Effects of high-fat diet on nutrient metabolism and cognitive functions in young APPKINL-G-F/NL-G-F mice

Wei Wang | Daisuke Tanokashira | Yudai Shibayama | Ryuhei Tsuji | Megumi Maruyama | Chiemi Kuroiwa | Takashi Saito | Takaomi C. Saido | Akiko Taguchi

1Department of Integrative Neuroscience, National Center for Geriatrics and Gerontology, Aichi, Japan
2Laboratory for Proteolytic Neuroscience, RIKEN Center for Brain Science, Saitama, Japan
3Department of Neurocognitive Science, Institute of Brain Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Correspondence
Akiko Taguchi, Department of Integrative Neuroscience, National Center for Geriatrics and Gerontology, Obu, Aichi, Japan.
Email: taguchia@ncgg.go.jp

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Abstract
Aim: Type 2 diabetes mellitus (T2DM) is an increased risk factor for Alzheimer’s disease (AD); however, the relationship between the 2 conditions is controversial. High-fat diet (HFD) causes cognitive impairment with/without Aβ accumulation in middle-aged or aged transgenic (Tg) and knock-in (KI) AD mouse models, except for metabolic disorders, which commonly occur in all mice types. Alternatively, whether HFD in early life has an impact on nutrient metabolism and neurological phenotypes in young AD mouse models is not known. In the present study, we examined the effects of HFD on young APPKINL-G-F/NL-G-F mice, one of the novel KI-AD mouse models.

Methods: The mice were categorized by diet into 2 experimental groups, normal diet (ND) and HFD. Four-week-old wild-type (WT) and APPKINL-G-F/NL-G-F mice were fed ND or HFD for 9 weeks. Both types of mice on ND and HFD were examined during young adulthood.

Results: HFD caused T2DM-related metabolic disturbances in both young WT and APPKINL-G-F/NL-G-F mice, whereas impaired thermoregulation and shortage of alternative energy sources specifically occurred in young APPKINL-G-F/NL-G-F mice. However, HFD had no impact on the cognitive function, Aβ levels, and phosphorylation of hippocampal insulin receptor substrate 1 (IRS1) at all the 3 Ser sites in both types of mice.

Conclusion: HFD is effective in causing metabolic disturbances in young WT and APPKINL-G-F/NL-G-F mice but is ineffective in inducing neurological disorders in both types of mice, suggesting that the aging effects, along with long-term HFD, facilitate neurological alterations.

KEYWORDS
Alzheimer’s disease, cognitive function, high-fat diet, hippocampus, insulin receptor substrate 1, knock-in mouse model, nutrient metabolism, type 2 diabetes mellitus

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1 | INTRODUCTION

Epidemiological evidence and experimental studies on animal models have suggested that type 2 diabetes mellitus (T2DM), characterized by impaired glucose metabolism and insulin resistance, is one of the risk factors for dementia, including Alzheimer’s disease (AD).\(^1,2\)

Aberrant alterations of the insulin signaling pathway mediated by insulin receptor substrate proteins correlate with the onset of T2DM.\(^3\)

Recently, the insulin receptor substrate 1 (IRS1) phosphorylation at specific serine (Ser) sites, such as human(h)Ser312/mouse(m) Ser307 implicated in metabolic dysfunctions, hSer616/mSer612 known as negative regulatory sites for IRS1 signaling, and hSer1101/mSer1097 associated with obesity and high-fat diet (HFD)-induced cognitive deficits, has been found in the brain of patients with AD and animal models of AD or A\(^\beta\)-unrelated cognitive decline.\(^4-7\)

Additionally, our recent studies have shown that these modifications of neural IRS1 via phosphorylation at those Ser sites occur in the brain of young amyloid precursor protein (APP) knock-in (KI) mouse model. This novel mouse model of AD uses a KI strategy, which overcomes the artificial effects of transgenes of transgenic (Tg) AD mouse models carrying humanized APP with Swedish NL, Beyreuther/Iberian F, and Arctic mutations. APPKINL-G-F/NL-G-F mice also exhibits normal energy metabolism, normal cognitive functions, and increased A\(^\beta\) levels from 2 months of age and cognitive deficits after 6 months.\(^8\)

Alternatively, previous clinical research has revealed a controversial relationship between T2DM and AD.\(^9-11\)

Similarly, basic research on conventional AD mouse models using Tg strategies has shown that a HFD that mimics the physiological status of T2DM leads to cognitive impairment, regardless of the extent of the disorder, with/without alteration of A\(^\beta\) accumulation in Tg-AD mouse models, such as Tg2576 and 3xTg-AD mice.\(^12-14\)

Moreover, in middle-aged APPKINL-G-N mice with Swedish NL mutation, a long-term HFD (60% calories from fat) from 2 to 18 months of age leads to mild behavioral impairment without A\(^\beta\) production and exhibits normal cognitive function in late middle ages without A\(^\beta\) deposition.\(^15\)

