Midlife milk consumption and substantia nigra neuron density at death

Robert D. Abbott, PhD
G. Webster Ross, MD
Helen Petrovitch, MD
Kamal H. Masaki, MD
Lenore J. Launer, PhD
James S. Nelson, MD
Lon R. White, MD
Caroline M. Tanner, MD, PhD

Correspondence to Dr. Abbott: rda3e@virginia.edu

ABSTRACT

Objective: To examine the relationship between midlife milk intake and Parkinson disease (PD) incidence through associations with substantia nigra (SN) neuron density and organochlorine pesticide exposure in decedent brains from the Honolulu-Asia Aging Study.

Methods: Milk intake data were collected from 1965 to 1968 in 449 men aged 45–68 years with postmortem examinations from 1992 to 2004. Neuron density (count/mm^2) was measured in quadrants from a transverse section of the SN. Additional measures included brain residues of heptachlor epoxide, an organochlorine pesticide found at excessively high levels in the milk supply in Hawaii in the early 1980s.

Results: Neuron density was lowest in nonsmoking decedents who consumed high amounts of milk (>16 oz/d). After removing cases of PD and dementia with Lewy bodies, adjusted neuron density in all but the dorsomedial quadrant was 41.5% lower for milk intake >16 oz/d vs intake that was less (95% confidence interval 22.7%–55.7%, $p < 0.001$). Among those who drank the most milk, residues of heptachlor epoxide were found in 9 of 10 brains as compared to 63.4% (26/41) for those who consumed no milk ($p = 0.017$). For those who were ever smokers, an association between milk intake and neuron density was absent.

Conclusions: Milk intake is associated with SN neuron loss in decedent brains unaffected by PD. Whether contamination of milk with organochlorine pesticides has a role in SN neurodegeneration warrants further study. ***Neurology*® 2016;86:512–519**

GLOSSARY

H&E = hematoxylin & eosin; **HAAS** = Honolulu-Asia Aging Study; **HHP** = Honolulu Heart Program; **PD** = Parkinson disease; **SN** = substantia nigra.

Evidence suggests that environmental organochlorines and other pesticides have a role in the development of Parkinson disease (PD).[1–4] Postmortem studies in humans have found elevated levels of organochlorines in brain tissue, and more specifically, in the substantia nigra (SN) of decedents with PD.[3,4] In vitro studies suggest that organochlorines may increase the susceptibility of dopaminergic neurons to cell death.[5] Several long-term population-based studies, including one from the Honolulu-Asia Aging Study (HAAS), have also shown an association between the intake of dairy products, including milk, and the future risk of PD.[6–10] Milk can bioconcentrate certain organic pollutants such as organochlorine pesticides.[11] This is potentially noteworthy in Hawaii, where excessively high levels of heptachlor epoxide were reported to be found in the milk supply during the time that participants were being followed in the HAAS.[12,13]

Nigral neurodegeneration in PD is thought to begin years prior to the development of the classic motor features of PD.[14–16] Demonstration that milk consumption is related to SN neuron loss would provide further evidence that the association of milk intake with PD incidence is causal. The unselected autopsy series from the HAAS, where brain organochlorine levels and SN

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From the Center for Epidemiologic Research in Asia (R.D.A.), Shiga University of Medical Science, Otsu, Japan; the Pacific Health Research and Education Institute (R.D.A., G.W.R., H.P., J.S.N., L.R.W.), Honolulu; the Department of Medicine (G.W.R.) and the John A. Hartford Foundation Center of Excellence in Geriatrics, Department of Geriatric Medicine (R.D.A., G.W.R., H.P., K.H.M.), John A. Burns School of Medicine, University of Hawaii, Honolulu; the Veterans Affairs Pacific Islands Health Care System (G.W.R., H.P., L.R.W.), Honolulu; Kuakini Medical Center (K.H.M.), Honolulu, HI; the National Institute on Aging (L.J.L.), Bethesda, MD; and the San Francisco Veterans Affairs Medical Center and the Department of Neurology (C.M.T.), University of California, San Francisco.

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neuron counts were performed on individuals with and without PD, provides a useful opportunity to explore the relationship among milk intake, organochlorine levels in brain tissue, and SN neurodegeneration.

