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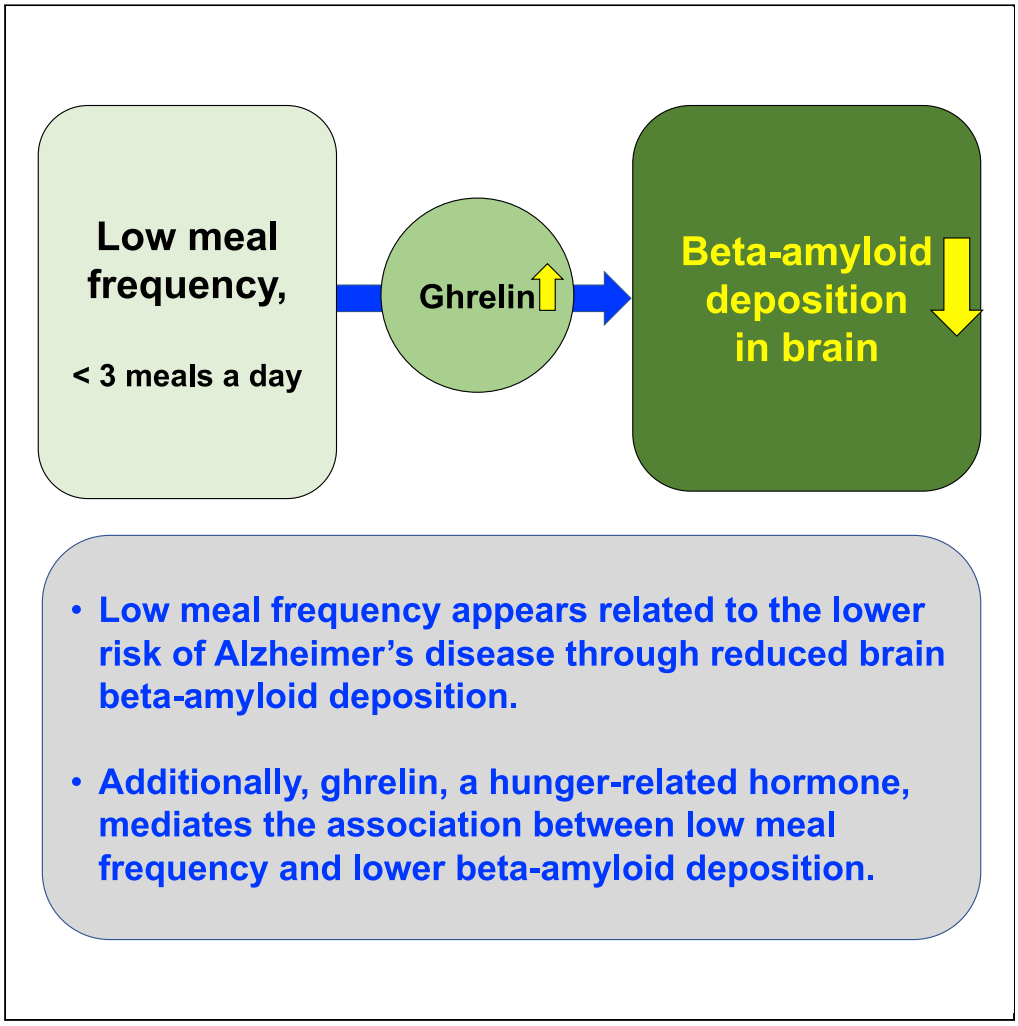

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Highlights

Low meal frequency (LMF) relates to lower brain amyloid deposition in human

Ghrelin mediates the association between LMF and lower amyloid deposition

LMF may decrease the risk of Alzheimer's disease (AD) by reducing amyloid deposition

This finding may be a clue for food intake-based preventive strategy against AD

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Association of low meal frequency with decreased *in vivo* Alzheimer's pathology

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SUMMARY

Little is known about the association between meal frequency and Alzheimer's disease (AD) in humans. We tested the hypothesis that low meal frequency (LMF) is associated with reduced *in vivo* AD pathology in human brain, and additionally investigated the mediation of serum ghrelin, a hunger-related hormone, for the association. A total of 411 non-demented older adults were systematically interviewed to identify their dietary patterns including meal frequency and underwent multi-modal neuroimaging for cerebral beta-amyloid (Aβ) and tau deposition, glucose metabolism, and cerebrovascular injury. LMF (less than three meals a day) was significantly associated with lower Aβ deposition compared to high meal frequency (HMF). In addition, both LMF and reduced Aβ deposition were significantly related to elevated serum ghrelin. Our findings suggest that LMF may be related to the lower risk of AD through reduced brain amyloid deposition. Additionally, ghrelin appears mediate the association between LMF and lower amyloid deposition.

INTRODUCTION

A growing body of evidence has suggested an association between food or nutritional intake, one of the most important modifiable lifestyle factors, and the risk of Alzheimer's disease (AD) and related cognitive decline (Jeon et al., 2019; Morris and Tangney, 2010). While many studies have focused on specific nutritional components or their combinations as AD and cognitive decline risk factors (Berti et al., 2018; Gu et al., 2010), other studies have indicated that dietary restriction (DR), either caloric restriction (CR) (Luchsinger et al., 2002) or intermittent fasting (IF) (de Cabo and Mattson, 2019), may be protective against AD or age-related neurodegeneration.

Several animal studies (Halagappa et al., 2007; Singh et al., 2012) implied that IF or diets with relatively long inter-meal intervals, independently of CR, may be protective against AD and related brain impairments. Studies using animal models of AD (Bruce-Keller et al., 1999; Zhu et al., 1999) also showed that low meal frequency (LMF) increases neuronal resistance to excitotoxic injury and reduces deficits in learning and memory. Moreover, a recent study (Gregosa et al., 2019) demonstrated that LMF, without CR, ameliorated beta-amyloid (Aβ) pathology and cognitive impairment in transgenic AD mice. Additionally, a couple of animal studies (Drazen et al., 2006; Sugino et al., 2002) suggested that LMF has a potential to increase ghrelin, a hunger-related hormone. Other studies (Jeon et al., 2019; Jeong et al., 2018) also indicated that the elevation of ghrelin has a protective effect against Aβ pathology and AD. These raise a possibility that LMF or relatively long inter-meal intervals may decrease the risk of AD via the change of ghrelin. Nevertheless, little is known about the association between meal frequency and AD or AD pathology in humans.