Chronic HFD (40% calories from fat) from 6 to 12 months of age promotes A\(^\beta\) aggregation and cognitive decline in middle-aged APPKINL-N mice, carrying humanized APP with Swedish NL and Beyreuther/Iberian F mutations at mouse APP locus. This model displays A\(^\beta\) accumulation from 6 months of age and very mild cognitive deficits at 18 months.\(^16\)

However, metabolic disorders are commonly observed in both Tg and KI AD mouse models on HFD.\(^8,12-17\)

Nevertheless, whether exposure to hyperalimentation in the early life of APPKI mice impacts the asymptomatic state in energy metabolism and cognitive function in addition to A\(^\beta\) levels and neural IRS1 modifications remains unknown. To address this question, we have examined the effects of HFD (60% calories from fat) on young APPKINL-G-F/NL-G-F mice. We found that HFD for 9 weeks leads to increased body weight, glucose intolerance, and dyslipidemia in both young wild-type (WT) and APPKINL-G-F/NL-G-F mice, whereas abnormal thermoregulation and impairment of ketogenesis occur only in young APPKINL-G-F/NL-G-F mice, regardless of the type of diet. However, HFD for 9 weeks has no impact on the cognitive functions, levels of A\(^\beta\), and phosphorylation of hippocampal IRS1 at all the 3 Ser sites in both young WT and APPKINL-G-F/NL-G-F mice. These results suggest that HFD for 9 weeks in early life is effective in causing metabolic dysfunctions but is ineffective to induce neurological manifestations in young APPKINL-G-F/NL-G-F mice as well as young WT mice.

2 | METHODS

2.1 | Animals

C57BL/6J male WT mice supplied by Japan SLC, Inc. were used to establish T2DM mice and their respective control mice. APPKINL-G-F/NL-G-F (Swedish (NL), Arctic (G), and Beyreuther/Iberian (F) mutations) homozygous mice were obtained from Dr. Saito at the Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, Saitama, Japan.\(^9\)

The WT and APPKINL-G-F/NL-G-F mice were categorized into 2 experimental groups: normal diet (ND) and HFD groups. The ND group was fed with ND (CE-2; CLEA Japan Inc) and HFD group was fed with HFD (D12492, 60% kcal from fat; Research Diets, Inc.) for 9 weeks, respectively. All the mice were housed at room temperature (25 ± 2°C) under a standard 12/12-hour light-dark cycle with free access to water and food. Animal experiments were conducted in compliance with the guidelines and approval of the ethics committee in Animal Care and Use of the National Center for Geriatrics and Gerontology in Japan.

2.2 | Measurement of metabolic parameters

Body weight and blood glucose were measured weekly throughout the study. Blood glucose was measured using a portable glucose meter (ACCU-CHEK® Aviva, Roche DC Japan K.K.). The plasma level of insulin in 6-h fasting was determined using an insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga, Yokohama, Japan). During random feeding and 3-h fasting, rectal temperature was measured using a rectal probe (Animal warmer, thermometer, Bio Research Center Co., LTD). The daily food intake was recorded after a 24-h fast. The biochemical parameters, including total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, free fatty acid, total ketone bodies, and triglyceride in 6-h fasting plasma, were measured using enzymatic methods (Oriental Yeast Co., Ltd.).

2.3 | Glucose tolerance tests and insulin tolerance tests

During the glucose tolerance tests, the mice were fasted overnight for 24 h and injected intraperitoneally with glucose at 1.5 g/kg body weight (Otsuka, Japan). Blood glucose was measured at 0, 15, 30,
60, and 120 min after injection. During the insulin tolerance tests, the mice were fasted briefly for 3 h and injected intraperitoneally with insulin at 0.75 units/kg body weight (Humulin R, Eli Lilly Japan K.K.). Blood glucose was collected at 0, 30, 60, 90, and 120 min after injection and normalized to the initial value at 0 min.

2.4 | Behavior

2.4.1 | Open field

The mice were gently placed in the center of a circular open field having a diameter of 100 cm and allowed to move freely for 5 min. The total distance a mouse traveled was recorded and analyzed via a video camera connected to the system software (O’HARA & Co., Ltd).

2.4.2 | Elevated plus maze

The elevated plus maze consists of two 25 × 5 cm open arms opposite to two 25 × 5 cm closed arms enclosed by a 20-cm wall. The arms extended from a 5 × 5 cm central platform, and the maze was had an elevation of 50 cm above the floor. The mice were first placed on the central platform of the maze and allowed to move freely for 5 min. Their behaviors were continuously recorded with a video and then analyzed with the system software (O’HARA & Co., Ltd). The time spent by the mice in the open arms was defined as anxiety-related behaviors.

2.4.3 | Passive avoidance

Passive avoidance was performed using a step-through box (Harvard Apparatus) consisting of a lighted chamber (20 × 20 × 25 cm) and a dark chamber (7 × 15 × 10 cm) connected with a sliding door. At first, mice were placed in the lighted compartment. When the mice entered the dark chamber, the sliding door was closed, and they received a light shock at the foot (0.15 mA, 1 s). Mice were trained up to criterion defined as the mice remaining in the lighted compartment for 120 s. The number of trials needed to reach this criterion was taken as the measure of task acquisition.

