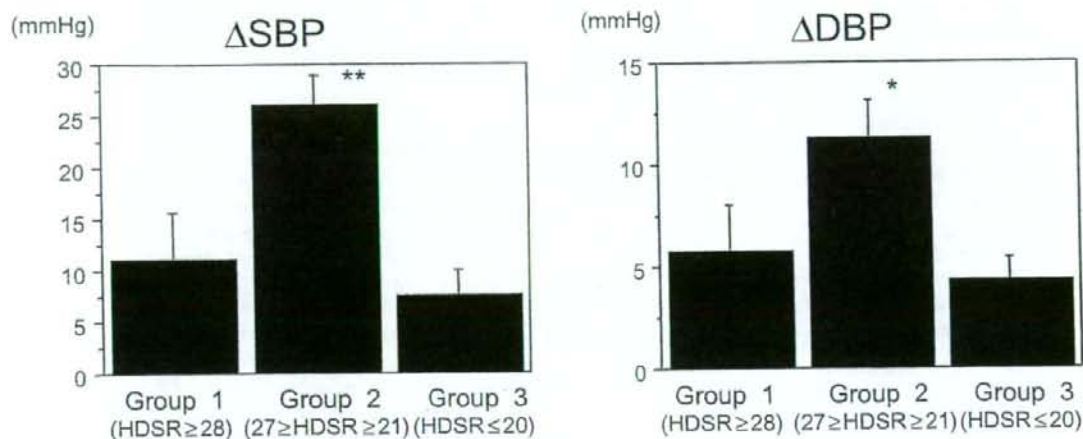
### Study I: Stress-induced BP elevation in the subjects as categorized by cognitive function

The characteristics of the subjects in the three groups are shown in Table 1. There were no significant differences

**Table 1** Characteristics of subjects in study I

	Group 1 (HDSR, ≥28 points)	Group 2 (HDSR, 21–27 points)	Group 3 (HDSR, ≤20 points)
No. of subjects (men/women)	8 (4/4)	18 (10/8)	13 (6/7)
HDSR, points	29.0 ± 1.0	24.6 ± 1.9 <sup>§</sup>	14.7 ± 3.8 <sup>†</sup>
Age, years	75 ± 8	78 ± 7	78 ± 7
SBP, mmHg	150 ± 14	148 ± 20	145 ± 20
DBP, mmHg	84 ± 12	77 ± 10	74 ± 11
Pulse rate (b.p.m.)	71 ± 11	73 ± 11	73 ± 11

Values are expressed as mean ± standard deviation. <sup>§</sup>*P* < 0.01 vs group 1; <sup>†</sup>*P* < 0.01 vs group 2. All other variables are not significantly different among the groups. DBP, diastolic blood pressure; HDSR, Hasegawa Dementia Scale-Revised; SBP, systolic blood pressure.



**Figure 1** Influence of cognitive function on stress-induced blood pressure elevation. The changes of systolic (SBP) and diastolic blood pressure (DBP) during the mental arithmetic test in study I are shown. Values are expressed as mean ± standard error of the mean. Group 1, Hasegawa Dementia Scale-Revised (HDSR) score of ≥28 points; group 2, 21–27 points; and group 3, ≤20 points. \**P* < 0.05, \*\**P* < 0.01 vs groups 1 and 3.

in sex, age and baseline BP between the groups. As shown in Figure 1, the responses of both systolic and diastolic BP to the mental arithmetic test were twice as large in group 2 as those in groups 1 and 3.

