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Original Contribution

Association Between Serum β-Alanine and Risk of Dementia

The Hisayama Study

Jun Hata*, Tomoyuki Ohara, Yoshinori Katakura, Kuniyoshi Shimizu, Shuntaro Yamashita, Daigo Yoshida, Takanori Honda, Yoichiro Hirakawa, Mao Shibata, Satoko Sakata, Takanari Kitazono, Satoru Kuhara, and Toshiharu Ninomiya

* Correspondence to Dr. Jun Hata, Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan (e-mail: junhata@eph.med.kyushu-u.ac.jp).

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We examined the association between serum concentrations of β-alanine, a metabolite of carnosine and anserine, and the risk of dementia in a general population of elderly Japanese persons. In 2007, 1,475 residents of Hisayama, Japan, aged 60–79 years and without dementia were divided into 4 groups according to quartiles of serum β-alanine concentrations (quartile 1, lowest; quartile 4, highest) and followed for a median of 5.3 years. During follow-up, 117 subjects developed all-cause dementia (Alzheimer in 77 cases and vascular dementia in 31). The risk of all-cause dementia decreased with increasing serum β-alanine levels after adjustment for potential confounding factors (quartile 2, hazard ratio (HR) = 0.73 (95% confidence interval (CI): 0.45, 1.18); quartile 3, HR = 0.50 (95% CI: 0.28, 0.89); quartile 4, HR = 0.50 (95% CI: 0.27, 0.92); $P = 0.01$ for trend). A similar inverse association was observed for Alzheimer disease (quartile 2, HR = 0.78 (95% CI: 0.44, 1.38); quartile 3, HR = 0.53 (95% CI: 0.26, 1.06); quartile 4, HR = 0.53 (95% CI: 0.25, 1.10); $P = 0.04$ for trend) but not for vascular dementia. We found that higher serum β-alanine levels were significantly associated with lower risks of all-cause dementia and Alzheimer disease. Because serum β-alanine levels reflect intakes of carnosine/anserine, higher intakes of carnosine/anserine might be beneficial for the prevention of dementia.

β-alanine; Alzheimer disease; cohort study; dementia; imidazole dipeptides

Abbreviations: AD, Alzheimer disease; MMSE, Mini-Mental State Examination; VaD, vascular dementia.

Dementia is one of the major causes of disability and mortality in the elderly (1). The prevalence and incidence of dementia have been increasing in Japan (2), and the medical and economic burden of dementia on society is a serious problem. However, the causes of dementia, especially Alzheimer disease (AD), remain unclear. Therefore, it is important to identify risk factors or protective factors in order to reduce the burden of dementia.

Growing epidemiologic evidence suggests that some modifiable risk factors—namely, diabetes (3), hypertension (4), cigarette smoking (5), and physical inactivity (6)—are associated with increased risk of dementia. In addition, dietary or nutritional factors are considered possible protective factors against dementia (7–10), and some nutraceuticals have been developed for the prevention and treatment of dementia (10). Recent studies have shown that carnosine and anserine, which are imidazole dipeptides contained in the skeletal muscles and brain, have some biological functions, including antioxidant, antiglycation, and antiinflammatory activities (11). The content of these imidazole dipeptides has been positively linked with muscle buffering capacity and performance capacity (12). Moreover, supplementation with these dipeptides has been reported to have a beneficial effect on cognitive function in humans (13, 14) and in mice (15). However, the influence of these dipeptides on cognitive function has not been fully investigated in a general population.

Carnosine and anserine are rapidly cleaved into β-alanine and histidine/methyl-histidine after intake. Thus, serum carnosine/anserine levels become undetectable within 6 hours after the intake of these dipeptides, while serum β-alanine levels increase

and are stable for more than 24 hours (16, 17). In addition, carnosine and anserine are also endogenously synthesized from β-alanine and histidine/methyl-histidine. Moreover, several clinical studies have revealed that supplementation with β-alanine increases muscle carnosine/anserine concentrations and improves performance capacity (18, 19). Therefore, serum β-alanine levels are a possible index of the amount of carnosine/anserine in the body.

