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Original Research

Brain Aging and Midlife Tofu Consumption

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Key words: brain, aging, nutrition, soy, cognition

Objective: To examine associations of midlife tofu consumption with brain function and structural changes in late life.

Methods: The design utilized surviving participants of a longitudinal study established in 1965 for research on heart disease, stroke, and cancer. Information on consumption of selected foods was available from standardized interviews conducted 1965–1967 and 1971–1974. A 4-level composite intake index defined "low-low" consumption as fewer than two servings of tofu per week in 1965 and no tofu in the prior week in 1971. Men who reported two or more servings per week at both interviews were defined as "high-high" consumers. Intermediate or less consistent "low" and "high" consumption levels were also defined. Cognitive functioning was tested at the 1991–1993 examination, when participants were aged 71 to 93 years (n = 3734). Brain atrophy was assessed using neuroimage (n = 574) and autopsy (n = 290) information. Cognitive function data were also analyzed for wives of a sample of study participants (n = 502) who had been living with the participants at the time of their dietary interviews.

Results: Poor cognitive test performance, enlargement of ventricles and low brain weight were each significantly and independently associated with higher midlife tofu consumption. A similar association of midlife tofu intake with poor late life cognitive test scores was also observed among wives of cohort members, using the husband's answers to food frequency questions as proxy for the wife's consumption. Statistically significant associations were consistently demonstrated in linear and logistic multivariate regression models. Odds ratios comparing endpoints among "high-high" with "low-low" consumers were mostly in the range of 1.6 to 2.0.

Conclusions: In this population, higher midlife tofu consumption was independently associated with indicators of cognitive impairment and brain atrophy in late life.

INTRODUCTION

Although aging-related declines in cognitive functioning have been identified in many diverse populations, there is reason to believe that environmental factors modulate brain aging, cognitive impairment and dementia. Research regarding the possible influences of diet during middle adult life on brain aging has been limited by the extreme rarity of study populations of substantial number for which both prospectively assessed dietary data and standardized, unbiased endpoint measurements are available. The data presented here are from the Honolulu-Asia Aging Study (HAAS), an ongoing epidemiologic investigation that utilizes the study population and data resources of the Honolulu Heart Program (HHP) [1,2]. The study provides a unique opportunity to examine midlife dietary risk factors for brain aging by virtue of: (1) characteristics of the study population—large, ethnically homogeneous, but with diversity in consumption of Western *vs.* traditional Japanese foods, (2) availability of prospectively collected dietary information from standardized interviews conducted on two occasions in midlife, (3) extensive health, social, and anthropometric information, (4) a standardized evaluation of cognitive functioning when subjects were aged 71 to 93 years, (5) an autopsy component designed specifically for research on brain aging, (6) standardized nuclear magnetic resonance (NMR) brain imaging of a sample of participants and (7) a caregiver

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component that included cognitive function testing of more than 500 spouses who had been married to HHP participants when their midlife dietary interviews were conducted.

MATERIALS AND METHODS

The Honolulu Heart Program (HHP) and Honolulu-Asia Aging Study (HAAS). The HHP began in 1965 with the examination of 8006 Japanese-American men living on Oahu and born 1900 through 1919. The cohort has been followed since its inception using surveillance for deaths and hospitalizations together with interval examinations focused on heart disease, hypertension, stroke, cancer and aging. Assessments of cognitive functioning were first done at the 1991–1993 examination. Of 4631 surviving HHP cohort members, 3734 (80%) agreed to participate and were tested using the Cognitive Abilities Screening Instrument (CASI) [3].

As part of an extended evaluation of the causes of cognitive impairment, a stratified random sample comprising approximately 27% of the men was invited to return for further evaluation; these men were asked to bring with them the persons who would care for them if they became ill. Of the 948 men evaluated at this second phase, 502 were accompanied by their spouses who had been living with the subjects at the times of the 1965–1967 and 1971–1974 HHP examinations. CASI testing was done on these women as part of an evaluation of their reliability as historians for the prior health and functioning of the cohort members.

Dietary Assessments. Information on frequency of consumption of 26 specific food and drink items (selected as characteristic of a Western or traditional Japanese diets) was collected at the baseline 1965–1967 examination from all but one of the 8006 original participants. Similar weekly or daily consumption questions were asked again at the 1971–1974 examination. Dietary patterns among the men in this cohort, as well as reliability of assessment instruments and within-individual variation on different days over one week have been described [4–6]. Although 6860 of the men were still alive at the 1971–74 examination, dietary data were ascertained for only 5944. This difference was due partly to non-response and occurred partly because the diet questions were added to the examination protocol a few weeks after the main examination had begun. To evaluate the possibility of a response bias, we examined relationships between participation at the 1971–1974 examination, tofu consumption at the 1965–1967 examination and cognitive test performance at the 1991–1993 examination. After controlling for the relevant co-variates, participation at the 1971–1974 examination was not significantly related to either baseline tofu consumption or to cognitive test scores.

For examination of associations of midlife tofu intake with cognitive function of wives of HHP participants, we used information provided by the husband as proxy for the wife's diet. All of the wives included in these analyses had been married to and were living with the HHP participants at the times of their 1965–1967 and 1971–1974 interviews. We were able to examine the correspondence of responses to tofu consumption questions in spouse pairs using data available for a subset of wives interviewed in 1975–1978 by virtue of their participation in the Japan-Hawaii Cancer Study [7]. At that examination, 1412 wives of HHP participants were interviewed using a food frequency instrument nearly identical with that administered to their husbands at the 1971–1974 HHP examination. For tofu consumption, the Pearson correlation coefficient measuring the association between wives' responses at the 1975–1978 interview and their husbands' responses at the 1971–1974 HHP interview was 0.292 ($p < 0.0001$). This value was nearly identical to the coefficient of correlation between responses to the tofu question for HHP participants (i.e., the husbands) interviewed at the 1965–1967 and 1971–1974 HHP examinations (husbands test-retest $r = 0.293$, $p < 0.0001$). Although correlations of this magnitude are generally considered modest or moderate, they are common in studies of dietary intake [8,9]. Thus responses to questions regarding frequency of tofu consumption in members of a spouse pair interviewed about four years apart (wife-husband test-test correspondence) were approximately as consistent as responses provided by the husbands on two occasions separated by a six-year interval (husbands' test-retest reliability).

Cognitive Testing. The Cognitive Abilities Screening Instrument (CASI) includes tasks assessing attention, concentration, orientation, short- and long-term memory, language ability, visual construction, list-generating fluency, abstraction and judgement [1,3]. Designed for use in comparative cross-national studies of dementia in the U.S. and Japan, it is a composite of the Hasegawa Dementia Screening Scale (widely used in epidemiologic studies in Japan), the Folstein Mini-Mental State Examination and the Modified Mini-Mental State Test (used in the U.S. and Canada). The CASI score range is 0–100. In this population, a score of 50 approximates a Mini-Mental State Exam (MMSE) score of 15 and a CASI score of 74 corresponds to a MMSE score of 21–22, representing mild cognitive impairment or dementia. The CASI has been validated as a screening instrument for dementia in the United States and Japan, in both English and Japanese languages. Testing was done by neuropsychology technicians and interviewers who had been fully trained and certified in its use. Sound amplification devices were used for persons with hearing impairment, and participants selected either Japanese or English for testing, depending on the language with which they were most comfortable.