#### Study II: Effects of cilnidipine on stress-induced BP elevation

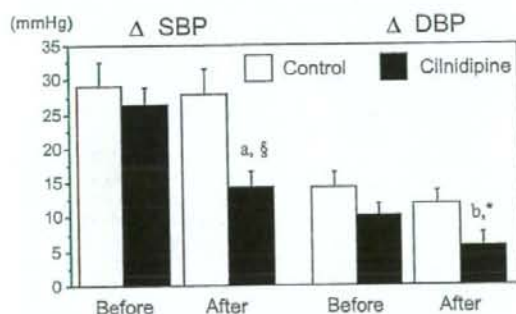
All the subjects completed the protocol of study II. The characteristics of the subjects are shown in Table 2. The average dose of cilnidipine used in the cilnidipine group was 13 ± 5 mg/day. There were no significant differences in cognitive function, age and resting BP between the control group and the cilnidipine group, although resting systolic and diastolic BP fell significantly by the treatment with cilnidipine for 4 weeks. Figure 2 shows

the BP responses to the mental arithmetic test. In the control group, BP responses did not change during the study period. In the cilnidipine group, however, BP responses to the mental arithmetic test were significantly decreased after 4 weeks. As a result, there were significant differences in the responses of systolic and diastolic BP to the mental arithmetic test between the control group and the cilnidipine group at the end of the study. We attempted to calibrate the reactivity by baseline BP. The percent changes of systolic BP during the mental arithmetic test were significantly smaller in the cilnidipine group than in the control group after treatment (12 ± 5% cilnidipine vs 18 ± 6% control, *P* < 0.05), although they were comparable in both groups before treatment (18 ± 6% cilnidipine vs 20 ± 9% control).

**Table 2** Characteristics of subjects in study II

	Control	Cilnidipine
No. of subjects (men/women)	10 (5/5)	14 (6/8)
HDSR, points	26.0 ± 3.0	25.0 ± 4.0
Age, years	81 ± 8	79 ± 8
Pretreatment drugs		
ACEI/ARB, n (%)	3 (30%)	3 (21%)
CCB, n (%)	3 (30%)	3 (21%)
Before treatment		
SBP, mmHg	153 ± 17	161 ± 20
DBP, mmHg	80 ± 7	85 ± 11
Pulse rate, b.p.m.	75 ± 9	74 ± 13
4 weeks after treatment		
SBP, mmHg	151 ± 18	144 ± 16 <sup>†</sup>
DBP, mmHg	79 ± 7	76 ± 9 <sup>†</sup>
Pulse rate, bpm	75 ± 8	77 ± 13

Values are expressed as mean ± standard deviation. <sup>†</sup> $P < 0.01$  vs baseline. No significant differences were found between the control and cilnidipine groups. ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; CCB, L-type calcium channel blocker; DBP, diastolic blood pressure; HDSR, Hasegawa Dementia Scale-Revised; SBP, systolic blood pressure.



**Figure 2** Effects of cilnidipine on stress-induced blood pressure elevation in elderly hypertensives with mild cognitive impairment. The changes of systolic (SBP) and diastolic blood pressure (DBP) during the mental arithmetic test before and after the treatment in study II are shown. Values are expressed as mean ± standard error of the mean. <sup>†</sup> $P < 0.01$ , <sup>b</sup> $P < 0.05$  vs before treatment. <sup>§</sup> $P < 0.01$ , <sup>\*</sup> $P < 0.05$  vs control.

## Discussion

In study I, we investigated whether mental stress-induced BP elevation was related to cognitive function in the elderly who were referred to our memory clinic. A few studies have shown the relationship between mental stress-induced BP responses and cognitive function.

Pierce *et al.*<sup>17</sup> have reported that BP responses during neuropsychological testing were unrelated to cognitive performance in college-aged subjects. Alternatively, Waldstein *et al.*<sup>18</sup> have reported that higher stress-induced BP reactivity is associated with poorer performance on tests of cognitive function in stroke- and dementia-free middle-aged and older adults (ages 54–79 years). In the present study, using older subjects with or without cognitive impairment, we found that the relation between mental stress-induced BP elevation and cognitive function was inverted U-shaped, and that stress-induced BP elevations were exaggerated in subjects with mild cognitive impairment.