2.4.4 | Water T maze

Hippocampal-dependent spatial memory of the mice was tested using a water T maze according to our previous reports.6,7,18 For the first screening step, the preference of each mouse was determined. The mice were placed in the start box and allowed to swim to the right or left arm. This screening step was repeated 3 times at 30-second intervals. The platform was placed on the side that the mice reached less often. Next, the mice were allowed to explore the right and left sides of the maze freely. If mice reached the platform (correct choice), they rested for 5 s on the platform. If not, the arm entry was closed with a board (incorrect choice), and they received a deterrent by being forced to swim for 15 s. This trial step was repeated 5 times at 5-min intervals. The mice were subjected to the trial step for 5 days. The percentage of correct responses per day was determined.

2.5 | Western blotting

Western blot analysis was conducted as previously described protocol.7 In summary, hippocampal tissue of 3-hour-fasted mice was homogenized in a lysis buffer T-PER® Tissue Protein Extraction Reagent (Thermo Fisher Scientific) containing a protease inhibitor cocktail (Nacalai Tesque) and a phosphatase inhibitor cocktail (Nacalai Tesque) with a pellet mixer. After incubation on ice for 15 min, the lysates were centrifuged for 5 min at 14200 × g and 4°C. Protein concentrations were determined using a BCA protein assay kit (Pierce). The protein samples were separated by SDS-PAGE and then transferred to a polyvinylidene fluoride membrane. The membranes were blocked using Block Ace (YukiJirushi Ltd.) and incubated with the indicated primary antibodies: rabbit anti-phospho-IRS1 (mouse Ser307/human Ser312 [mSer307/hSer312]), mouse Ser1097/human Ser1101 [mSer1097/hSer1101], 1:400; Cell Signaling Technology [CST]) and rabbit anti-IRS1 (1:1000; CST). Immunodetection was performed with horseradish peroxidase-conjugated secondary antibodies (1:5000; CST). The chemiluminescent signals were detected using Chemi-Lumi One (Nacalai Tesque) or ImmunoStar LD (FUJIFILM Wako). The images were scanned using Amersham Imager 680 (GE Healthcare Life Sciences).

2.6 | ELISA quantitation of Aβ

Quantitation of Aβ levels was conducted as previously described.7 In summary, the levels of T-PER-extractable Aβ40 and Aβ42 in the hippocampus tissues were determined using the Human/Rat/Mouse βAmyloid (1-40) ELISA Kit Wako II (#294-64701; Fujifilm Wako Pure Chemical Corp., Osaka, Japan) and the Human/Rat/Mouse βAmyloid (1-42) ELISA Kit Wako, High Sensitivity (#292-64501, Fujifilm Wako Pure Chemical Corp.) as per the manufacturers’ instructions.

2.7 | Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM) in the text. Statistical analyses were performed using Prism7 for Mac OS X v.7.0d (GraphPad Software Inc.). The comparisons of the means from multiple groups were analyzed using two-way repeated measure analysis of variance (ANOVA) or two-way ANOVA followed by post hoc Bonferroni’s (if n in each group was the same) or Tukey-Kramer’s (if n in each group was different) multiple
comparison tests. $P$ values of less than 0.05 were considered as statistically significant.

3 | RESULTS

3.1 | HFD leads to metabolic disturbance in both young WT and APPKINL-G-F/NL-G-F mice