Apolipoprotein E Typing. Apolipoprotein E 4 zygosity was determined at Duke University under the direction of Dr. Ann Saunders, using DNA extracted from leukocytes. In the few cases in which blood was unavailable, phenotyping was done using plasma, at the Northwest Lipid Laboratories under the direction of Dr. S. Marcovina. Apolipoprotein E typing was available for 97% of the full population, but for only 92% of the

participants with cognitive impairment. The resulting association between missing data for apoE 4 zygosity and cognitive impairment reflects greater logistic problems with obtaining blood samples from homebound, cognitively impaired subjects. To avoid bias in multivariate regression modeling, we used four categories for apoE4 zygosity: homozygosity (two copies of the gene), heterozygosity (one copy), negative (no copies; the reference category) and missing.

Autopsies and Neuropathology Procedures. A research autopsy program began in 1991. Although there was a special focus on deaths in subjects who suffer from neurodegenerative diseases, efforts were made to obtain an autopsy in all deaths of cohort members, and autopsied subjects were similar to non-autopsied decedents with regard to the majority of characteristics, including cancer, cardiovascular diseases and age. Autopsies were usually conducted within 18 hours of death and began with removing the brain, emptying the ventricular system, weighing, removal of samples for freezing and transfer of the whole brain to neutral formalin. After approximately two months fixation the brain was re-weighed and dissected. Infarcts identified from one centimeter thick coronal sections were measured and photographed. Tissue blocks from multiple regions were sectioned and stained using hematoxylin and eosin, a modified Bielschowsky silver stain, a Gallius stain, an alpha synuclein immunostain and an anti-beta amyloid immunostain. Neurofibrillary tangle (NFT), senile plaque (SP) and neuritic plaque (NP) counts were done on Bielschowsky-stained sections from seven areas: middle frontal gyrus, inferior parietal lobule, middle temporal gyrus, occipital cortex, entorhinal cortex and from the CA1 and subiculum regions of the hippocampus. The three neuropathologists (W.R.M., D.D., and J.S.N.) who read slides are blinded to all clinical information. Since we began measuring the internal bitemporal calvarium diameter after about 50 autopsies had been completed, skull measurements are unavailable for approximately 20% of the autopsied decedents. In early analyses we noted that internal skull diameter was significantly associated with brain weight ($r = 0.357$, $p < 0.0001$), that height was significantly but less strongly associated with brain weight ($r = 0.225$, $p < 0.0001$) and that internal skull diameter and height were correlated ($r = 0.201$, $p = 0.0013$). In analysis of variance models, the association of height with brain weight was largely attributable to the correlation of height with skull diameter. Based of these findings we elected to include only the skull diameter as a control variable in multivariate regression models. For the 48 decedents for whom the actual skull measure was unavailable, it was imputed based on the pattern of correlation of skull diameter with height. Thus, in the multivariate regression models in which internal skull diameter was included as a controlling covariate, the value was a true measure in 80% of cases and was imputed based on height for the remaining 20%. When both height and skull diameter (non-imputed) were included in models, only the skull measurement remained significantly associated with brain weight. We interpret this as indicating that, while both height and skull diameter reflect growth differences among individuals, the skull measurement is the more proximate indicator of brain size attained in adult life. We believe that by controlling for skull size we are effectively controlling for adult brain size. This implies that in models having brain weight as the dependent variable, controlling for internal skull diameter focuses the analysis on factors that correlate with or predict brain atrophy, rather than on adult attained brain size.

Nuclear Magnetic Resonance Neuroimaging. Scans were done using a GE Signa Advantage, 1.5 Tesla machine at Kuakini Medical Center, Honolulu. The acquisition protocol typically required 20 minutes and included four pulse sequences: (1) Sagital, 24 cm FOV, TR = 500, TE = minimum, 5mm contiguous interleaved sections, 192 views, 1 repetition, (2) a 3D oblique spoiled gradient recalled echo sequence (SPGR). 22 cm FOV, minimum TR and TE, 1.6 mm slice thickness, 124 slices, 1 repetition, 45 degree flip angle, (3) An axial proton density weighted fast-spin echo sequence, 3mm (up to 5mm allowed) interleaved sections, minimum TE, TR = 2300 msec, 24 cm FOV, 256 views, 1 repetition, 4 echo train length, minimum inter-echo spacing, (4) another axial fast-spin echo sequence, T2-weighted. 3 mm (up to 5 mm allowed) interleaved sections, TR 4000 msec or more, 24 cm FOV, 256 views, 1 repetition, echo train length equal to 8, minimum inter-echo spacing. Semi-quantitative readings were done at the Johns Hopkins Neuroradiology Reading center using a protocol developed for use in the NHLBI Community Health Study and described elsewhere, with readers shielded from knowledge of the subject's risk factors and health at the time of the scan [10].

RESULTS

We began our analyses with the expectation that aculturation might be a risk factor for poor cognitive function in late life and that traditional Japanese lifestyle, culture and diet might be protective. Instead, we found that poorer cognitive test performance in later life was weakly associated with a more oriental midlife diet. As this finding was explored it became evident that the relationship with oriental diet could be attributed almost entirely to a single food item: tofu.

Association of Prospectively Ascertained Midlife Tofu Consumption with Poor Performance on a Test of Cognitive Function in Late Life

At the 1965–1967 examination participants were asked, "How often do you eat tofu?" Possible response categories were limited to "almost never," "less than twice weekly," "2–4 times weekly," "almost daily" or "more frequently." Table 1 provides a description of the study population when it was established and again at the 1991–1993 examination, with data presented for four strata of tofu consumption based on their

Table 1. The HAAS Cohort, 1965–68, and 1991–93; Characteristics at Baseline and at the Time of Cognitive Assessment, according to Response at Baseline to the Question: "How Often do You Eat Tofu?"

	How often do you eat tofu? Servings/week, 1965 interview			
	almost never	<twice	2–4 times	>4 times
1965–68 examination				
Number examined	89	6218	1531	167
age (mean, s.d.)	53.7, 5.2	54.0, 5.5	55.8, 5.6	57.5, 5.9
born Japan	4.5%	9.2%	20.4%	32.9%
1991–93 examination				
N still alive	53	3215	808	88
N examined	38	3003	628	65
age (mean, s.d.)	78.4, 4.87	78.5, 5.0	78.9, 5.1	80.2, 5.2
education (years; mean, s.d.)	10.1, 3.0	10.6, 3.2	9.7, 3.0	9.8, 3.5
apolipoprotein E4 zygosity				
blood not available for testing	2.6%	2.8%	4.1%	4.5%
negative (2:2, 2:3, or 3:3)	76.3%	78.9%	79.3%	78.7%
heterozygous (2:4 or 3:4)	21.1%	17.8%	16.1%	15.3%
homozygous (4:4)	0%	0.5%	0.5%	1.5%
stroke*				
hospitalized	0%	3.9%	4.0%	6.9%
history only	1.0%	7.2%	7.6%	7.0%
cancer* (ever, by hx)	2.6%	9.2%	9.1%	7.3%
CASI test performance*				
Mean score (age and education adjusted)	83.4	82.4	81.3	80.7
<50 (mod/severe cogn. impairment)	2.0%	4.4%	5.3%	4.9%
50–74 (mild cognitive impairment)	2.0%	10.4%	14.2%	14.1%
74+ (normal)	96.0%	85.2%	80.5%	81.0%

* values for stroke, cancer, and CASI score adjusted for age (single year) and years of schooling completed.

responses at the 1965–1967 interview. The apparent associations of tofu consumption with older age, fewer years of education and birth in Japan reflect historical patterns of migration of young men from Japan to work on Hawaii's pineapple and sugarcane plantations in the early decades of the century. Those men who had spent their childhood years in Japan tended to be older at the 1991 examination and tended to have retained traditional dietary preferences. Prevalence values for stroke, cancer and cognitive impairment, as well as mean CASI score, are given after adjustment for age and education.

