3.2. Glutamate increased production of NO and memantine decreased it

Glutamate leads to over-activation of NMDA receptors, and the excessive influx of Ca^{2+} into neuronal cells changes nNOS activity. Treatment with 10–40 mM glutamate for 10 h increased nNOS expression in a dose-dependent manner (Fig. 2A). Production of NO was also increased by treatment with 10 mM glutamate in the culture medium. However, pretreatment with 100 μM memantine, 100 μM MK801, or 100 μM N^{ω}-propyl-L-arginine, a potent selective inhibitor of nNOS, inhibited the production of NO (Fig. 2B, C, and D). To investigate whether memantine decreased the content of intracellular NO through nNOS, DAF-2 was used. Pretreatment with 100 or 200 μM memantine decreased the number of DAF-2-stained PC12 cells compared with glutamate-treated cells for 10 h (Fig. 3A and B). Furthermore, the number of SA-ßgal-positive PC12 cells was decreased by pretreatment with 100 μM N^{ω}-propyl-L-arginine (Fig. 1E). These results indicate that memantine inhibits the activation of nNOS and decreases NO induced by glutamate, leading to a reduction of senescence.

3.3. Memantine treatment improved cognitive decline in SAMP8

In order to assess the effects of memantine on cognitive function, we used an in vivo model of AD, SAMP8, and a control counterpart strain, SAMR1. SAMP8 was originally derived from the AKR/J strain, which is characterized by cognitive decline. SAMP8 showed a marked age-related deterioration of memory and learning as early as 2 months of age compared to SAMR1 (Yagi et al., 1988; Flood and Morley, 1998; Miyamoto et al., 1992). These mice exhibit age-related deficits in learning and memory at an early age, and are considered a suitable animal model to study the pathology of AD. By determining the time required to find the platform (escape latency) as a function of the number of days of training in the Morris water maze, we observed a marked decline in performance in SAMP8 (N = 5) compared with that in SAMR1 (N = 5) (Fig. 4A). SAMP8 treated with 20 mg/kg memantine for 3 weeks showed significantly reduced escape latency time compared with that in untreated SAMP8 on training day 10 (Fig. 4A). However, SAMP8 treated with 10 mg/kg MK801 did not show a change in escape latency time compared with untreated SAMP8 (Fig. 4A). The spatial probe trial helps to determine whether the mice would use a spatial learning strategy to locate the platform in the target quadrant. There was no difference in swim speed between the groups (Fig. 4B); the time was spent in the target quadrant where the platform was located was greatly reduced in SAMP8 compared to SAMR1 on day 10 (Fig. 4C). Memantine-treated SAMP8 showed a markedly longer time searching for the platform in the target quadrant (Fig. 4C). These results indicate that memantine, but not MK801, ameliorated spatial learning in SAMP8. The water maze is appropriate for hippocampus-dependent paradigms. However, memantine and MK801 administration may affect behavior and how animals respond to different stimuli. Therefore, we performed an open field test to examine locomotion, exploratory behavior, and anxiety. No significant effect of memantine (20 mg/kg) and MK801 (10 mg/kg) on locomotor performance was observed in SAMR1 and SAMP8, whereas SAMR1 showed significantly more movement compared with SAMP8 (Fig. 4D). The ratio of the distance traveled in the central area to that in the total area in the open field, an indirect measure of exploratory behavior and anxiety, was also determined. In SAMP8, memantine (20 mg/kg) and MK801 (10 mg/kg) increased this ratio (Fig. 4E), suggesting that both memantine and MK801 promoted exploratory behavior and diminished anxiety.

3.4. Memantine treatment inhibits senescence in hippocampus of SAMP8

Next, we assessed the number of SA-ßgal-positive cells in the CA1 and CA3 areas of the hippocampus in these mice. The number of SA-βgal-stained cells was significantly increased in SAMP8 compared with SAMR1, but treatment with memantine (10, 20 mg/kg) prevented this change in SAMP8 (Fig. 5A and B).

Glutamate stimulation increases the production of NO and O_2^- and has the capacity to generate $ONOO^-$ in the CNS (Eliasson et al., 1999). Therefore, we investigated the level of oxidative stress, using the SAMR1 and SAMP8 hippocampus at 12 weeks of age. SAMP8 hippocampus showed an increase in the level of oxidative stress compared with SAMR1 as judged by detection of carbonylated proteins. Memantine treatment (10 or 20 mg/kg) decreased carbonylated proteins in the

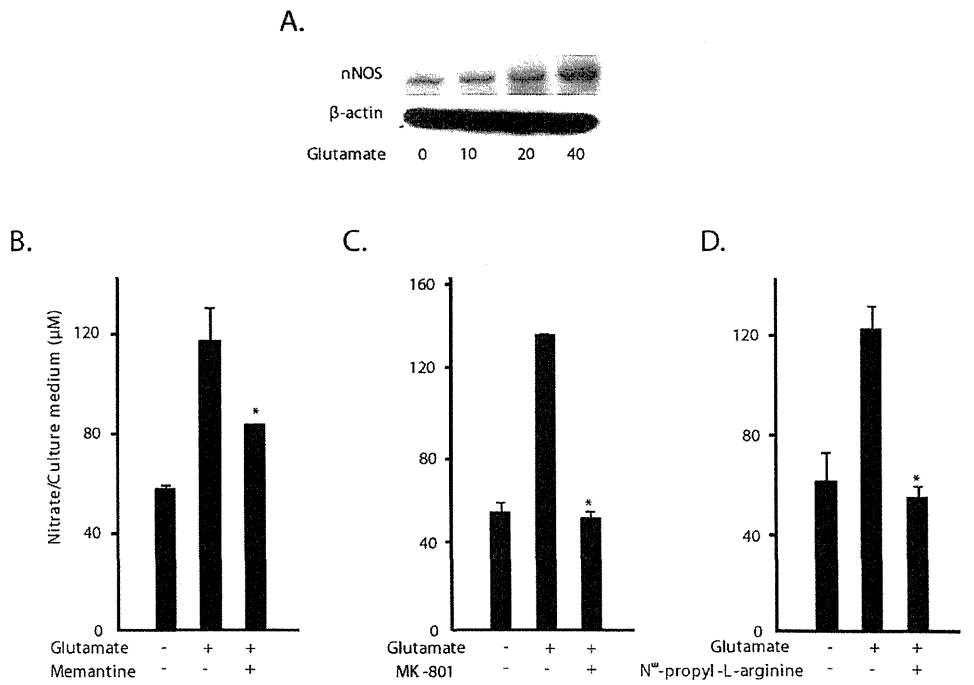

Fig. 2. A. Expression of nNOS in glutamate (10–40 mM)-treated PC12 cells. (N = 3, representative shown). Production of nitrate in PC12 cells treated with memantine (100 μM) (B), MK801 (100 μM) (C), or N^{ω}-propyl-L-arginine (100 μM) (D). (*$p < 0.05$, N = 3).

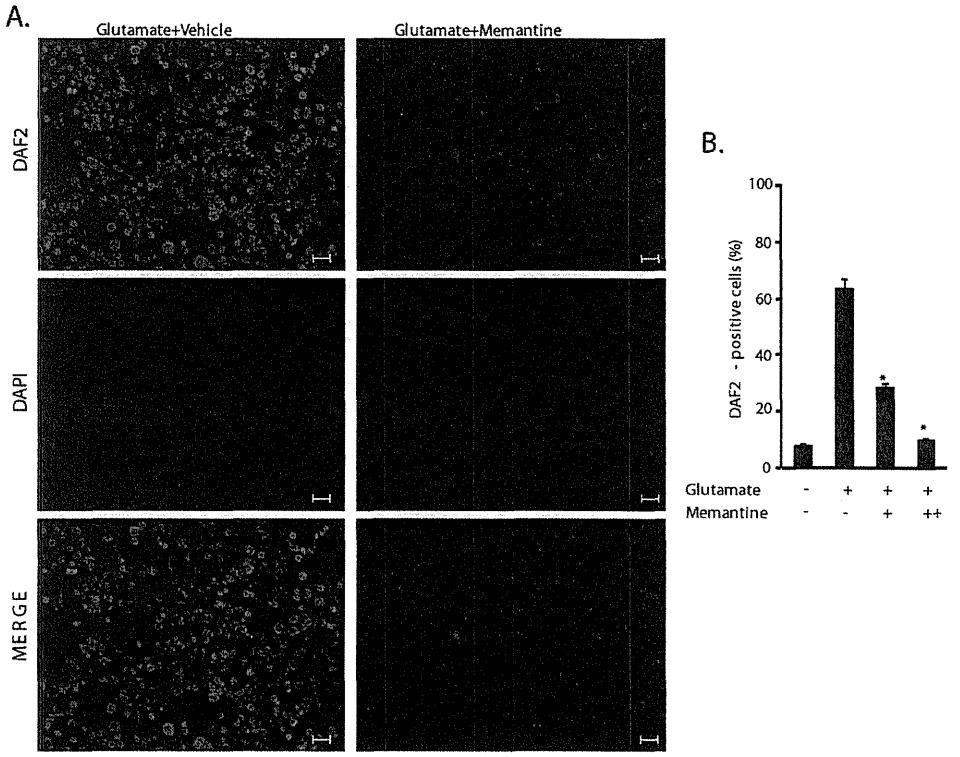

Fig. 3. A. NO production detected using DAF-2 (green) in glutamate (10 mM)-treated PC12 cells under treatment with or without memantine (200 μM). DAPI (blue) staining for nuclei. B. Number of DAF-2-stained PC12 cells treated with memantine (+: 100 μM, ++: 200 μM). (*$p < 0.05$, N = 3).

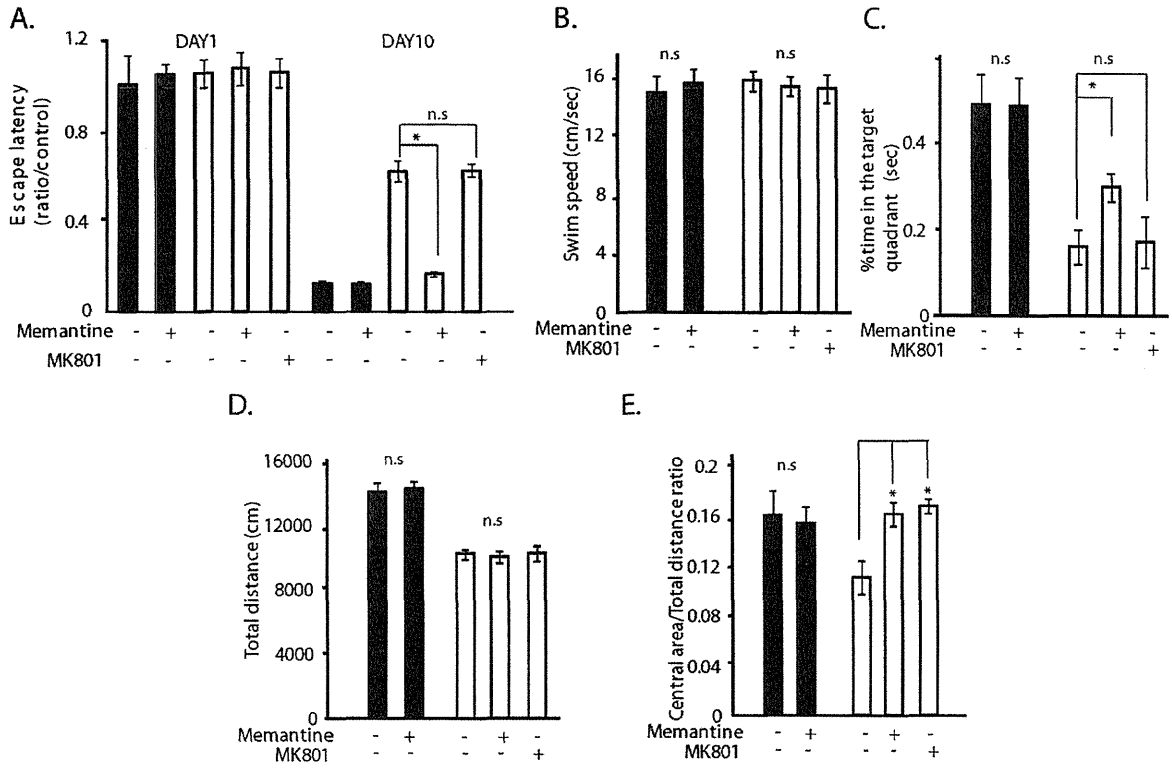

Fig. 4. A. Escape latency ratio on day 1 and 10 in SAMR1 (black bar) and SAMP8 (gray bar) mice (N = 5). Male mice were treated daily for 3 weeks with memantine (20 mg/kg s.c) or MK801 (10 mg/kg s.c) before trials. B. Swim speed during quadrant test on day 10. C. % time in target quadrant in memantine (20 mg/kg s.c)- and MK801 (10 mg/kg s.c)- treated mice on day 10. Total distance (D) and ratio of central/total distance (E) were measured in open field tests. (*$p < 0.05$, n.s: not significant).