To examine the effects of HFD on young APPKINL-G-F/NL-G-F mice from an early age to young adulthood, 4-week-old WT and APPKINL-G-F/NL-G-F mice were fed a ND (12% calories from fat) or HFD (60% calories from fat) for 9 weeks. While body weight was comparable between the WT and APPKINL-G-F/NL-G-F mice on ND, HFD significantly increased the body weight of both WT and APPKINL-G-F/NL-G-F mice despite the decreased food intake in the HFD groups (Figure 1A: repeated two-way ANOVA, group: $F_{3,18} = 16.20, P < 0.0001$; time: $F_{8,144} = 124.7, P < 0.0001$; interaction: $F_{24,144} = 4.595, P < 0.0001$ and Figure 1F: two-way ANOVA, genotype: $F_{1,12} = 2.26, P = 0.1522$; diet: $F_{3,16} = 405.8, P < 0.0001$; interaction: $F_{1,16} = 5.785, P < 0.05$). There were significant differences in the random blood glucose levels among the 4 groups (Figure 1B: repeated two-way ANOVA, group: $F_{3,18} = 8.105, P < 0.01$; time: $F_{8,144} = 12.78, P < 0.0001$; interaction: $F_{24,144} = 1.816, P < 0.05$). Consistent with these results, the WT and APPKINL-G-F/NL-G-F mice on HFD displayed glucose intolerance (HFD-APPKINL-G-F/NL-G-F mice showed more severe glucose intolerance than HFD-WT mice) and hyperinsulinemia compared with the ND groups, whereas insulin sensitivity was significantly reduced only in the HFD-WT mice (Figure 1C: repeated two-way ANOVA, group: $F_{3,17} = 22.03, P < 0.0001$; time: $F_{4,68} = 705, P < 0.0001$; interaction: $F_{12,68} = 5.967, P < 0.0001$; Figure 1E: two-way ANOVA, genotype: $F_{1,12} = 1.874, P = 0.6728$; diet: $F_{3,12} = 29.67, P < 0.0001$; interaction: $F_{1,12} = 0.08376, P = 0.7772$; Figure 1D: repeated two-way ANOVA, group: $F_{3,17} = 1.66, P = 0.2131$; time: $F_{4,68} = 70.17, P < 0.0001$; interaction: $F_{12,68} = 3.949, P = 0.0001$). Also, ND-APPKINL-G-F/NL-G-F mice exhibited a significant decrease in core body temperature correlated with energy metabolism; however, it was no longer observed under HFD and/or 3-hour fasting conditions (Figure 1G: two-way ANOVA, random-fed: genotype: $F_{1,16} = 17.21, P < 0.01$; diet: $F_{3,16} = 1.076, P = 0.3151$; interaction: $F_{3,16} = 8.134, P < 0.05$; 3-hour fasting: genotype: $F_{1,16} = 2.172, P = 0.16$; diet: $F_{1,16} = 3.063, P = 0.0993$; interaction: $F_{1,16} = 0.1357, P = 0.7174$). Interestingly, we found that APPKINL-G-F/NL-G-F mice displayed a consistent reduction in the levels of fasting total blood ketone bodies regardless of diet type (Figure 1H: two-way ANOVA, genotype: $F_{1,18} = 19.84, P < 0.01$; diet: $F_{1,18} = 0.00059, P = 0.9808$; interaction: $F_{1,18} = 0.2698, P = 0.6098$). Alternatively, while the levels of total LDL and HDL blood cholesterol were comparable between the WT and APPKINL-G-F/NL-G-F mice on ND, the levels of total and HDL blood cholesterol conspicuously increased in both the WT and APPKINL-G-F/NL-G-F mice on HFD, and LDL blood cholesterol levels significantly increased only in the HFD-APPKINL-G-F/NL-G-F mice compared with the ND groups (Figure 1I: two-way ANOVA, total cholesterol: genotype: $F_{1,18} = 2.092, P = 0.1653$; diet: $F_{3,18} = 92.64, P < 0.0001$; interaction: $F_{1,18} = 1.133, P = 0.3011$; LDL cholesterol: genotype: $F_{1,18} = 0.01134, P = 0.9164$; diet: $F_{3,18} = 24.53, P = 0.0001$; interaction: $F_{1,18} = 2.067, P = 0.1676$; HDL cholesterol: genotype: $F_{1,18} = 3.644, P = 0.0723$; diet: $F_{1,18} = 138.5, P < 0.0001$; interaction: $F_{1,18} = 0.9975, P = 0.3312$). Corresponding to decreased food intake of both mice on HFD (Figure 1J), only the HFD-APPKINL-G-F/NL-G-F mice exhibited significant reduction in blood triglyceride levels compared with that in ND-APPKINL-G-F/NL-G-F mice, whereas these levels were comparable between ND-WT mice and ND-APPKINL-G-F/NL-G-F mice or both mice on HFD (Figure 1J: two-way ANOVA, genotype: $F_{1,18} = 4.448, P < 0.05$; diet: $F_{3,18} = 12.43, P < 0.01$; interaction: $F_{1,18} = 0.5745, P = 0.4583$). Meanwhile, the levels of plasma free fatty acid were comparable between all the groups (Figure 1K: two-way ANOVA, genotype: $F_{1,18} = 5.037, P < 0.05$; diet: $F_{3,18} = 2.569, P = 0.1264$; interaction: $F_{1,18} = 0.7885, P = 0.3863$). These results indicate that HFD in early life causes typical T2DM-related metabolic disorders in both WT and APPKINL-G-F/NL-G-F mice, but impaired thermoregulation and specific reduction in an alternative energy source occur only in young APPKINL-G-F/NL-G-F mice.

3.2 | HFD is inadequate to impair hippocampus-associated behaviors in young APPKINL-G-F/NL-G-F mice