METHODS

Background and study sample. The HAAS was launched in 1991 as an extension of the Honolulu Heart Program (HHP) with a focus on cognitive impairment and associated neuropathologic abnormalities.[17] Initiation of the HHP began with enrollment of 8,006 men of Japanese ancestry from 1965 to 1968.[18] Subjects were aged 45–68 years. All received a comprehensive physical examination following a protocol documenting cardiac and neurologic risk factors and conditions related to cardiovascular disease. Subjects were followed through a series of repeat examinations and a review of hospital discharges, medical records, death certificates, and autopsy reports.

There were 3,734 participating HHP cohort members at the beginning of the HAAS, when an autopsy study was initiated following a rigorous protocol of postmortem brain examination. Microscopic surveys and measurement of SN neuron density are available on deaths that occurred from 1992 to 2004. During this period, there were 3,086 deaths among surviving members of the HHP. There were 2,502 without an autopsy. Among the 584 with an autopsy, neuron density measurement is complete for 485 brains.

Standard protocol approvals, registrations, and patient consents. Procedures were in accordance with institutional guidelines and approved by an institutional review board. Written informed consent was obtained from the study participants.

Measurement of milk intake and confounding information. At initiation of the HHP (1965–1968), information on midlife food intake was collected by a dietitian through the use of 24-hour dietary recall methods.[19] Standardized methods were used to gather individual recall of dietary intake based on food models and serving utensils to illustrate portion sizes.[19,20] Nutrient intake was measured by grouping foods into standard portions in 54 categories. Nutrient intake within a category was then obtained from the US Department of Agriculture Handbook No. 8 and from a food table specifically designed for the HHP.[21]

The amount of milk consumed in the previous 24 hours was recorded in oz/d. Other midlife dietary data included the intake of coffee, total kilocalories, and fat. Data were validated against a 7-day dietary record in 329 members of the original HHP cohort.[22] There were no significant differences between the 7-day and 24-hour assessment methods for 15 nutrient categories. Day-to-day variation was less than in typical Western cultures, suggesting that intake recorded by the 24-hour recall method is representative of usual or habitual intake. Additionally, only dietary intake reported as fairly typical or not unusual (defined in instructions for data collection by the dietician) is included for analysis in this article. Based on this criterion, 36 of 485 brains with measurements of neuron density were excluded (7.4%), leaving a sample of 449 decedents. Using the same criterion in those without an autopsy, 142 of 2,502 decedents were excluded (5.7%), leaving a sample of 2,360. The remaining samples are later compared to show that study characteristics at the time of dietary assessment (1965–1968) were similar between decedents with and without an autopsy.

Along with dietary factors, other potential confounders that existed at the time of dietary assessment were considered. Variables included age, pack-years of cigarette smoking, a measure of physical activity, and triceps-skinfold thickness. Adjustments were made for triceps-skinfold thickness based on earlier findings of an association between midlife adiposity and the future risk of PD.[23] As a correlate of body fat, physical activity was measured through the use of a physical activity index, a well-established index of overall metabolic output in a typical 24-hour period with strong relationships with cardiovascular disease.[24] High indices are associated with active lifestyles. As a surrogate for pesticide exposure, adjustments were made for years worked on a pineapple or sugarcane plantation.[25,26]

Autopsy methods and diagnostic criteria. Diagnoses of PD and dementia with Lewy bodies were based on clinical criteria and confirmed pathologically by the presence of Lewy bodies in either the substantia nigra or locus ceruleus.[27,28] All diagnoses were made after the dietary interview. Since 1991, clinical diagnosis of PD considered data from repeat physical and neurologic examinations that were given from 1991 to 2001. Participants with parkinsonian symptoms, those receiving PD medications, and those with a history of PD were referred to a study neurologist. Clinical diagnosis is based on consensus from at least 2 of the study neurologists trained in movement disorders using published criteria.[27,29]

Autopsy consent was obtained from a legal authorized representative according to Hawaii State law. Neuron counts in the SN were performed on a 30×, scaled, microprojector tracing of a single transverse hematoxylin & eosin (H&E)–stained 10-μm-thick section of the nucleus at the level of the roots of the oculomotor nerve.[16] Neuropathologists were blinded to clinical information.[30] Midbrain tegmentum and the crus cerebri formed dorsal and ventral borders of the nucleus. The lateral mesencephalic sulcus and the margin of the cerebral peduncle next to the oculomotor nerve roots marked the medial and lateral extent of the nucleus. The nucleus was divided into medial and lateral halves by a line drawn perpendicular to the midpoint of the transverse dimension. The tracing further divided the nucleus into dorsal and ventral halves forming the dorsolateral, ventrolateral, dorsomedial, and ventromedial quadrants. Neurons were counted in each quadrant. The area of each quadrant was determined from planimetric measurement of the traced quadrant. Neuron density was calculated as count/mm^2.