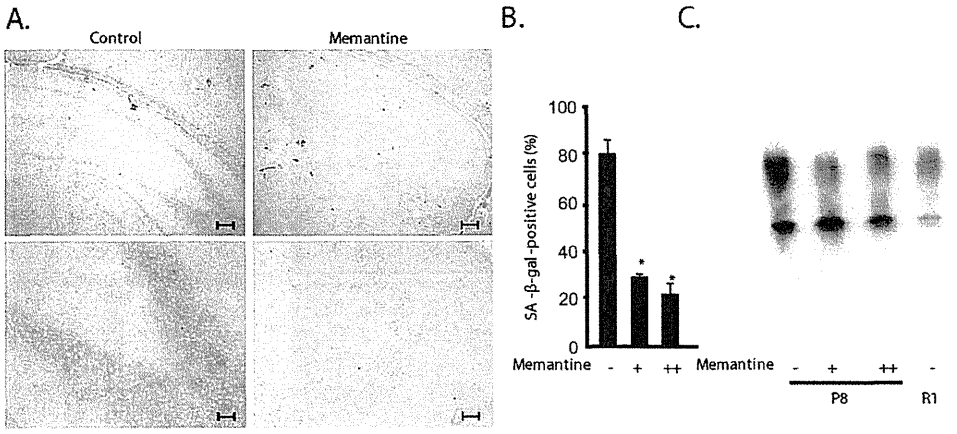

Fig. 5. A. B. SA-βgal-stained cells in CA1 and CA3 areas of hippocampus in SAMP8 with or without memantine (+: 10 mg/kg, ++: 20 mg/kg) treatment. (*$p < 0.05$, $N = 3$) (size scale 50 μm). C. Oxidative stress level was measured by detection of carbonyl groups introduced into proteins (R1: SAMR1, P8: SAMP8).

SAMP8 hippocampus (Fig. 5C). These results indicate that memantine inhibits senescence in the hippocampus of SAMP8 and suggest that reduction of oxidative stress involving NO may play an important role in the protective effect of memantine against senescence of the hippocampus.

3.5. Memantine and donepezil showed combined protective effect against senescent phenotype

Recently, the usefulness of combination therapy with memantine and donepezil has been investigated in many clinical studies. Because memantine and donepezil have different mechanisms of action, donepezil has been used together with memantine in many patients with AD. Therefore, we investigated the combined effect of memantine and donepezil. Combination treatment with memantine (50 μM) and donepezil (1 μM) significantly decreased the number of SA-ßgal-positive PC12 cells compared with only memantine or donepezil treatment (Fig. 6A). Moreover, SAMP8 treated with the combination showed significantly reduced escape latency at earlier days (Fig. 6B), and the time spent in the target quadrant was increased compared with that in memantine (10 mg/kg)- or donepezil (0.3 mg/kg)-treated SAMP8

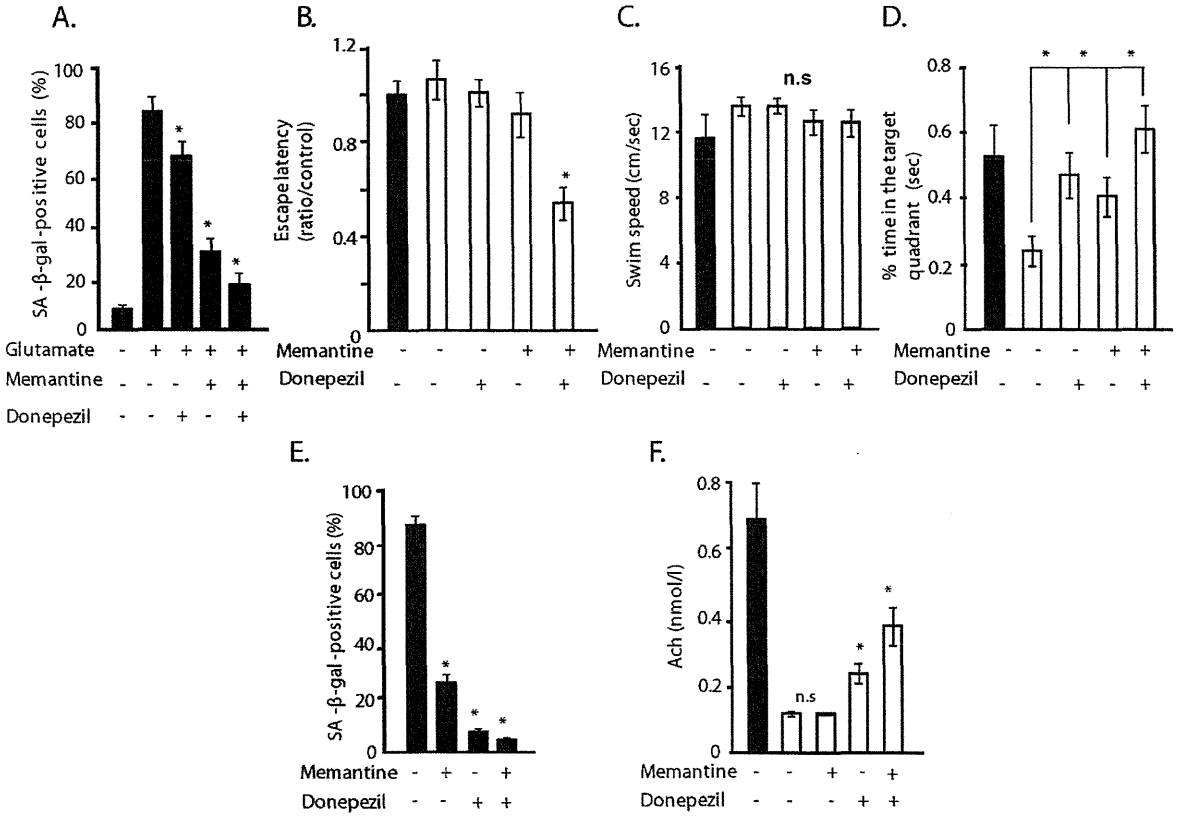

Fig. 6. A. A combination of memantine (50 μM) and donepezil (1 μM) more markedly reduced SA-βgal activity of PC12 cells compared with memantine or donepezil alone (*$p < 0.05$, $N = 3$). B. Escape latency ratio on day 1 in SAMR1 (black bar) ($N = 5$) and SAMP8 (gray bar) mice ($N = 5$) treated with donepezil (0.3 mg/kg), memantine (10 mg/kg), or donepezil + memantine. C. Swim speed during quadrant test on day 10. D. % time in target quadrant in donepezil, memantine, and donepezil + memantine-treated mice. E. Number of SA-βgal-stained cells in CA1 and CA3 areas of hippocampus (*$p < 0.05$, $N = 3$). F. Acetylcholine concentration was measured by a colorimetric method (*$p < 0.05$, $N = 3$).

(Fig. 6D). There was no difference in swim speed between these groups (Fig. 6C). The number of SA-βgal-stained cells in the CA1 and CA3 areas of the hippocampus was also significantly decreased in combination-treated compared with memantine- or donepezil-treated SAMP8 (Fig. 6E). Finally, the concentration of Ach in hippocampal lysates was increased in SAMP8 treated with donepezil but not memantine, but it was further increased by memantine in combination with donepezil (Fig. 6F).

4. Discussion

In this study, we showed that glutamate induced the senescence of PC12 cells and treatment with memantine inhibited it in accordance with restoration of the expression of SIRT1 and p53. Furthermore, memantine treatment reduced hippocampal senescence and led to improved spatial memory in SAMP8.

The Morris water maze results are often confounded by the fact that it is a potent anxiety/stress test (Morley et al., 2012). Therefore, there is a possibility that SAMP8 could show less anxiety than SAMR1, and the alteration in protein carbonyls could be due to stress.

Axonopathy observed in AD is considered to be the result of accumulation of Aß-peptide in glutamatergic synaptosomes, which leads to excessive release of glutamate with consequently more axonal degeneration in neurons through NMDA receptors (glutamate hypothesis) (Harrison, 1986). Indeed, recent findings indicate that increased levels of Aß-peptide and glutamate have a detrimental impact on neurons via interactions with NMDA receptors (Hiruma et al., 2003; Miguel-Hidalgo et al., 2002; Rammes et al., 2008), resulting in large Ca^{2+} entry into neurons and subsequent axonal and neuronal damage (Parsons et al., 2007). NMDA receptors are glutamate-gated Ca^{2+} channels that play pivotal roles in fundamental aspects of neuronal function. Glutamate treatment caused intracellular Ca^{2+} concentration to remain elevated up to 1 h in vitro (Dubinsky, 1993). Glutamate causes failure of the Ca^{2+} homeostasis early, and the degeneration primarily involves the activation of catabolic enzyme such as endonucleases, endopeptidases, and phospholipases which lead to production of ROS and $ONOO^-$ (Choi, 1992; Lazarewicz et al., 1990; Chan and Fishman, 1978). Therefore, we examined the cell viability in the course in early time. We found that when treated with 10 mM glutamate for 5 h, cell viability was not altered (Supplementary Fig. 1A). In accordance with this, it was reported that glutamate levels between 1 μM and 10 mM did not affect cell viability over a 24 h period in brain endothelial cells (Scott et al., 2007). In this study, we decided to use a glutamate concentration of 10 mM mainly because at this concentration it was easy to detect senescence phenotype at 10 days. However, glutamate, at mM concentrations, is known to exert toxic effect on CNS-derived preparation, including endothelial cells (Sharp et al., 2003; Parfenova et al., 2006). Because senescent cells were not detectable at an early time (24 h) and were detected at least 72 h after the start of treatment with glutamate, accumulation of the oxidative stress may occur, causing in cell damage, and it is thought that it gradually results in cellular senescence.

Moreover, it has been well documented that glutamate induces nNOS over-activation, secondary to elevation of extracellular glutamate, and leads to neuronal damage (Darra et al., 2009). Therefore, glutamate shows excitotoxicity at an early time, but treatment with memantine antagonized the NMDA receptor and resulted in attenuation of the senescence of PC12 cells via inhibition of nNOS activation. N^5-(1-amino-3-butenyl)-L-ornithine (L-VNIO), a selective nNOS inhibitor, was applied to examine the involvement of NOS in native testosterone-deficient SAMP8 with supplementation. L-VNIO did not show any effect on cognitive function (Ota et al., 2012). Moreover, we investigated cognitive function in aged endothelial NOS (eNOS) KO mice (96 weeks old). They did not show a cognitive decline compared with wild mice of the same age (data not shown). Unlike our in vitro experiments, these results suggest the possibility of no participation of NOS in vivo. However, it was reported that NOS inhibitors of nNOS, inducible NOS (iNOS), and eNOS had favorable effects on cognitive function in several AD mouse models (Maher et al., 2014; Santhanam et al., 2015). Given the differences observed in vivo and in vitro, because nNOS expression is naturally downregulated in the hippocampus of SAMP8 (Han et al., 2010), it could be difficult to evaluate the effect of nNOS as shown in vitro. Further studies are needed to clarify the participation of NOS.

In this study, similarly to memantine, treatment with MK-801 attenuated NO production and inhibited the senescence of PC12 cells. However, administration of MK-801 to SAMP8 did not alleviate learning deficit. Memantine has moderate affinity for NMDA receptors, with rapid blocking/unblocking kinetics and strong voltage dependency. In contrast, MK-801 has strong affinity for NMDA receptors and inhibits the induction of long-term potentiation at a concentration blocking NMDA receptors and worsens the impairment of spatial memory (Zajaczkowski et al., 1996). As described (Olney et al., 1989), noncompetitive NMDAR antagonists may by themselves induce excitotoxicity through inducing receptor hypoactivity. That is, blocking NMDA receptors on GABAergic/other inhibitory neurons leads to hypoactivity, resulting in overactivation/excitotoxicity of neurons downstream of these inhibitory neurons.

In another study, treatment of rats with memantine at the same concentrations as those used in this study (10, 20 mg/kg/day) showed serum levels of 52–64 ng/ml and 150–199 ng/ml respectively (Nakamura et al., 2006). These serum concentrations of memantine are similar to the levels seen in the serum of dementia patients treated with memantine, and the concentration of memantine in mice in our study may have been higher, assuring inhibition of the NMDA receptor based on in vitro data.

Memantine has been approved for the treatment of moderate to severe AD in Europe since 2002, in USA since 2003, and in Japan since 2011 (Allgaier and Allgaier, 2014), and has been shown in clinical trials to be a safe and effective treatment for vascular dementia (Orgogozo et al., 2002). Because memantine showed a protective effect on PC12 cells, we then examined its effect on vascular endothelial cells. Although the effect was weaker than that on neuronal cells, memantine inhibited glutamine-induced endothelial senescence (Supplementary Fig. 1B), and NMDA receptors were found in endothelial cells (Supplementary Fig. 1C). These results suggest that memantine may have a protective effect on not only neuronal function, but also vascular function.