The aim of the present study was to elucidate whether increased concentrations of serum β-alanine (as a prespecified surrogate marker of the amount of carnosine/anserine in the body) were associated with a lower risk of dementia using a prospective longitudinal data set from a general elderly population of Japanese.

METHODS

Study participants

The Hisayama Study is a prospective cohort study of cerebrocardiovascular diseases that was begun in 1961 in the town of Hisayama, a suburban community of the Fukuoka metropolitan area on Kyushu Island, Japan. Health examinations for residents have been repeated every year since 1961 (20). In addition, dementia-screening surveys for the elderly residents have been repeated every 6–7 years (1985, 1992, 1998, 2005, 2012), and a follow-up survey of dementia was performed in the town (2). In 2007, a total of 1,560 residents aged 60–79 years underwent a health examination (participation rate, 85.5%). After exclusion of 6 individuals who refused to participate in the epidemiologic research, 46 with dementia at baseline, 19 with no measurement of serum β-alanine concentration, and 14 for whom fasting blood samples were lacking, the remaining 1,475 residents (655 men and 820 women) were enrolled in the present study. The study was approved by the Kyushu University Institutional Review Board for Clinical Research, and written informed consent was obtained from all subjects.

Quantitation of serum β-alanine concentration

At the screening examination, portions of the serum specimens were stored at −80°C until used for the measurements of β-alanine by liquid-chromatograph mass spectrometry in 2017. Sera mixed with 5% sulfosalicylic acid at a ratio of 1:1 were applied to an Ostro Protein Precipitation and Phospholipid Removal Plate (Waters, Milford, Massachusetts) and eluted with 0.2% trifluoroacetic acid/acetonitrile according to the manufacturer's instructions (21, 22). All solvents used below (acetonitrile, formic acid, 1 mol/L ammonium formate solution) were purchased from Wako Pure Chemical (Osaka, Japan) and were liquid-chromatograph mass spectrometry grade. Chromatography was performed on an Agilent 6495 Triple Quadrupole LC/MS System (Agilent Technologies, Santa Clara, California). A 1.0-μL aliquot of the extracted serum sample was injected onto an Intrada Amino Acid column (50 × 3 mm; Imtakt, Kyoto, Japan). The gradient elution buffers were A (0.1% formic acid/acetonitrile) and B (100 mM ammonium formate), and the flow rate was 0.6 mL/minute. The elution gradient (A:B, v/v) was as follows: initial conditions A:B = 86:14 for 3 minutes, followed by a linear gradient to 100% B over 7 minutes, a further 5 minutes in 100% B, and finally a switch back to the initial conditions and re-equilibration for 5 minutes. β-alanine was analyzed in the positive ion mode and detected by scheduled selective reaction monitoring. Data were acquired using MassHunter Workstation Software, version B.08.00 (Agilent). Quantitative values were obtained by relating chromatographic peak areas to those derived from external stable isotope-labeled internal standard β-alanine (0.1 μg/mL, 490822; Merck, Kenilworth, New Jersey). The coefficient of variation for the serum β-alanine concentration from 100 repeated measurements by this method was 0.083. The study participants were divided into 4 groups according to quartiles of serum β-alanine levels (quartile 1, 0.29–0.76 μmol/L; quartile 2, 0.77–0.96 μmol/L; quartile 3, 0.97–1.22 μmol/L; quartile 4, 1.23–8.34 μmol/L).

Other baseline variables

Blood pressure was measured 3 times in a sitting position using an automated sphygmomanometer after a 5-minute rest, and the mean of the 3 measurements was used for the analysis. Hypertension was defined as blood pressure ≥140/90 mm Hg or use of antihypertensive medications. Plasma glucose concentrations were measured by the hexokinase method. Diabetes mellitus was defined as fasting plasma glucose ≥7.0 mmol/L, 2-hour postload or postprandial plasma glucose ≥11.1 mmol/L, or use of antidiabetic medications. Serum total cholesterol concentrations were measured enzymatically. Body height and weight were measured in light clothing without shoes, and body mass index was calculated. Obesity was defined as body mass index ≥25.0 (calculated as weight (kg)/height $(m)^2$). History of stroke was defined as any preexisting event of symptomatic stroke including ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage. All stroke events were adjudicated on the basis of physical examinations and a review of all available clinical information, including medical records and imaging as described previously (20). Information on educational levels, smoking habits, alcohol intake, physical activity, and medication for hypertension and diabetes was obtained using a standardized questionnaire. A low educational level was defined as ≤9 years of formal education. Smoking and drinking habits were categorized as current use or not. The subjects engaging in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group. Total energy intake per day was estimated using a brief self-administered diet history questionnaire (23). Participants who needed someone's help to walk or who needed a wheelchair or a stretcher to move were categorized as having physical disability. Cognitive function based on the Mini-Mental State Examination (MMSE) (24) was evaluated in the dementia-screening survey in 2005 (i.e., approximately 2 years before baseline) for 806 participants.