To evaluate the effect of HFD-induced metabolic disturbances on cognitive functions in young APPKINL-G-F/NL-G-F mice, hippocampus-associated behavioral tests were conducted. There were no statistically significant differences in spontaneous activity or learning and memory between all groups (Figure 2A: two-way ANOVA, genotype: $F_{1,15} = 0.8553, P = 0.3697$; diet: $F_{1,15} = 4.787, P < 0.05$; interaction: $F_{1,15} = 2.764, P = 0.1171$ and Figure 2B: two-way ANOVA, genotype: $F_{1,15} = 0.4693, P = 0.5037$; diet: $F_{1,15} = 0.4693, P = 0.5037$; interaction: $F_{1,15} = 0.169, P = 0.6868$). Similarly, hippocampus-dependent spatial memory was comparable between ND and HFD groups, whether WT mice or APPKINL-G-F/NL-G-F mice (Figure 2C: repeated two-way ANOVA, group: $F_{3,39} = 0.8124, P = 0.4947$; time: $F_{4,156} = 36.59, P < 0.0001$; interaction: $F_{12,156} = 0.8709, P = 0.5778$). Furthermore, no statistically significant differences in anxiety-like behavior between all groups were observed (Figure 2D: two-way ANOVA, genotype: $F_{1,15} = 1.653, P = 0.2181$; diet: $F_{3,15} = 0.003036, P = 0.9568$; interaction: $F_{1,15} = 1.847, P = 0.1942$). Meanwhile, HFD had no impact on the levels of Aj40 and Aj42 in the T-PER fractions of the hippocampus in APPKINL-G-F/NL-G-F mice, where the levels of Aj40 consistently decreased regardless of the type of diet (Figure 2E: two-way ANOVA, genotype: $F_{1,15} = 671.1, P < 0.0001$; diet: $F_{3,15} = 0.2224, P = 0.6440$; interaction: $F_{1,15} = 5.64, P < 0.05$ and Figure 2F: two-way ANOVA, genotype: $F_{1,15} = 24.04, P < 0.01$; diet: $F_{1,15} = 2.418, P = 0.1408$; interaction: $F_{1,15} = 0.02402, P = 0.8789$).
These data suggest that HFD-induced metabolic disorders are insufficient to exert a negative impact on cognitive functions and Aβ levels in APPKINL-G-F/NL-G-F and WT mice during young adulthood.

3.3 | HFD has no impact on specific Ser phosphorylation of hippocampal IRS1 in young APPKINL-G-F/NL-G-F mice

Lastly, whether HFD influences the phosphorylation levels of hippocampal IRS1 at 3 residues, such as mSer307, mSer612, and mSer1097 sites, that seem to be associated with the original elevation of Aβ42 in APPKINL-G-F/NL-G-F mice was assessed. Consistent with the unchanged levels of Aβ42 between ND- and HFD-APPKINL-G-F/NL-G-F mice, no statistically significant differences in the IRS1 phosphorylation at mSer307 and mSer612 sites between both mice on ND and HFD were observed, although these phosphorylation levels in the HFD groups were on the rise (Figure 3A, B: two-way ANOVA, p-IRS1(S307)/IRS1: genotype: F_{1,16} = 0.1423, P = 0.7110; diet: F_{1,16} = 7.767, P < 0.05; interaction: F_{1,16} = 8.37, P < 0.05). Regardless of the Aβ42 level and cognitive function, HFD led to elevated levels of IRS1 phosphorylation at mSer1097 site in WT mice, which is consistent with our previous studies, whereas the IRS1 mSer1097 site in APPKINL-G-F/NL-G-F mice was resistant to HFD, indicating the monotonous level of IRS1 phosphorylation.
IRS1: genotype: $F_{1,16} = 0.7704, P = 0.3931$; diet: $F_{1,16} = 7.573, P < 0.05$; interaction: $F_{1,16} = 5.002, P < 0.05$. These results indicate that HFD, which has no impact on the Aβ42 level in young APPKI NL-G-F/NL-G-F mice, does not modify the hippocampal IRS1 via phosphorylation at 3 Ser sites in mice exhibiting normal cognitive functions.

**FIGURE 2** No effect of high-fat diet (HFD) on hippocampus-related behaviors in young APPKI NL-G-F/NL-G-F mice. (A) Open field, (B) passive avoidance test, (C) the water T maze test, and (D) elevated plus maze in WT and APPKI NL-G-F/NL-G-F mice on normal diet (ND) and HFD (12 weeks, n = 9-12 per group). Levels of T-PER-extractable (E) Aβ40 and (F) Aβ42 in the hippocampi in the respective mouse lines (12 weeks, n = 4-5 per group). Results are presented as mean ± standard error of the mean (SEM). A post hoc Tukey-Kramer’s multiple comparison test showed the following results: ND-WT vs ND-APPKI NL-G-F/NL-G-F, *$P < 0.05$, **$P < 0.01$; ND-WT vs HFD-APPKI NL-G-F/NL-G-F, ‡‡$P < 0.01$; HFD-WT vs HFD-APPKI NL-G-F/NL-G-F, ¶$P < 0.05$, ‡‡$P < 0.01$. N.S., not significant.

**FIGURE 3** Young APPKI NL-G-F/NL-G-F mice on high-fat diet (HFD) display no alteration in IRS1 Ser phosphorylation in the hippocampus of young APPKI NL-G-F/NL-G-F mice. Western blot analysis: (A) the phosphorylation levels of mouse Ser307 (mSer307), and mSer1097 sites on IRS1, the respective total protein levels, and β-tubulin in the hippocampi of 3-h-fasted WT and APPKI NL-G-F/NL-G-F mice on normal diet (ND) and HFD (12 weeks, n = 5 per group). (B) Graphs show relative quantification of Ser phosphorylation, respectively. An arrow indicates the position of IRS1 (170 kDa) in (A). The phosphorylation of their respective molecules was normalized to their respective total protein content. Results are presented as mean ± standard error of the mean (SEM). A post hoc Bonferroni’s multiple comparison test showed the following result: ND-WT vs HFD-WT, †$P < 0.05$.