In this study, memantine and donepezil showed an additive protective effect against neuronal senescence and improved cognitive function at an earlier time compared with monotherapy. Clinically, the combination of memantine and donepezil has demonstrated efficacy for treating the symptoms of AD (Gareri et al., 2014). Consistent with our results, several preclinical studies also have indicated cognition-enhancing effects of memantine and donepezil with repeated administration in other mouse models of AD (Nagakura et al., 2013). Because memantine and donepezil have different and complementary mechanisms of action, together they potentially offer additional benefits in relation to the etiology of AD. The detailed mechanism of the combined effect needs further study.

Memantine combined with environmental enrichment improves spatial memory in SAMP8 (Dong et al., 2012). Moreover, it has been recently reported that memantine can prevent Aß-peptide production (Miguel-Hidalgo et al., 2002). Although other mechanism may exist, the findings of the present study indicate that neuronal senescence may constitute an important target for AD and memantine may prevent it. Our results appear to support arguments justifying the presence of a "glutamate-associated excitotoxic insult" as a mechanism of the neurodegenerative changes observed in AD. We believe that the application of memantine as anti-excitotoxic treatment chronically may act as a defense against brain/neuronal aging and senescence causing AD.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.exger.2015.09.016.

Disclaimer

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References

Allgaier, M., Allgaier, C., 2014. An update on drug treatment options of Alzheimer's disease. Front. Biosci. (Landmark Ed.) 19, 1345–1354.
Areosa, S.A., Sherriff, F., McShane, R., 2005. Memantine for dementia. Cochrane Database Syst. Rev. 2, CD003154.
Benveniste, H., Drejer, J., Schousboe, A., Diemer, N.H., 1984. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J. Neurochem. 43, 1369–1374.
Cao, D., Lu, H., Lewis, T.L., Li, L., 2007. Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. J. Biol. Chem. 282, 36275–36282.
Chan, P.H., Fishman, R.A., 1978. Brain edema: induction in cortical slices by polyunsaturated fatty acids. Science 201, 358–360.
Choi, D.W., 1992. Excitotoxic cell death. J. Neurobiol. 23, 1261–1276.
Darra, E., Ebner, F.H., Shoji, K., Suzuki, H., Mariotto, S., 2009. Dual cross-talk between nitric oxide and D-serine in astrocytes and neurons in the brain. Cent. Nerv. Syst. Agents Med. Chem. 4, 289–294.
Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., Peacocke, M., Campisi, J., 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U. S. A. 92, 9363–9367.
Dong, J., Zhou, M., Wu, X., Du, M., Wang, X., 2012. Memantine combined with environmental enrichment improves spatial memory and alleviates Alzheimer's disease-like pathology in senescence-accelerated prone-8 (SAMP8) mice. J. Biomed. Res. 26, 439–447.
Doucet, M.V., O'Toole, E., Connor, T., Harkin, A., 2015. Small-molecule inhibitors at the PSD-95/nNOS interface protect against glutamate-induced neuronal atrophy in primary cortical neurons. Neuroscience 301, 421–438.
Dubinsky, J.M., 1993. Effects of calcium chelators on intracellular calcium and excitotoxicity. Neurosci. Lett. 150, 129–132.
Eliasson, M.J., Huang, Z., Ferrante, R.J., Sasamata, M., Molliver, M.E., Snyder, S.H., Moskowitz, M.A., 1999. Neuronal nitric oxide synthase activation and peroxynitrite formation in ischemic stroke linked to neural damage. J. Neurosci. 19, 5910–5918.
Flood, J.F., Morley, J.E., 1998. Learning and memory in the SAMP8 mouse. Neurosci. Biobehav. Rev. 22, 1–20.
Gareri, P., Putignano, D., Castagna, A., Cotroneo, A.M., De Palo, G., Fabbo, A., Forgione, L., Giacummo, A., Lacava, R., Marino, S., Simone, M., Zurlo, A., Putignano, S., 2014. Retrospective study on the benefits of combined memantine and cholinesterase inhibitor treatment in aged patients affected with Alzheimer's disease: the MEMAGE study. J. Alzheimers Dis. 41, 633–640.
Han, S., Rudd, J.A., Hu, Z.Y., Zhang, L., Yew, D.T., Fang, M., 2010. Analysis of neuronal nitric oxide synthase expression and increasing astrogliosis in the brain of senescence-accelerated-prone 8 mice. Int. J. Neurosci. 120, 602–608.
Harrison, P.J., 1986. Pathogenesis of Alzheimer's disease—beyond the cholinergic hypothesis: discussion paper. J. R. Soc. Med. 79, 347–352.
Hiruma, H., Katakura, T., Takahashi, S., Ichikawa, T., Kawakami, T., 2003. Glutamate and amyloid beta-protein rapidly inhibit fast axonal transport in cultured rat hippocampal neurons by different mechanisms. J. Neurosci. 23, 8967–8977.
Ingram, D.K., Shimada, A., Spangler, E.L., Ikari, H., Hengemihle, J., Kuo, H., Greig, N., 1996. Cognitive enhancement. New strategies for stimulating cholinergic, glutamatergic, and nitric oxide systems. Ann. N. Y. Acad. Sci. 786, 348–361.
Lazarewicz, J.W., Wroblewski, J.T., Costa, E., 1990. N-methyl-D-aspartate-sensitive glutamate receptors induce calcium-mediated arachidonic acid release in primary cultures of cerebellar granule cells. J. Neurochem. 55, 1875–1881.
Lukacs, H., Hiatt, E.S., Lei, Z.M., Rao, C.V., 1995. Peripheral and intracerebroventricular administration of human chorionic gonadotropin alters several hippocampus-associated behaviors in cycling female rats. Horm. Behav. 29, 42–58.
Maher, A., El-Sayed, N.S., Breitinger, H.G., Gad, M.Z., 2014. Overexpression of NMDAR2B in an inflammatory model of Alzheimer's disease: modulation by NOS inhibitors. Brain Res. Bull. 109, 109–116.
Mattson, M.P., 2004. Pathways towards and away from Alzheimer's disease. Nature 430, 631–639.
Miguel-Hidalgo, J.J., Alvarez, X.A., Cacabelos, R., Quack, G., 2002. Neuroprotection by memantine against neurodegeneration induced by beta-amyloid (1–40). Brain Res. 958, 210–221.
Miyamoto, M., Kiyota, Y., Nishiyama, M., Nagaoka, A., 1992. Senescence accelerated mouse (SAM): age-related reduced anxiety-like behavior in the SAM-P/8 strain. Physiol. Behav. 51, 979–985.
Morley, J.E., Farr, S.A., Kumar, V.B., Armbrecht, H.J., 2012. The SAMP8 mouse: a model to develop therapeutic interventions for Alzheimer's disease. Curr. Pharm. Des. 18, 1123–1130.
Nagakura, A., Shitaka, Y., Yarimizu, J., Matsuoka, N., 2013. Characterization of cognitive deficits in a transgenic mouse model of Alzheimer's disease and effects of donepezil and memantine. Eur. J. Pharmacol. 703, 53–61.
Nakamura, S., Murayama, N., Noshita, T., Katsuragi, R., Ohno, T., 2006. Cognitive dysfunction induced by sequential injection of amyloid-beta and ibotenate into the bilateral hippocampus; protection by memantine and MK-801. Eur. J. Pharmacol. 548, 115–122.
Naylor, R.M., Baker, D.J., van Deursen, J.M., 2013. Senescent cells: a novel therapeutic target for aging and age-related diseases. Clin. Pharmacol. Ther. 93, 105–116.
Ogawa, M., Ishikawa, T., Irimajiri, A., 1984. Adrenal chromaffin cells form functional cholinergic synapses in culture. Nature 307, 66–68.
Olney, J.W., Labruyere, J., Price, M.T., 1989. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244, 1360–1362.
Orgogozo, J.M., Rigaud, A.S., Stöffler, A., Möbius, H.J., Forette, F., 2002. Efficacy and safety of memantine in patients with mild to moderate vascular dementia: a randomized, placebo-controlled trial (MMM 300). Stroke 33, 1834–1839.
Ota, H., Akishita, M., Akiyoshi, T., Kahyo, T., Setou, M., Ogawa, S., Iijima, K., Eto, M., Ouchi, Y., 2012. Testosterone deficiency accelerates neuronal and vascular aging of SAMP8 mice: protective role of eNOS and SIRT1. PLoS ONE 7, e29598.
Parfenova, H., Basuroy, S., Bhattacharya, S., Tcheranova, D., Qu, Y., Regan, R.F., Leffler, C.W., 2006. Glutamate induces oxidative stress and apoptosis in cerebral vascular endothelial cells: contributions of HO-1 and HO-2 to cytoprotection. Am. J. Physiol. Cell Physiol. 290, C1399–C1410.
Parsons, C.G., Stöffler, A., Danysz, W., 2007. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system—too little activation is bad, too much is even worse. Neuropharmacology 53, 699–723.
Rammes, G., Danysz, W., Parsons, C.G., 2008. Pharmacodynamics of memantine: an update. Curr. Neuropharmacol. 6, 55–78.
Santhanam, A.V., d'Uscio, L.V., He, T., Das, P., Younkin, S.G., Katusic, Z.S., 2015. Uncoupling of endothelial nitric oxide synthase in cerebral vasculature of Tg2576. J. Neurochem. (Epub ahead of print).
Scott, G.S., Bowman, S.R., Smith, T., Flower, R.J., Bolton, C., 2007. Glutamate-stimulated peroxynitrite production in a brain-derived endothelial cell line is dependent on N-methyl-D-aspartate (NMDA) receptor activation. Biochem. Pharmacol. 73, 228–236.
Sharp, C.D., Hines, I., Houghton, J., Warren, A., Jackson IV, T.H., Jawahar, A., Nanda, A., Elrod, J.W., Long, A., Chi, A., Minagar, A., Alexander, J.S., 2003. Glutamate causes a loss in human cerebral endothelial barrier integrity through activation of NMDA receptor. Am. J. Physiol. Heart Circ. Physiol. 285, H2592–H2598.
Tacutu, R., Budovsky, A., Yanai, H., Fraifeld, V.E., 2011. Molecular links between cellular senescence, longevity and age-related diseases – a systems biology perspective. Aging 12, 1178–1191.
Yagi, H., Katoh, S., Akiguchi, I., Takeda, T., 1988. Age-related deterioration of ability of acquisition in memory and learning in senescence accelerated mouse; SAM-P/8 as an animal model of disturbances in recent memory. Brain Res. 474, 86–93.
Zajaczkowski, W., Quack, G., Danysz, W., 1996. Infusion of (+)-MK-801 and memantine – contrasting effects on radial maze learning in rats with entorhinal cortex lesion. Eur. J. Pharmacol. 296, 239–246.

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Original Study

Favorable Effect of Sympathetic Nervous Activity on Rehabilitation Outcomes in Frail Elderly

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A B S T R A C T

Objectives: Previous studies have suggested the relationship between physical function, mortality, and autonomic nervous activity in frail elderly and that maintaining sympathetic nervous activity might lead to improved physical function and mortality in the elderly population. The aim of this study was to investigate the utility of sympathetic nervous activity measured by heart rate variability in frail elderly patients undergoing inpatient rehabilitation, further focusing the nervous activity on the effect of rehabilitation therapy.
Design: Prospective cohort study.
Participants: Sixty-one subjects aged 75 years or older were recruited after treatment of acute phase illness.
Measurements: Before undergoing rehabilitation, data of 24-hour Holter monitoring and a blood venous sample were obtained. From RR intervals in the electrocardiogram, heart rate and SDs of all NN intervals in all 5-minute segments of the entire recording, power spectral density, low frequency (LF), high frequency (HF), and low frequency/high frequency (LF/HF) were calculated. Functional Independence Measure (FIM) and Barthel index were used to measure physical function.
Results: FIM score and Barthel index were 46.8 ± 25.4 and 32.8 ± 31.7, respectively. Serum total protein, albumin, hemoglobin, and total cholesterol were all significantly related to FIM score and Barthel index before rehabilitation. Heart rate variability indices did not show a significant relationship with physical function, whereas the high LH/HF group showed significant improvement in physical function compared with the low LH/HF group. Moreover, LF/HF frequency was a predictive factor for improvement of physical function after 2 months of rehabilitation.
Conclusion: A favorable effect of preserved LF/HF on rehabilitation outcome was observed in elderly undergoing rehabilitation. Preservation of sympathetic nervous activity may lead to improved physical function in the elderly.