Follow-up survey

The subjects were followed prospectively from when they underwent a baseline examination in 2007 to November 2012. Details of the follow-up survey on dementia were

published previously (2, 25). In brief, information about new events of dementia, stroke, and death was collected through a daily monitoring system established by the study team, local physicians, and members of the town's Health and Welfare Office. In this system, the physicians in the study team visited clinics, hospitals, and the town's office regularly to collect information on events of dementia and stroke, including suspected cases. Regular health examinations, including physical and neurological examinations, were also repeated every year to obtain information on new events of dementia, stroke, and death. Health information was checked annually by letter or telephone for any subjects who did not undergo regular examination or who had moved away from town. In addition, comprehensive assessment of cognitive function, including neuropsychological tests such as the MMSE (24) and the Hasegawa Dementia Scale, revised version (26), were conducted in 2012 to precisely detect dementia cases to the greatest extent possible. A total of 1,343 subjects (91.1%) participated in this assessment. When a subject was suspected of having new neurological symptoms, including cognitive impairment, he or she was carefully evaluated by the study team. This team, which consisted of stroke physicians and psychiatrists, conducted various investigations, including physical and neurological examinations, interviews of the family and attending physician, and a review of the clinical records. In addition, when a subject died, we reviewed all the available clinical information, interviewed the attending physician and the family of the deceased, and tried to obtain permission for autopsy from the family. During the follow-up period, 80 subjects died, of whom 51 underwent autopsy examination. Except for the deceased individuals, no subject was lost to follow-up through November 2012.

Study outcomes

The primary outcome for the present study was all-cause dementia and the secondary outcomes included 2 major subtypes of dementia: AD and vascular dementia (VaD). The guidelines of the *Diagnostic and Statistical Manual of Mental Disorders*, *Third Edition*, *Revised* (27), were used to define the diagnosis of dementia. The criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (28) were used to define subjects with AD. The criteria of the National Institute of Neurological Disorders and Stroke–Association International pour la Recherche et l'Enseignement en Neurosciences (29) were used to make a diagnosis of VaD. Every dementia case was adjudicated by expert stroke physicians and psychiatrists. The diagnosis of possible or probable dementia subtypes was based on clinical information and morphological examination from neuroimaging. Definite dementia subtypes were also decided on the basis of clinical and neuropathological information in subjects with dementia who underwent autopsy. The diagnostic procedure for autopsy cases was reported previously (30).

The other secondary outcomes for the present study included all-cause mortality, changes in body weight, and development of physical disability during the follow-up. Mortality events were collected through the above-mentioned daily monitoring system by the study team until November 2012. Body weight was measured at the baseline (in 2007) and follow-up (in 2012) examinations for 1,257 participants. Body weight change (%) was defined as ((body weight (kg) in 2012) – (body weight (kg) in 2007)) / (body weight (kg) in 2007) × 100. Development of physical disability was defined when a participant without physical disability at baseline had physical disability in the follow-up examination in 2012. After excluding the subjects who already had physical disability at baseline and the subjects without enough information on physical function, 1,241 participants were analyzed for this outcome.