Single H&E-stained sections from both midbrain and pons were examined for Lewy bodies in the SN and locus ceruleus. For those with Lewy bodies, α-synuclein staining was performed and Lewy bodies were counted in insula, cingulate cortex, amygdala, entorhinal cortex, and the 4 neocortical regions. In addition to the clinical diagnosis of PD and dementia with Lewy bodies, postmortem findings were used to identify decedents with neuropathologic evidence of PD (based on the finding of Lewy bodies and neuronal loss in either the SN or locus ceruleus) and dementia with Lewy bodies.[28] Residues of heptachlor epoxide were measured in frozen occipital lobes as previously described.[31]

Statistical methods. Comparisons of the study characteristics at the time of dietary assessment were made between autopsied and nonautopsied decedents based on general linear regression models. For those who received autopsies, similar models were used to compare the study characteristics and postmortem correlates of SN neuron density across common strata of milk intake. For dichotomous postmortem correlates, logistic regression was used with exact testing methods for correlates that occurred infrequently.[32] As a test for trend, milk intake was modeled in its original continuous format.

Assessments of the association between milk intake and neuron density were made separately for nonsmokers and for those

who were ever smokers. This was done in large part because smoking is associated with a lower risk of PD,[33] and there is a tendency for smoking to reduce PD susceptibility from environmental factors[25,26] and other attributes linked to PD.[29,34]

Average neuron densities (and standard deviations) are reported for each quadrant. To examine the association between neuron density and milk intake, neuron density was modeled as an over dispersed integer response following a negative binomial distribution.[35] Milk intake was modeled as a continuous independent variable. Comparisons of neuron density were also made between milk intake strata. Regression coefficients yield a percent deficit (a minus sign represents a deficit) or excess in neuron density between the strata. Adjustments were made for age at death and other characteristics. All reported p values are based on 2-sided tests of significance.

RESULTS Decedent characteristics with and without an autopsy are shown in table 1. Other than a slightly higher age at death among autopsied decedents, there were no other significant group differences.

The relationship between midlife milk intake and SN neuron density is described in table 2. For ever smokers (n = 304), and separately for past and current smokers, there were no associations between milk intake and SN neuron density. In contrast, for nonsmokers (n = 143), there was a tendency for SN neuron density to decline in the ventromedial and dorsolateral quadrants with increasing amounts of milk consumed ($p = 0.006$ and $p < 0.001$). For all quadrants, neuron density was maximized in those who consumed no milk and lowest in those who consumed the most milk. The greatest effect of milk intake on neuron density occurred for amounts exceeding 16 oz/d. Except for the dorsomedial quadrant, neuron density was significantly lower in those who consumed >16 oz/d vs those whose intake was less ($p < 0.01$). Neuron density was unrelated to milk intake ≤16 oz/d.

Table 3 further describes the association of milk intake with the study characteristics measured at the time of dietary assessment (1965–1968). For nonsmokers, associations between milk intake and age, coffee intake, and years worked on a plantation were absent. Although not significant, physical activity increased with greater amounts of milk consumed while triceps-skinfold thickness declined. Increasing intake of milk was associated with greater intake of total calories ($p = 0.002$) and fat ($p < 0.001$). For ever smokers, patterns of association were similar. Exceptions include a significant decline in triceps-skinfold thickness with greater amounts of milk consumed ($p = 0.039$). As with nonsmokers, total caloric and fat intake tended to rise with increasing milk intake.

The relationship between milk intake and postmortem correlates of SN neuron density is described in table 4. Milk intake was unrelated to time to death in both nonsmokers and ever smokers. Because dementia with Lewy bodies is rare, its association with milk intake is difficult to assess and not significant. Although the number of PD cases is also small, the percent of nonsmoking decedents with PD increased with amounts of milk consumed ($p = 0.020$). Prevalence of PD in ever smokers declined as milk intake increased ($p = 0.031$).