The number of disabled elderly is increasing in Japan, and the number of elderly needing long-term care reached more than 5 million in 2011.[1] It is important for disabled elderly to maintain or improve their physical function. Rehabilitation is a well-established approach to improve physical function after treatment of acute phase illness.[2] In Japan, the underlying causes of physical dysfunction necessitating rehabilitation are cerebrovascular disease, fracture, disuse syndrome, and other diseases that cause physical dysfunction.[3] It is our most urgent task to prevent and treat these diseases, and also to improve physical function after the occurrence of these diseases. Recent studies have indicated a relationship between these underlying diseases and sympathetic nervous activity.[4–8]

With regard to cerebrovascular disease, which is the most frequent cause of elderly becoming frail or needing long-term care, Muslumanoglu et al[4] demonstrated that low sympathetic nervous

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activity measured by skin sympathetic reactivity reflected greater severity of paralysis and severe limited motor function in the chronic phase of ischemic cerebrovascular disease in the elderly. In addition, RR intervals in the electrocardiogram are used to evaluate heart rate variability (HRV),[9] and low frequency/high frequency (LF/HF), which was reported to be a marker of sympathovagal balance or sympathetic modulation, showed a positive correlation with both respiratory and skeletal muscle strength in chronic obstructive pulmonary disease.[5] Moreover, it was reported that low LF/HF value was related to overall mortality in not only frail elderly and elderly in long-term care, but also community-dwelling elderly.[6–8] In addition to these previous reports, we recently showed that elderly in long-term care aged 75 years or older had lower LF/HF than in physically intact elderly controls. Moreover, in only long-term care elderly, the low LF/HF group was significantly associated with high mortality after adjustment for age, sex, cardiovascular risk factors, and physical function. These findings suggest that preserved sympathetic nervous activity might have some favorable effects in long-term care elderly.[8]

Dementia is known to be a limiting factor in rehabilitation.[10–12] Patients with Lewy body dementia and Parkinson disease dementia show low sympathetic activity.[13,14] The same relationship is observed in fractures. β-blockers are widely accepted for the treatment of hypertension and chronic heart failure through reducing sympathetic nervous activity in middle age.[15,16] However, most studies concerning the benefit of β-blockers targeted only middle age, and there have been few studies of elderly aged 75 years or older.[17] In contrast to the benefit of β-blockers in middle age, they were found to have a relationship with incident fracture in elderly people aged 80 years or older.[18]

These findings suggest that it might be necessary to maintain sympathetic nervous activity in very elderly people, especially in frail or long-term care elderly. However, few studies have examined the relationship between sympathetic nervous activity and the effect of rehabilitation therapy in very elderly people. Therefore, we investigated whether sympathetic nervous activity affects physical function increment in elderly aged 75 years or older undergoing rehabilitation therapy. The aim of this study was to determine whether the high sympathetic nervous activity group would show greater improvement in physical function, and whether sympathetic nervous activity could predict the effect of rehabilitation.

Methods

Setting and Participants

This observational study analyzed 61 consecutive elderly persons aged 75 years or older who were admitted to a rehabilitation unit. The hospital was located in Nagano prefecture, Japan. Inclusion criteria were elderly aged 75 years or older undergoing rehabilitation. Exclusion criteria were treatment of acute phase disease within the last 2 weeks, arrhythmia, administration of anti-arrhythmia drugs or β-blockers, malignancy, and neurodegenerative disease.[9] Medical records were reviewed to obtain information on history of cardiovascular disease, such as hypertension, diabetes mellitus, hyperlipidemia, chronic heart failure, or ischemic heart disease, which was confirmed by the patient or family. This study protocol was approved by the institutional review board of the Keijinkai Kikyougahara Hospital. Written informed consent was obtained from all participants or their families.

Heart Rate Variability

Ambulatory Holter recording was performed for 24 hours using QR2100 (Fukuda ME, Kogyo, Tokyo, Japan) and processed with HS1000VL (Fukuda ME Kogyo). For time domain analysis, the SDs of all NN intervals in all 5-minute segments of the entire recording (SDANN) were calculated, and frequent domain analysis was performed with fast Fourier transform. From the power spectral density, LF (0.04∼0.15 Hz), HF (0.15∼0.40 Hz), and LF/HF were determined.

Anthropometric, Physical Function, and Hematologic Measures

Height, weight, and body mass index (BMI) were measured before Holter monitoring. FIM score[19] and Barthel index[20] were determined to assess physical function. Venous blood samples were obtained from individuals in the morning after an overnight fast. Blood cell counts and serum levels of chemical parameters were determined by a commercial laboratory (Health Science Research Institute, Yokohama, Japan).

Statistical Analysis

Data were analyzed using SPSS software (Ver.11.0.1J; SPSS Japan Inc., Tokyo, Japan). Pearson correlation coefficient was calculated to determine the relationship between physical function and blood nutritional data and HRV indices. Standardized multiple regression analysis of FIM and Barthel index was performed with age, sex, and the disease necessitating rehabilitation as covariates. One-way analysis of variance was used for the effect of 2 months of rehabilitation on each HRV index. Age, sex, BMI, the disease necessitating rehabilitation, FIM before undergoing rehabilitation (pre-FIM), blood nutritional data, and HRV indices, including heart rate, SDANN, LF, HF, and LF/HF were used as covariates in stepwise regression analysis to determine independent predictors of increment of FIM after 2 months of rehabilitation (ΔFIM). For selected predictive factors, the standardized regression coefficient (β) was calculated by multiple regression analysis.

Results

We registered 61 elderly people who received rehabilitation, and assessed physical function and HRV from 24-hour Holter monitoring. The background data of this study are shown in Table 1.

The underlying diseases necessitating rehabilitation were cerebrovascular disease (n = 37, 60.7%), disuse syndrome (n = 14, 23.0%), and fracture (n = 10, 16.3%). Mean age was 86 ± 5 years, blood nutritional data including total protein, albumin, total cholesterol,

Table 1
Characteristics of Elderly Individuals

Category	Measurement Items	Results
Background data	Number	61
	Age	86 ± 6 (75–100)
	Sex, male (%)	20 (32.8)
	BMI, kg/m^2	19.7 ± 3.2
Type of disease, n (%)	Cerebrovascular disease	37 (60.7)
	Disuse syndrome	14 (23.0)
	Fracture	10 (16.3)
Blood nutritional data	Total protein, g/dL	6.4 ± 0.7
	Albumin, g/dL	3.4 ± 0.5
	Hemoglobin, g/dL	12.1 ± 1.7
	Total cholesterol, mg/dL	177 ± 44
Physical function	FIM	46.8 ± 25.4
	Barthel index	32.8 ± 31.7
HRV indices	Heart rate (beats per minute)	74.8 ± 12.8
	SDANN, ms	85.7 ± 35.5
	LF, ms^2	35.8 ± 27.5
	HF, ms^2	63.8 ± 57.8
	LF/HF	0.70 ± 0.26

Values are mean ± SD.

and hemoglobin were at the lower limit of the normal range. FIM and Barthel index, representing physical function, were 46.8 ± 25.4 and 32.8 ± 31.7, respectively. Scores for each FIM item were as follows: eating 3.8 ± 2.2, grooming 2.7 ± 1.8, bathing 1.5 ± 1.2, upper body dressing 2.5 ± 1.7, lower body dressing 2.3 ± 1.5 toileting 2.4 ± 1.7, bladder management 2.8 ± 2.2, bowel management 2.8 ± 2.1, bed-to-chair transfer 3.2 ± 1.8, toilet transfer 2.7 ± 1.9, shower transfer 1.2 ± 0.9, locomotion (ambulatory or wheelchair level) 1.7 ± 1.5, stairs 1.2 ± 0.7, cognitive comprehension 3.7 ± 1.8, expression 3.8 ± 2.1, social interaction 3.3 ± 2.2, problem solving 2.8 ± 1.8, and memory 2.8 ± 1.8. In terms of HRV indices, heart rate, SDANN, LF, HF, and LF/HF were 74.8 ± 12.8 beats per minute, 85.7 ± 35.5 ms, 35.8 ± 27.5 ms^2, 63.8 ± 57.8 ms^2, and 0.7 ± 0.26, respectively. Of these indices, heart rate was higher and the others were lower than those in healthy controls matched for age, sex, and cardiovascular risk factors, which we previously reported.

Pearson correlation coefficient and multiple regression analysis revealed an association among total protein, albumin, total cholesterol, hemoglobin, and physical function (both FIM and Barthel index). Although low heart rate was significantly related to high Barthel index, we did not find a significant relationship between other HRV indices and physical function in multiple regression analysis (Table 2). After 2 months of rehabilitation, FIM was significantly increased from 47 to 54 points. We investigated the relationship between HRV indices and change in physical function (ΔFIM). Two months of rehabilitation did not show a significant difference in SDANN, heart rate, LF, and HF between the high-value group and low-value group (Figure 1A–D). Only the high LF/HF group showed significantly improved ΔFIM compared with the low LF/HF group; 9.0 versus 4.5 points (Figure 1E). FIM is constructed of 13 physical items and 5 cognitive items, and physical items were significantly improved in the high LF/HF group compared with the low LF/HF group after 2 months of rehabilitation (Table 3).

In age, sex, pre-FIM, BMI, cardiovascular disease risk factors, blood nutritional data, and HRV indices, stepwise regression analysis revealed that high ΔFIM was significantly related to high pre-FIM ($\beta = 0.341$, $P = .006$) and high LF/HF ($\beta = 0.296$, $P = .015$) (ΔFIM = [pre-FIM] × 0.127 + [LF/HF] × 11.054 − 6.994) (Table 4).

Discussion

The present longitudinal study demonstrated that the frail elderly patients undergoing inpatient rehabilitation with high LF/HF represented a greater increase in FIM than those in the low LF/HF group, and that ΔFIM was predicted by LF/HF as well as pre-FIM at baseline. Previous studies have shown that increment of physical function with rehabilitation was predicted by pre-FIM and age.[21–24] Based on our findings, it was also suggested that FIM was the strongest predictive factor for increment of physical function in frail elderly, which was consistent with several studies about clinical characteristics of rehabilitation therapy.[21–23]

Sarcopenia[25] and frailty[26] in the elderly, whose criteria include low extremity muscle strength, such as low walking speed and low handgrip strength, have been shown to be associated with high mortality. Slow walking speed is one of the criteria of frailty that was reported by Fried et al.[26] Although we did not measure walking speed in this study, we evaluated the locomotion score in FIM. The average score of locomotion in the study population was 1.7 ± 1.5 (range 1–6) and 59 of 61 participants gained fewer than 5 points, suggesting that most participants were mobility-dependent. In this point of view, it is suggested that the individuals in our study might belong to a frail population. Frailty index was also reported by Searle et al,[27] in which procedures for selecting variables related to frailty were applied to yield 40 deficits. We measured 15 variables (help bathing, help dressing, help getting in/out of chair, help walking around house, help eating, help grooming, help using toilet, help up/down stairs, high blood pressure, heart attack, chronic heart failure, stroke, cancer, diabetes, BMI) among a total of 40 deficits, and the average deficit score in our study was 9.45, corresponding to frailty index as 0.24 ± 0.04 at least. Based on these observations, it was suggested that individuals in this study might meet the criteria of frailty. These concepts and findings of sarcopenia and frailty suggest that it is important to improve physical function and musculoskeletal property in the elderly population. Sympathetic nervous activity has the potential to improve physical function in the elderly, and activation of the sympathetic nervous system might lead to an increase in blood pressure, muscle blood flow, and muscle strength by inducing muscle protein synthesis. In practice, it has been suggested that high sympathetic nervous activity is related to improvement of physical function.[28–30] Taken together with our findings and previous studies, it would be important to maintain sympathetic nervous activity in frail elderly whose physical performance is not preserved. Age was also reported to predict increment of physical function in younger individuals,[21–23] whereas the effect of age on rehabilitation has been considered to be not so strong.[11,12,24] In addition, studies limited to only elderly individuals have not shown a significant relationship between age and increment of physical function.[11,12] This relationship was not observed in our study, and one possible explanation might be that inclusion criteria of this study were limited to age of 75 years or older.