Statistical analysis

Age- and sex-adjusted mean values or frequencies of risk factors across the serum β-alanine quartiles were estimated and tested by linear or logistic regression analysis, respectively. The cumulative incidence of dementia across the β-alanine levels was calculated by the Kaplan-Meier method and compared by the log-rank test. The Cox proportional hazards model was used to estimate the age- and sex-adjusted and multivariable-adjusted hazard ratios and their 95% confidence intervals for dementia and death among the β-alanine categories. The multivariable-adjusted model included age, sex, educational level, systolic blood pressure, antihypertensive medication, diabetes, serum total cholesterol, body mass index, history of stroke, current smoking, current drinking, regular exercise, and total energy intake. In addition, the following sensitivity analyses were performed for the estimation of dementia risk: an analysis using Fine and Gray's model for the adjustment of competing risk of death, an analysis after the exclusion of participants with cognitive impairment (MMSE score ≤24) or missing MMSE data in 2005, and an analysis after the exclusion of subjects who developed dementia during the initial 2 years of follow-up. The heterogeneity in the influence of the β-alanine levels on dementia among the subgroups (age, sex, hypertension, diabetes, and obesity) was evaluated by adding interaction terms to the relevant statistical model. The age- and sex-adjusted body weight changes over 5 years were compared by the analysis of covariance. The age- and sex-adjusted odds ratios and their 95% confidence intervals for the development of physical disability over 5 years were analyzed by a logistic regression. All statistical analyses were performed with SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina). Two-sided values of $P < 0.05$ were considered statistically significant.

RESULTS

Figure 1 presents the histogram of serum β-alanine concentrations. The distribution of serum β-alanine concentrations was right-skewed. The baseline characteristics of the study population according to the quartiles of serum β-alanine concentrations are shown in Table 1. The proportion of men, the prevalence of hypertension, obesity, and current drinking increased significantly, and the mean age decreased significantly, with increasing serum β-alanine levels.

During the follow-up (median, 5.3 years), 117 subjects developed all-cause dementia. Among them, 113 subjects (97%) underwent some type of morphological examination

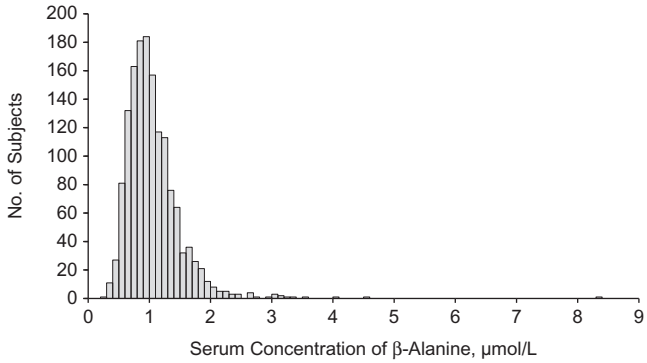

Figure 1. Distribution of serum β-alanine concentrations, the Hisayama Study, Japan, 2007. Median of serum β-alanine concentration, 0.97 (interquartile range, 0.77–1.23) μmol/L.

for dementia (8 received both brain autopsy and brain imaging, and 105 underwent brain imaging alone). Among dementia cases, 5 cases were a mixed type of AD and VaD and counted as events in the analysis for each subtype. Taking these results together, 77 subjects experienced AD and 31 experienced VaD. Figure 2 and Web Tables 1–3 (available at https://academic.oup.com/aje) show the cumulative risk of all-cause dementia and its subtypes according to the serum β-alanine quartiles. The 5-year risk of all-cause dementia decreased with increasing serum β-alanine levels (quartile 1, 9.9%; quartile 2, 8.6%; quartile 3, 4.9%; quartile 4, 4.7%; log rank $P = 0.006$; Web Table 1). A similar association was observed for AD (quartile 1, 6.8%; quartile 2, 5.5%; quartile 3, 3.5%; quartile 4, 2.5%; log rank $P = 0.02$; Web Table 2). In contrast, there was no clear association between serum β-alanine levels and the risk of VaD (quartile 1, 1.8%; quartile 2, 2.4%; quartile 3, 1.5%; quartile 4, 2.0%; log rank $P = 0.64$; Web Table 3).