In this context, we set out to test the hypothesis that LMF, defined as less than 3 meals per day (Paoli et al., 2019), is associated with reduced *in vivo* AD pathologies, including cerebral Aβ and tau deposition, and AD-related neurodegeneration, as measured on multi-modal brain imaging, in non-demented older adults. We also investigated the association between LMF and white matter hyperintensities (WMHs) on MRI as a measure of cerebrovascular white matter injury referring to previous studies that reported an association between DR and decreased vascular risk factors or cerebrovascular diseases (Brandhorst and

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Longo, 2019). Additionally, we investigated the mediation of serum ghrelin for the association between LMF and *in vivo* brain pathology.

RESULTS

Participant characteristics

Table 1 presents the demographic and clinical characteristics of the participants according to meal frequency categories. Of all the participants, 272 were categorized into the HMF group and 139 into the LMF group. No participants were malnourished (*i.e.,* serum albumin <3.5 g/dL (Cabrerizo et al., 2015)).

Association between meal frequency and *in vivo* brain pathologies

As shown in Table 2 and Figures 1A and 1B, LMF was associated with a lower global Aβ retention and Aβ positivity rate compared to HMF, regardless of the models tested. In contrast, we did not find any significant difference in other pathological markers between the meal frequency groups. Sensitivity analyses only for CN participants showed similar results (see Table S3).

Moderation for the association between meal frequency and Aβ deposition

The interaction between meal frequency and LPA was significant, indicating that LPA moderates the association between meal frequency and global Aβ retention (or Aβ positivity) (see Table S4). Further subgroup analyses showed that LMF was significantly associated with lower global Aβ retention (or Aβ positivity) only in the low LPA subgroup, not in high LPA one (see Table 3, Figures 1C and 1D). In contrast, the interactions between meal frequency and variables such as age, sex, APOE4, depression, BMI status, inter-meal snack intake, and clinical diagnosis were not significant (see Table S4).

Mediation by ghrelin for the association between meal frequency and Aβ deposition

The LMF group had higher serum ghrelin level than the HMF group, regardless of the models tested (see Table 4 and Figure 1E). Serum ghrelin showed a significant inverse association with brain Aβ deposition (see Table 4 and Figure 1F). When serum ghrelin was controlled as an additional covariate, the association between meal frequency groups and Aβ deposition was no longer significant (see Table 4).

DISCUSSION

The present study revealed that LMF (less than three meals a day) was significantly associated with decreased Aβ deposition in non-demented older adults, while the meal frequency had no associations with other brain pathologies.

This finding on the association between LMF and a decreased Aβ deposition is in line with the results from a preclinical study using an AD animal model,[20] which demonstrated that diet with long inter-meal intervals ameliorated Aβ pathology and reduced cognitive impairment. Other preclinical studies (Halagappa et al., 2007; Shin et al., 2018; Singh et al., 2012) also suggested that longer fasting time or diets with relatively long inter-meal intervals, independently of CR, are protective against AD or age-related brain impairment.

We found significant relationships not only between LMF and increased serum ghrelin but also between increased serum ghrelin and lower brain Aβ deposition. In addition, the association of LMF with lower Aβ deposition was no longer significant when serum ghrelin was controlled as an additional covariate in the model. These findings indicate that ghrelin may be a link between LMF and decreased Aβ deposition. The relatively longer fasting times associated with LMF may contribute to lower Aβ deposition by inducing the hunger-related hormone ghrelin. Ghrelin is an orexigenic hormone that binds to its growth hormone secretagogue-receptor (GHS-R) and regulates food intake (Jeon et al., 2019). Animal studies using AD-transgenic mice have shown significant decreases in cerebral Aβ accumulation by the administration of ghrelin receptor agonist (*i.e.*, LY444711) or GHS-R agonist (*i.e.,* MK-0677) (Jeon et al., 2019). As an alternative mechanism for the association between LMF and lower brain Aβ, LMF-associated reduction of the total caloric intake may play a role. A couple of preclinical studies (Patel et al., 2005; Schafer et al., 2015) have shown that CR reduced Aβ deposition in an AD mouse model. However, this possibility appears less likely given that we found no significant difference in body weight or BMI between the two meal frequency

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Table 1. Participant characteristics