It was also demonstrated in this study that serum levels of total protein, albumin, hemoglobin, and total cholesterol were all related to both FIM and Barthel index at baseline, whereas these blood nutritional data were not correlated with the increment of FIM in a longitudinal analysis. Although previous cross-sectional studies suggested that low serum albumin level was related to low skeletal muscle mass, grip strength, and leg power,[31–33] this relationship remains controversial in other longitudinal studies. In practice, it was reported that lower serum albumin was associated with muscle strength decline over 3 and 6 years,[32] whereas another did not represent this association.[34] Instead, the combination of low serum albumin and low total cholesterol could predict future decline of functional performance.[34,35] The prognostic role of total cholesterol varies among types of cerebrovascular disease, such as hemorrhagic stroke, noncardiac ischemic stroke, and cardiac ischemic stroke.[36,37] Although low total cholesterol at baseline was reported to be able to predict future low physical performance,[34,36] the relationship was not revealed in this study, and the discrepancy might be derived from differences in age and underlying diseases of individual study. Serum

Table 2
Relationship Among FIM, Barthel Index, and Blood Nutritional Data and HRV Indices

	FIM (r)	Barthel Index (r)	FIM (β)	Barthel Index (β)
Total protein	0.44‡	0.48‡	0.49‡	0.50‡
Albumin	0.54‡	0.56‡	0.55‡	0.55‡
Total cholesterol	0.46‡	0.46‡	0.52‡	0.49‡
Hemoglobin	0.33†	0.37†	0.34*	0.38†
BMI	0.16	0.16	0.26	0.23
Heart rate	−0.29*	−0.29*	−0.25	−0.27*
SDANN	0.27*	0.25	0.22	0.22
LF	0.02	−0.08	0.02	−0.06
HF	0.02	−0.11	0.04	−0.07
LF/HF	−0.07	0.10	−0.09	0.04

Pearson correlation coefficient (r) and standardized regression coefficient (β) were calculated. Standardized regression coefficient (β) was adjusted for age, sex, and disease necessitating rehabilitation.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

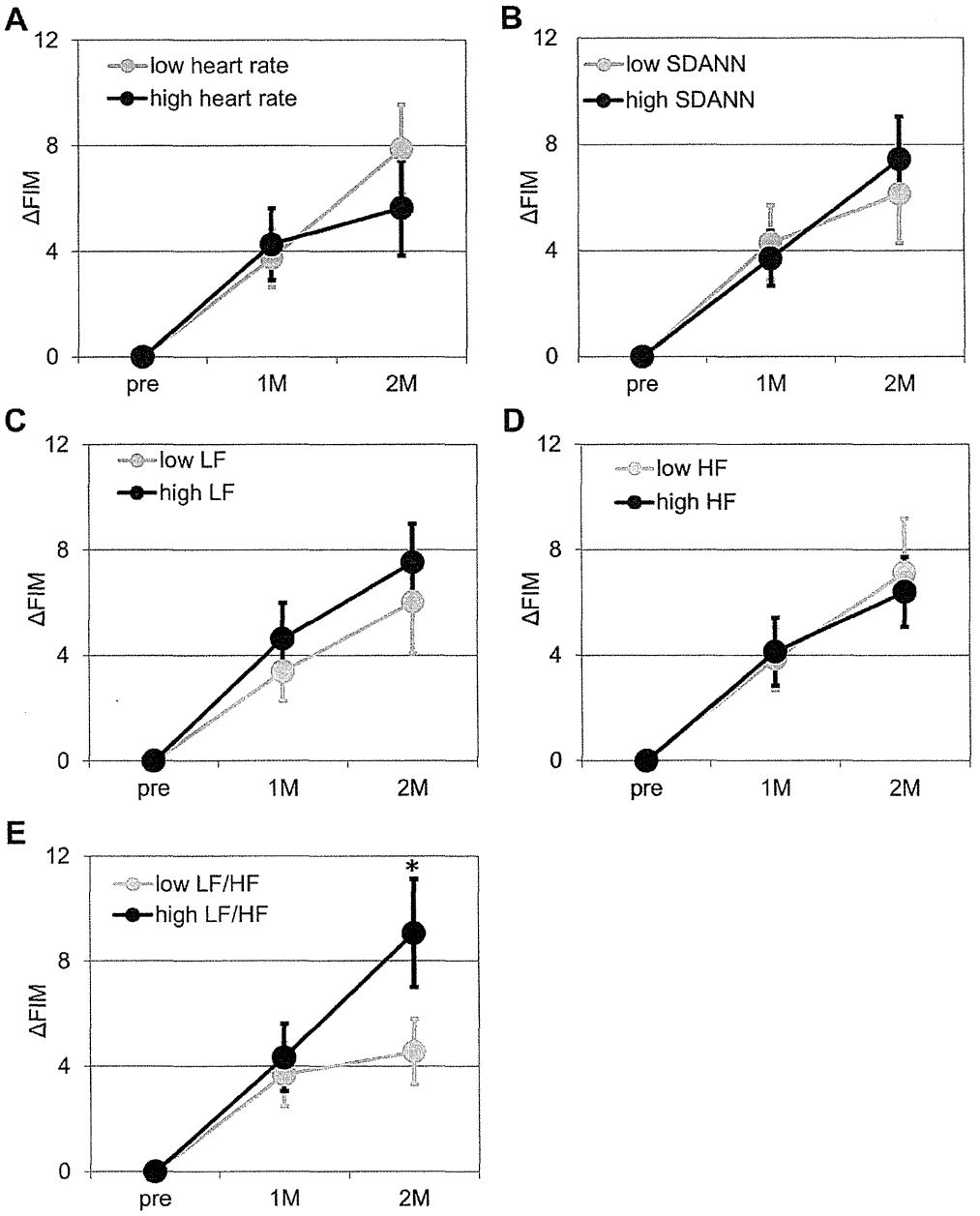

Fig. 1. HRV indices and ΔFIM before and after 2 months of rehabilitation. Data are mean ± SE. All HRV indices were divided into a high group (n = 31) and low group (n = 30) by the median. Standardized regression coefficient (β) was adjusted for age, sex, and disease necessitating rehabilitation. 1M, 1 month after start of rehabilitation; 2M, 2 months after start of rehabilitation. A, Heart rate and ΔFIM; B, SDANN and ΔFIM; C, LF and ΔFIM; D, HF and ΔFIM; E, LF/HF and ΔFIM. *$P < .05$.

hemoglobin level was related to physical function in a cross-sectional study, but no relationship was found in a longitudinal study in fracture patients,[38] which was also supported by our study results. Taken together, blood nutritional data were correlated with physical function in cross-sectional studies in the elderly based on both our results and previous studies, whereas in a longitudinal study, they could not predict the improvement of physical performance at least after 2 months of rehabilitation. Hence, it might be important to consider long-term nutritional support of elderly patients with regard to the underlying diseases as well.

To maintain or increase sympathetic nervous activity, a recent study has suggested that daylight exposure is the most simple and powerful rhythmic regulator in the environment.[39] A sitting or standing position also activates sympathetic nervous activity compared with the supine position.[40] These findings indicate that it may be possible to improve physical and cognitive function in elderly patients hospitalized in rehabilitation units by incorporating these environmental settings.

Drug intervention to induce sympathetic nervous activation has been applied to aging, disuse, and sarcopenic model rats.[41–47] Administration of β2-adrenergic agents, such as clenbuterol and formoterol, was reported to prevent muscle weakness and aging. In addition, β2-agonist administration was reported to improve not only myodystrophy[48] but also heart failure.[49] At the same time, further investigation is necessary for its clinical application because few studies have demonstrated a favorable effect of β-agonist administration in elderly people. Development of more specific drugs for skeletal muscle is expected to lead to application of such drugs in humans.

Table 3
Each Item of ΔFIM Increment in the High LF/HF Group and Low LF/HF Group

	Δ High LF/HF	Δ Low LF/HF
Physical items	8.37*	4.29
Eating	0.44	0.27
Grooming	0.44	0.44
Bathing	0.59	0.32
Upper body dressing	0.70	0.47
Lower body dressing	0.78*	0.27
Toileting	0.74	0.59
Bed to chair transfer	0.56	0.47
Toilet transfer	0.74	0.59
Shower transfer	0.70*	0.12
Bladder management	0.59*	0.12
Bowel management	0.59	0.18
Locomotion	1.00	0.35
Stairs	0.48	0.12
Cognitive items	1.07	0.35
Cognitive comprehension	0.11	0.09
Expression	0.19	0.09
Social interaction	0.15	−0.03
Problem solving	0.19	0.09
Memory	0.44	0.12

*$P < .05$.

Several limitations of this study should be considered. First, only HRV was conducted before rehabilitation therapy. The improvement of sympathetic nervous activity and physical and cognitive function needs to be shown simultaneously. Intervention therapy to improve sympathetic nervous activity should be conducted to support our study finding that increment of sympathetic nervous activity relates to good outcome. Second, as the sample size was not enough to perform multiple regression analysis with these covariates in this study, we performed a stepwise selection method to specify the explanatory variables first, and investigated subsequent multiple regression analysis with 2 selected covariates, pre-FIM and LF/HF.[50] Statistical power of the study might still not be enough to conclude the relationship between ΔFIM and LF/HF, and a large-scale study as well as direct evidence for a causal relationship should be investigated in the near future. Third, this study did not consider the effect of diseases that would affect HRV. Although the effect of LF/HF on stroke is still controversial, stroke might alter LF/HF.[51–53] Some studies also demonstrated that LF/HF was higher in patients with ischemic stroke compared with healthy controls,[51,52] but others showed it was lower in patients with stroke.[53] Multiple regression analysis in this study demonstrated no significant relationship between HRV and underlying diseases subject to rehabilitation therapy, and future study would clarify the contribution of each disease to HRV indices.

Conclusion

In summary, our study revealed that LF/HF was one of the strongest predictive factors of physical and cognitive functional recovery in rehabilitation, and that high LF/HF was related to good outcome in terms of physical function among frail elderly patients undergoing rehabilitation, further suggesting that appropriate sympathetic nervous activity may lead to well-being and successful aging.

Table 4
Independent Predictive Factors for ΔFIM

	Standardized Regression Coefficient (β)	*P*
Pre-FIM	0.341	.006
LF/HF	0.298	.015

References

1. Ministry of Health, Labour and Welfare. Health and Welfare Services for the Elderly. Available at: http://www.mhlw.go.jp/english/wp/wp-hw6/dl/10e.pdf. Accessed May 21, 2015.
2. Roach ES, Bettermann K, Biller J. Toole's Cerebrovascular Disorders. 6th ed. New York: Cambridge University Press; 2010.
3. Ministry of Health, Labour and Welfare. Available at: http://www.mhlw.go.jp/shingi/2009/11/dl/s1110-5d03.pdf. Accessed May 21, 2015.
4. Muslumanoglu L, Aki S, Turkdogan D, et al. Involvement of sympathetic reflex activity in patients with acute and chronic stroke: A comparison with functional motor capacity. Arch Phys Med Rehabil 2004;85:470–473.
5. Camillo CA, Pitta F, Possani HV, et al. Heart rate variability and disease characteristics in patients with COPD. Lung 2008;186:393–401.
6. Varadhan R, Chaves PH, Lipsitz LA, et al. Frailty and impaired cardiac autonomic control: New insights from principal components aggregation of traditional heart rate variability indices. J Gerontol A Biol Sci Med Sci 2009;64:682–687.
7. Tsuji H, Venditti FJ Jr, Manders ES, et al. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. Circulation 1994;90:878–883.
8. Shibasaki K, Ogawa S, Yamada S, et al. Association of decreased sympathetic nervous activity with mortality of older adults in long-term care. Geriatr Gerontol Int 2014;14:159–166.
9. Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology. Heart rate variability. Circulation 1996;93:1043–1065.
10. Hershkovitz A, Kalandariov Z, Hermush V, et al. Factors affecting short-term rehabilitation outcomes of disabled elderly patients with proximal hip fracture. Arch Phys Med Rehabil 2007;88:916–921.
11. Beloosesky Y, Grinblat J, Epelboym B, et al. Functional gain of hip fracture patients in different cognitive and functional groups. Clin Rehabil 2002;16:321–328.
12. Heinemann AW, Linacre JM, Wright BD, et al. Prediction of rehabilitation outcomes with disability measures. Arch Phys Med Rehabil 1994;75:133–143.
13. Oka H, Morita M, Onouchi K, et al. Cardiovascular autonomic dysfunction in dementia with Lewy bodies and Parkinson's disease. J Neurol Sci 2007;254:72–77.
14. Oka H, Yoshioka M, Morita M, et al. Reduced cardiac 123I-MIBG uptake reflects cardiac sympathetic dysfunction in Lewy body disease. Neurology 2007;69:1460–1465.
15. Lucini D, Mela GS, Malliani A, et al. Impairment in cardiac autonomic regulation preceding arterial hypertension in humans: Insights from spectral analysis of beat-by-beat cardiovascular variability. Circulation 2002;106:2673–2679.
16. Burns J, Sivananthan MU, Ball SG, et al. Relationship between central sympathetic drive and magnetic resonance imaging-determined left ventricular mass in essential hypertension. Circulation 2007;115:1999–2005.
17. Flather MD, Shibata MC, Coats AJ, et al. SENIORS Investigators. Randomized trial to determine the effect of nebivolol on mortality and cardiovascular hospital admission in elderly patients with heart failure (SENIORS). Eur Heart J 2005;26:215–225.
18. Peters R, Beckett N, Burch L, et al. The effect of treatment based on a diuretic (indapamide) +/- ACE inhibitor (perindopril) on fractures in the Hypertension in the Very Elderly Trial (HYVET). Age Ageing 2010;39:609–616.
19. Keith RA, Granger CV, Hamilton BB, et al. The functional independence measure: A tool for rehabilitation. Adv Clin Rehabil 1987;1:6–18.
20. Mahoney FI, Barthel DW. Functional evaluation: The Barthel index. Md State Med J 1965;14:61–65.
21. Alexander MP. Stroke rehabilitation outcome. A potential use of predictive variables to establish levels of care. Stroke 1994;25:128–134.
22. Masiero S, Avesani R, Armani M, et al. Predictive factors for ambulation in stroke patients in the rehabilitation setting: A multivariate analysis. Clin Neurol Neurosurg 2007;109:763–769.
23. Ng YS, Stein J, Salles SS, et al. Clinical characteristics and rehabilitation outcomes of patients with posterior cerebral artery stroke. Arch Phys Med Rehabil 2005;86:2138–2143.
24. Bagg S, Pombo AP, Hopman W. Effect of age on functional outcomes after stroke rehabilitation. Stroke 2002;33:179–185.
25. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al, European Working Group on Sarcopenia in Older People. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing 2010;39:412–423.
26. Fried LP, Tangen CM, Walston J, et al, Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: Evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001;56:M146–M156.
27. Searle SD, Mitnitski A, Gahbauer EA, et al. A standard procedure for creating a frailty index. BMC Geriatr 2008;8:24.
28. Koopman R, Ryall JG, Church JE, et al. The role of β-adrenoceptor signaling in skeletal muscle: Therapeutic implications for muscle wasting disorders. Curr Opin Clin Nutr Metab Care 2009;12:601–606.
29. Nakamura T, Mizushima T, Yamamoto M, et al. Muscle sympathetic nerve activity during isometric exercise in patients with cerebrovascular accidents. Arch Phys Med Rehabil 2005;86:436–441.
30. Astrand PO, Cuddy TE, Saltin B, et al. Cardiac output during submaximal and maximal work. J Appl Physiol 1964;19:268–274.
31. Visser M, Kritchevsky SB, Newman AB, et al. Lower serum albumin concentration and change in muscle mass: The Health, Aging and Body Composition Study. Am J Clin Nutr 2005;82:531–537.