Subjects with mild cognitive impairment can recognize their cognitive decline,<sup>19</sup> thus anxiety and irritation during the mental arithmetic test may arouse the accelerated BP response. By contrast, the mental arithmetic test is not likely to impose a heavy burden on demented subjects, because they display deficits in executive function,<sup>20</sup> often associated with depression and apathy.<sup>21</sup> Recently, greater variability in BP on 24-h ambulatory monitoring has been associated with poorer cognitive performance or cognitive impairment in samples of older adults.<sup>15,22</sup> Murakami *et al.*<sup>23</sup> investigated the relation between pressor responses to mental arithmetic tests and 24-h BP variability in normotensive subjects and hypertensive patients. They reported that the pressor response during the mental arithmetic test was significantly correlated with the value of 24-h BP variability in both subjects. Taken together, it is possible that mental stress-induced BP elevation in daily life is a strong determinant of BP variability in subjects with mild cognitive impairment.

In study II, we found that cilnidipine had inhibitory effects on stress-induced BP elevation in subjects with hypertension and mild cognitive impairment. Cilnidipine is a 1,4-dihydropyridine derivative calcium antagonist with potent inhibitory actions against not only L-type but also N-type voltage-dependent calcium channels.<sup>24</sup> Fujita *et al.*<sup>25</sup> have recently reported that cilnidipine is superior to amlodipine in preventing the progression of proteinuria in hypertensive patients with chronic kidney disease. The N-type voltage-dependent calcium channel plays an important role in sympathetic neurotransmission and regulates the release of norepinephrine from sympathetic nerve endings.<sup>26</sup> Accordingly, several studies have reported the inhibitory effects of cilnidipine on ambulatory BP and "white coat effect" in patients with essential hypertension.<sup>27–30</sup> The present results found in elderly hypertensives with mild cognitive impairment are consistent with these studies, and may provide a therapeutic implication in elderly hypertension.

Controversy exists as to prognostic significance of stress-induced BP elevation, typically known as the white coat effect, clinic-ambulatory BP difference.<sup>31</sup>



Verdecchia *et al.*<sup>32</sup> reported that white coat effect does not predict cardiovascular morbidity and mortality in subjects with essential hypertension. Also, Khattar *et al.*<sup>33</sup> reported that white coat hypertensives had a significantly lower incidence of cardiovascular events than sustained hypertensives. In contrast, Mulè *et al.*<sup>34</sup> reported that left ventricular mass was greater in the group with higher systolic and diastolic white coat effect, suggesting an end-organ damage in white coat hypertension. Amado *et al.*<sup>35</sup> reported that elderly hypertensives with white coat effect have more previous history of ischemic cardiovascular or cerebrovascular disease than those with no white coat effect. Other numerous studies also found a considerable association between the white coat effect<sup>36,37</sup> or the exaggerated cardiovascular reactivity to mental stress<sup>38-44</sup> and cardiovascular mortality or target organ damage. Recent studies suggest that hypertension is associated with the onset and exacerbation of Alzheimer's disease.<sup>38-42</sup> Thus, the control of stress-induced hypertension may be a therapeutic target in the management of elderly hypertensives.

This study has some limitations. First, the number of subjects examined was small, and, therefore, the results of the present study should be carefully interpreted and be confirmed in large-scale studies. Second, we studied only the response to a mental stress. BP is influenced by all kinds of stress associated with daily activities, such as mental stress, static exercise, dynamic exercise and temperature variation.<sup>23</sup> We also assessed the responses to hand grip exercise and found similar results to the mental arithmetic test, but did not show the data because of low reproducibility of the responses to the modified and weakened hand grip load. Third, significant increases in PR in response to the mental arithmetic test were not observed in this study. It has been known, however, that the heart rate reactivity to mental stress was attenuated in older adults compared to younger adults and children.<sup>43-45</sup> This phenomenon is attributable, at least in part, to the age-related decline of  $\beta$ -adrenergic sensitivity.<sup>46</sup> Consequently, it is not surprising that PR reactivity was dissociated from BP reactivity in elderly subjects in the present study. Fourth, we only measured BP and PR as physiological responses to stress. There are many indicators of the quantity of stress received, such as BP, heart rate, sympathetic activity, and catecholamine and cortisol levels.<sup>47-49</sup> Particularly, many investigators evaluated plasma norepinephrine and epinephrine concentrations together with BP and heart rate.<sup>50,51</sup> The lack of PR responses to the mental arithmetic test in the present study may have been recompensed if we had measured plasma catecholamine.