Characteristic	Meal frequencies			t or χ^2	p
	3 meals per day	<3 meals per day	total		
n	272	139	411		
Age, y	71.28 (7.9)	69.76 (7.5)	70.77 (7.8)	1.878	0.061
Female, No. (%)	135 (49.6)	97 (69.8)	232 (56.4)	15.195	<0.001
Education, y	11.39 (4.9)	10.65 (4.6)	11.14 (4.8)	1.457	0.146
MMSE	25.42 (3.6)	25.63 (3.0)	25.49 (3.4)	−0.632	0.528
APOE4 positivity, No. (%)	60 (22.1)	35 (25.4)	95 (23.2)	0.561	0.454
Clinical diagnosis, CN, No. (%)	189 (69.5)	89 (64.0)	278 (67.6)	1.251	0.263
Lifetime physical activity, MET-h/week (n = 330)	74.00 (55.6)	75.28 (55.1)	74.40 (55.4)	0.193	0.847
Lifetime cognitive activity score	2.30 (0.6)	2.20 (0.7)	2.27 (0.6)	1.500	0.134
Occupational complexity, No. (%)				1.629	0.804
None	49 (18.0)	26 (18.8)	75 (18.3)		
Skill level 1	18 (6.6)	11 (8.0)	29 (7.1)		
Skill level 2	86 (31.6)	48 (34.8)	134 (32.7)		
Skill level 3	40 (14.7)	15 (10.9)	55 (13.4)		
Skill level 4	79 (29.0)	38 (27.5)	117 (28.5)		
Annual income, No. (%)				0.085	0.958
<MCL	22 (8.1)	12 (8.6)	34 (8.3)		
≥MCL, <2× MCL	121 (44.5)	63 (45.3)	184 (44.8)		
≥2× MCL	129 (47.4)	64 (46.0)	193 (47.0)		
Vascular risk score, %	17.71 (16.3)	18.35 (16.7)	17.92 (16.4)	−0.371	0.711
Geriatric depression scale				1.765	0.184
Normal (<9), No. (%)	210 (77.2)	99 (71.2)	309 (75.2)		
Depressed (≥10), No. (%)	62 (22.8)	40 (28.8)	102 (24.8)		
Fluid intake				2.609	0.271
More than 5 cups	172 (63.2)	85 (62.0)	257 (62.8)		
3 to 5 cups	87 (32.0)	40 (31.5)	127 (31.1)		
Less than 3 cups	13 (4.8)	12 (8.8)	25 (6.1)		
Alcohol intake, SD per week, lifetime	6.16 (16.2)	4.86 (13.4)	5.72 (15.3)	0.814	0.416
Smoking, pack per day, lifetime	0.33 (0.6)	0.23 (0.5)	0.30 (0.5)	1.801	0.073
Dietary pattern and nutritional assessment					
Malnutrition (%)	0 (0.0)	0 (0.0)	0 (0.0)		
Dietary pattern including food types					
Protein, No (%)				0.861	0.650
High	37 (13.6)	15 (10.9)	52 (12.7)		
Moderate	109 (40.1)	53 (38.7)	162 (39.6)		
Low	126 (46.3)	69 (50.4)	195 (47.7)		
Fruit & Vegetables, No (%)				3.529	0.060
High	175 (64.3)	75 (54.7)	250 (61.1)		
Low	97(35.7)	62 (45.3)	159 (38.9)		
Fried foods, No (%)				3.483	0.175
High (Always)	16 (5.9)	7 (5.0)	23 (5.6)		
Moderate (Sometimes)	70 (25.7)	48 (34.5)	118 (28.7)		

(*Continued on next page*)

Table 1. *Continued*

Characteristic	Meal frequencies			t or χ^2	p
	3 meals per day	<3 meals per day	total		
Low (Seldom)	186 (68.4)	84 (60.4)	270 (65.7)		
Fatty foods, No (%)				0.864	0.649
High (Always)	106 (39.0)	48 (34.5)	154 (37.5)		
Moderate (Sometimes)	78 (28.7)	41 (29.5)	119 (29.0)		
Low (Seldom)	88 (32.4)	50 (36.0)	138 (33.6)		
Salty foods, No (%)				5.736	0.057
High (Always)	22 (8.1)	12 (8.6)	34 (8.3)		
Moderate (Sometimes)	70 (25.7)	51 (36.7)	121 (29.4)		
Low (Seldom)	180 (66.2)	76 (54.7)	256 (62.3)		
Inter-meal snack, No (%)				2.025	0.363
High (Always)	6 (2.2)	2 (1.4)	8 (1.9)		
Moderate (Sometimes)	27 (9.9)	20 (14.4)	47 (11.4)		
Low (Seldom)	239 (87.9)	117 (84.2)	356 (86.6)		
Serum nutritional markers					
Albumin,	4.49 (0.2)	4.45 (0.2)	4.47 (0.2)	1.402	0.162
Glucose, fasting	105.24 (22.5)	103.59 (22.7)	104.68 (22.5)	0.702	0.483
HDL-Cholesterol	54.03 (13.2)	55.10 (15.5)	54.39 (36.1)	−0.736	0.462
LDL-Cholesterol	108.30 (31.1)	109.46 (36.1)	108.69 (32.8)	−0.337	0.736
Iron	120.45 (41.1)	112.66 (33.4)	117.83 (38.8)	1.936	0.054
Ferritin	127.22 (100.9)	109.24 (74.4)	121.16 (93.1)	1.743	0.082
Zinc	86.19 (11.3)	86.80 (12.9)	86.40 (11.8)	−0.462	0.644
Vitamin B_{12}	597.76 (380.8)	552.88 (220.4)	582.66 (335.9)	1.509	0.132
Folic acid	10.39 (5.8)	9.45 (5.3)	10.07 (5.6)	1.609	0.108
Body index					
Body weight, kg	61.29 (9.2)	61.40 (10.4)	61.33 (9.6)	−0.114	0.909
Body mass index, kg/m^2				5.914	0.052
Underweight (<18.5)	5 (1.8)	3 (2.2)	8 (2.0)		
Normal weight (18.5–24.9)	170 (62.5)	69 (50.0)	239 (58.3)		
Overweight (≥25)	97 (35.7)	66 (47.8)	163 (39.8)		
Serum ghrelin, pg/ml (n = 357)	151.39 (110.43)	185.53 (125.95)	162.96 (116.87)	6.940	0.009
Cerebral Aβ deposition					
Aβ positivity, No. (%)	74 (27.2)	28 (20.1)	102 (24.8)	2.459	0.117
Aβ retention, SUVR	1.30 (0.4)	1.26 (0.3)	1.29 (0.4)	1.138	0.256
Cerebral tau deposition* (n = 117)					
AV-1451, SUVR	1.61 (0.8)	1.58 (0.7)	1.60 (0.8)	0.195	0.846
Neurodegeneration					
AD-CM, SUVR	1.39 (0.1)	1.40 (0.1)	1.39 (0.1)	−0.335	0.738
AD-CT, mm	2.79 (0.2)	2.82 (0.2)	2.80 (0.2)	−1.295	0.196
WMH volume, cm^3 (n = 367)	6.28 (5.6)	5.45 (5.0)	6.01 (5.4)	1.442	0.150

APOE4, apolipoprotein ε4; CN, cognitively normal; MCL, minimum cost of living; Aβ, beta-amyloid; AD, Alzheimer's disease; AD-CM, Alzheimer's disease signature cerebral glucose metabolism; AD-CT, Alzheimer's disease signature cortical thickness; SUVR, standardized uptake value ratio; WMH, white matter hyperintensities.
Unless otherwise indicated, data are expressed as means (standard deviations).