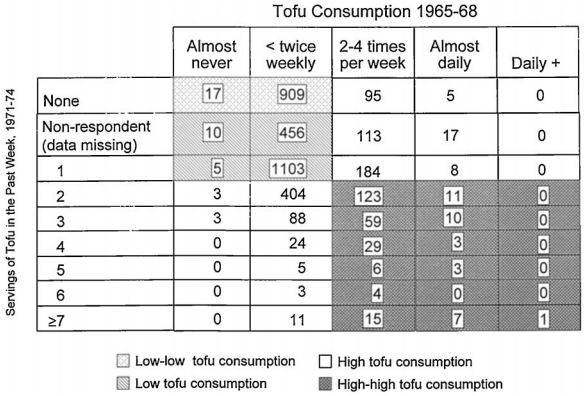
As expected, given these demographic correlational patterns, survival to the time of the 1991–1993 examination was inversely related to age and tofu intake. Survival analyses by proportional hazards modeling indicated that this could be entirely attributed to the older age of the higher consumers of tofu, and no independent influence of tofu consumption on survival was evident. After adjustment for age and education, no significant associations with tofu intake were noted for lifetime history of stroke or cancer.

Although non-participation in the 1991–1993 examination was somewhat greater among those men who had reported consuming tofu more often at the 1965–1967 interview, the association did not reach statistical significance. Data at the bottom of Table 1 indicate that men who consumed tofu more frequently in midlife had higher rates of cognitive impairment at the 1991 examination, compared with their peers who ate tofu less frequently (test for trend $p = 0.006$, controlling for age and education).

At the 1971–1974 examination study participants again received a battery of food frequency questions. At that time they were asked: "During the past week how many servings of tofu did you have?" Responses at this examination were moderately correlated with responses to the tofu intake question asked at the first examination (Pearson $r = 0.293$, $p < 0.0001$). Fig. 1 presents the conjoint distribution of tofu intake in 1965–1967 and 1971–1974 for all participants whose cognitive function was subsequently evaluated. The number in each cell represents the actual number of men evaluated at the 1991–1993 examination according to their answers to the tofu intake question asked first at the 1965–1967 examination and then again at the 1971–1974 examination. As shown, men in the "low-low" ($n = 926$) and "high-high" ($n = 271$) categories reported consistently low or consistently high tofu intakes at the 1965–1967 and 1971–1974 examinations, while the "low" ($n = 1574$) and "high" ($n = 962$) groups gave less consistent answers, or were classified solely on the basis of their 1965–1967 responses when the 1971–1974 information was missing.

As shown in Fig. 2, an increase in the prevalence of cognitive impairment with greater tofu consumption (based on the 4-level 1965–1971 composite index) remained apparent after stratifying by age. The bars at the right side of the figure, summarizing prevalence levels for cognitive impairment after adjustment for single years of age, illustrate a statistically significant, increasing trend in total cognitive impairment with increasing mid-life tofu intake ($p < 0.0001$).

To investigate alternative explanations for the association of



Servings of Tofu in the Past Week, 1971-74	Almost never	< twice weekly	2-4 times per week	Almost daily	Daily +
None	17	909	95	5	0
Non-respondent (data missing)	10	456	113	17	0
1	5	1103	184	8	0
2	3	404	123	11	0
3	3	88	59	10	0
4	0	24	29	3	0
5	0	5	6	3	0
6	0	3	4	0	0
≥7	0	11	15	7	1

Fig. 1. Conjoint distribution of responses to questions concerning consumption of tofu asked at the 1965–1968 and 1971–1974 interviews among 3734 HAAS participants tested with the Cognitive Abilities Screening Instrument at the 1991–1993 examination. Each participant is categorized as low-low (low intake at both times), low (<twice weekly at the 1965 interview, no information or only one serving in the prior week at the 1971 interview), high (2+ servings per week at either interview), or high-high (2+ servings per week at both interviews).

high mid-life tofu intake with poorer cognition in late life, analyses were conducted to identify possible confounding factors, i.e., factors correlated both with higher tofu consumption in middle life and with impaired cognitive functioning in later life. Thirty-one factors were examined, including eight other dietary items, using composite indicators based on their self-reported intakes at the 1965–1967 and 1971–1974 interviews: miso soup, rice, fish, green tea, milk, meat, coffee and black tea. Total calories, grams of protein, grams of fat and grams of carbohydrate were estimated from a 24-hour intake diary also completed at the 1965–1967 examination. Other factors included age, midlife body mass index (BMI), years of formal

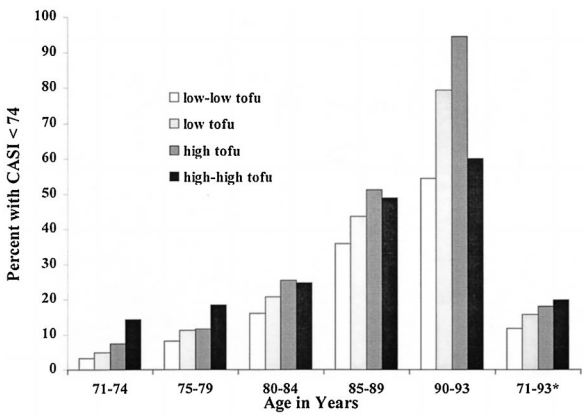


Fig. 2. Prevalence of a CASI score <74 among study participants stratified according to 5-year age groups and four levels of midlife tofu consumption, based on the composite 1965/1971 tofu intake index.
* The bars for ages 71–93 represent the entire study population (n = 3734), with prevalence levels of CASI score <74 for each level of tofu intake shown after adjustment for single years of age.

education, occupation (six strata ordered according to average educational attainment), birth in Japan, years of childhood lived in Japan, history of sugarcane or pineapple plantation employment, years of agricultural field work, height, average midlife systolic blood pressure (s.BP, measured 1965–1967, 1967–1969, and 1971–1974), average midlife FEV-1 (one second forced expiratory volume, a test of pulmonary function), average midlife serum cholesterol, serum uric acid, usual alcohol consumption, cigarette smoking history, apolipoprotein E epsilon 4 zygosity, lifetime history of stroke and lifetime history of cancer. These analyses utilized logistic regression methods and were conducted first with univariate models, examining each independent variable for its association with the 1965–1972 composite index and in a separate model with poor performance on the CASI. Variables were then tested in stepwise models in which the other candidate confounders were considered.