IRS1: genotype: $F_{1,16} = 0.7704, P = 0.3931$; diet: $F_{1,16} = 7.573, P < 0.05$; interaction: $F_{1,16} = 5.002, P < 0.05$. These results indicate that HFD, which has no impact on the Aβ42 level in young APPKI NL-G-F/NL-G-F mice, does not modify the hippocampal IRS1 via phosphorylation at 3 Ser sites in mice exhibiting normal cognitive functions.
In the present study, we have shown that HFD leads to T2DM- and obesity-related abnormalities of nutrient metabolism, such as weight gain, impaired glucose metabolism, and dyslipidemia in both young WT and APPKINL-G/F/NL-G-F mice, while thermoregulatory disorder and impaired ketogenesis specifically occur only in young APPKINL-G/F/NL-G-F mice. Nevertheless, both WT and APPKINL-G/F/NL-G-F mice on HFD display normal cognitive function accompanied by unchanged levels of Aβ and phosphorylation at specific 2 or 3 Ser sites on neural IRS1, respectively.

HFD treatment can cause T2DM- and obesity-associated metabolic disorders in WT mice, although the differences in HFD protocols, such as mouse age and treatment duration, may produce diverse outcomes.6,19–21 Alternatively, several Tg-AD mouse models, although the differences in HFD protocols are not clearly defined, show metabolic disorders similar to WT mice (whether young APPKINL-G/F/NL-G-F mice respond to HFD is not reported). Given that APPKINL-G/F/NL-G-F mice carry 3 mutations at mouse APP locus than none in WT mice, 1 mutation in APPKINL/N mice, or 2 mutations in APPKINL-NL/F mice, APPKINL-G/F/NL-G-F mice are likely to have increased susceptibility to HFD, leading to more severe metabolic dysfunctions than others on HFD. However, practically there are no significant differences in metabolic alterations (i) between young WT and APPKINL-G/F/NL-G-F mice on HFD, (ii) between young or middle-aged WT and APPKINL-NL/F mice on HFD, (iii) between middle-aged WT and APPKINL-NL/F mice on HFD, regardless of age, treatment stage, or dietary fat ratio.15,16 These unexpected outcomes may be due to the low effect of HFD in young APPKINL-G/F/NL-G-F mice, middle-aged APPKINL-NL/F mice, and APPKINL-G/F/NL-G-F mice. Alternatively, the impact of age accompanied by HFD is supposed to deteriorate further energy metabolism and neurological functions than individual effects of aging or HFD.

Thermoregulatory disorder, attributed as the cause for aberrant changes in the core body temperature, is observed even before the onset of neurodegenerative diseases, such as AD,22 although the correlation between thermoregulation associated with lipogenesis and neurodegenerative diseases is completely unknown. While the rectal temperature is comparable between the WT and APPKINL-G/F/NL-G-F mice on HFD under fed conditions and on both ND and HFD under 3-hour-fasting conditions, ND-APPKINL-G/F/NL-G-F mice in fed conditions exhibit significant decrease in core body temperature compared with that in ND-WT mice, suggesting that, in the fed state, thermoregulatory function may be decreased in young APPKINL-G/F/NL-G-F mice despite normal lipid metabolism (comparable levels of blood triglyceride and plasma free fatty acid between WT and APPKINL-G/F/NL-G-F mice on ND).

Ketone bodies are the lipid-derived molecules produced during fasting or on T2DM as an alternative energy fuel for the brain. The ketone bodies can compensate for the deterioration of brain energy metabolism, such as the lower brain glucose levels observed in AD and other neurological diseases.23–25 Although blood ketone bodies normally rise in T2DM or during a fasting state, both ND- and HFD-APPKINL-G/F/NL-G-F mice display significant reduction in blood total ketone bodies regardless of the presence or absence of glucose intolerance, which is consistent with previous studies showing that beta-hydroxybutyrate/beta-hydroxybutyric acid (a ketone body) decreased in the blood and brain parenchyma of patients with AD compared with non-AD subjects.26,27 Additionally, other previous studies, including our report, have shown that the persistent activation of adenosine monophosphate-activated protein kinase (AMPK), which has been correlated with energy depletion and AD, occurs in the brain of AD mouse models, including young and middle-aged APPKINL-G/F/NL-G-F mice on ND.7,28,29 Hence, APPKINL-G/F/NL-G-F mice showing activation of hippocampal AMPK may possess deteriorating brain energy metabolism accompanied by impaired ketogenesis. To clarify this, further studies on alterations in ketogenesis in the liver and neural energy metabolism-related factors, such as metabolic intermediates and energy productions in the brain of young and aged APPKINL-G/F/NL-G-F mice, need to be conducted.