Table 2. Results of multiple linear and logistic regression analyses for the associations between meal frequency categories and Aβ, AV-1451, AD-CM, AD-CT, or WMH volume in non-demented older adults

	Aβ retention, SUVR		Aβ positivity	
Meal frequencies	β	p	OR (95% CI)	p
Model 1[a]				
3 meals per day	Reference		Reference	
<3 meals per day	−0.121	0.015	0.294 (0.130–0.668)	0.003
Model 2[b]				
3 meals per day	Reference		Reference	
<3 meals per day	−0.127	0.011	0.253 (0.103–0.620)	0.003
Model 3[c]				
3 meals per day	Reference		Reference	
<3 meals per day	−0.125	0.015	0.191 (0.073–0.501)	0.001

	AV-1451, SUVR		AD-CM, SUVR		AD-CT, mm		WMH volume, cm^3	
	β	p	β	p	β	p	β	p
Model 1[a]								
3 meals per day	Reference		Reference		Reference		Reference	
<3 meals per day	−0.136	0.127	0.064	0.272	−0.012	0.810	−0.011	0.864
Model 2[b]								
3 meals per day	Reference		Reference		Reference		Reference	
<3 meals per day	−0.171	0.097	0.098	0.095	−0.004	0.936	−0.016	0.808
Model 3[c]								
3 meals per day	Reference		Reference		Reference		Reference	
<3 meals per day	−0.145	0.146	0.088	0.128	−0.006	0.905	−0.026	0.694

Aβ, beta-amyloid; AD-CM, Alzheimer's disease signature cerebral glucose metabolism; AD-CT, Alzheimer's disease signature cortical thickness; WMH, white matter hyperintensity; OR, odds ratio; CI, confidence interval.

[a]Adjusted for age, sex, education, apolipoprotein ε4, geriatric depression scale status, and clinical diagnosis.

[b]Adjusted for covariates in Model 1 plus, vascular risk score, body mass index status, lifetime physical activity score, lifetime cognitive activity score, occupational complexity, annual income status, fluid intake, alcohol intake, smoking, dietary pattern including food types (protein, fruits or vegetables, fried foods, fatty foods, and salty foods), and inter-meal snack.

[c]Adjusted for covariates in Model 2 plus, serum nutritional markers (albumin, glucose, HDL-cholesterol, LDL-cholesterol, iron, ferritin, zinc, vitamin B_{12}, and folate).

groups. Nevertheless, as whether a reduced caloric intake affects the association between LMF and decreased Aβ deposition remains unclear, the total dietary caloric intake amounts need to be measured in a future study.

LMF was significantly associated with Aβ deposition only in the low LPA group, but not in the high one, indicating moderation effect of physical activity. This finding implies that LMF needs to be more recommended for physically inactive individuals, compared to physically active ones. Chronic physical activity has been suggested to alter the sensitivity of the appetite control systems by balancing the increased drive to eat with an improved satiety response to a meal (King et al., 2009). Such influence of physical activity on the appetite control system may change the condition under which LMF plays a role in the Aβ reduction. As an alternative possibility, relatively reduced Aβ deposition of the high LPA subgroup, compared to low LPA one, in the HMF state (Figures 1C and 1D) may give a floor effect to make further decrease of Aβ difficult. Many preclinical studies indicated that physical activity likely contributes to the reduction of brain Aβ accumulation, although data from human cohorts are relatively limited yet (Dorling et al., 2018).

In addition to LMF, other dietary patterns may affect Aβ or tau depositions. A study from the Women's Health Aging Project reported that a Mediterranean diet with low "junk-food" intake provides a beneficial effect on Aβ deposition (Hill et al., 2018). Moreover, a longitudinal brain imaging study at New

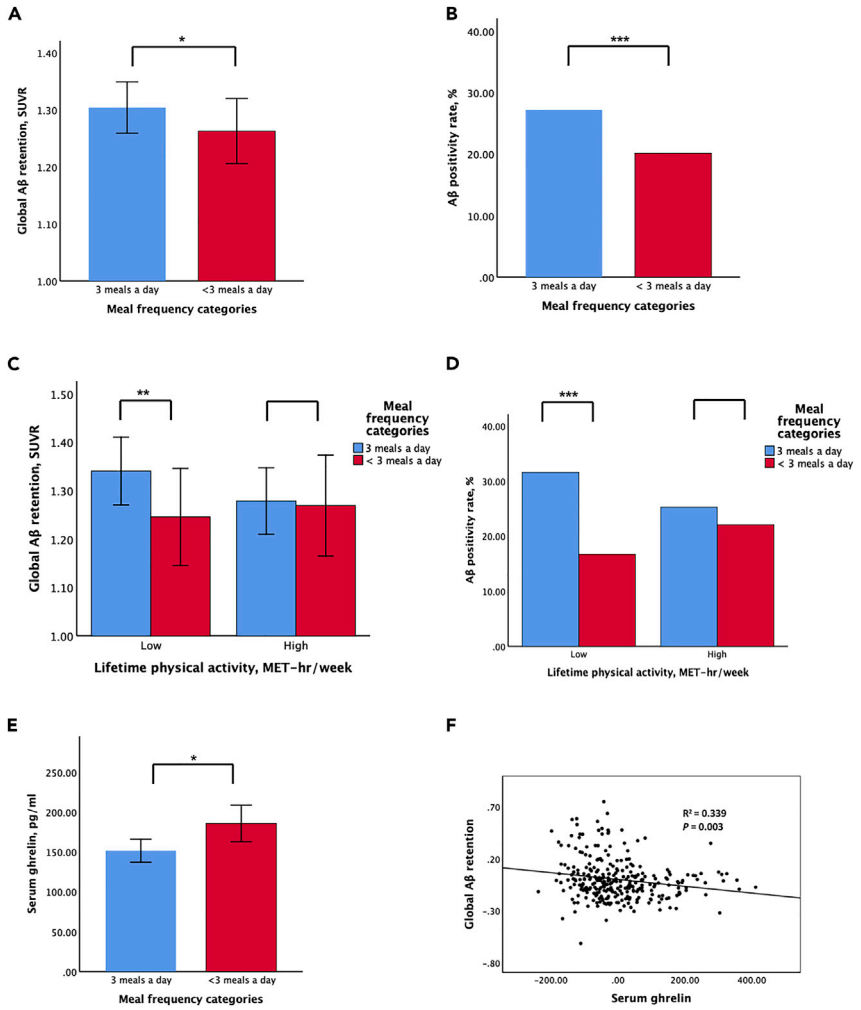

Figure 1. Plots of the associations between meal frequency categories, Aβ deposition, and serum ghrelin