Candidate confounding factors identified as independently associated with poorer cognitive test performance and with midlife tofu intake were older age, lesser education, occupation of lesser complexity, more years of childhood lived in Japan, apolipoprotein E4 homozygosity (marginal), height (marginal) and high midlife fish consumption (marginally protective relationship with CASI score, marginal direct correlation with tofu intake). History of prior stroke and non-availability of the apolipoprotein E genotype (because blood was not obtained) were associated with the CASI score but not with midlife tofu intake.

Initial multivariate analyses were done using linear regression modeling with the participant's CASI score as the dependent variable. Controlling covariates included all of the candidate confounders and predictors of CASI score mentioned above, together with midlife FEV-1 (a pulmonary function indicator previously reported to predict CASI scores) and midlife BMI (a predictor of brain weight in subsequent analyses). In a model using the 1965–1967 tofu intake question as the primary predictor variable, while controlling for all other independent variables, there was a significant inverse association with CASI score (beta = -1.4, $p < .006$). Similar associations were seen with the 1971–1974 tofu intake variable (beta = -0.74, $p = .0005$), and with the composite variable (beta = -0.86, $p = .0025$). When the 933 subjects who had lived one or more years of their childhood in Japan were excluded from the analysis, the corresponding values were beta = -1.69, $p = 0.01$ for 1965–67 tofu intake; beta = -0.65, $p = 0.01$ for 1971–74 tofu intake, and beta = -0.72, $p = 0.015$ for the composite tofu intake index. There was no evidence of interaction between apolipoprotein E genotype and tofu in their associations with cognitive test performance.

Linear regression analyses using the CASI score as the dependent variable can be criticized because the distribution of the endpoint is moderately skewed toward higher scores, thereby violating the assumption of a normal distribution. To address this problem and to facilitate examination of dose

response relationships, logistic regression analyses were carried out with a dichotomized dependent variable (cognitive impairment, based on a CASI score <74) and with each of the four levels of the composite tofu intake variable considered as an independent (predictor) variable. Table 2 shows the model first with the entire study cohort and then after excluding participants with a history of prior stroke. These results show a statistically significant, increasing effect (odds for cognitive impairment) with increasing dose of the exposure (level of tofu intake). The relationship was more apparent among participants without prior stroke, indicating that the effect was not mediated through an association of tofu intake with clinically apparent stroke.

Replication of the Finding in a Panel of Wife-Caregivers

As part of the 1991–1993 HAAS examination, approximately 27% of the men were invited to return for further evaluation and were asked to bring with them the person who currently provided their care or who would care for them were they to become ill or disabled. Those invited back included all men with low CASI scores, a sample of those with intermediate scores and a sample with normal CASI scores [1]. Of caregiver informants interviewed, 502 were wives who had been living with the participant at the time of their 1965–1967 and 1971–1974 dietary interviews. The CASI was administered to 495 of these wife-caregivers as part of a structured interview designed to provide proxy information about the primary participant and to examine aspects of health and functioning of the caregiver herself. Husbands' and wives' CASI scores were not significantly correlated (Pearson r = −.04, $p = 0.38$). CASI scores among wives showed influences of age and education similar to those observed in male participants, except that moderate or severe cognitive impairment (scores below 74 or 60) was substantially less common. The better cognitive test scores achieved by the wives was expected, since their identification as the responsible caregiver implied some degree of functional competence.

In this population it is likely that meals in midlife had been shared by the spouse pairs, and that responses to food frequency questions provided by a male HHP participant would reflect the wife's diet at the same time, a presumption on which previous analyses have been based [7]. This expectation is supported by a correlation of wives' and husbands' responses to similar tofu intake questions (spouse-spouse correspondence) that is very similar in magnitude to the test-retest reliability of tofu consumption questions administered to HHP men on two separate occasions (described in methods).

We examined relationships between the wife's CASI score and her husband's midlife composite (1965–1971) tofu intake index, using that index as proxy for her midlife tofu intake. As previously observed in the male HAAS subjects, lower CASI scores were associated with higher levels of midlife tofu intake (Table 3). To determine statistical significance and to control

Table 2. Results of Multivariate Logistic Regression Models for Predictors of Cognitive Impairment (CASI <74) in the Full Study Cohort and after Excluding Participants with Prior Stroke

	FULL COHORT			EXCLUDING STROKE		
N cognitively impaired	555			420		
normal	3081			2818		
	OR	95% CI	*p*	OR	95% CI	*p*
Age (single years)	1.16	(1.14–1.19)	<.0001	1.17	(1.14–1.20)	<.0001
Education (single years)	0.82	(0.79–0.85)	<.0001	0.82	(0.78–0.86)	<.0001
Occupation (6 categories, increasing cmplx)	0.89	(0.80–0.99)	=0.03	0.90	(0.80–1.02)	=0.09
Years of childhood lived in Japan	1.04	(1.02–1.06)	<.0001	1.04	(1.02–1.06)	=.0005
Stroke (none, history only, or hosp records)	2.67	(2.23–3.20)	<.0001		(not in model)	
Fish intake (4 levels, 1965–71 composite)	0.89	(0.79–1.00)	=0.05	0.87	(0.77–0.99)	=0.03
Midlife height (inches)	0.95	(0.90–1.01)	=0.08	0.93	(0.88–0.99)	=0.03
Midlife FEV-1 (liters)	0.79	(0.61–1.02)	=0.07	0.78	(0.59–1.05)	=0.10
Apolipoprotein E4						
Negative (type 2:2, 2:3, or 3:3)	1.0	[reference]		1.0	[reference]	
Blood sample not obtained	1.74	(1.08–2.80)	=0.02	1.70	(0.99–2.91)	=0.05
Heterozygous (2:4 or 3:4)	1.22	(0.93–1.61)	=0.15	1.23	(0.90–1.66)	=0.19
Homozygous (4:4)	5.73	(1.94–16.92)	=0.002	5.27	(1.70–16.7)	=.004
1965/71 tofu intake index						
low-low	1.0	[reference]		1.0	[reference]	
low	1.42	(1.06–1.90)	=0.02	1.51	(1.08–2.11)	=0.02
high	1.74	(1.28–2.37)	=0.0004	1.99	(1.41–2.82)	=.0001
high-high	1.62	(1.06–2.46)	=0.03	1.87	(1.17–3.00)	=0.009

Cognitive impairment based on a CASI score <74, compared with > or = 74. Odds ratios expressed in terms of changing odds of the endpoint per single unit change in the independent variable. For apolipoprotein E and tofu intake, each stratum of the variable is compared with the reference stratum. A small number of participants could not be included because of non-availability of pulmonary function data (FEV-1).

Table 3. Characteristics of a panel of wife-caregivers to HAAS participants according to midlife tofu consumption reported by their husbands at interviews in 1965–68 and 1971–74

	1965–71 COMPOSITE TOFU INTAKE INDEX			
	Low-Low	Low	High	High-High
Number	100	194	139	62
Wife-caregiver's:				
Age (mean, s.d.)	76.0, 5.3	75.6, 4.7	75.8, 4.9	76.5, 4.9
Education (years; mean, s.d.)	10.0, 2.7	9.7, 2.7	9.8, 8.1	9.5, 2.8
Born in Japan	3.0%	1.6%	6.5%	11.3%
Elementary school in Japan	8.0%	9.3%	15%	19%
CASI score (mean, s.d.)	86.8, 7.6	85.7, 8.5	83.6, 9.8	83.8, 8.6
% CASI <74	6.0%	9.3%	14.4%	12.9%
% CASI 74+	94%	90.7%	85.6%	87.1%

for other factors, multivariate modeling was done in two ways: first using conventional linear regression with the CASI score as the dependent variable and then using logistic modeling with cognitive impairment (CASI score <74) as the endpoint. To be certain that the association of CASI score with midlife tofu intake was independent of the spouse's cognitive functioning, the husband's CASI score was included as an additional covariate, together with her age and education.