Table 2 shows the hazard ratios for the development of each outcome across the serum β-alanine levels. The age- and sex-adjusted hazard ratios for all-cause dementia and AD decreased with increased β-alanine levels (P for trend < 0.05 for both). The inverse association between serum β-alanine levels and the risks of all-cause dementia and AD remained significant even after adjustment for potential confounding factors (P for trend < 0.05 for both). However, serum β-alanine levels were not associated with the risk of VaD. A sensitivity analysis with

Table 1. Baseline Characteristics According to Quartiles of Serum β-Alanine Concentration[a], Age- and Sex-Adjusted[b], the Hisayama Study, Japan, 2007

Variable	Serum β-Alanine Level								*P* for Trend
	Quartile 1 ($n = 353$)		Quartile 2 ($n = 385$)		Quartile 3 ($n = 358$)		Quartile 4 ($n = 379$)		
	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%	Mean (SE)	%	
Age, years[c]	69 (0.3)		69 (0.3)		69 (0.3)		68 (0.3)		0.045
Men[d]		15.9		33.5		53.9		73.1	<0.001
Education, ≤9 years		37.3		43.2		40.6		40.0	0.69
Hypertension		53.2		58.0		57.4		69.3	<0.001
Systolic blood pressure, mm Hg	132 (1.0)		134 (0.9)		134 (0.9)		136 (1.0)		0.009
Diastolic blood pressure, mm Hg	79 (0.5)		80 (0.5)		80 (0.5)		81 (0.5)		0.01
Antihypertensive medication		40.2		41.7		36.0		48.6	0.13
Diabetes		18.7		24.2		19.3		22.4	0.58
Serum total cholesterol, mmol/L	5.51 (0.05)		5.37 (0.04)		5.39 (0.04)		5.40 (0.05)		0.16
Obesity		23.4		20.5		29.1		40.1	<0.001
Body mass index[e]	22.9 (0.18)		22.7 (0.17)		23.4 (0.17)		24.1 (0.18)		<0.001
History of stroke		3.1		6.3		2.6		6.4	0.25
Current smoking		9.6		11.5		9.6		8.9	0.40
Current drinking		34.7		45.4		47.7		46.4	0.01
Regular exercise		14.6		12.1		13.4		15.7	0.54
Total energy intake, kcal/day	1,855 (30)		1,840 (27)		1,854 (28)		1,818 (29)		0.48
MMSE score of ≤24[f]		11.3		7.6		8.9		5.8	0.12

Abbreviations: MMSE, Mini-Mental State Examination; SE, standard error.

[a] Range of serum β-alanine concentrations: quartile 1, 0.29–0.76 μmol/L; quartile 2, 0.77–0.96 μmol/L; quartile 3, 0.97–1.22 μmol/L; quartile 4, 1.23–8.34 μmol/L.

[b] Values are shown as age- and sex-adjusted mean (SE) or percentages.

[c] Adjusted for sex.

[d] Adjusted for age.

[e] Calculated as weight (kg)/height $(m)^2$.

[f] MMSE was administered in 2005 (approximately 2 years before baseline) to 806 participants.