Detection of heptachlor epoxide was also associated with milk intake, although only in nonsmokers. Among 116 brains screened for heptachlor epoxide, frequency of detectable residues was highest in nonsmokers who drank the most amounts of milk (test for trend $p < 0.017$). For those who consumed >16 oz/d of milk,

Table 1 Study characteristics at the time of dietary assessment of milk intake (1965–1968) in decedents with and without an autopsy

Study characteristics[a]	Autopsied (n = 449), mean ± SD	Not autopsied (n = 2,360), mean ± SD
Age, y	54.1 ± 4.9	54.1 ± 5.1
Age at death, y	85.7 ± 5.2	85.0 ± 5.3
Pack-years of smoking	29.9 ± 29.3	30.2 ± 28.6
Coffee intake, oz/d[b]	13.5 ± 12.6	13.8 ± 12.9
Physical activity index	33.1 ± 4.9	32.9 ± 4.5
Triceps-skinfold, mm	7.9 ± 3.1	8.0 ± 3.3
Total caloric intake	2,341 ± 684	2,308 ± 700
Total fat intake, g	90.0 ± 36.9	86.5 ± 38.1
Years of plantation work	3.5 ± 7.8	3.6 ± 8.6
Midlife milk intake, oz/d	5.7 ± 7.5	5.1 ± 7.0

Autopsied decedents were significantly older at death than decedents without an autopsy ($p = 0.014$). There are no other significant differences in study characteristics between decedents with and without an autopsy.
[a] For autopsied decedents, 2 are missing data on cigarette smoking, 3 are missing data on the physical activity index, and 1 is missing data on plantation work. For those without an autopsy, 21 are missing data on cigarette smoking, 14 are missing data on the physical activity index, and 3 are missing data on plantation work.
[b] 1 oz = 29.6 mL.

Table 2 Neuron density (count/mm^2) by midlife milk intake and smoking status within each quadrant of the substantia nigra

	Midlife milk intake, oz/d[a]				
Quadrant	0	>0–8	>8–16	>16	Test for trend
Nonsmoker, sample size[b]	52	54	25	12	
Ventrolateral	20.3 ± 11.5	19.2 ± 9.9	20.3 ± 10.0	9.9 ± 7.2[c]	0.021
Ventromedial	17.5 ± 8.5	17.0 ± 8.3	15.6 ± 7.6	10.1 ± 4.8[d]	0.006
Dorsolateral	14.3 ± 6.9	12.1 ± 6.4	12.6 ± 5.8	7.3 ± 5.8[c]	<0.001
Dorsomedial	20.9 ± 10.6	17.6 ± 8.4	20.4 ± 11.9	14.6 ± 8.9	0.108
Total	18.3 ± 7.3	16.5 ± 6.0	17.0 ± 6.0	10.7 ± 5.7[c]	0.001
Ever smoker, sample size	105	128	45	26	
Ventrolateral	21.1 ± 9.0	18.8 ± 10.0	19.4 ± 9.6	18.7 ± 6.8	0.617
Ventromedial	19.4 ± 9.6	18.4 ± 10.0	18.4 ± 10.9	20.9 ± 11.8	0.737
Dorsolateral	14.1 ± 8.6	14.1 ± 7.8	14.2 ± 9.7	15.8 ± 7.4	0.132
Dorsomedial	21.4 ± 9.2	18.5 ± 9.7	19.2 ± 9.2	20.3 ± 7.7	0.722
Total	19.1 ± 6.8	17.6 ± 7.5	18.0 ± 7.2	19.1 ± 6.7	0.817

Values are mean ± SD. Neuron density is unrelated to milk intake ≤16 oz/d.
[a] 1 oz = 29.6 mL.
[b] Smoking data are missing on 2 decedents.
[c] Neuron density is significantly lower in those whose milk intake exceeded 16 oz/d vs those whose intake was ≤16 oz/d ($p < 0.001$).
[d] Neuron density is significantly lower in those whose milk intake exceeded 16 oz/d vs those whose intake was ≤16 oz/d ($p = 0.002$).

heptachlor epoxide residues were found in 9 of 10 brains. For those who drank no milk, residues were found in 63.4% of brains (26/41).