In summary, stress-induced BP elevations were exaggerated in elderly subjects with mild cognitive impairment. Cilnidipine decreased the BP responses to the mental arithmetic test in elderly hypertensives with mild cognitive impairment. These findings may provide

insight into the mechanistic link between hypertension and cognitive impairment, and therapeutic implication on the management of elderly patients.

## References

- Pickering TG. The effects of environmental and lifestyle factors on blood pressure and the intermediary role of the sympathetic nervous system. *J Hum Hypertens* 1997; 11 (Suppl. 1): S9-S18.
- McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med* 1998; 338: 171-179.
- Rozanski A, Blumenthal JA, Kaplan J. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* 1999; 99: 2192-2217.
- Everson SA, McKey BS, Lovallo WR. The effect of trait hostility on cardiovascular responses to harassment in young men. *Int J Behav Med* 1995; 2: 172-181.
- Kamarck TW, Peterman AH, Raynor DA. The effects of the social environment on stress-related cardiovascular activation: current findings, prospects, and implications. *Ann Behav Med* 1998; 20: 247-256.
- Smith TW, Christensen AJ. Cardiovascular reactivity and interpersonal relations: psychosomatic processes in social context. *J Soc Clin Psychol* 1992; 11: 279-301.
- Steptoe A, Cropley M, Joeekes K. Job strain, blood pressure and response to uncontrollable stress. *J Hypertens* 1999; 17: 193-200.
- Markovitz JH, Raczynski JM, Wallace D, Chettur V, Chesney MA. Cardiovascular reactivity to video game predicts subsequent blood pressure increases in young men: the CARDIA study. *Psychosom Med* 1998; 60: 186-191.
- Carroll D, Smith GD, Sheffield D, Shipley MJ, Marmot MG. Pressor reactions to psychological stress and prediction of future blood pressure: data from the Whitehall II Study. *BMJ* 1995; 310: 771-776.
- Kamarck TW, Everson SA, Kaplan GA *et al.* Exaggerated blood pressure responses during mental stress are associated with enhanced carotid atherosclerosis in middle-aged Finnish men: findings from the Kuopio Ischemic Heart Disease Study. *Circulation* 1997; 96: 3842-3848.
- Barnett PA, Spence JD, Manuck SB, Jennings JR. Psychological stress and the progression of carotid artery disease. *Hypertension* 1997; 15: 49-55.
- Nakashima T, Yamano S, Sasaki R *et al.* White-coat hypertension contributes to the presence of carotid arteriosclerosis. *Hypertens Res* 2004; 27: 739-745.
- Everson SA, Lynch JW, Kaplan GA, Lakka TA, Sivenius J, Salonen J. Stress-induced blood pressure reactivity and incident stroke in middle-aged men. *Stroke* 2001; 32: 1263-1270.
- Waldstein SR, Siegel EL, Lefkowitz D *et al.* Stress-induced blood pressure reactivity and silent cerebrovascular disease. *Stroke* 2004; 35: 1294-1298.
- Kanemaru A, Kanemaru K, Kuwajima I. The effects of short-time blood pressure variability and nighttime blood pressure levels on cognitive function. *Hypertens Res* 2001; 24: 19-24.
- Muneta S, Dazai Y, Iwata T *et al.* Baroreceptor reflex impairment in climacteric and ovariectomized hypertensive women. *Hypertens Res* 1992; 15: 27-32.
- Pierce TW, Elias MF. Cognitive function and cardiovascular reactivity in subjects with a parental history of hypertension. *J Behav Med* 1993; 16: 277-294.