Although long- or short-term HFD-induced metabolic dysfunctions that cause Aβ-unrelated behavioral alterations in WT mice have no impact on hippocampus-associated behaviors in young APPKINL-G/F/NL-G-F mice, a simple comparison in these different impacts of HFD on behaviors between WT and APPKINL-G/F/NL-G-F mice is practically impossible. This is because behavioral alterations, above all on short-term HFD, are observed on hippocampus-independent behavioral tests or different test batteries used in APPKINL mice groups.7,15,16 Nevertheless, chronic HFD for 6 months or more that causes metabolic disorders induces hippocampus-associated behavioral abnormalities in middle-aged APPKINL-NL/F and APPKINL-NL/F mice regardless of alterations in the Aβ levels, differences in APPKINL lines, and initiation age of HFD, suggesting that the correlation between T2DM and AD neuropathology remains controversial.7,13-16,30 Given that HFD for 9 weeks in early life impairs nutrient metabolism but does not alter cognitive function in young APPKINL-G/F/NL-G-F mice, it is likely that aging effects accompanied by long-term HFD accelerate the onset of cognitive dysfunction.

Our previous studies have shown that the phosphorylation of hippocampal IRS1 at 3 residues, such as mSer307, mSer612, and mSer1097 sites, observed in young APPKINL-G/F/NL-G-F mice on ND may be attributed to originally increased level of Aβ42 but not cognitive deficits in those mice, while chronic HFD leads to increased phosphorylation of hippocampal IRS1 at those Ser sites accompanied by Aβ-unrelated cognitive deficits in middle-aged WT mice.7 Given that HFD increases the phosphorylation at mSer1097 site and tends to increase the phosphorylation levels at mSer307 and mSer612 sites on hippocampal IRS1 in young WT mice without cognitive dysfunctions, it is reasonable to assume that HFD can promote IRS1 phosphorylation
levels at these Ser sites in young WT mice prior to the onset of HFD-induced cognitive decline. Corresponding to the unchanged levels of Aβ42 in HFD-APPKI^NL-G-F/NL-G-F mice compared with that in ND-APPKI^NL-G-F/NL-G-F mice, the phosphorylation levels at mSer307 and mSer612 sites on hippocampal IRS1 have been on the rise but have not significantly increased in the HFD-APPKI^NL-G-F/NL-G-F mice; however, the increase in IRS1 phosphorylation at mSer612 is probably partially due to the impact of HFD. By contrast, it is like that mSer307 and mSer1097 residues on hippocampal IRS1 in APPKI^NL-G-F/NL-G-F mice are resistant to HFD-induced activation.

In conclusion, in young APPKI^NL-G-F/NL-G-F mice, HFD is sufficient to provoke metabolic dysfunctions but insufficient to cause neurological alterations, which are likely to be triggered by the aging effect with chronic HFD. Further studies need to be conducted to determine the impact of long-term HFD on nutrient metabolism and neurological phenotypes in APPKI^NL-G-F/NL-G-F mice after middle age.

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AUTHOR CONTRIBUTIONS
W.W., D.T., Y.S., R.T., M.M., and C.K. researched data. W.W. and D.T. analyzed data and wrote the manuscript. T.S. and T.C.S. provided animal experiments and interpreted the data. A.T. designed experiments, analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available in Supporting information.

ANIMAL STUDIES
Animal experiments were conducted in compliance with the guidelines and approval of the ethics committee in Animal Care and Use of the National Center for Geriatrics and Gerontology in Japan.

ORCID
Wei Wang https://orcid.org/0000-0002-6718-4954
Akiko Taguchi https://orcid.org/0000-0003-2893-8685