(A–F) (A) Meal frequency categories vs. global Aβ retention for overall participants, (B) meal frequency categories vs. Aβ positivity rates for overall participants, (C) meal frequency categories vs. global Aβ retention for each lifetime physical activity subgroup, (D) meal frequency categories vs. Aβ positivity rates for each lifetime physical activity subgroup, (E) meal frequency categories vs. serum ghrelin levels, and (F) serum ghrelin levels vs. global Aβ retention. *Aβ*, beta-amyloid. For (A), (C), and (E), values are presented as the mean of global Aβ retention and error bars represent standard errors. For (B) and (D), values are presented as percentages of Aβ positivity. For (F), partial regression plot controlling all potential covariates is presented. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.

York University revealed that a dietary pattern characterized by a high intake of fresh fruit, vegetables, whole grains, and fish, and a low intake of high-fat dairy, sweets, fried potatoes, processed meat, and butter was negatively associated with *in vivo* Aβ deposition (Berti et al., 2015). However, our results did not change even after controlling for such dietary patterns, indicating that LMF is associated with reduced Aβ deposition independently of specific nutritional components.

Unlike the association between LMF and Aβ deposition, LMF did not show associations with tau, AD-signature neurodegeneration, or WMH. No studies have investigated the association between LMF and AD-signature neurodegeneration or cerebrovascular injury in humans, but a couple of experimental studies using animal models of AD (Bruce-Keller et al., 1999; Zhu et al., 1999) have revealed that LMF or DR increase the resistance of neurons to excitotoxic injury and reduce learning and memory deficits. In addition, several experimental studies using animal models have shown that LMF or DR improve cerebrovascular diseases and protect neurons against cerebrovascular injury (Brandhorst and Longo, 2019). However, in animal

Table 3. Results of multiple linear and logistic regression analyses for the associations between meal frequency categories and Aβ retention in each lifetime physical activity subgroup

	Aβ retention, SUVR		Aβ positivity	
Meal frequencies	β	*p*	OR (95% CI)	*p*
High physical activity (n = 165)				
Model 1[a]				
3 meals per day	Reference		Reference	
<3 meals per day	0.042	0.553	0.572 (0.197–1.666)	0.306
Model 2[b]				
3 meals per day	Reference		Reference	
<3 meals per day	0.069	0.349	0.283 (0.070–1.137)	0.075
Model 3[c]				
3 meals per day	Reference		Reference	
<3 meals per day	0.071	0.368	0.193 (0.037–1.008)	0.051
Low physical activity (n = 165)				
Model 1[a]				
3 meals per day	Reference		Reference	
<3 meals per day	0.194	0.007	0.070 (0.014–0.366)	0.002
Model 2[b]				
3 meals per day	Reference		Reference	
<3 meals per day	0.194	0.009	0.053 (0.007–0.373)	0.003
Model 3[c]				
3 meals per day	Reference		Reference	
<3 meals per day	0.223	0.005	<0.001 (<0.001 to 0.127)	0.005

Aβ, beta-amyloid; OR, odds ratio; CI, confidence interval.

[a]Adjusted for age, sex, education, apolipoprotein ε4, geriatric depression scale status, and clinical diagnosis.

[b]Adjusted for covariates in Model 1 plus, vascular risk score, body mass index status, lifetime cognitive activity score, occupational complexity, annual income status, fluid intake, alcohol intake, smoking, dietary pattern including food types (protein, fruits or vegetables, fried foods, fatty foods, and salty foods), and inter-meal snack.

[c]Adjusted for covariates in Model 2 plus, serum nutritional markers (albumin, glucose, HDL-cholesterol, LDL-cholesterol, iron, ferritin, zinc, vitamin B_{12}, and folate).

studies, controls are usually fed *ad libitum*, get little exercise, and are overweight, therefore, the potential impact of obesity on neurodegeneration cannot be ruled out (Mattson, 2005). Thus, comparing animal study findings with the results from human studies like ours is difficult. In addition, our participants were not demented and lacked a history of stroke or severe cerebrovascular lesions; thus, the resulting reduced variability in tau, neurodegeneration, and WMHs may have masked potential associations with LMF. Moreover, in terms of tau deposition, our null finding may be related to low statistical power due to the relatively smaller size of our population.

The present study has a strong point in that the findings were based on data from a relatively large number of well-characterized participants, who underwent comprehensive clinical assessments, laboratory blood tests including serum ghrelin, and multi-modal brain imaging for *in vivo* AD pathologies and WMHs in the brain. We also evaluated potential confounders extensively and controlled the statistical models for them to investigate the association between LMF and AD brain pathologies as clearly as possible.

Taken together, the present findings suggest that less than three meals a day may be related to the lower risk of AD through reduced brain amyloid deposition, especially in physically less active individuals. Additionally, hunger-related hormone ghrelin appears mediate the association between LMF and lower amyloid deposition.

Table 4. Results of multiple linear or logistic regression analyses for the associations between meal frequency categories, serum ghrelin hormone, and Aβ deposition in non-demented individuals

	Serum ghrelin, pg/mL	
Meal frequencies	β	*p*
Model 1[a]		
3 meals per day	Reference	
<3 meals per day	0.130	0.030
Model 2[b]		
3 meals per day	Reference	
<3 meals per day	0.147	0.016
Model 3[c]		
3 meals per day	Reference	
<3 meals per day	0.142	0.021

	Aβ retention, SUVR		Aβ positivity	
	β	*p*	OR (95% CI)	*p*
Serum ghrelin				
Model 1[a]	−0.178	0.001	0.995 (0.991–0.998)	0.002
Model 2[b]	−0.159	0.003	0.995 (0.991–0.998)	0.005
Model 3[c]	−0.167	0.003	0.994 (0.991–0.998)	0.003
Meal frequencies				
3 meals per day[d]	Reference		Reference	
<3 meals per day[d]	−0.104	0.056	0.396 (0.153–1.023)	0.056

Aβ, beta-amyloid; OR, odds ratio; CI, confidence interval.