- 18 Waldstein SR, Katzel LI. Stress-induced blood pressure reactivity and cognitive function. *Neurology* 2005; **64**: 1746-1749.
- 19 Crowe M, Andel R, Wadley V *et al*. Subjective cognitive function and decline among older adults with psychometrically defined amnesic MCI. *Int J Geriatr Psychiatry* 2006; **21**: 1187-1192.
- 20 Levinoff EJ, Phillips NA, Verret L *et al*. Cognitive estimation impairment in Alzheimer disease and mild cognitive impairment. *Neuropsychology* 2006; **20**: 123-132.
- 21 Lyketsos CG, Lopez O, Jones B, Fitzpatrick AL, Breitner J, Dekosky S. Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. *JAMA* 2002; **288**: 1475-1483.
- 22 Bellelli G, Pezzini A, Bianchetti A, Trabucchi M. Increased blood pressure variability may be associated with cognitive decline in hypertensive elderly subjects with no dementia. *Arch Intern Med* 2002; **162**: 483-484.
- 23 Murakami E, Matsuzaki K, Sumimoto T, Mukai M, Kazatani Y, Kodama K. Clinical significance of pressor responses to laboratory stressor testing in hypertension. *Hypertens Res* 1996; **133**-137.
- 24 Fujii S, Kameyama K, Hosono M, Hayashi Y, Kitamura K. Effect of cilnidipine, a novel dihydropyridine Ca<sup>++</sup> channel antagonist, on N-type Ca<sup>++</sup> channel in rat dorsal root ganglion neurons. *J Pharmacol Exp Ther* 1997; **280**: 1184-1191.
- 25 Fujita T, Ando K, Nishimura H *et al*. Cilnidipine versus Amlodipine Randomised Trial for Evaluation in Renal Disease (CARTER) Study Investigators. Antiproteinuric effect of the calcium channel blocker cilnidipine added to renin-angiotensin inhibition in hypertensive patients with chronic renal disease. *Kidney Int* 2007; **72**: 1543-1549.
- 26 Hirning LD, Fox AP, McCleskey EW *et al*. Dominant role of N-type Ca<sup>2+</sup> channels in evoked release of norepinephrine from sympathetic neurons. *Science* 1988; **239**: 57-61.
- 27 Yamagishi T. Beneficial effect of cilnidipine on morning hypertension and white-coat effect in patients with essential hypertension. *Hypertens Res* 2006; **29**: 339-344.
- 28 Hoshida S, Kario K, Ishikawa J, Eguchi K, Shimada K. Comparison of the effects of cilnidipine and amlodipine on ambulatory blood pressure. *Hypertens Res* 2005; **28**: 1003-1008.
- 29 Morimoto S, Takeda K, Oguni A *et al*. Reduction of white coat effect by cilnidipine in essential hypertension. *Am J Hypertens* 2001; **10**: 1053-1057.
- 30 Mansour G, Mansour J. Cilnidipine in management of patients with uncontrolled hypertension and white-coat effect. *Curr Hypertens Rep* 2007; **9**: 489-490.
- 31 Lantelme P, Milon H, Gharib C, Gayet C, Fortrat JO. White coat effect and reactivity to stress: cardiovascular and autonomic nervous system responses. *Hypertension* 1998; **31**: 1021-1029.
- 32 Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Porcellati C. Prognostic significance of the white coat effect. *Hypertension* 1997; **29**: 1218-1224.
- 33 Khattar RS, Senior R, Lahiri A. Cardiovascular outcome in white-coat versus sustained mild hypertension: a 10-year follow-up study. *Circulation* 1998; **98**: 1892-1897.
- 34 Mulè G, Nardi E, Cottone S *et al*. Relationships between ambulatory white coat effect and left ventricular mass in arterial hypertension. *Am J Hypertens* 2003; **16**: 498-501.
- 35 Amado P, Vasconcelos N, Santos I, Almeida L, Nazaré J, Carmona J. Arterial hypertension difficult to control in the elderly patient. The significance of the "white coat effect". *Rev Port Cardiol* 1999; **18**: 897-906.
- 36 Munakata M, Saito Y, Nunokawa T, Ito N, Fukudo S, Yoshinaga K. Clinical significance of blood pressure response triggered by a doctor's visit in patients with essential hypertension. *Hypertens Res* 2002; **25**: 343-349.
- 37 Strandberg TE, Salomaa V. White coat effect, blood pressure and mortality in men: prospective cohort study. *Eur Heart J* 2000; **21**: 1714-1718.
- 38 Skoog I, Lernfelt B, Landahl S *et al*. 15-year longitudinal study of blood pressure and dementia. *Lancet* 1996; **347**: 1141-1145.
- 39 Wu C, Zhou D, Wen C, Zhang L, Como P, Qiao Y. Relationship between blood pressure and Alzheimer's disease in Linxian County, China. *Life Sci* 2003; **72**: 1125-1133.
- 40 Kivipelto M, Helkala EL, Laakso MP *et al*. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ* 2001; **322**: 1447-1451.
- 41 Sakurai T, Yokono K. Comprehensive studies of cognitive impairment of the elderly with type 2 diabetes. *Geriatr Gerontol Int* 2006; **6**: 159-164.
- 42 Kim KI, Cho YS, Choi DJ, Kim CH. Optimal treatment of hypertension in the elderly: a Korean perspective. *Geriatr Gerontol Int* 2008; **8**: 5-11.
- 43 Kudielka BM, Buske-Kirschbaum A, Hellhammer DH, Kirschbaum C. Differential heart rate reactivity and recovery after psychosocial stress (TSST) in healthy children, younger adults, and elderly adults: the impact of age and gender. *Int J Behav Med* 2004; **11**: 116-121.
- 44 Barnes RF, Raskind M, Gumbrecht G, Halter JB. The effects of age on the plasma catecholamine response to mental stress in man. *J Clin Endocrinol Metab* 1982; **54**: 64-69.
- 45 Steptoe A, Fieldman G, Evans O, Perry L. Cardiovascular risk and reactivity to mental stress: the influence of age, gender and risk factors. *J Cardiovasc Risk* 1996; **3**: 83-93.
- 46 Boutcher SH, Stocker D. Cardiovascular response of young and older males to mental challenge. *J Gerontol B Psychol Sci Soc Sci* 1996; **51**: 261-267.
- 47 Yoshiuchi K, Nomura S, Ando K *et al*. Hemodynamic and endocrine responsiveness to mental arithmetic task and mirror drawing test in patients with essential hypertension. *Am J Hypertens* 1997; **10**: 243-249.
- 48 Sawai A, Ohshige K, Yamasue K, Hayashi T, Tochikubo O. Influence of mental stress on cardiovascular function as evaluated by changes in energy expenditure. *Hypertens Res* 2007; **30**: 1019-1027.
- 49 Hiramatsu R. Direct assay of cortisol in human saliva by solid phase radioimmunoassay and its clinical applications. *Clin Chim Acta* 1981; **117**: 239-249.
- 50 Paran E, Neumann L, Cristal N. Effects of mental and physical stress on plasma catecholamine levels before and after beta-adrenoceptor blocker treatment. *Eur J Clin Pharmacol* 1992; **43**: 11-15.
- 51 Grossman E, Oren S, Garavaglia GE, Schmieder R, Messerli FH. Disparate hemodynamic and sympathoadrenergic responses to isometric and mental stress in essential hypertension. *Am J Cardiol* 1989; **64**: 42-44.