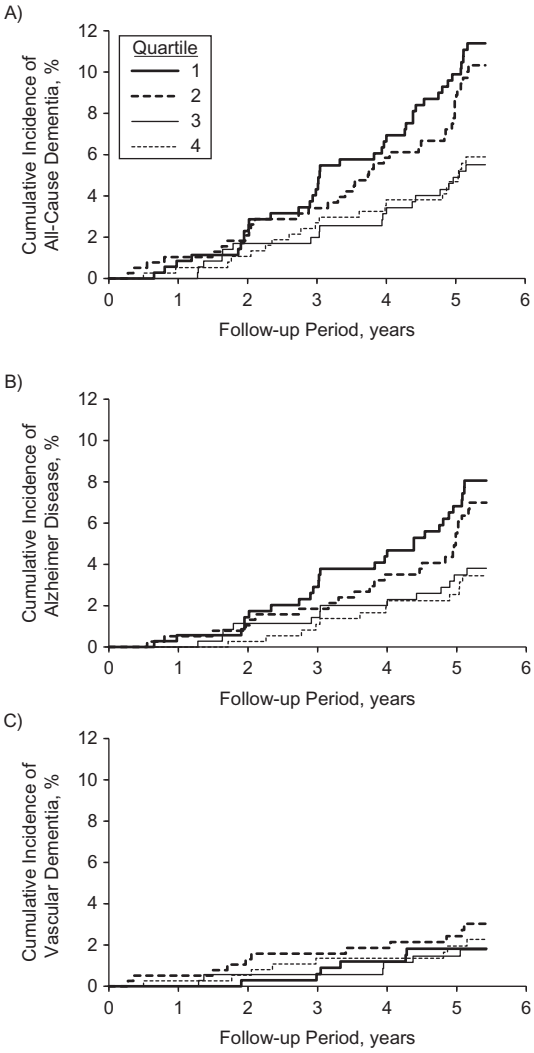

Figure 2. Cumulative incidences of dementia according to the quartiles of serum β-alanine concentrations, the Hisayama Study, Japan, 2007–2012. A) All-cause dementia (log-rank $P = 0.006$); B) Alzheimer disease (log-rank $P = 0.02$); C) vascular dementia (log-rank $P = 0.64$). Range of serum β-alanine concentrations: quartile 1, 0.29–0.76 μmol/L; quartile 2, 0.77–0.96 μmol/L; quartile 3, 0.97–1.22 μmol/L; quartile 4, 1.23–8.34 μmol/L.

adjustment for competing risk of death (Web Table 4), after excluding participants with low cognitive function (Web Table 5) or participants who developed dementia during the initial 2 years (Web Table 6), did not make any material difference in the findings, although some *P* values failed to reach the level of significance.

The subgroup analyses for the association between serum β-alanine levels and the risk of all-cause dementia are summarized in Table 3. An inverse association was commonly observed in all subgroups stratified by age, sex, hypertension, diabetes, and obesity, although some *P* values failed to reach the level of statistical significance. No evidence of heterogeneity was observed in the association between β-alanine levels and the risk of all-cause dementia among the subgroups (*P* for heterogeneity > 0.1 for all).

Finally, we examined the associations of serum β-alanine levels with other secondary outcomes, such as all-cause mortality, body weight changes, and the development of physical disability (Web Table 7). There were no clear associations between serum β-alanine levels and these outcomes.

DISCUSSION

We found that the risk of developing all-cause dementia and AD decreased significantly with increasing serum β-alanine levels, even after adjustment for possible risk factors in a general population of elderly Japanese persons. Serum β-alanine levels are a possible indicator of the amount of carnosine/anserine in the body, because β-alanine is a metabolite of carnosine/anserine and serum β-alanine levels are more stable than serum carnosine/anserine levels. To the best of our knowledge, this is the first study showing a clear association between serum β-alanine levels and the risk of dementia using prospective longitudinal data from a general elderly population. Our findings suggest that the dietary intake of foods containing carnosine/anserine or β-alanine might have a beneficial role in the prevention of dementia.

Thus far, there remains limited epidemiologic evidence regarding the association of serum β-alanine levels with the risk of dementia, or the influence of dietary intake of carnosine/anserine on cognitive function. In an animal study, transgenic AD mice fed a high-fat diet showed an increased expression of receptors for advanced glycation end products in blood vessels, but the expression of these receptors was inhibited by carnosine supplementation, resulting in a reduced memory deficit (15). Randomized controlled trials in Poland (13) and Japan (14) reported that carnosine/anserine supplementation in healthy elderly volunteers had beneficial effects on cognitive function. In the present study we found that higher serum β-alanine levels were significantly associated with lower risks of all-cause dementia and AD. This finding might support the hypothesis that these imidazole dipeptides have a protective role against cognitive impairment. In the present study, β-alanine levels showed no clear association with the risk of VaD. The exact explanation for this negative finding is unclear, but it might have been due to the small number of VaD events in our cohort.

Carnosine is an imidazole dipeptide consisting of β-alanine and histidine and is present in the skeletal muscles and brain of vertebrates (11). Anserine is a methylated form of carnosine and is present at high levels in the breast skeletal muscles of chickens (11). These imidazole dipeptides have many biochemical functions, such as antioxidant, antiglycation, and antiinflammatory activities (11). Oxidative stress, advanced protein glycation, and inflammation are all known to be underlying mechanisms for dementia (31). Increased oxidative stress might precede the accumulation of amyloid-β peptide and neurofibrillary tangles (31, 32), resulting in an elevated risk of AD. Advanced protein glycation also affects brain tissue directly and leads to microvascular changes (31). Moreover, serum proinflammatory cytokines are known to be risk factors for AD (33). Recently, we demonstrated that carnosine/