In the absence of heptachlor epoxide (n = 40 brains), associations between milk intake and neuron density were absent in all but one quadrant. Although

Table 3 Study characteristics at the time of dietary assessment of milk intake (1965–1968) by midlife milk intake and smoking status

	Midlife milk intake, oz/d[a]				
Study characteristics	0	>0–8	>8–16	>16	Test for trend
Nonsmoker, sample size	52	54	25	12	
Age, y	55.8 ± 4.9	54.9 ± 5.1	55.0 ± 4.4	53.5 ± 5.6	0.161
Coffee intake, oz/d	8.7 ± 8.8	10.1 ± 7.9	11.8 ± 10.0	4.7 ± 8.2	0.293
Physical activity index	32.1 ± 3.8	33.7 ± 5.5	33.9 ± 5.0	34.3 ± 3.7	0.132
Triceps-skinfold, mm	8.1 ± 3.2	7.9 ± 2.5	7.6 ± 2.4	7.5 ± 3.7	0.500
Total caloric intake	2,039 ± 737	2,331 ± 561	2,524 ± 596	2,612 ± 563	0.002
Total fat intake, g	76.9 ± 36.0	89.5 ± 32.8	94.6 ± 32.4	118.7 ± 40.0	<0.001
Years of plantation work	1.9 ± 5.4	4.1 ± 7.9	7.4 ± 14.0	3.3 ± 8.1	0.289
Ever smoker, sample size	105	128	45	26	
Age, y	53.6 ± 4.8	53.3 ± 5.0	54.2 ± 5.0	54.7 ± 4.5	0.239
Coffee intake, oz/d	13.9 ± 13.1	15.2 ± 11.0	19.3 ± 20.2	17.1 ± 12.9	0.228
Physical activity index	32.8 ± 4.5	33.2 ± 5.0	33.2 ± 5.6	33.7 ± 5.7	0.822
Triceps-skinfold, mm	8.7 ± 3.1	7.5 ± 3.3	7.5 ± 2.7	7.0 ± 3.4	0.039
Total caloric intake	2,308 ± 734	2,419 ± 687	2,317 ± 510	2,526 ± 804	0.276
Total fat intake, g	85.4 ± 40.6	92.2 ± 35.2	94.8 ± 32.8	101.2 ± 40.8	0.027
Years of plantation work	3.3 ± 6.8	3.4 ± 8.2	3.9 ± 7.6	2.5 ± 6.1	0.856

Values are mean ± SD.
[a] 1 oz = 29.6 mL.

Table 4 Postmortem correlates of neuron density by midlife milk intake and smoking status

	Midlife milk intake, oz/d[a]				
Postmortem characteristic	0	>0–8	>8–16	>16	Test for trend
Nonsmoker, sample size	52	54	25	12	
Time to death, y	31.5 ± 3.1	32.2 ± 3.2	31.7 ± 3.2	33.2 ± 2.2	0.290
Parkinson disease	5.8 (3/52)	1.9 (1/54)	16.0 (4/25)	25.0 (3/12)	0.020
Dementia with Lewy bodies	3.9 (2/52)	3.7 (2/54)	0.0 (0/25)	0.0 (0/12)	0.408
Detectable levels of heptachlor epoxide[b]	63.4 (26/41)	54.6 (24/44)	81.0 (17/21)	90.0 (9/10)	0.017
Ever smoker, sample size	105	128	45	26	
Time to death, y	31.1 ± 3.0	31.7 ± 3.4	31.2 ± 3.0	31.0 ± 3.8	0.769
Parkinson disease	7.6 (8/105)	5.5 (7/128)	0.0 (0/45)	0.0 (0/26)	0.031
Dementia with Lewy bodies	1.9 (2/105)	4.7 (6/128)	2.2 (1/45)	0.0 (0/26)	0.877
Detectable levels of heptachlor epoxide[b]	70.2 (59/84)	67.7 (67/99)	86.1 (31/36)	53.3 (8/15)	0.507

Values are mean ± SD or % (n/sample size).
[a] 1 oz = 29.6 mL.
[b] Measurement of heptachlor epoxide is incomplete in 27 nonsmokers and 70 ever smokers.

data are limited (with only 1 decedent in the highest milk intake stratum), neuron density in the dorsolateral quadrant declined consistently from 14.3/mm^2 to 1/mm^2 as milk intake increased from 0 to >16 oz/d ($p = 0.013$). For those with measurable levels of heptachlor epoxide in the highest milk intake strata (>16 oz/d), 3 of 9 brains (33.3%) had PD. For brains with measurable levels of heptachlor epoxide in the other milk intake strata, 7.7% (2 of 26), 4.2% (1 of 24), and 17.7% (3 of 17) had PD as milk intake increased from 0 to >8–16 oz/d. In the absence of detectable levels of heptachlor epoxide, there was only one case of PD (in a decedent whose milk intake was >8–16 oz/d).