Although both linear and logistic models indicated an association between higher midlife tofu intake and poorer cognitive test scores in later life among wives, the association reached statistical significance only in the linear regression model (Table 4). This was likely due to a low prevalence of cognitive impairment among the wives attributed to the selection for cognitive competence mentioned above. Nonetheless, the magnitude of the odds ratios for increasing levels of tofu intake were similar to those observed among the male HAAS participants.

A linear regression model identical to that presented in Table 4, but with tofu intake entered as a single, 4-category independent variable, was examined as a test for trend. This analysis demonstrated a statistically significant association of higher midlife tofu intake with lower CASI scores (n = 495, beta = −1.13, $p = 0.004$) after controlling for the wife's age, education, birth and elementary schooling in Japan and the husband's CASI score. As observed among the male HHP participants, women who had received elementary education in Japan tended to perform less well on the CASI even with tofu intake as a covariate. When women born in Japan or who had attended elementary school in Japan were excluded, the association was essentially unchanged (n = 426, beta = −1.14, $p = 0.006$).

Association of Midlife Tofu with Low Brain Weight Determined at Autopsy

Between February 1992 and January 1999, 290 research autopsies were conducted on cohort members. All had provided information concerning weekly tofu consumption at the 1965 interview. Although the results of gross brain examinations were available for all, complete information from the microscopic evaluations was complete for only 248 at the time of these analyses. As shown in Table 5, autopsied participants who had consumed tofu more frequently in midlife were slightly older at death, slightly shorter and had slightly higher

Table 4. Multivariate Regression Analyses of the Predictors of Cognitive Test Performance in a Panel of Wife-Caregivers to HAAS Participants

	LINEAR REGRESSION MODEL Dependent Variable = CASI Score N=502		LOGISTIC REGRESSION MODEL Cognitive Impairment (CASI<74) N=448 normal, 54 cognitively impaired		
	Beta	*p*	OR	95% CI	*p*
Age (single years)	−0.40	0.0001	1.1	1.03–1.18	0.006
Education (single years)	0.42	0.0001	0.69	0.60–0.80	0.0001
Birth in Japan	ns		ns		
Elem school in Japan	−4.1	0.0003	2.7	1.25–5.94	0.01
Husband's CASI score	−0.01	ns	1.02	1.00–1.04	0.03
1965–71 tofu intake index:					
Low-low	[reference]		1.0	[reference]	
Low	−1.2	ns	1.67	0.60–4.62	ns
High	−3.0	0.005	2.08	0.75–5.78	ns
High-High	−2.2	0.10	2.13	0.65–7.01	ns

Table 5. Characteristics of HAAS/HHP Men Autopsied 1991–1999, Stratified according to Midlife Tofu Consumption

	1965–71 COMPOSITE TOFU INTAKE INDEX			
	Low-Low	Low	High	High-High
Number	58	126	83	25
Age at death (mean, sd)	83.9, 5.7	83.7, 5.2	85.2, 5.4	85.8, 5.7
Born Japan	5%	8%	18%	36%
Height (inches; mean, sd)	64.2, 1.9	64.4, 2.4	63.6, 2.4	63.1, 2.1
Midlife BMI (mean, sd)	23.6, 3.0	23.7, 3.0	23.2, 2.9	23.6, 2.8
Midlife d.BP (mean, sd)	83.1, 9.2	83.9, 9.9	81.5, 10.0	84.4, 13.5
Midlife s.BP (mean, sd)	131.2, 14.2	134.9, 17.1	133.0, 16.6	143.0, 13.5
CASI (mean, sd)	78.3, 21.1	72.4, 24.9	63.9, 25.1	73.2, 20.8
Brain weight (g., mean, sd)	1248, 124	1236, 117	1220, 114	1166, 113
Less than 1120 grams	12%	15%	21%	40%
neuritic plaque density				
elevated, neocortex	17%	17%	14%	29%
elevated, hippocampus	17%	28%	26%	29%
neurofibrillary tangle density				
elevated, neocortex	21%	20%	26%	24%
elevated, hippocampus	21%	21%	21%	14%

midlife systolic blood pressures than decedents who had consumed tofu less frequently. None of these differences were statistically significant. As previously noted, higher tofu consumption was associated with birth and childhood residence in Japan, indicating a tendency for these men to have retained traditional dietary preferences. While the density of neuritic plaques appeared to be somewhat elevated among the high-high tofu consumers, this association failed to reach statistical significance once age at death was taken into account.

Small body size (short height and a correspondingly small head) is rather common among the oldest HHP participants, especially those who migrated from Japan during late childhood or early adult life in search of a better life in Hawaii. Since skull growth is largely determined by brain growth, a small calvarium volume implies a small brain. Thus, internal skull measurements can serve as indicators of childhood brain growth and adult-achieved brain size. Based on this idea, internal skull diameter was included as a co-variate in all linear and logistic models as an indirect means to control for attained adult brain size, thereby emphasizing the contribution of atrophy as an explanation for low brain weight at death.

Fig. 3 illustrates a consistent increase in the prevalence of low brain weight (less than 1120 grams, corresponding to the 15th percentile in this autopsy sample) with increasing midlife tofu intake. This trend is somewhat more evident in persons whose average midlife BMI was below the mean. In a multivariate linear regression analysis, lower brain weight was significantly associated with high-high midlife tofu consumption, controlling for age at death, average midlife BMI, internal skull diameter and average midlife diastolic blood pressure ($p < 0.017$, Table 6). In a logistic model the association of high-high midlife tofu consumption with low brain weight was marginally significant ($p = .055$).

After controlling for the variables mentioned, none of the following were found to be significantly associated with low brain weight: apolipoprotein E epsilon 4 zygosity, education, occupation, birth in Japan, number of years of childhood lived in Japan, height, large vessel infarcts, lacunar infarcts, or midlife consumption of miso, fish, rice, meat, milk, green tea, black tea or coffee. No interaction was observed between apolipoprotein E type and midlife tofu intake. Analyses also failed to support a statistically significant interaction between lower adiposity and higher tofu consumption in determining brain weight.

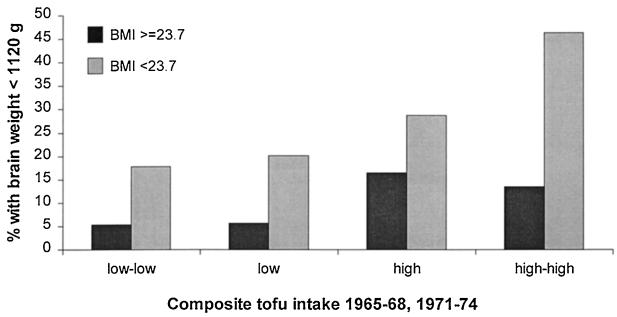


Fig. 3. Adjusted prevalence of brain weight less than 1120 grams among autopsied HAAS decedents stratified according to midlife BMI (below or above the mean) and midlife tofu consumption. Adjustment was done using multivariate regression methods, controlling for age at death, internal diameter of the skull, and midlife blood pressure.