Table 2. Hazard Ratios for the Development of Dementia and Its Subtypes According to Quartiles of Serum β-Alanine Concentration[a], the Hisayama Study, 2007–2012

Outcome	No. of Events	No. at Risk	Age- and Sex-Adjusted			Multivariable-Adjusted[b]		
			HR	95% CI	*P* Value	HR	95% CI	*P* Value
All-cause dementia								
Quartile 1	39	353	1.00	Referent		1.00	Referent	
Quartile 2	38	385	0.85	0.54, 1.34	0.48	0.73	0.45, 1.18	0.20
Quartile 3	19	358	0.48	0.27, 0.84	0.01	0.50	0.28, 0.89	0.02
Quartile 4	21	379	0.57	0.32, 1.01	0.05	0.50	0.27, 0.92	0.03
P for trend					0.01			0.01
Alzheimer disease								
Quartile 1	27	353	1.00	Referent		1.00	Referent	
Quartile 2	25	385	0.85	0.49, 1.47	0.55	0.78	0.44, 1.38	0.39
Quartile 3	13	358	0.53	0.27, 1.05	0.07	0.53	0.26, 1.06	0.07
Quartile 4	12	379	0.55	0.27, 1.15	0.11	0.53	0.25, 1.10	0.09
P for trend					0.049			0.04
Vascular dementia								
Quartile 1	6	353	1.00	Referent		1.00	Referent	
Quartile 2	11	385	1.47	0.54, 4.03	0.46	1.16	0.41, 3.33	0.78
Quartile 3	6	358	0.81	0.25, 2.63	0.72	0.97	0.30, 3.19	0.96
Quartile 4	8	379	1.07	0.34, 3.35	0.91	0.81	0.22, 2.92	0.75
P for trend					0.78			0.67

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Range of serum β-alanine concentrations: quartile 1, 0.29–0.76 μmol/L; quartile 2, 0.77–0.96 μmol/L; quartile 3, 0.97–1.22 μmol/L; quartile 4, 1.23–8.34 μmol/L.

[b] Adjusted for age, sex, educational level, systolic blood pressure, antihypertensive medication, diabetes, serum total cholesterol, body mass index, history of stroke, current smoking, current drinking, regular exercise, and total energy intake.

anserine supplementation reduces the expression of inflammatory cytokines in serum (14, 34). Therefore, the dietary intake of carnosine/anserine is likely to be beneficial for the prevention of cognitive impairment and dementia through the antioxidant, antiglycation, and antiinflammatory activities of these dipeptides.

The present study had some limitations. First, our findings were based on a single measurement of serum β-alanine concentrations at baseline. The variability of serum β-alanine concentrations during follow-up was not taken into consideration. Second, β-alanine concentrations were determined using serum samples frozen in long-term storage. Long-term storage might result in the degradation of β-alanine and thus decreased concentrations. These limitations might weaken the association observed in the present study, biasing the results toward the null hypothesis. Third, the present study could not investigate the association between serum carnosine/anserine levels and the risk of dementia, because carnosine and anserine were hardly detected in the sera from fasting blood samples (data not shown). Moreover, dietary intake levels of carnosine/anserine have not been estimated from the data of current nutritional questionnaires such as the brief self-administered diet history questionnaire (23). Therefore, it is hard to speculate as to which of carnosine/anserine or β-alanine had the greater impact on cognitive function in the present study. In addition, daily intakes of meat (4 categories: chicken, beef/pork, ham/bacon/sausage, and liver) and seafood (6 categories: high-fat fish, low-fat fish, fish with bones, canned tuna, dried or salted fish, and other seafood), which were estimated by using the brief self-administered diet history questionnaire, did not show meaningful correlations with serum β-alanine concentration in our cohort (Pearson's correlation coefficient, −0.2 to +0.2 for all). Further studies screening for foods that contain meaningful amounts of carnosine/anserine or β-alanine will be necessary. Fourth, reverse causality in the association between serum β-alanine levels and the risk of dementia was possible, because the follow-up period of the present study was relatively short (median, 5.3 years). Subjects with mild cognitive impairment, who were at higher risk of dementia, were more likely to have unfavorable dietary habits, resulting in lower serum concentrations of β-alanine. However, sensitivity analyses after the exclusion of participants with low MMSE score or the exclusion of participants who developed dementia during the initial 2 years of follow-up did not make any material difference in the findings. Fifth, it was difficult to perform subgroup analyses of each risk factor for AD and VaD due to the limited number of dementia events. Sixth, because the age of the participants was between 60 and 79 years, it remains unclear whether a higher serum β-alanine concentration is protective against dementia in very old people (i.e., ≥80 years). Finally, the present study was performed in a single Japanese population.