32. Schalk BW, Deeg DJ, Penninx BW, et al. Serum albumin and muscle strength: A longitudinal study in older men and women. J Am Geriatr Soc 2005;53: 1331–1338.
33. Snyder CK, Lapidus JA, Cawthon PM, et al, Osteoporotic Fractures in Men (MrOS) Research Group. Serum albumin in relation to change in muscle mass, muscle strength, and muscle power in older men. J Am Geriatr Soc 2012;60: 1663–1672.
34. Schalk BW, Visser M, Deeg DJ, et al. Lower levels of serum albumin and total cholesterol and future decline in functional performance in older persons: The Longitudinal Aging Study Amsterdam. Age Ageing 2004;33:266–272.
35. Reuben DB, Ix JH, Greendale GA, et al. The predictive value of combined hypoalbuminemia and hypocholesterolemia in high functioning community-dwelling older persons: MacArthur Studies of Successful Aging. J Am Geriatr Soc 1999;47:402–406.
36. Pan SL, Lien IN, Chen TH. Is higher serum total cholesterol level associated with better long-term functional outcomes after noncardioembolic ischemic stroke? Arch Phys Med Rehabil 2010;91:913–918.
37. Iso H, Jacobs DR Jr, Wentworth D, et al. Serum cholesterol levels and six-year mortality from stroke in 350,977 men screened for the multiple risk factor intervention trial. N Engl J Med 1989;320:904–910.
38. Gruson KI, Aharonoff GB, Egol KA, et al. The relationship between admission hemoglobin level and outcome after hip fracture. J Orthop Trauma 2002;16: 39–44.
39. Appenzeller O. The Autonomic Nervous System. An Introduction to Basic and Clinical Concepts. 3rd ed. New York: Elsevier Biomedical Press; 1982.
40. Huikuri HV, Niemelä MJ, Ojala S, et al. Circadian rhythms of frequency domain measures of heart rate variability in healthy subjects and patients with coronary artery disease. Effects of arousal and upright posture. Circulation 1994;90: 121–126.
41. Carter WJ, Dang AQ, Faas FH, et al. Effects of clenbuterol on skeletal muscle mass, body composition, and recovery from surgical stress in senescent rats. Metabolism 1991;40:855–860.
42. Carter WJ, Lynch ME. Effect of clenbuterol on recovery of muscle mass and carcass protein content following dietary protein depletion in young and old rats. J Gerontol 1994;49:B162–B168.
43. Chen KD, Alway SE. Clenbuterol reduces soleus muscle fatigue during disuse in aged rats. Muscle Nerve 2001;24:211–222.
44. Ryall JG, Plant DR, Gregorevic P, et al. Beta 2-agonist administration reverses muscle wasting and improves muscle function in aged rats. J Physiol 2004;555: 175–188.
45. Ricart-Firinga C, Stevens L, Canu MH, et al. Effects of beta(2)-agonist clenbuterol on biochemical and contractile properties of unloaded soleus fibers of rat. Am J Physiol Cell Physiol 2000;278:C582–C588.
46. Stevens L, Firinga C, Gohlsch B, et al. Effects of unweighting and clenbuterol on myosin light and heavy chains in fast and slow muscles of rat. Am J Physiol Cell Physiol 2000;279:C1558–C1563.
47. Ryall JG, Scherzer JD, Lynch GS. Attenuation of age-related muscle wasting and weakness in rats after formoterol treatment: Therapeutic implications for sarcopenia. J Gerontol A Biol Sci Med Sci 2007;62A:813–823.
48. Fowler EG, Graves MC, Wetzel GT, et al. Pilot trial of albuterol in Duchenne and Becker muscular dystrophy. Neurology 2004;62:1006–1008.
49. Kamalakkannan G, Petrilli CM, George I, et al. Clenbuterol increases lean muscle mass but not endurance in patients with chronic heart failure. J Heart Lung Transplant 2008;27:457–461.
50. Lang TA, Secic M. How To Report Statistics in Medicine, Annotated Guidelines for Authors, Editors, and Reviewers. 2nd ed. Philadelphia: American College of Physicians; 2006. p. 87–99.
51. Dütsch M, Burger M, Dörfler C, et al. Cardiovascular autonomic function in poststroke patients. Neurology 2007;69:2249–2255.
52. Colivicchi F, Bassi A, Santini M, et al. Cardiac autonomic derangement and arrhythmias in right-sided stroke with insular involvement. Stroke 2004;35: 2094–2098.
53. Korpelainen JT, Sotaniemi KA, Huikuri HV, et al. Circadian rhythm of heart rate variability is reversibly abolished in ischemic stroke. Stroke 1997;28:2150–2154.

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RESEARCH ARTICLE

The State of Health in Older Adults in Japan: Trends in Disability, Chronic Medical Conditions and Mortality

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Data Availability Statement: Data are available from the Japanese government's official website (URL: http://www.mhlw.go.jp/english/database/db-hw/index.html; http://www.mhlw.go.jp/english/database/db-hss/ps.html; http://www.mhlw.go.jp/english/database/db-hss/cslc-index.html).

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Abstract

Both life expectancy and healthy life expectancy in Japan have been increasing and are among the highest in the world, but the gap between them has also been widening. To examine the recent trends in old age disability, chronic medical conditions and mortality in Japan, we retrospectively analyzed three nationally representative datasets: Comprehensive Survey of Living Conditions (2001–2013), Patient Survey (1996–2011) and Vital Statistics (1995–2010). We obtained the sex- and age-stratified trends in disability rate, treatment rates of nine selected chronic medical conditions (cerebrovascular diseases, joint disorders, fractures, osteoporosis, ischemic heart disease, diabetes mellitus, hypertension, pneumonia and malignant neoplasms), total mortality rate and mortality rates from specific causes (cerebrovascular diseases, heart diseases, pneumonia and malignant neoplasms) in both sexes in four age strata (65–69, 70–74, 75–79, 80–84 years). Disability rates declined significantly in both sexes. Treatment rates of all selected medical conditions also decreased significantly, except for fractures in women and pneumonia. Both total mortality rate and cause-specific mortality rates decreased in both sexes. We concluded that the recent decline in disability rates, treatment rates of chronic medical conditions and mortality rates points toward overall improvement in health conditions in adults over the age of 65 years in Japan. Nonetheless, considering the increase in the number of older adults, the absolute number of older adults with disability or chronic medical conditions will continue to increase and challenge medical and long-term care systems.

Introduction

Japan's life expectancy has increased steadily over the past several decades and is one of the highest in the world [1–3]. Despite the gradual and continuous rise in life expectancy, the definition of old age has not changed and the age of 65 has been considered the beginning of old age [3, 4]. Consequently, the increase in life expectancy, coupled with the falling birth rate, has resulted in a dramatic increase in the proportion of the "old age" population, from 14.6% in 1995 to 25.1% in 2013 [3]. The shift in demography to older ages has had a large impact on

society and the economy, which has led to discussion of the definition of old age [5], since the timing of old age is roughly equivalent to the age of retirement and receiving pension benefit.

The Survey on the Senior Citizens' Attitude toward Daily Life, conducted by the Cabinet Office of the Government of Japan in 2009, reported that the majority of respondents considered that the threshold for old age should be higher than the current one, 65 years, and more than 40% thought it should be 70 years [6]. This survey also showed that more than a quarter of the respondents answered that an even higher threshold of 75 years is appropriate for old age [6]. This may reflect the change in people's perception towards aging, and it is possible that the biological age, which involves multitudinous factors including not only elapsed time but also nutrition, living environment and medical conditions, may be going down compared with the chronological age. This hypothesis is compatible with the increase in healthy life expectancy that has occurred simultaneously with the increase in life expectancy [1, 3, 7].

Although both life expectancy and healthy life expectancy have been increasing, the gap between them has also been widening [1, 3]. In order to prevent disability and extend healthy life expectancy, a number of epidemiological studies have been conducted, and comorbid chronic medical conditions have been identified as significant risk factors for disability or the requirement for long-term care [8–13]. However, the prevalence of disability does not necessarily change in parallel with the prevalence of chronic medical conditions. Indeed, studies in some developed countries have reported an increase in the prevalence of chronic medical conditions but a stable or declining disability rate [14–17]. Therefore, examination of the trends in disability and chronic medical conditions in Japan, whose proportion of the old age population is the highest in the world, may facilitate our understanding of the relationship between chronic medical conditions and disability, and the medical and nursing care needs in the old adult population.

In this study, we aimed to characterize the trends in disability and chronic medical conditions over the age of 65 years, the current threshold for old age. We hypothesized that the prevalence of disability, along with the prevalence of chronic medical conditions, has been declining over time, consistent with increasing healthy life expectancy.

Materials and Methods

All the data analyzed in the current study are publicly available on the Japanese government's official website, and therefore ethical review is deemed not to be required [18].

We retrospectively analyzed three databases, Comprehensive Survey of Living Conditions, Patient Survey, and Vital Statistics, all of which are conducted by the Ministry of Health, Labour and Welfare and whose details and tabulated data are available on the website of the Statistics Bureau, Ministry of Internal Affairs and Communications [2, 19, 20].

The Comprehensive Survey of Living Conditions is a series of cross-sectional national surveys on a random stratified sample of households and their members [20]. A long-term care questionnaire has been administered every three years starting from 2001, covering persons requiring long-term care (approximately 6000 persons) in 2,500 districts from the National Census. Results from the long-term care questionnaire include the rate of persons certified for long-term care under the Long-Term Care Insurance System per 100,000 population (hereafter referred to as the disability rate), which is approximately equivalent to the prevalence of disability.

The Patient Survey is a series of cross-sectional national surveys on a random sample of medical institutions (including hospitals and outpatient clinics) [19]. All hospitals with more than 500 beds were included in the surveys. Data from medical institutions in Fukushima prefecture and the Ishinomaki and Kesennnuma medical areas of Miyagi prefecture were not

included in the 2011 survey data due to the Tohoku earthquake and tsunami on March 11th, 2011. The surveys were conducted on one designated date set for each medical institution from three days in October. Physicians filled out the questionnaire and collected information on patients who attended the participating medical institutions on the date of the survey. Approximately 8 million patients were included in each survey. The International Statistical Classification of Diseases and Related Health Problems, ninth Revision (ICD-9), published by the World Health Organization, was applied to classify diseases and injuries in the surveys until 1996, and the ICD-10 thereafter, which prevented direct comparison of the data prior to and after 1996.

For each disease or injury, the rate of estimated number of patients per 100,000 population (hereafter referred to as the treatment rate) was calculated as the estimated number of patients divided by the estimated population x 100,000. The estimated number of patients who continuously received medical care was calculated using the following formula [19]:

Estimated number of patients receiving medical treatment = Estimated number of inpatients + Estimated number of initial visit outpatients + (Estimated number of return visit outpatients × Average interval since last visit × Adjustment factor (6/7))

Therefore, the estimated number of patients included those who did not receive medical care at medical institutions on the date of the survey, and the treatment rate can be considered a rough approximation of the prevalence.