Association of Midlife Tofu Intake with Ventricular Enlargement on NMR Brain Imaging

During the 1994–1996 HAAS examination cycle a stratified sample of 575 HAAS participants received an NMR brain scan. Subjects were invited to receive a scan based on a CASI score below 74, a diagnosis of dementia (excluding those with severe dementia), clinically recognized stroke and/or apolipoprotein

Table 6. Multivariate Regression Analyses of the Association of Midlife Tofu Consumption with Brain Weight Determined at Autopsy Using Weight as a Continuous Measure (Linear Regression) or Dichotomized into Low and Normal Weight Categories (Logistic Analysis)

	LINEAR REGRESSION MODEL Dependent variable=brain weight N=294		LOGISTIC REGRESSION MODEL Dependent variable=low brain weight N=241 br.wt. >=1120 g; 53 br.wt. <1120 g		
	Beta	*p*	OR	95% CI	*p*
Age (single years)	−5.09	0.0002	1.12	1.05–1.20	0.0004
Midlife BMI	4.95	0.0001	1.00	0.89–1.13	ns
Int. skull diameter (mm)	6.02	0.031	0.91	0.86–0.97	0.003
Midlife d.BP (mm Hg)	−1.14	0.083	1.034	1.00–1.07	0.05
1065–71 tofu intake index					
Low-low	[reference]		1.0	[reference]	
Low	−13.46	ns	1.23	0.47–3.24	ns
High	−19.25	ns	1.66	0.61–4.55	ns
High-High	−61.04	.017	2.08	0.97–11.47	0.055

E4 heterozygosity or homozygosity. In addition, a probability sample of men with none of these conditions was also invited to receive a scan. Semi-quantitative readings were done using methods described elsewhere [10]. Ventricular size and shape were graded on a scale of 0–9 with values of 0–3 indicating no ventricular enlargement, 4–6 borderline and values of 7–9 indicating a definite increase in ventricular volume. Characteristics of the men in this sample categorized according to midlife tofu intake are shown in Table 7 for the 574 fully adequate scans. Preliminary analyses identified poor cognitive functioning and infarcts (identified on the scan) as significantly associated with ventricular enlargement. Multivariate analyses included age, CASI score, apolipoprotein E4 type (positive or no), numbers of large and small infarcts and internal skull diameter as controlling covariates. Logistic regression analysis was conducted with sampling weights taken into account.

In linear regression analyses, high-high midlife tofu consumption was significantly associated with larger ventricular size (beta = 0.62, $p < 0.03$, Table 8). When a linear regression analysis was limited to the 248 subjects without evidence of either large vessel or lacunar infarcts, the strength of association between high-high tofu consumption and ventricular enlargement was stronger (beta = 0.88), though the smaller number of observations resulted in a reduction in statistical significance to a marginal level ($p = 0.076$). Although the odds ratio estimated from the logistic model for an association between ventricular enlargement and high-high midlife tofu consumption was 2.85, the association failed to reach statistical significance ($p = 0.11$). No interactions were observed between midlife tofu intake and either apolipoprotein E genotype or the number of cerebrovascular lesions.

Table 7. Characteristics of HAAS/HHP Men who Received a Research NMR Brain Scan 1994–1996, Stratified according to Midlife Tofu Consumption

	1965–71 COMPOSITE TOFU INTAKE INDEX			
	Low-Low	Low	High	High-High
Number	142	243	149	40
Age (mean, sd)	81.2, 4.8	81.3, 4.8	81.9, 5.2	84.1, 5.4
Born in Japan	4.9%	7.8%	12.1%	20%
Height (inches, mean, sd)	64.4, 2.0	64.2, 2.3	63.8, 2.5	63.6, 2.7
Midlife BMI (mean, sd)	24.0, 2.6	23.8, 2.9	23.7, 2.6	24.0, 2.4
Midlife d.BP (mean, sd)	84.0, 10.1	83.3, 9.3	82.0, 8.6	82.3, 9.3
Midlife s.BP (mean, sd)	133.1, 16.7	132.3, 15.9	129.8, 15.6	131.5, 18.7
CASI (mean, sd)	74.4, 15.7	73.4, 15.3	72.5, 17.8	69.0, 15.1
1 or more large infarct (>=1 cm)	51.4%	45.9%	49.7%	55%
Small infarctions (<1 cm volume)				
1	14.8%	16.4%	11.4%	12.5%
2 or more	10.6%	9%	6.7%	20%
ventricular enlargement				
marginal (scale score = 6)	14.9%	9.8%	12.8%	17.5%
definite (score >6)	8.5%	11.9%	7.4%	22.5%

Table 8. Multivariate Regression Analysis of the Association between Midlife Tofu Intake and Ventricular Enlargement as Assessed by NMR Brain Scan. An Unweighted General Linear Analysis was used for the Linear Model. The Logistic Model was Adjusted for Sampling with the Use of Sampling Weights

	LINEAR REGRESSION MODEL Dependent variable=ventricle grade N=574		LOGISTIC REGRESSION MODEL Dependent variable=ventricular enlargement N=442 with grade <6; 132 with grade 6+		
	Beta	*p*	OR	95% CI	*p*
Age (single years)	0.00	ns	1.035	0.96–1.11	ns
CASI score	−.023	<.0001	0.97	0.95–0.99	0.001
Int. skull diameter (mm)	0.97	ns	1.41	0.87–2.28	ns
Apolipoprotein E4 zygosity	0.17	ns	1.08	0.58–2.02	ns
Large infarcts (>=1 cm)	0.46	0.0002	2.22	1.06–4.64	0.001
Small infarcts (<1 cm)	0.24	0.013	1.39	0.87–2.23	ns
1965–71 tofu intake index:					
Low-low	1.0	[reference]	1.0	[reference]	
Low	−0.02	ns	1.11	0.45–2.71	ns
High	−0.18	ns	0.94	0.35–2.48	ns
High-High	−0.56	0.03	2.85	0.73–11.16	0.11

Based on our Analyses, How Important is the Adverse Influence of High Midlife Tofu Consumption on Brain Aging?

Midlife tofu consumption by itself (employing both the 1965–1967 and 1971–1974 tofu intake information and controlling for no other factors) explained 2.3% of the variance in CASI scores. A linear regression model that included only age, education and history of a prior stroke explained 27.8% of the variance. After controlling for these three most important factors, midlife tofu consumption remained statistically significant even though it then explained only an additional 0.8% of the variance in test scores. Thus the influence of midlife tofu intake on late life cognitive functioning appeared to be far less than age, education and history of stroke in this population.