[a]Adjusted for age, sex, education, apolipoprotein ε4, geriatric depression scale status, and clinical diagnosis.

[b]Adjusted for covariates in Model 1 plus, vascular risk score, body mass index status, lifetime cognitive activity score, occupational complexity, annual income status, fluid intake, alcohol intake, smoking, and dietary pattern including food ingredients (protein, fruits or vegetables, fried foods, fatty foods, and salty foods).

[c]Adjusted for covariates in Model 2 plus, serum nutritional markers (albumin, glucose, HDL-cholesterol, LDL-cholesterol, iron, ferritin, zinc, vitamin B_{12}, and folate).

[d]Adjusted for covariates in Model 3 plus, serum ghrelin.

Limitations of the study

We are aware of the limitations of our study. First, because this was a cross-sectional study, inferring causal associations from the findings is difficult. However, the significant association between LMF and Aβ pathology suggests the possible causal nature of the association, considering cognitively and physically healthy individuals usually maintain their dietary habits unchanged throughout their lifetime (Kalmijn et al., 1997). Second, a retrospective recall bias may have affected the association between dietary history and AD pathologies. In the present study, approximately one-third of the study participants were diagnosed as having MCI, which may raise concerns about the accuracy of self-reports for dietary history. However, although individuals with MCI present recent memory impairments, their remote memory is well-preserved. Therefore, it is unlikely that individuals with MCI reported erroneous dietary histories because these self-reports rely mainly on remote memory rather than on recent memory. In addition, we obtained similar results after controlling for the clinical diagnoses (CN vs. MCI) as an additional covariate in Model 1. Also, our sensitivity analysis, after excluding the participants with MCI, showed similar results. The consistency of meal frequency assessments between baseline and two-year follow-ups, as measured using the gamma coefficient, was also very high. Lastly, we did not measure the meal timings and daily caloric intake. Meal timings may affect Aβ deposition either via circadian rhythm disruption or via metabolic responses to food intake under the circadian rhythm system (Mattson et al., 2014). Restriction of calorie intake may also be protective against AD-related brain changes (Luchsinger et al., 2002). Therefore, further investigations with strict control of meal timings and daily calorie intake are needed.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105422.

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AUTHOR CONTRIBUTIONS

JWK and DYL conceived and designed the study. MSB, DY, JHL, KS, DH, GB, MJK, JHJ, YYC, GJ, JYL, Y-SL, YKK, KMK, C-HS, and DYL were involved in acquisition, or analysis and interpretation of the data and helped to draft the article. JWK, MSB, DY, JHL, and DYL were major contributors to writing the article and critically revising the article for intellectual content. DYL served as a principal investigator and supervised the study. All authors read and approved the final article.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Biograph mMR PET-MR scanner	Siemens; Washington DC, WC, USA	https://www.siemens-healthineers.com/
Biograph True point 40 PET/CT scanner	Siemens; Washington DC, WC, USA	https://www.siemens-healthineers.com/
Software and algorithms		
Matlab 2015b	Mathworks, Natick, MA, USA	https://www.mathworks.com/
SPM 12	FIL	https://www.fil.ion.ucl.ac.uk/spm/software/spm12/
FreeSurfer version 5.3	FreeSurfer	https://surfer.nmr.mgh.harvard.edu/
IBM SPSS Statistics 27 software	IBM, Armonk, NY, USA	https://www.ibm.com/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dong Young Lee (selfpsy@snu.ac.kr).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

As of February 2017, 411 individuals (232 female and 139 male) [278 cognitively normal (CN) adults (135 female and 137 male), and 133 adults with mild cognitive impairment (MCI) (97 female and 42 male)], between 56 and 90 years of age had been enrolled in the study.

Participants were recruited through four recruitment sites around Seoul, South Korea. Potentially eligible individuals who participated in a dementia screening program at two public centers for dementia prevention and management or visited memory clinics at two university hospitals [i.e., Seoul National University Hospital (SNUH) and Seoul National University-Seoul Metropolitan Government (SNU-SMG) Boramae Medical Center] around Seoul, South Korea, were informed about study participation and those who volunteered were invited for an assessment of eligibility. In addition, volunteers from the community were recruited through advertisements at an online homepage, posters, and brochures provided at main recruitment sites and word of mouth (recommended by other participants, family members, friends, or acquaintances).

The CN group consisted of participants with a Clinical Dementia Rating (CDR) score of 0 and no diagnosis of MCI or dementia. All participants with MCI met the current consensus criteria for amnestic MCI, including: 1) memory complaints confirmed by an informant; 2) objective memory impairments; 3) preservation of global cognitive function; 4) independence in functional activities; and 5) absence of dementia. Regarding Criterion 2, the age-, education-, and gender-adjusted z-score was < -1.0 for at least one of four episodic memory tests: Word List Memory, Word List Recall, Word List Recognition, and Constructional Recall tests included in the Korean version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD-K) neuropsychological battery. All MCI individuals had a CDR score of 0.5. The exclusion criteria were the following: 1) presence of a major psychiatric illness; 2) significant neurological or medical condition or comorbidity that could affect mental functioning; 3) contraindications for an MRI

scan (*e.g.*, pacemaker or claustrophobia); 4) illiteracy; 5) presence of significant visual/hearing difficulties and/or severe communication or behavioral problems that would make clinical examinations or brain scans difficult; 6) pregnancy or lactation; and, 7) use of an investigational drug.