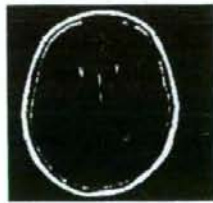
### Attenuation of Brain White Matter Hyperintensities after Cerebral Infarction

Brain white matter hyperintensities (WMHs) on MR imaging are reported to increase with age<sup>1</sup> and do not decrease,<sup>2</sup> though their pathogenesis is not well understood. Here, we report an interesting case in which a patient showed attenuation of WMHs at 1 year after the incidence of cerebral infarction.

A 78-year-old female outpatient with hypertension and dyslipidemia presented with sudden onset of left homonymous hemianopia. She showed no other neurologic abnormality. The next day, brain MR imaging scans showed an acute infarct in the right occipital lobe. The symptom persisted thereafter with a little amelioration. When follow-up MR imaging scans were performed 1 year later, it was found that the WMHs attenuated (Fig 1). Measurement of the WMH area with MetaMorph 6.2 (Universal Imaging, Downingtown, Pa) confirmed this finding (Fig 2). Because the temporal decrease in WMHs was consistent in 2 different regions of the brain (fluid-attenuated inversion recovery [FLAIR] images 1 and 2), we could rule out a "chance appearance" of the attenuation of WMHs because of the difference in the levels of the 2 temporal images. Because the area of WMHs was apart from the infarct lesion, the change of WMHs was not considered to be from the direct effect of infarction.