Table 5 further describes the association between milk intake and SN neuron density after removing decedents with PD and dementia with Lewy bodies. Since SN neuron density was unrelated to milk intake ≤16 oz/d, neuron density in those who consumed >16 oz/d was compared to those whose intake was less. Adjustments were also made for age at death and other factors. For nonsmokers, milk intake continued to be related to SN neuron density in ventrolateral, ventromedial, and dorsolateral quadrants. After covariate adjustment, neuron density in the 3 affected quadrants was 39.4%–43.5% lower in those in the highest intake strata vs intake that was less ($p < 0.01$). For ever smokers, milk intake remained unrelated to SN neuron density. Although not removed from table 5, any confounding effects from incidental Lewy bodies were absent.

DISCUSSION While past reports have found an association between dairy products, including milk intake, and higher PD risk,[6–10] current findings suggest that the relationship can begin with early nigral neurodegeneration prior to the onset of clinical PD, and may involve a link with heptachlor epoxide. Although generalizability to women and other ethnicities is difficult to confirm, the link is reasonable for Hawaii, where contamination of the milk supply with heptachlor epoxide is well-documented.[12,13] We lack evidence that the actual milk consumed by our study decedents was contaminated with heptachlor epoxide other than to note that it occurred at a critical time of midlife dietary intake for the current sample. Although contamination was reported to have occurred from 1981 to 1982, when it began is unclear. While difficult to confirm, the fairly typical measure of milk intake in the current study could be a surrogate for continued intake in the years that followed. Intake of milk exceeding 16 oz/d may also be a marker of intake that is less sporadic than for other food items. Alternative explanations for the presence of heptachlor epoxide in the brains of decedents who consumed large amounts of milk are not apparent.

There is also evidence that organochlorine pesticides have a role in the etiology of PD. Organochlorine pesticides are more commonly found in the brains of patients with PD than in cases of Alzheimer disease and controls.[3,4] In a recent report from Hawaii, combinations of heptachlor epoxide, methoxychlor, and benzene hexachloride were more common in the presence vs the absence of Lewy pathology.[31] In addition to other organochlorine compounds, many organic environmental chemicals can bioconcentrate in milk and adipose tissue.[11,36] Whether the milk

Table 5 Percent deficit in neuron density (a minus sign represents a deficit) for decedents whose midlife milk intake exceeded 16 oz/d[a] vs lower intake in the absence of Parkinson disease and dementia with Lewy bodies by smoking status and substantia nigra quadrant

	Percent deficit in neuron density	
Quadrant	Unadjusted	Adjusted[b]
Nonsmoker		
Ventrolateral[c]	−39.8 (−58.2, −13.1)	−39.4 (−58.7, −11.2)
Ventromedial[c]	−39.8 (−57.0, −15.7)	−40.3 (−58.4, −14.4)
Dorsolateral[c]	−37.9 (−56.4, −11.4)	−43.5 (−61.4, −17.2)
Dorsomedial	−17.7 (−40.4, 13.6)	−17.2 (−41.2, 16.6)
Total[c]	−32.0 (−47.0, −12.9)	−32.5 (−48.0, −12.4)
Ever smoker		
Ventrolateral	−9.6 (−25.2, 9.3)	−10.7 (−25.9, 7.5)
Ventromedial	7.2 (−12.6, 31.4)	9.7 (−10.3, 34.3)
Dorsolateral	8.5 (−12.3, 34.3)	9.4 (−11.5, 35.4)
Dorsomedial	−0.8 (−17.4, 19.2)	1.5 (−15.3, 21.5)
Total	0.7 (−12.9, 16.2)	1.6 (−11.8, 17.1)

Values are % (95% confidence interval).
[a] 1 oz = 29.6 mL.
[b] Adjusted for age at death, coffee intake, physical activity index, triceps-skinfold thickness, total caloric intake, total fat intake, and years of plantation work. Decedents with missing data were deleted from the analysis.
[c] Except for the dorsomedial quadrant, the percent deficit in neuron density in nonsmokers is significantly higher in those whose milk intake exceeded 16 oz/d vs intake that was less ($p < 0.01$).

supply in Hawaii was contaminated with these compounds is unknown.