Odds ratios provide an estimate of the importance of a factor as it may influence risk in individuals. Based on odds ratios, the magnitude of the association of high midlife tofu with late life cognitive test performance was rather similar across all four endpoints. Compared with low-low midlife consumption of tofu, high or high-high consumption was associated with odds ratios in the range of 1.6 to 2.0 for having poor cognitive test scores (observed independently in the male participants and in their wife caregivers), low brain weight or ventricular enlargement in later life. For cognitive impairment, this increment is comparable to the effect of a four-year difference in age or a three-year difference in education. Thus, a high-high consumer of tofu who had completed high school would have a probability for cognitive impairment similar to that of another high school graduate who had been a low-low consumer of tofu but who was four years older.

To assess the possible importance of midlife tofu consumption in terms of its impact on the prevalence of cognitive impairment in the population, we computed an estimate of the population attributable fraction. This was defined as the difference between the observed prevalence of cognitive impairment in the study population and the expected prevalence had all members of the cohort consumed tofu at the low-low level, with no other change. Computations were based on values and coefficients for each of the predictor variables shown in Table 2 (logistic model, CASI scores below 74) and from a similar model for more severe cognitive impairment (CASI score <50), without excluding participants with prior stroke. Applied to each subject in the cohort, the computation generates an estimate of the probability of moderate or severe cognitive impairment based on the individual's age, education, apolipoprotein E type, years of childhood lived in Japan, history of stroke, occupational complexity, midlife fish consumption, midlife height, midlife pulmonary function and midlife tofu intake. The sum of the probabilities for all subjects corresponds exactly to the observed prevalence in the study population. The expected prevalence of cognitive impairment (conditioned upon tofu intake having no influence) is then computed by repeating the process, substituting for each participant's tofu intake a value of zero (equivalent to low-low consumption).

When each individual's actual data for all predictors (including tofu) were used in the model, the resultant estimates were 4.4% for severe cognitive impairment (CASI score below 50) and 15.3% for all cognitive impairment (CASI score below 74)—corresponding perfectly to the observed prevalence levels. When identical models were run, but with values for tofu intake set to "low-low" for all participants, corresponding prevalence estimates were 3.3% and 12.2%. Thus 20% to 25% of the cognitive impairment observed in the HAAS population appears to have been attributable to tofu consumption, or to some other undefined factor for which tofu consumption served as a surrogate.

DISCUSSION

The findings described here are interpreted as suggesting steeper age-related declines in brain structure and function in

late life among study participants who had habitually consumed tofu more frequently in middle life. Patterns were remarkably consistent, with generally apparent dose-response patterns in men and in women and across subsets of the study population stratified by age or by body mass index. We have identified only one previously published study directly bearing on these issues, a report on the incidence of dementia in 272 age-, gender-, and zip code-matched California residents (half vegetarians) and in a second group of 2984 unmatched participants who consumed variable amounts and types of meat. In the smaller, matched group, Giem and co-workers identified a barely significant association of dementia with meat eating. No associations of dementia with dietary factors (including meat consumption) were observed in the larger group [11].

Is it Reasonable to Consider our Examination of Two Functional and Two Structural Endpoints as Providing Four Independent Indications of an Adverse Influence of Midlife Tofu Intake on Brain Aging in this Study Population? An inverse relationship between midlife tofu intake and the wife's cognitive performance similar in magnitude to that in the male subjects was observed despite an opposing selective force due to the requirement that she be sufficiently competent to act as actual or potential caregiver. No correlation between CASI test scores of husbands and wives was apparent, and the association of the wives' test performance with midlife tofu intake was largely due to poor CASI scores among women whose husbands were not cognitively impaired. The independence of the relationships between midlife tofu intake and late life cognitive function in these two groups (cohort members and their wives) was demonstrated by failure of the husband's CASI score (entered in the model as a controlling covariate) to explain the observed association between poor cognitive function and midlife tofu intake among the wives.

Even though cognitive impairment (a functional endpoint) and brain atrophy (a structural endpoint identified by neuroimaging or autopsy) may well represent related expressions of a common underlying mechanism, they did not always occur in the same individual. Among the decedent subjects for whom autopsy data were available, CASI scores determined at the 1991–1993 examination were only modestly (not significantly) correlated with brain weight after age, midlife blood pressure and skull size were taken into account. Since brain weights were available only for decedents, it was not possible to determine if the association of a low CASI score with midlife tofu intake in the full study population was dependent on brain atrophy. The autopsy and NMR subsets were nearly non-overlapping, with only a single participant in both. In analyses of the NMR data, we included the subject's CASI score as a covariate in order to ensure independent evaluation of the influence of tofu consumption, since men who had received low CASI scores were over-sampled to receive scans. Ventricular enlargement and cognitive test performance were correlated, possibly reflecting and adverse influence of brain atrophy on cognitive functioning. Inclusion of CASI score as a controlling covariate may have resulted in an underestimation of the true association of tofu intake with ventricular enlargement.

Is it Possible that the Apparent Influence of Midlife Tofu Intake on Brain Structure and Function in Late Life was Actually a Consequence of Privation and Suboptimal Brain Development in Childhood? This concern reflects the strong correlations of midlife tofu consumption with birth in Japan, the number of years of childhood lived in Japan, short stature and small skull size. These correlational patterns are thought to reflect preservation of Japanese culture and identity by men who migrated from Japan during late childhood or early adult life, often to escape privation and economic disadvantage by accepting work on Hawaii's sugarcane and pineapple plantations. This possibility has been addressed for each of the four endpoints by controlling for years of childhood lived in Japan, primary education in Japan, height or skull diameter. In every case tofu intake was independently predictive of the endpoint. In addition, limiting analyses to men who had been born and lived all of their lives in Hawaii or to wives who had been born and attended elementary school in Hawaii resulted in no lessening of the strengths of correlation of midlife tofu intake with the endpoints. We interpret these findings as supporting independence of the influences of childhood privation and adult tofu consumption. Structural brain reserve, an important determinant of cognitive function in late life, may be independently affected by suboptimal brain development during childhood and by factors acting to aggravate aging-related atrophy in late life.

If Midlife Tofu Consumption Leads to an Exaggeration of Brain Aging in Late Life, Why are Rates of Cognitive Impairment not Higher in Japan and Other Asian Nations where Soyfoods are a Staple? Our findings are counter-intuitive, given the great life expectancy of the Japanese, reports suggesting lower rates of Alzheimer's disease (but higher rates of vascular dementia) in Japanese elderly [1,3], and the well-established belief that tofu is a health promoting food. While no definitive explanation is possible, possibilities include (1) our findings are spurious or are peculiar to this population or (2) the widespread consumption of tofu in Japan and other Asian nations has contributed to the total burden of aging-related cognitive decline in that population, but its impact is relatively modest, compared with the influences of age, education, cerebrovascular disease and possibly other factors.

The recognition of aging-related cognitive decline, a relatively subtle phenomenon, is likely to be strongly influenced by social, cultural, educational and demographic factors, as well as by differences in how cognitive impairment is diagnosed and recorded by health care providers and researchers. The elevated prevalence of cognitive impairment we observed in the highest compared with the lowest midlife consumers of tofu was roughly of the magnitude as would be caused by a four year difference in age or a three year difference in education. In this study population, 20% to 25% of the burden of cognitive impairment appears attributable to midlife tofu consumption—an effect size of enormous public

health importance, yet not readily discernable in comparisons across populations of diverse education, occupation, age distribution and genetic composition, especially when studied using different methods. Even if high consumption of soy foods in a country such as Japan were to be associated with an increase in the age-specific prevalence of cognitive impairment in later life, a higher prevalence of cognitive impairment might not be apparent, given the many cross-national differences in age, education, apolipoprotein E type, cerebrovascular disease, differences in case ascertainment methods, and other factors.