This study protocol was approved by the institutional review boards of the Seoul National University Hospital (C-1401-027-547) and the Seoul Metropolitan Government-Seoul National University (SMG-SNU) Boramae Medical Center (26-2015-60), in Seoul, South Korea; and we conducted it in accordance with the recommendations of the current version of the Declaration of Helsinki. The subjects or their legal representatives gave written informed consent.

METHOD DETAILS

Assessment of meal frequency and other dietary habits

All participants were systematically interviewed to identify their overall dietary patterns using the items for dietary and food patterns (see Table S1) selected from the mini-dietary assessment (MDA) (Kim et al., 2003) and the mini nutritional assessment (MNA) tools.(Vellas et al., 1999) The MDA tool was devised based on dietary guidelines and the food pyramid for Koreans and it provides a valid evaluation of meal frequency and overall dietary habits including food components or dietary regulation.(Jang et al., 2008) The MNA is also a brief, valid nutritional evaluation tool for older populations.(Vellas et al., 1999) The meal frequency for each participant was assessed based on participant's response to an item related to meal skipping or intermittent fasting from the MDA assessment; the specific item asks, "have you regularly had three regular meals a day?", for which the options for the response were either (a) always (with neither meal skipping nor intermittent fasting), or (b) not always (with meal skipping and intermittent fasting).(Kim et al., 2003) To test the hypothesis that LMF—as defined as less than 3 meals a day(Paoli et al., 2019) —is associated with reduced *in vivo* AD pathologies, we categorized the participants into two groups: HMF (for individuals who replied "always") and LMF (for individuals who replied "not always") based on the response to the abovementioned item on the MDA. We re-applied the same question to the participants approximately two-years after the first interview (n = 315) to verify the reliability of their responses. Regarding the consistency for the grouping (HMF *vs.* LMF) between the initial and two-year follow-up responses, the gamma coefficient was 0.827 ($p < 0.001$), indicating good reliability. We also assessed inter-meal snacks and dietary patterns including food types, such as protein, fruit or vegetables, fried foods, fatty foods, or salty foods using the items from the MDA and MNA.

Measurement of cerebral Aβ deposition

All participants underwent simultaneous three-dimensional [^{11}C] Pittsburg compound B (PiB)-positron emission tomography (PET) and T1-weighted MRI scans using a 3.0T Biograph mMR (PET-MR) scanner (Siemens; Washington DC, WC, USA) according to the manufacturer's guidelines. The details of PiB-PET acquisition and preprocessing were described in our previous report.(Park et al., 2019) An AAL algorithm and a region-combining method were applied to determine the regions of interest (ROIs) for characterization of PiB retention levels in the frontal, lateral parietal, posterior cingulate-precuneus, and lateral temporal regions. The standardized uptake value ratio (SUVR) values for each ROI were calculated by dividing the mean value for all voxels within each ROI by the mean cerebellar uptake value on the same image. We defined a global cortical ROI consisting of the four ROIs and generated a global Aβ retention value by dividing the mean value for all voxels of the global cortical ROI by the mean cerebellar uptake value in the same image.(Reiman et al., 2009) We classified each participant as Aβ positive (Aβ+) if the SUVR value was >1.4 in at least one of the four ROIs.(Reiman et al., 2009).

Measurement of cerebral tau deposition

A subset of subjects (n = 117) underwent [^{18}F] AV-1451 PET scans (Siemens) using a Biograph True point 40 PET/CT scanner (Siemens), in accordance with the manufacturer's guidelines. While all the other neuroimaging scans were performed during the baseline visit, the AV-1451 PET imaging was performed at an average of 2.6 (standard deviation 0.3) years after the baseline visit. The details of AV-1451 PET imaging acquisition and preprocessing have been described (Park et al., 2019). To estimate cerebral tau deposition, we quantified the AV-1541 SUVR of an *a priori* ROI of "AD-signature regions" of tau accumulation, which comprised a size-weighted average of partial volume-corrected uptake in entorhinal, amygdala, parahippocampal, fusiform, inferior temporal, and middle temporal ROIs. We used the AV-1541 SUVR of the above-mentioned ROI as an outcome variable for cerebral tau deposition (Park et al., 2019).

Measurement of AD-related neurodegeneration

All subjects underwent [^{18}F] fluorodeoxyglucose (FDG)-PET imaging using the above-described PET-MR machine. The details of FDG-PET acquisition and preprocessing were described in our previous report (Park et al., 2019). We determined AD-signature FDG ROIs that are sensitive to the changes associated with AD, such as the angular gyri, posterior cingulate cortex, and inferior temporal gyri (Park et al., 2019). We defined AD-CM as the voxel-weighted mean SUVR extracted from the AD-signature FDG ROIs. All T1-weighted images were acquired in the sagittal orientation using the above-described 3.0T PET-MR machine. MR image acquisition and preprocessing were described in our previous report (Park et al., 2019). We defined AD-CT as the mean cortical thickness values obtained from AD-signature regions including the entorhinal, inferior temporal, middle temporal, and fusiform gyrus (Park et al., 2019).

Measurement of WMH

All participants underwent MRI scans with fluid-attenuated inversion recovery using the abovementioned 3.0T PET-MR scanner. We followed the validated automatic procedure reported previously.(Tsai et al., 2014) Briefly, the procedure consisted of 11 steps, i.e., spatial coregistration of T1 and FLAIR images, fusion of T1 and FLAIR images, segmentation of T1, attainment of transformation parameters, deformation and obtainment of the white matter mask, obtainment of FLAIR within the white matter mask, intensity normalization of the masked FLAIR, nomination of candidate WMH with a designated threshold, creation of a junction map, and elimination of the junction. The current processing procedure had two modifications compared to the original study: (a) an optimal threshold of 70 was applied, as it was more suitable for our data than the threshold of 65 used in the original study; and, (b) given that individuals with acute cerebral infarcts were not enrolled in our sample, we did not use diffusion weighted imaging in the current automated procedure. Using the final WMH candidate image, the WMH volume was extracted in the native space in each subject. More specifically, the lobar ROIs template was adapted from a previously published minimal deformation template (MDT3).(Kochunov et al., 2001) The acquired transformation parameter for each subject from the automated procedure was applied to the template to transform the lobar ROIs template into native space to be used for extracting WMH volumes in each lobe.