Although the sample is limited, neuron density continued to decline with increasing milk intake in the absence of heptachlor epoxide. Associations were weaker, however, and only occurred in a single SN quadrant. If real, other contributing factors need to be considered. One possibility includes uric acid. Low uric acid is a risk factor for PD and uric acid is lower in high consumers of milk.[37,38] In the current sample, there was no association between serum uric acid and SN neuron density. Explanatory effects from calcium, cheese, butter, ice cream, and yogurt were also not apparent, although consumption is low and possibly sporadic.

The timing when risk factors are measured could also be important for identifying relationships with diseases that seldom occur until later in life. While risk factors measured early in life can change with time, those measured later could have limited opportunity to exert an influence on a disease outcome. Since PD is known to include a long preclinical period of neurodegeneration, the use of milk intake in midlife for the current sample could be an advantage. Prior to PD onset, neurodegeneration can begin as early as the third decade of life.[14] It takes a 50% decline in SN neuron density before parkinsonian symptoms and motor signs begin to appear.[15,16] Intake of milk in later life could also be influenced by effects of preclinical PD processes on liquid and dietary intake.[39]

The quantity of milk consumed might also be important. Support for this is provided by the current sample and our earlier article on clinical PD.[6] For milk intake that exceeded 16 oz/d, neuron density was significantly lower and PD risk was significantly higher vs intake that was less. For both outcomes, neuron density and the incidence of PD were unrelated to milk intake ≤16 oz/d.

The possibility that cigarette smoking is neuroprotective also warrants emphasis. Among ever smokers, a relationship between milk intake and SN neuron density is noticeably absent. Similar effects of smoking have been observed for other factors. Infrequent bowel movements and elevated levels of hemoglobin in late life are associated with low neuron density, but only in nonsmokers.[29,34] Cigarette smoking also reduces susceptibility to clinical PD from pesticide exposure and the intake of carbohydrates and polyunsaturated fatty acids.[26]

It should be noted that while prevalent PD rose with increasing milk intake in the sample of autopsied men who were nonsmokers (table 4), PD prevalence declined with increasing milk intake for ever smokers. In our earlier article on clinical PD,[6] while we failed to examine the effects of milk separately within smoking stratum, the presence of significant interaction between milk intake and pack-years of cigarette smoking was absent. In a reanalysis, high levels of milk intake (>16 oz/d) in nonsmokers continued to be strongly associated with an increased risk of PD. For ever smokers, an association was absent. While this is an important omission, it adds support for a neuroprotective effect of cigarette smoking against factors that would otherwise be associated with an increased risk of PD. Its role in the current study and in other studies involving environmental exposures and preclinical features of PD warrants consideration.

AUTHOR CONTRIBUTIONS

R.D.A., G.W.R., H.P., L.R.W., and C.M.T. participated in study conception, interpretation of findings, and drafting the report. R.D.A. conducted the statistical analysis. K.H.M., L.J.L., and J.S.N. helped draft the report. J.S.N. participated in data acquisition.

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This Week's *Neurology*® Podcast

Midlife milk consumption and substantia nigra neuron density at death **(see p. 512)**

This podcast begins and closes with Dr. Robert Gross, Editor-in-Chief, briefly discussing highlighted articles from the February 9, 2016, issue of *Neurology*. In the second segment, Dr. Binit Shah talks with Dr. Robert Abbott about his paper on midlife milk consumption and substantia nigra neuro density at death. Dr. Robert Gross interviews Dr. Ray Dorsey about being the section editor and curator of our new *Neurology* site, *Innovations in Care Delivery*, in our "What's Trending" feature of the week. In the next part of the podcast, Dr. Ted Burns focuses his interview with Dr. Thomas Bird on a *Neurology Today*® story on whether there should be a moratorium on human germline editing.

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Midlife milk consumption and substantia nigra neuron density at death

Robert D. Abbott, G. Webster Ross, Helen Petrovitch, et al.

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