WMHs have not been reported to regress,<sup>3</sup> though their pathogenesis is not well understood. The proposed mechanisms of their appearance are ischemia; blood-brain barrier dysfunction; and endothelial injury, which are not mutually exclusive.<sup>3</sup> In this case, 1 possible explanation for the attenuation of WMHs could be that the blood-brain barrier was disturbed immediately after the onset of infarction, causing leakage of cerebral fluid into the white matter and this recovered afterward.

#### FLAIR1



Onset of infarction      One year follow-up

#### FLAIR2

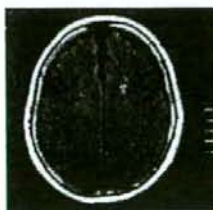
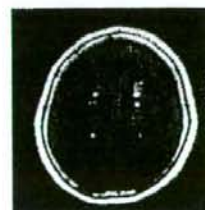


Fig 1. Attenuation of WMHs at 2 different levels (FLAIR 1 and FLAIR 2).

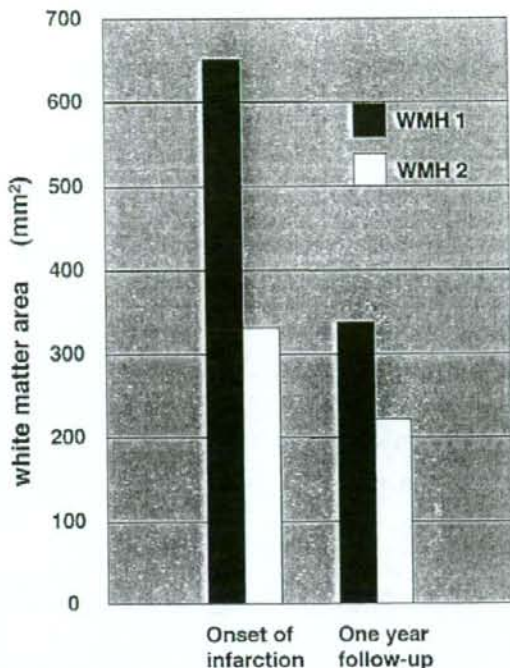


Fig 2. WMH area was measured with use of MetaMorph 6.2. WMH 1 refers to the area of WMHs at the FLAIR 1 level (Fig 2), and WMH 2 refers to the area of WMHs at the FLAIR 2 level.

#### Image Analysis

The WMH area was quantified by a technician (who was not a physician) in a blind manner with use of MetaMorph 6.2. On acquired FLAIR images, 1) the scale was set; 2) unnecessary white areas such as bone were removed; 3) the average number of pixels in "normal white matter" fields in each image were measured (79 and 82 pixels), and the highest number of pixels in the 2 temporal images was set as the threshold for the ensuing measurement of WMHs; and 4) all of the areas above the threshold were selected, and their size was measured.

#### Sources of Funding

This study was supported by the Mitsui Sumitomo Insurance Welfare Foundation (2006) and the Japan Health Foundation.

#### References

- Sachdev P, Wen W, Chen X, et al. Progression of white matter hyperintensities in elderly individuals over 3 years. *Neurology* 2007;68:214-22
- Schmidt R, Fazekas F, Kapeller P, et al. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. *Neurology* 1999;53:132-39
- O'Sullivan M. Leukoaraiosis. *Pract Neurol* 2008;8:26-38

Y. Moriya  
K. Kozaki  
K. Nagai  
K. Toba

Department of Geriatric Medicine  
Kyorin University School of Medicine  
Tokyo, Japan

DOI 10.3174/ajnr.A1340

## 活力度指標の信頼性、妥当性および、活力度指標と加齢、運動との関連性に関する検討

神崎 恒一<sup>1)</sup> 村田 久<sup>2)</sup> 菊地 令子<sup>1)</sup> 杉山 陽一<sup>1)</sup>  
長谷川 浩<sup>3)</sup> 井形 昭弘<sup>3)</sup> 鳥羽 研二<sup>1)</sup>