Our analysis focused on the trends in the prevalence of medical conditions that can cause disability and lower healthy life expectancy. The Comprehensive Survey of Living Conditions in 2013 reported that the most common cause of disability was cerebrovascular accident, followed by dementia, frailty due to aging, joint disorders, bone fracture and cardiac disease [20]. These six categories accounted for more than 70% of causes of disability. In the current analysis, we chose to investigate cerebrovascular diseases, osteoarthritis, inflammatory polyarthropathies, fractures, osteoporosis, ischemic heart disease, diabetes mellitus, hypertension, pneumonia and malignant neoplasms based on their clinical significance, their potential to cause disability, and the availability of data. Osteoarthritis and inflammatory polyarthropathies were combined into a single category, joint disorders. We initially planned to investigate Alzheimer's disease as well, but our preliminary analysis suggested that the treatment rate had been much lower than the prevalence previously reported [21] despite recent increase in the treatment rate, presumably because of under-diagnosis (S1 Fig and S1 Table) [22]. We therefore decided not to include Alzheimer's disease in our analysis because our primary aim was to examine the actual prevalence of chronic medical conditions.

Vital Statistics is based on the Family Registry, and collects data on birth, marriage and death registrations [2]. The data on total mortality rate and mortality rates from specific causes (cerebrovascular diseases, heart diseases, pneumonia and malignant neoplasms) were obtained from Vital Statistics. The cause of death was obtained from the death certificate issued by physicians, and classified using ICD-9 until 1995, and ICD-10 thereafter, and therefore we included the data after 1995 in our analysis.

Statistical Analysis

We stratified the data by sex and examined the trends in disability rate, treatment rates of selected medical conditions and mortality rates in the four 5-year age strata over the age of 65 years (65–69, 70–74, 75–79 and 80–84 years). The overall increasing or decreasing trend in the rates in each sex was evaluated by the linear trend test using PROC GLM of SAS (SAS Institute, Inc., Cary, NC, USA) adjusted for the age groups. Provided the initial trend test was statistically significant, linear regression was performed to evaluate the trend in each age stratum. Two-sided $p<0.05$ was considered statistically significant.

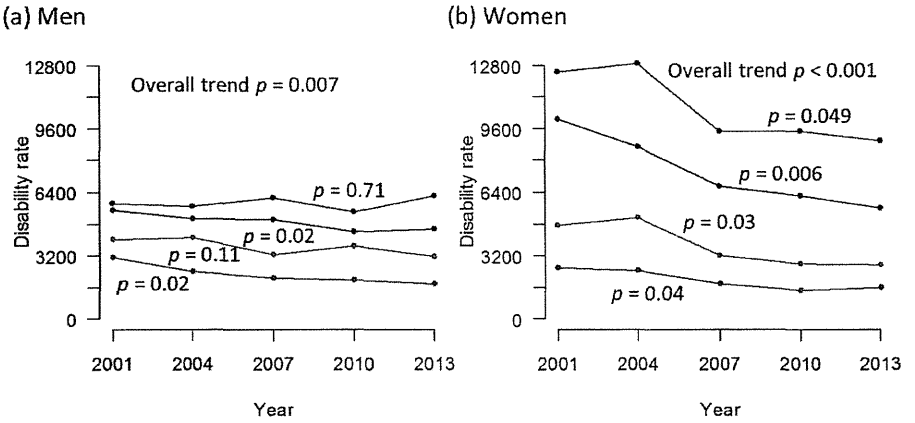

Fig 1. Trends in disability rate in men and women from 2001 to 2013. (a) men. (b) women. The disability rate is the rate of persons certified for long-term care under the Long-Term Care Insurance System per 100,000 population. The black line represents those aged 80–84 years, the blue line represents those aged 75–79 years, the green line represents those aged 70–74 years and the red line represents those aged 65–69 years. The p values signify statistical significance for the trends in each age stratum.

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Results

Trends in Disability

The trends in the disability rates from 2001 to 2013 are displayed in Fig 1 (Tabulated data available in S2 Table). The overall trend was downward and statistically significant in each sex. The trend in each age stratum was also statistically significant except for men aged 70–74 and 80–84 years.

Trends in Chronic Medical Conditions

Figs 2 and 3 show the trends in the treatment rates for the nine selected medical conditions from 1996 to 2011 (Tabulated data available in S3 Table). The overall treatment rate declined significantly for all medical conditions in each sex, except for fractures in women and pneumonia.

For cerebrovascular diseases, ischemic heart disease and osteoporosis, the downward trend was statistically significant for all age strata (65–69, 70–74, 75–79 and 80–84) in each sex.

For diabetes mellitus and hypertension, the treatment rate decreased over time with statistical significance for all age strata in women, but in men the downward trend was statistically significant in two younger age strata only (65–69 and 70–74).

For fractures and malignant neoplasms, the downward trend was statistically significant in two younger age strata of 65–69 and 70–74 years in men. In women, statistical significance was observed only in the age stratum of 70–74 years for malignant neoplasms.

For joint disorders, the downward trend was statistically significant in two younger age strata of 65–69 and 70–74 years in each sex, but the significance decline was not observed in older age strata except for the age stratum of 75–79 years in men.

Trends in Mortality

Figs 4 and 5 show the trends in total mortality rate and mortality rates from specific causes from 1995 to 2010 (Tabulated data available in S4 Table). Both total mortality rate and cause-specific mortality rates declined significantly in all age strata in both sexes, except for mortality

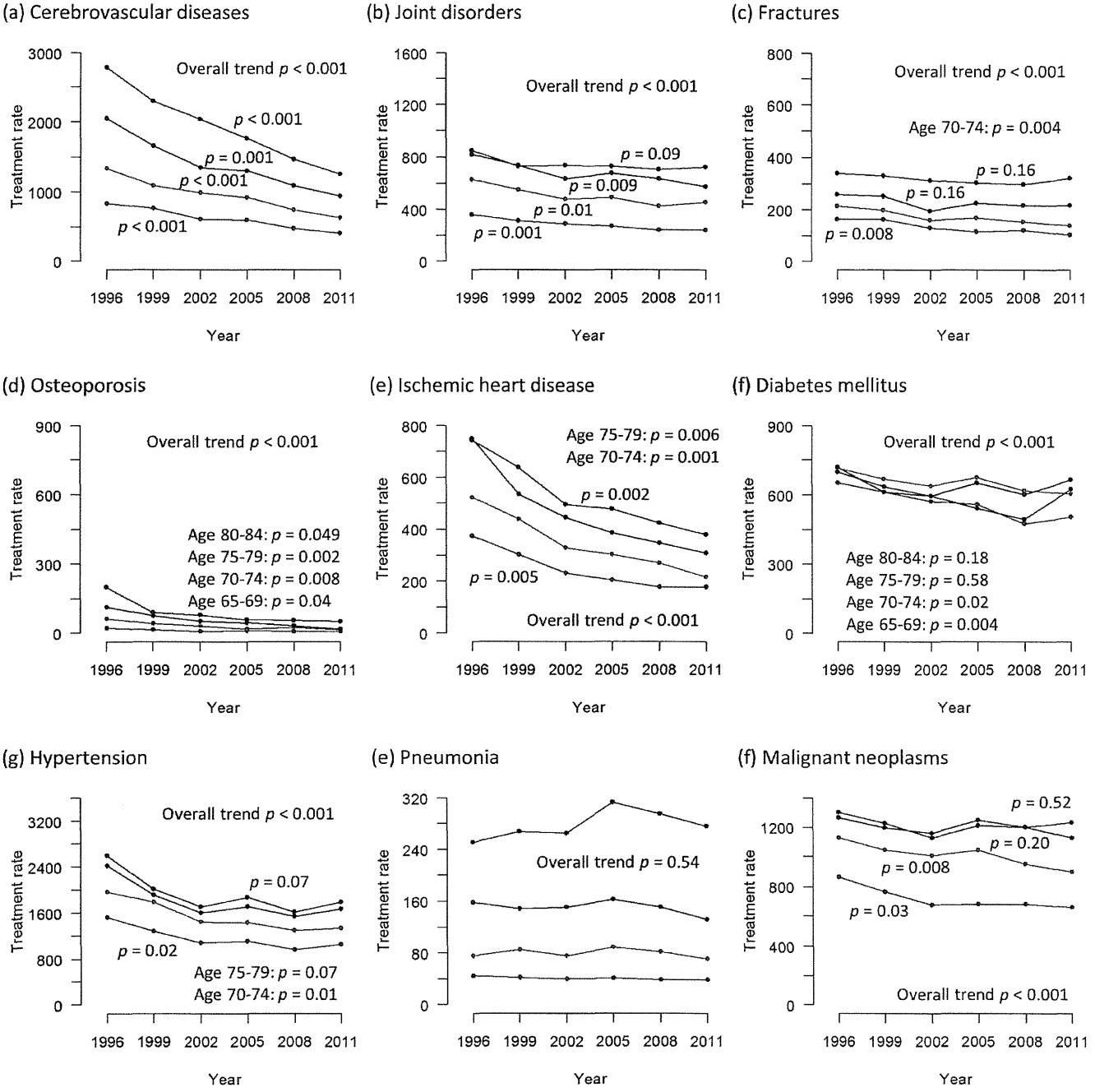

Fig 2. Trends in treatment rates of nine selected medical conditions in men from 1996 to 2011. (a) cerebrovascular diseases (b) joint disorders (c) fractures (d) osteoporosis (e) ischemic heart disease (f) diabetes mellitus (g) hypertension (h) pneumonia (i) malignant neoplasms The treatment rate is calculated as the estimated number of patients divided by the estimated population x 100,000. The black line represents those aged 80–84 years, the blue line represents those aged 75–79 years, the green line represents those aged 70–74 years and the red line represents those aged 65–69 years. The p values signify statistical significance for the trends in each age stratum.

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rate from malignant neoplasms in the age stratum of 70–74 years and heart diseases in the age stratum of 80–84 years in men in which the change was not statistically significant.

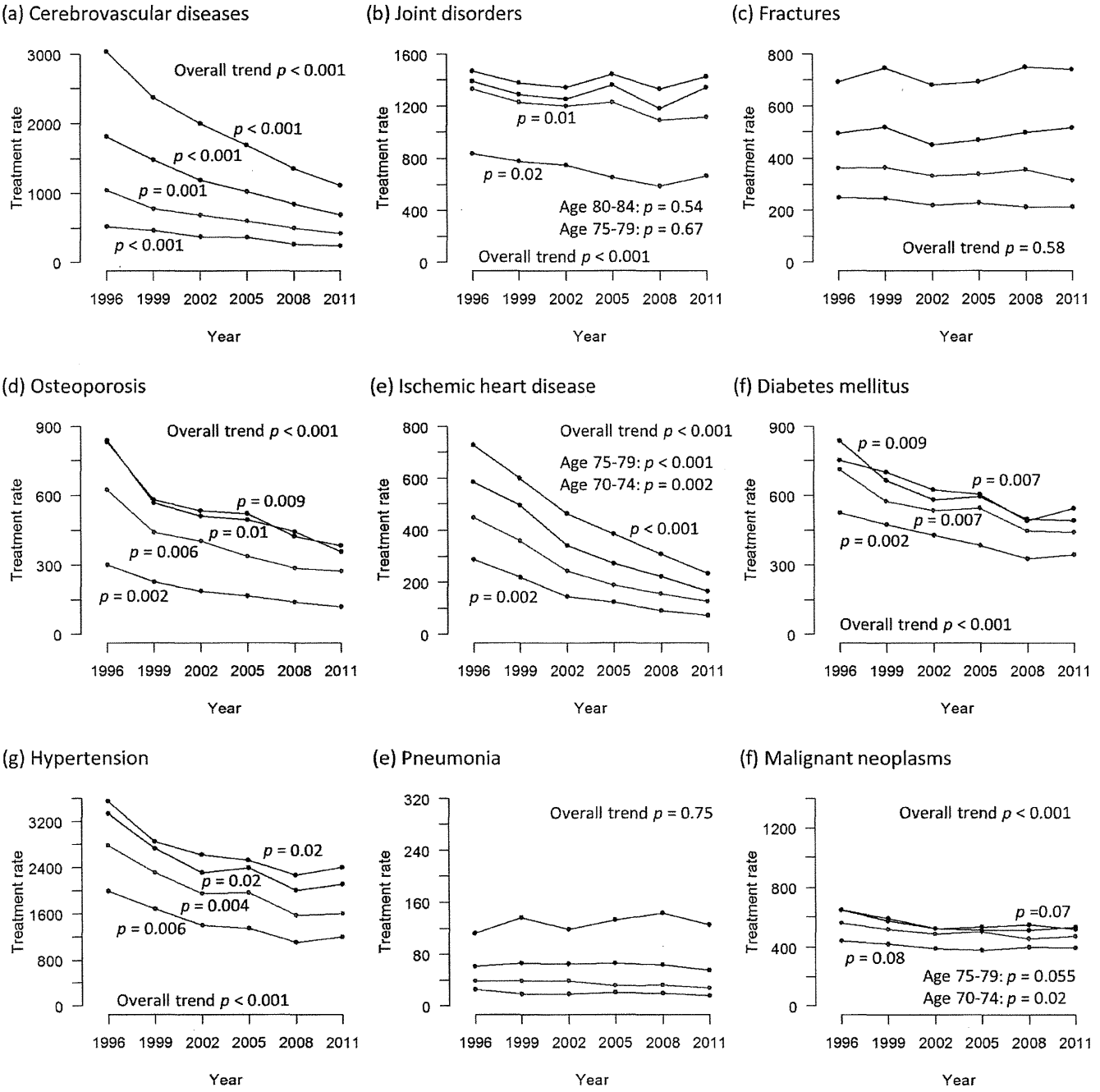

Fig 3. Trends in treatment rates of nine selected medical conditions in women from 1996 to 2011. (a) cerebrovascular diseases (b) joint disorders (c) fractures (d) osteoporosis (e) ischemic heart disease (f) diabetes mellitus (g) hypertension (h) pneumonia (i) malignant neoplasms The treatment rate is calculated as the estimated number of patients divided by the estimated population x 100,000. The black line represents those aged 80–84 years, the blue line represents those aged 75–79 years, the green line represents those aged 70–74 years and the red line represents those aged 65–69 years. The p values signify statistical significance for the trends in each age stratum.