Is it Possible that Isoflavone Phytoestrogens, Widely Viewed as Key Health Promoting Constituents of Soyfoods, might have an Adverse Influence on Brain Aging? Although low concentrations of isoflavone phytoestrogens are also found in sprouts, wheat, lentils, beans, chickpeas and other foods, soyfoods are the dominant human dietary source in the United States and most Asian nations [12–17]. Tofu is the major soyfood in Asian and in American vegetarian diets. Concentrations of isoflavones in the blood or urine of persons consuming soy foods have been shown to be substantial in persons consuming a tradition Asian diet [18,19]. Nonetheless, concentrations vary greatly among individuals, possibly reflecting differences in intestinal flora [20,21].

The pharmacologic properties of these molecules allow them to interact with estrogen receptors and with enzymes involved in estrogen metabolism. In some circumstances they act as weak estrogens; under other conditions their actions are anti-estrogenic [22,23]. Genistein, the most thoroughly studied soy phytoestrogen, is an inhibitor of aromatase, an enzyme found in the brain and other tissues that acts to convert androgenic hormones to estrogens [24,25]. Dietary phytoestrogens, including genistein and coumesterol, are potent inhibitors of estrogen-specific 17-beta-hydroxysteroid dehydrogenase and oxidoreductase type 1, enzymes involved in the biosynthesis and metabolism of endogenous estrogen [26]. Soy isoflavones have also been shown to inhibit tyrosine kinase [27] and to modulate topoisomerases I and II activities [28].

The physiologic impact of dietary phytoestrogens in a person who eats soyfoods regularly is not trivial, and nearly all studies reported to date confirm that regular consumption of a diet high in soyfoods results in pharmacologically significant blood levels of biologically active isoflavone phytoestrogens [12,29–31]. Although we have found no published information documenting an influence of dietary phytoestrogens on the central nervous system of adult humans, several studies have identified definite influences on endocrine, breast and gonadal tissues. Daily consumption of 60 grams of soy protein for one month was found to increase the follicular phase, lengthen the menstrual cycle and suppress the mid-cycle surges of luteinizing hormone and follicle-stimulating hormone in premenopausal women, thereby demonstrating effects similar to those reported for tamoxifen [32]. Recent studies suggest that premenopausal women consuming a diet high in isoflavone phytoestrogens may experience a drop in serum levels of endogenous estrogens in conjunction with alterations in the menstrual cycle [33,34]. In post-menopausal women, a six-week supplementation of the normal diet with soya flower (45 g daily), red clover sprouts and linseed induced modest changes in levels of follicle stimulating hormone and substantial vaginal cytology changes, indicating estrogenicity [35]. In other studies of post-menopausal women the effects of a high-phytoestrogen diet have been somewhat more variable [30,36,37]. Consumption of 38 grams of soy protein daily for five months was found to increase the secretion of breast fluid in women of childbearing age, with 29% of the women showing cytological evidence of epithelial hyperplasia [38].

The idea that phytoestrogens may affect late life brain structure and function rests in part on the role of endogenous (or replacement) estrogen as a modulator of brain aging. A growing body of information suggests that estrogens may be needed for optimal repair and replacement of neural structures eroded with aging, including synapses in the neocortex and hippocampus [39–42]. Chronic sub-optimal synaptic plasticity might be a factor in aging-related cognitive decline and could influence the clinical expression of dementing diseases, including Alzheimer's disease [43].

Although there are certainly estrogen receptors in the neurons of males, the importance of estrogens or androgens as modulators of plasticity and synaptic connectivity in aging men has received little attention [44]. Adipose cells make substantial amounts of estrogen, and estrogen blood levels are strongly related to adiposity in men and in women [45]. While androgens may have a direct effect on plasticity, their influence could be mediated through an estrogen-dependent mechanism, since estrogens are generated in the brain by conversion of testosterone to estradiol-17 beta through the action of an enzyme complex comprised of cytochrome P450 aromatase and NADPH-dependent cytochrome P450 reductase [46]. Aromatase activity in the brain seems to be of two types, one that is modulated by sex hormone blood levels (localized to the hypothalamus) and a second that is uninfluenced by blood levels of sex hormones (in the amygdala, hippocampus and other areas of the neocortex), suggesting two separate neural functions [24,47]. Thus the male brain's exposure to endogenous estrogen depends both on adiposity and the androgen-aromatase system.

There are now several reports indicating that oral intake of soy isoflavones by experimental animals results in significant alterations in brain metabolism, including (1) alterations in 5 alpha reductase activity in the amygdala and hypothalamic-preoptic area of adult male rats [48], (2) marginally elevated levels of nerve growth factor message RNA in the hippocampus of young ovariectomized rats and significantly elevated choline acetyl transferase message RNA in the frontal cortex of ovariectomized retired breeder rats [49,50], (3) apparently permanent alterations of calbindin-D28k levels and in the hypothalamus and preoptic areas of male and female rats resulting from dietary phytoestrogen exposure during pregnancy [51] and (4) up regulation in estrogen receptor beta RNA expression (in contrast to down regulation with 17-beta estradiol) in the hypothalamus [52].

In addition to pharmacologic mechanisms involving modulation of estrogen-related metabolic processes, certain soy isoflavones are potent inhibitors of tyrosine kinase, an enzyme

known to be involved in neuronal plasticity. In a study of the effects of five tyrosine kinase inhibitors on neuron electrophysiological functioning, genistein showed high specificity for hippocampal tyrosine kinase. Presumably by inhibiting the activity of this enzyme, genistein selectively blocked the induction of long-term potentiation (LPT) in post-synaptic cells [53]. LPT is widely viewed as centrally involved in learning and memory, particularly as they occur in the hippocampus and related brain areas, and normal postsynaptic LPT may be required for long-term synaptic plasticity in the hippocampus [54]. Genistein has also been shown to block voltage-sensitive sodium channels in cultured rat brain neurons, possibly by allosteric interaction with neurotoxin binding site 2 or possibly by virtue of its ability to inhibit tyrosine kinase [55]. Isoflavones might also effect plasticity by interfering with the regulation by tyrosine kinase of NMDA channels located at neuronal synapses [56].

The results presented here demonstrate an association of self-reported tofu consumption frequency in midlife with functional and structural indicators of brain aging. Because the effects were apparent at relatively modest levels of intake (two or more servings weekly), an adverse pharmacologic mechanism seems more likely than a nutritional pathogenesis. While our study cannot directly impune a specific constituent with certainty, isoflavone phytoestrogens are obvious candidates. We hypothesize that regular dietary exposure to soy isoflavones over many years during middle life may be associated with the appearance of accelerated brain aging in later life attributable to chronically sub-optimal neural plasticity. The specific means by which soy phytoestrogens might exert such influence could involve competition with endogenous estrogens for estrogen receptors in neurons and/or reduction in estrogen concentration in the brain by inhibition of the aromatization of androgens. Alternatively, isoflavones in tofu and other soyfoods might exert their influence through interference with tyrosine kinase dependent mechanisms required for optimal hippocampal function, structure and plasticity.

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