**要約 目的：**地域高齢者のQOLに対する運動の効果を検討するため、我々は日常生活の活力度を評価する指標（活力度指標）を作成し、加齢及び運動との関連性を検討した。方法：Lawtonの階段型評価をもとに36項目からなるVS原案を作成し、全国の体操三井島システム会員4,701名と、東京都住民579名の計5,280名を対象に調査を行い、因子分析、信頼性の検討を行った。VSと加齢及び運動との関連性については各下位尺度と加齢、運動との相関係数及び活力度指標を従属変数とし加齢、運動を独立変数とする分散分析によって検討した。結果：活力度指標原案の質問36項目について因子分析を行った結果、因子数4、20項目を活力度指標（ASE）として設定した。5,280名の対象者のASEの平均値は27.18±5.28であり、男女に有意差はなかった。4因子構造を確認するための確認的因子分析では適合度指標において十分な値（GFI=0.91 AGFI=0.88 RMSEA=0.04）が得られ、因子の安定性が示された。また、各下位尺度における $\alpha$ 係数の算出では一定の内的一貫性が認められた。ASEと年齢、運動との関連性の検討では、ASE得点の差が運動群—非運動群間、年代間で有意であった（ $p<0.01$ ）。2要因分散分析の結果、運動と年齢で有意な交互作用（ $p<0.01$ ）がみられ、非運動群ではASE得点が60歳代26.3から70歳代23.9と下降したが、運動群では下降は見られなかった。また、50歳代から80歳代の各年代において、運動群の方が非運動群よりも有意（ $p<0.01$ ）にASE得点が高かった。結論：地域高齢者のQOL評価のために、下位尺度の内的一貫性と安定性をもつ活力度指標は有用であり、運動は加齢による活力度低下を防ぐ効果をもつことが期待される。

**Key words：**活力度、加齢、運動、QOL

（日老医誌 2008；45：188-195）

### 緒 言

運動は糖尿病、高血圧、脂質代謝異常、骨粗鬆症などの生活習慣病に対して治療効果をもたらす<sup>1)</sup>ほか、うつ、間歇性跛行、虚血性心疾患、多臓器疾患を有する患者のQOLに対して良い効果をもたらすことが報告されている。しかしながら、地域高齢者に対して運動がどのようにQOL（身体機能、気分・意欲、社会活動等）に影響を与えるかについての報告はほとんどない<sup>2)</sup>。そこで我々は、地域高齢者の日常生活の活力度を評価する指標（活力度指標）の作成を試み、その因子構造と安定性を検討した。原案となる活力度指標項目はLawtonの階

階の概念<sup>3)</sup>を拡張した36項目からなる活力度調査票原案である。本研究ではまず36項目の活力度調査票原案について因子構造の検討を行い、続いて作成された20項目よりなる活力度指標と加齢及び運動との関連を検討し、指標の実用性と妥当性について検証した。

### 方 法

#### 活力度指標原案

Lawtonの階段型評価は、社会的役割を最上位に据え、その下位に状況対応、手段的自立、身体的自立、知覚—認知、機能的健康度、生命維持が段階的に並べられており、各下位尺度の内部は上部に複雑な要素、下部に単純な要素が配置されている。活力度指標原案ではLawtonの階段型評価を参考に生命維持、認知、身体的・手段的自立、気分/意欲、社会参加の5つの領域（ドメイン）と、8つの下位尺度、36の質問項目（附表）を設定した。下位尺度は生命維持について健康に関する4項目健康活動（質問項目1、2）、健康感（質問項目3、4）と老年症候

1) K. Kozaki, R. Kikuchi, Y. Sugiyama, H. Hasegawa, K. Toba : 杏林大学医学部高齢医学

2) H. Murata : 東京大学総括プロジェクト機構ジェロントロジー寄附研究部門

3) A. Igata : 名古屋学芸大学

受付日：2007.6.25、採用日：2007.10.17