Measurement of serum ghrelin

After an overnight fast, blood samples were obtained via venipuncture in the morning (8–9 a.m.). Serum ghrelin was measured with the EZGRT-89K human ghrelin (total) enzyme-linked immunosorbent assays (ELISA) kit (Merck Millipore, Darmstadt, Germany).

Assessment of potential confounders

The association between dietary patterns and AD may be influenced by various factors. Therefore, we systematically evaluated all participants to identify potential confounders, such as lifetime physical activity (LPA), lifetime cognitive activity (LCA), occupational complexity, annual income, depression, vascular risk, body mass index (BMI), apolipoprotein E (APOE) 4 positivity, albumin, glucose, cholesterol [high-density lipoprotein (HDL), and low-density lipoprotein (LDL)], iron, ferritin, vitamin B_{12}, folate, zinc, fluid intake, alcohol intake, and smoking. We assessed physical activity using the interviewer-administered Lifetime Total Physical Activity Questionnaire, a tool with demonstrated reliability(Friedenreich et al., 1998, 2004) and validity.(Gill et al., 2015) This questionnaire assesses occupational, household, and leisure activities separately throughout a respondent's lifetime. We assessed the frequency and duration of these activities by recording the number of years, months per year, weeks per month, days per week and hours per day that each activity was performed. The intensity of activity was estimated by the participant as sedentary, light, moderate or heavy. A metabolic equivalent (MET) value was assigned to each activity based on the Compendium of Physical Activities.(Ainsworth et al., 2011) We calculated LPA scores as the sum of the MET-h/week spent on occupational, household, and leisure activities over the lifetime, and we categorized the LPA status into two groups based on the median quartile (high LPA group vs. low LPA group). We measured the cognitive activity participation frequency using 39-item structured questionnaires.(Wilson et al., 2005, 2007) The details of the cognitive activity measurement are described in our previous report (Ko et al., 2018). We averaged item scores to yield separate values for each age period and then calculated the composite LCA score to use in the subsequent analysis, which was an average of all 4-epoch averages. In terms of occupational complexity, we considered only the longest-held occupation and classified participants into four levels based on the skill levels described in the International Standard Classification of Occupations (http://www.ilo.org/public/english/bureau/stat/isco/). Occupations typically involving simple

and routine physical or manual tasks were classified as skill level 1; occupations with performance of tasks, such as operating machinery and electronic equipment, driving vehicles, maintenance and repair of electrical and mechanical equipment, and manipulation, and ordering and storing information are skill level 2; those with performance of complex technical and practical tasks that require complex problem solving, reasoning, and decision making in a specialized field are skill level 3; and, those with performance of tasks that require complex problem-solving, decision-making, and creativity based on an extensive body of theoretical and factual knowledge in a specialized field are skill level 4. We obtained information about occupations from participants' self-reports and confirmation by reliable informants. We assessed annual incomes and categorized them into three groups (below the minimum cost of living [MCL], more than MCL but below two MCLs, and two or more MCLs (http://www.law.go.kr). The MCL was determined according to the administrative rule published by the Ministry of Health and Welfare, Republic of Korea, in November 2012. The MCL is 572,168 Korea Won (KRW) for single-person household and adds 286,840 KRW for each additional housemate. We used the Geriatric Depression Scale (GDS) (Kim et al., 2008; Yesavage et al., 1982) to measure the severity of depressive symptoms and categorized participants into two groups according to their results (0 to 9 as normal, 10 and above as depressed) (Yesavage et al., 1982). The comorbidity rates of vascular risk factors were assessed by interviewing the participants and their reliable informants; we calculated a vascular risk score (VRS) based on the number of vascular risk factors present and reported the VRS as a percentage (DeCarli et al., 2004). The BMI was calculated using the weight in kilograms divided by the height in meters squared and we categorized participants into three groups (<18.4 as underweight, 18.5 to 24.9 as normal weight, 25 as overweight to obese) according to the World Health Organization (WHO) guideline (https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle—who-recommendations). We assessed daily fluid intake (usually more than five cups, three to five cups, or less than three cups) using the MNA tool (Vellas et al., 1999), and a nurse obtained the average lifetime alcohol intake status (standard drinks per week), and average lifetime smoking amount (packs per day) after interviewing the participants (see Table S2). To acquire accurate information, reliable informants were interviewed, and medical records were reviewed.

QUANTIFICATION AND STATISTICAL ANALYSIS

To examine the associations between meal frequency groups and neuroimaging variables, we performed multiple logistic or linear regression analyses as appropriate. For these analyses, we used the HMF group as a reference. We tested three models for controlling the covariates in a stepwise manner. The first model included age, sex, education, APOE4, GDS status, and clinical diagnosis as covariates; the second model included the covariates in the first model plus VRS, BMI status, LPA score, LCA score, occupational complexity, annual income status, fluid intake, alcohol intake, smoking, dietary pattern including food types (such as protein, fruit or vegetables, fried foods, fatty foods, and salty foods), and inter-meal snacks; and, the third model included the covariates in the second model plus serum nutritional markers (such as albumin, glucose, HDL-/LDL-cholesterol, iron, ferritin, zinc, vitamin B_{12}, and folate). To investigate the mediation of serum ghrelin on the association between meal frequency groups and the biomarker(s) that showed significant association with meal frequency, we first conducted the same regression analyses for the association between meal frequency groups and ghrelin, and then for the association between ghrelin and the neuroimaging biomarker(s). We also analyzed the association between meal frequency groups and the neuroimaging biomarker(s) using the same regression model while controlling for ghrelin as an additional covariate.

For these analyses, $p < 0.05$ was served as a statistical threshold. All statistical analyses were performed using IBM SPSS Statistics 27 software (IBM, Armonk, NY, USA).

ADDITIONAL RESOURCES

This study is a part of the Korean Brain Aging Study for the Early Diagnosis and Prediction of Alzheimer's disease (ClinicalTrials.gov Identifier: NCT02137460, www.kbase.kr), which is an ongoing prospective cohort study that began in 2014.(Byun et al., 2017).