REFERENCES
1. Biessels GJ, Kofman A, Scheltens P. Diabetes and cognitive impairment. Clinical diagnosis and brain imaging in patients attending a memory clinic. J Neurol. 2006;253(4):477–82.
2. Ninomiya T. Epidemiological evidence of the relationship between diabetes and dementia. Adv Exp Med Biol. 2019;1128:13–25.
3. Copp KD, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. Diabetologia. 2012;55(10):2565–82.
4. Moloney AM, Griffin RJ, Timmons S, O’Connor R, Ravid R, O’Neill C. Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer’s disease indicate possible resistance to IGF-1 and insulin signalling. Neurobiol Aging. 2010;31(2):224–43.
5. Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, et al. Demonstrated brain insulin resistance in Alzheimer’s disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. J Clin Invest. 2012;122(4):1316–38.
6. Tanokashira D, Kurata E, Fukuokaya W, Kadowaki K, Kashiwada M, Takeuchi H, et al. Metformin treatment ameliorates diabetes-associated decline in hippocampal neurogenesis and memory via phosphorylation of insulin receptor substrate 1. FEBS Open Bio. 2018;8(7):1104–18.
7. Wang W, Tanokashira D, Fuku Y, Maruyama M, Kuroiwa C, Saito T, et al. Serine phosphorylation of IRS1 correlates with Aβ-unrelated memory deficits and elevation in Aβ level prior to the onset of memory decline in AD. Nutrients. 2019;11(8):1942.
8. Saito T, Matsuba Y, Mihira N, Takano J, Nilsson P, Itohara S, et al. Single app knock-in mouse models of Alzheimer’s disease. Nat Neurosci. 2014;17(5):661–3.
9. Beeer MS, Silverman JM, Davis KL, Marin D, Grossman HZ, Schmeidler J, et al. Type 2 diabetes is negatively associated with Alzheimer’s disease neuropathology. J Gerontol A Biol Sci Med Sci. 2005;60(4):471–5.
10. Matsuzaki T, Sasaki K, Tanizaki Y, Hata J, Fujiki M, Matsui Y, et al. Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama study. Neurology. 2010;75(9):764–70.
11. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang HY, Ahima RS, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nat Rev Neurosci. 2018;14(3):168–81.
12. Ho L, Qin W, Pompl PN, Xiang Z, Wang J, Zhao Z, et al. Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer’s disease. FASEB J. 2004;18(7):902–4.
13. Vandal M, White PJ, Tremblay C, St-Amour I, Chevrier G, Emond V, et al. Insulin reverses the high-fat diet-induced increase in brain Aβ and improves memory in an animal model of Alzheimer disease. Diabetes. 2014;63(12):4291–301.
14. Knight EM, Martins IV, Gümüsgöz S, Allan SM, Lawrence CB. High-fat diet-induced memory impairment in triple-transgenic Alzheimer’s disease (3xTgAD) mice is independent of changes in amyloid and tau pathology. Neurobiol Aging. 2014;35(8):1821–32.
15. Salas IH, Weerasekera A, Ahmed T, Callaerts-Vegh Z, Himmelreich U, D’Hooge R, et al. High fat diet treatment impairs hippocampal long-term potentiation without alterations of the core neuropathological features of Alzheimer disease. Neurobiol Dis. 2018;113:82–96.
16. Mazzei G, Ikegami R, Abolhassani N, Haruyama N, Sakumi K, Saito T, et al. A high-fat diet exacerbates the Alzheimer’s disease pathology in the hippocampus of the appNL-F/NL-F knock-in mouse model. Aging Cell. 2021;20(8):e13429.
17. Petrov D, Pedrós I, Artiach G, Sureda FX, Barroso E, Pallás M, et al. High-fat diet-induced deregulation of hippocampal insulin signaling and mitochondrial homeostasis deficiencies contribute to Alzheimer disease pathology in rodents. Biochim Biophys Acta. 2015;1852(9):1687–99.
18. Tanokashira D, Wang W, Maruyama M, Kuroiwa C, White MF, Taguchi A. Irs2 deficiency alters hippocampus-associated behaviors during young adulthood. Biochem Biophys Res Commun. 2021;559:148–54.
19. Gainey SJ, Kwakwa KA, Bray JK, Pillote MM, Tir VL, Towers AE, et al. Short-term high-fat diet (HFD) induced anxiety-like behaviors and cognitive impairment are improved with treatment by glyburide. Front Behav Neurosci. 2016;10:156.

20. Johnson LA, Zuloaga KL, Kugelman TL, Mader KS, Morrè JT, Zuloaga DG, et al. Amelioration of metabolic syndrome-associated cognitive impairments in mice via a reduction in dietary fat content or infusion of non-diabetic plasma. EBioMedicine. 2016;3:26–42.

21. McLean FH, Grant C, Morris AC, Horgan GW, Polanski AJ, Allan K, et al. Rapid and reversible impairment of episodic memory by a high-fat diet in mice. Sci Rep. 2018;8(1):11976.

22. Cheshire WP Jr. Thermoregulatory disorders and illness related to heat and cold stress. Auton Neurosci. 2016;196:91-104.

23. Jensen NJ, Wodschow HZ, Nilsson M, Rungby J. Effects of ketone bodies on brain metabolism and function in neurodegenerative diseases. Int J Mol Sci. 2020;21(22):8767.

24. Cunnane SC, Courchesne-Loyer A, St-Pierre V, Vandenberghe C, Pierotti T, Fortier M, et al. Can ketones compensate for deteriorating brain glucose uptake during aging? Implications for the risk and treatment of Alzheimer’s disease. Ann N Y Acad Sci. 2016;1367(1):12–20.

25. García-Rodríguez D, Giménez-Cassina A. Ketone bodies in the brain beyond fuel metabolism: from excitability to gene expression and cell signaling. Front Mol Neurosci. 2021;14:732120.

26. Shippy DC, Wilhelm C, Viharkumar PA, Raife TJ, Ulland TK. β-Hydroxybutyrate inhibits inflammasome activation to attenuate Alzheimer’s disease pathology. J Neuroinflammation. 2020;17(1):280.

27. Newman JC, Verdin E. β-Hydroxybutyrate: a signaling metabolite. Annu Rev Nutr. 2017;37:51-76.

28. Liu YJ, Chern Y. AMPK-mediated regulation of neuronal metabolism and function in brain diseases. J Neurogenet. 2015;29(2–3):50–8.

29. Wang X, Zimmermann HR, Ma T. Therapeutic potential of AMP-activated protein kinase in Alzheimer’s disease. J Alzheimer’s Dis. 2019;68(1):33–8.

30. Rabinovici GD, Furst AJ, Alkalay A, Racine CA, O’Neil JP, Janabi M, et al. Increased metabolic vulnerability in early-onset Alzheimer’s disease is not related to amyloid burden. Brain. 2010;133(Pt 2):512-28.

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