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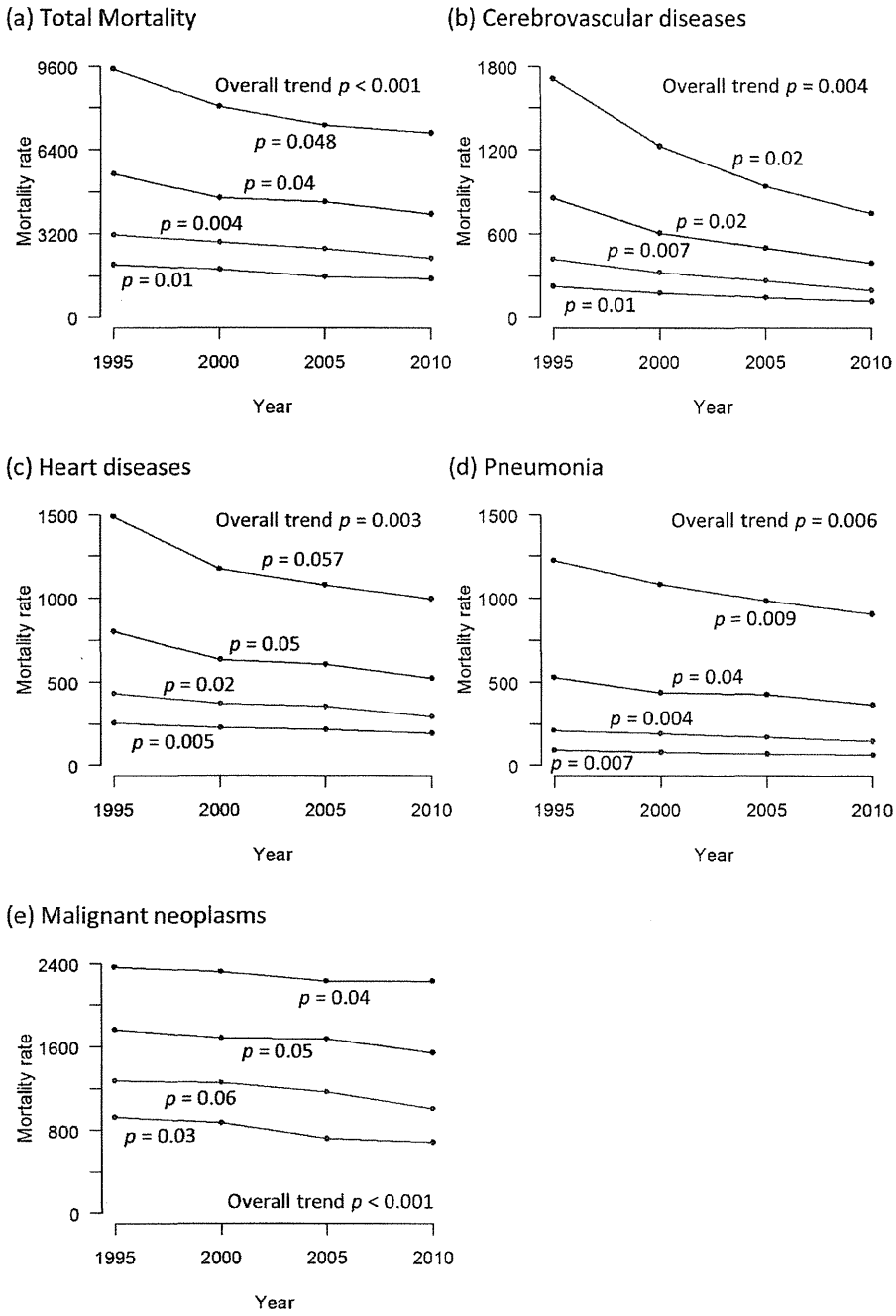

Fig 4. Trends in total mortality rate and mortality rates from specific causes in men from 1995 to 2010. (a) total mortality rate. Mortality rate from (b) cerebrovascular diseases (c) heart diseases (d) pneumonia (e) malignant neoplasms. The mortality rate is calculated as the number of deceased divided by the estimated population x 100,000. The black line represents those aged 80–84 years, the blue line represents those aged 75–79 years, the green line represents those aged 70–74 years and the red line represents those aged 65–69 years. The p values signify statistical significance for the trends in each age stratum.

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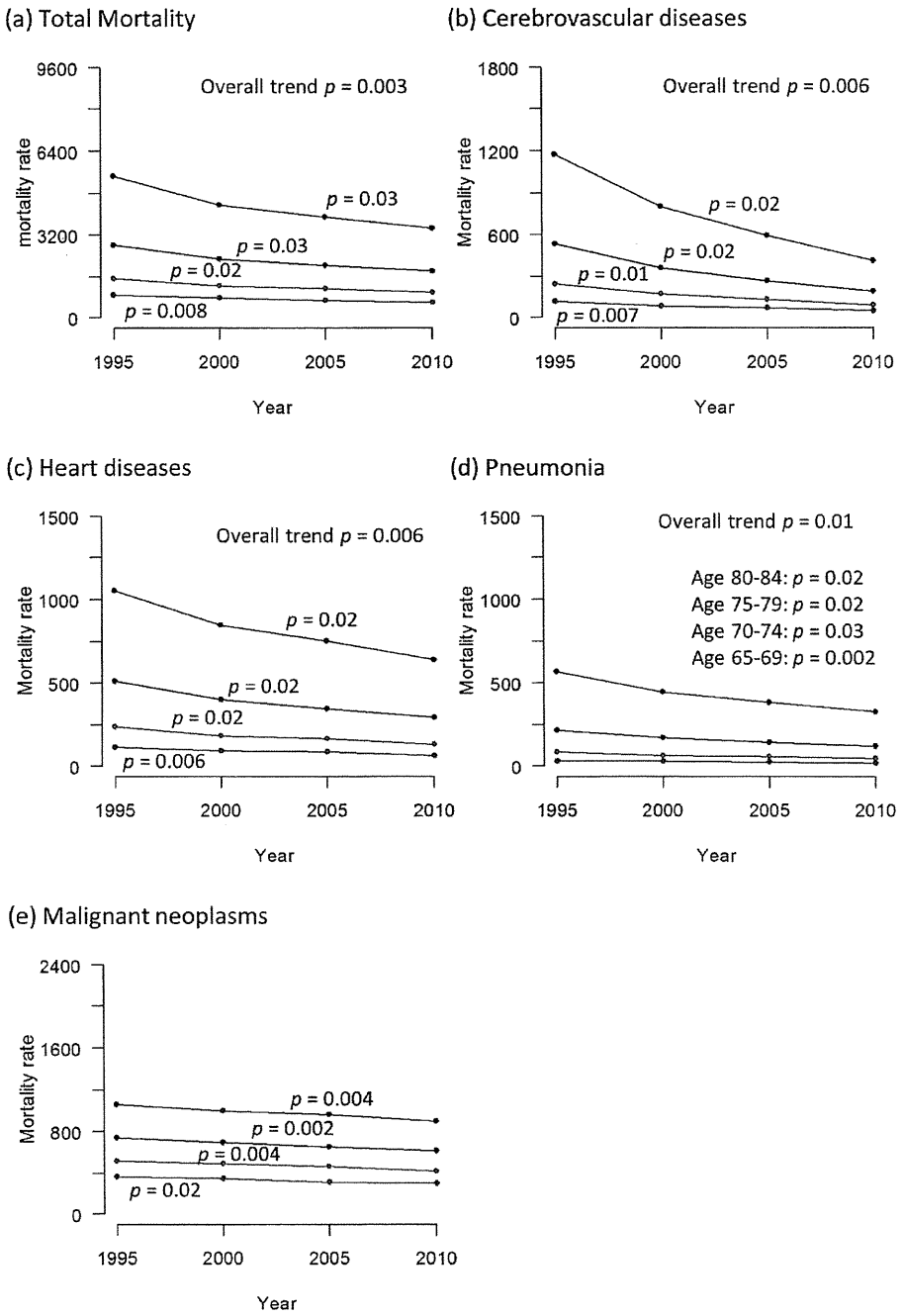

Fig 5. Trends in total mortality rate and mortality rates from specific causes in women from 1995 to 2010. (a) total mortality rate. Mortality rate from (b) cerebrovascular diseases (c) heart diseases (d) pneumonia (e) malignant neoplasms. The mortality rate is calculated as the number of deceased divided by the estimated population x 100,000. The black line represents those aged 80–84 years, the blue line represents those aged 75–79 years, the green line represents those aged 70–74 years and the red line represents those aged 65–69 years. The p values signify statistical significance for the trends in each age stratum.

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Discussion

In the analysis of nationally representative datasets, we demonstrated that the disability rate and mortality rate declined significantly over time in old adults aged between 65 and 84 in Japan. We also observed a decrease in treatment rates of many chronic medical conditions over approximately the same time period. This finding suggests a decline in the prevalence of these conditions, which is in concordance with the decline in disability rate. The concurrent decrease in mortality implies that the decrease in treatment rates is not attributable to attrition of sicker persons or survival bias. Hence, combined together, our findings indicate overall improvement in health conditions among adults entering "old age"; that is, people reaching the age of 65 years have recently enjoyed better health compared to preceding cohorts.

Among conditions whose treatment rates declined, three medical conditions, namely cerebrovascular diseases, ischemic heart disease and osteoporosis, showed a particularly substantial decline. The treatment rates of these medical conditions in each age group in 2011 were roughly equivalent to or even lower than the treatment rates of the group 5 years younger in 1996, and were consistent in each sex. Public health organizations have made efforts to increase people's awareness of the importance of a healthy lifestyle and avoiding major risk factors for these medical conditions, such as smoking, hypertension and diabetes. These efforts have gradually altered people's behavior. The smoking rate was 44.7% for men over 60 and 7.8% for women over 60 in 1996, but in 2011 it fell to 23.9% and 6.4%, respectively [23]. People, particularly those older than 60, exercise more than previously [23]. The Specific Health Checkup, which focuses on screening for metabolic syndrome and lifestyle-related diseases, was introduced in 2008 against a backdrop of increased awareness of such medical conditions, and led to early screening, diagnosis and treatment [24]. In addition, advances in medical technology, medication and care and an improving standard of living have helped facilitate prevention and management of these conditions. It should be noted that cerebrovascular diseases and ischemic heart disease are important causes of both mortality and disability.

Interestingly, the change in the overall treatment rate for fracture in women was not statistically significant, whereas the treatment rate for osteoporosis declined significantly. The observed disparity between the treatment rates of fractures and osteoporosis may be due to limited predictive ability of bone mineral density measurements, which have been widely used to diagnose osteoporosis. It is true that fracture is more likely to occur when bone mineral density is lower, but multiple factors play a role in determining fracture risk, and bone mineral density alone accounts for only 1.7 to 7.4 percent of fracture risk [25, 26]. Another possible explanation for the disparity may be under-diagnosis of osteoporosis. In primary care settings, osteoporosis is often undiagnosed and untreated. However, health checkups for old adults in Japan include screening for osteoporosis, and it is unlikely that the diagnostic rate of osteoporosis has significantly changed recently. The time gap between osteoporosis and fractures may also help explain the disparity. People tend to suffer fracture at an older age than the age when they receive a diagnosis of osteoporosis. Therefore, even though the treatment rate for osteoporosis has declined, the effects on the treatment rate of fracture may be delayed and take some more years to be observable.

The treatment rates for diabetes mellitus and hypertension declined significantly for all age strata in women, but the improvement in men was mostly restricted to those younger than 75 years. The reason for this apparent disparity between men and women is not clear, but it may reflect sex differences in age-related changes or a cohort effect. Those older than 75 years experienced the Second World War in their childhood when chronic malnutrition was widespread and started their occupational career in the period of rapid growth in 1960s when men were expected to earn their livings and women to stay home and raise a family.