Table 3. Subgroup Analyses for the Association of Serum β-Alanine Concentration[a] With the Risk of All-Cause Dementia, Multivariable-Adjusted[b], the Hisayama Study, 2007–2012

Subgroup	Serum β-Alanine Level								*P* for Trend	*P* for Heterogeneity
	Quartile 1 (*n* = 353)		Quartile 2 (*n* = 385)		Quartile 3 (*n* = 358)		Quartile 4 (*n* = 379)			
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI		
Age group, years										
60–69	1.00	Referent	0.89	0.25, 3.14	0.32	0.06, 1.76	0.34	0.06, 1.79	0.11	0.72
70–79	1.00	Referent	0.81	0.48, 1.36	0.55	0.30, 1.03	0.57	0.29, 1.13	0.05	
Sex										
Men	1.00	Referent	0.91	0.31, 2.63	0.71	0.24, 2.10	0.67	0.22, 2.03	0.39	0.52
Women	1.00	Referent	0.69	0.39, 1.21	0.43	0.20, 0.94	0.47	0.20, 1.14	0.02	
Hypertension										
No	1.00	Referent	1.17	0.47, 2.92	0.66	0.21, 2.08	0.74	0.22, 2.48	0.44	0.25
Yes	1.00	Referent	0.58	0.33, 1.04	0.39	0.20, 0.78	0.39	0.19, 0.81	0.005	
Diabetes										
No	1.00	Referent	0.82	0.48, 1.40	0.48	0.25, 0.94	0.41	0.20, 0.87	0.006	0.19
Yes	1.00	Referent	0.47	0.15, 1.45	0.52	0.15, 1.88	0.51	0.15, 1.72	0.41	
Obesity										
No	1.00	Referent	0.87	0.47, 1.59	0.50	0.24, 1.06	0.54	0.24, 1.22	0.05	0.57
Yes	1.00	Referent	0.45	0.19, 1.08	0.40	0.15, 1.04	0.40	0.15, 1.04	0.05	

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Range of serum β-alanine concentrations: quartile 1, 0.29–0.76 μmol/L; quartile 2, 0.77–0.96 μmol/L; quartile 3, 0.97–1.22 μmol/L; quartile 4, 1.23–8.34 μmol/L.

[b] Adjusted for age, sex, educational level, systolic blood pressure, antihypertensive medication, diabetes, serum total cholesterol, body mass index, history of stroke, current smoking, current drinking, regular exercise, and total energy intake. The variable relevant to the subgroup was excluded from each model.

Thus, the generalizability of the findings is limited. Our results should be verified among other populations with different genetic and nutritional backgrounds.

In conclusion, the present study clearly demonstrated that higher serum β-alanine was significantly associated with lower incidence of all-cause dementia and AD, suggesting that serum β-alanine levels can be an indicator for good health. These findings raise the possibility that carnosine/anserine or β-alanine has a beneficial role in cognitive function in the elderly. Further experimental, epidemiologic, and clinical studies will be needed to reveal the precise roles of these dipeptides and β-alanine in the prevention of dementia.

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Author affiliations: Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan (Jun Hata, Daigo Yoshida, Takanori Honda, Toshiharu Ninomiya); Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan (Jun Hata, Mao Shibata, Satoko Sakata, Takanari Kitazono, Toshiharu Ninomiya); Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan (Tomoyuki Ohara); Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan (Yoshinori Katakura, Shuntaro Yamashita, Satoru Kuhara); Department of Agro-Environmental Sciences, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan (Kuniyoshi Shimizu); and Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan (Yoichiro Hirakawa, Takanari Kitazono).

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