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Effects of high-fat diet on nutrient metabolism and cognitive functions in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice

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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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, whereas impaired thermoregulation and shortage of alternative energy sources specifically occurred in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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

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 Aβ-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)$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ (APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$) 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. APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice also exhibits normal energy metabolism, normal cognitive functions, and increased Aβ 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 Aβ accumulation in Tg-AD mouse models, such as Tg2576 and 3xTg-ADmice.[12–14] Moreover, in middle-aged APPKI$^{NL/N}$ mouse model 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 Aβ production and exhibits normal cognitive function in late middle ages without Aβ deposition.[15] Chronic HFD (40% calories from fat) from 6 to 12 months of age promotes Aβ aggregation and cognitive decline in middle-aged APPKI$^{NL\text{-}F/NL\text{-}F}$ mice, carrying humanized APP with Swedish NL and Beyreuther/Iberian F mutations at mouse APP locus. This model displays Aβ 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 Aβ levels and neural IRS1 modifications remains unknown. To address this question, we have examined the effects of HFD (60% calories from fat) on young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice. We found that HFD for 9 weeks leads to increased body weight, glucose intolerance, and dyslipidemia in both young wild-type (WT) and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, whereas abnormal thermoregulation and impairment of ketogenesis occur only in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, regardless of the type of diet. However, HFD for 9 weeks has no impact on the cognitive functions, levels of Aβ, and phosphorylation of hippocampal IRS1 at all the 3 Ser sites in both young WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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. APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ (Swedish (NL), Arctic (G), and Beyreuther/Iberian (F) mutations) homozygous mice were obtained from Dr. Saido at the Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, Saitama, Japan.[8] The WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 at 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 β Amyloid (1-40) ELISA Kit Wako II (#294-64 701; Fujifilm Wako Pure Chemical Corp., Osaka, Japan) and the Human/Rat/Mouse β Amyloid (1-42) ELISA Kit Wako, High Sensitivity (#292-64 501, 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice

To examine the effects of HFD on young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice from an early age to young adulthood, 4-week-old WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on ND, HFD significantly increased the body weight of both WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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: $F_{1,16} = 2.26$, $P = 0.1522$; diet: $F_{1,16} = 405.8$, $P < 0.0001$; 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on HFD displayed glucose intolerance (HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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: $F_{1,12} = 0.1874$, $P = 0.6728$; diet: $F_{1,12} = 29.67$, $P = 0.0001$; 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-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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: $F_{1,16} = 1.076$, $P = 0.3151$; interaction: $F_{1,16} = 8.134$, $P < 0.05$; 3-h 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on ND, the levels of total and HDL blood cholesterol conspicuously increased in both the WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on HFD, and LDL blood cholesterol levels significantly increased only in the HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice compared with the ND groups (Figure 1I: two-way ANOVA, total cholesterol: genotype: $F_{1,18} = 2.092$, $P = 0.1653$; diet: $F_{1,18} = 92.64$, $P < 0.0001$; interaction: $F_{1,18} = 1.133$, $P = 0.3011$; LDL cholesterol: genotype: $F_{(1,18)} = 0.01134$, $P = 0.9164$; diet: $F_{1,18} = 24.53$, $P = 0.0001$; 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 1F), only the HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice exhibited significant reduction in blood triglyceride levels compared with that in ND-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, whereas these levels were comparable between ND-WT mice and ND-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice or both mice on HFD (Figure 1J: two-way ANOVA, genotype: $F_{1,18} = 4.448$, $P < 0.05$; diet: $F_{1,18} = 12.43$, $P < 0.01$; 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: $F_{1,18} = 2.569$, $P = 0.1264$; 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, but impaired thermoregulation and specific reduction in an alternative energy source occur only in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice.

3.2 | HFD is inadequate to impair hippocampus-associated behaviors in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice

To evaluate the effect of HFD-induced metabolic disturbances on cognitive functions in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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: $F_{1,15} = 0.003036$, $P = 0.9568$; interaction: $F_{1,15} = 1.847$, $P = 0.1942$). Meanwhile, HFD had no impact on the levels of Aβ40 and Aβ42 in the T-PER fractions of the hippocampus in APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, where the levels of Aβ40 consistently decreased regardless of the type of diet (Figure 2E: two-way ANOVA, genotype: $F_{1,15} = 671.1$, $P < 0.0001$; diet: $F_{1,15} = 0.2224$, $P = 0.6440$; 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: $F_{1,15} = 0.02402$, $P = 0.8789$).

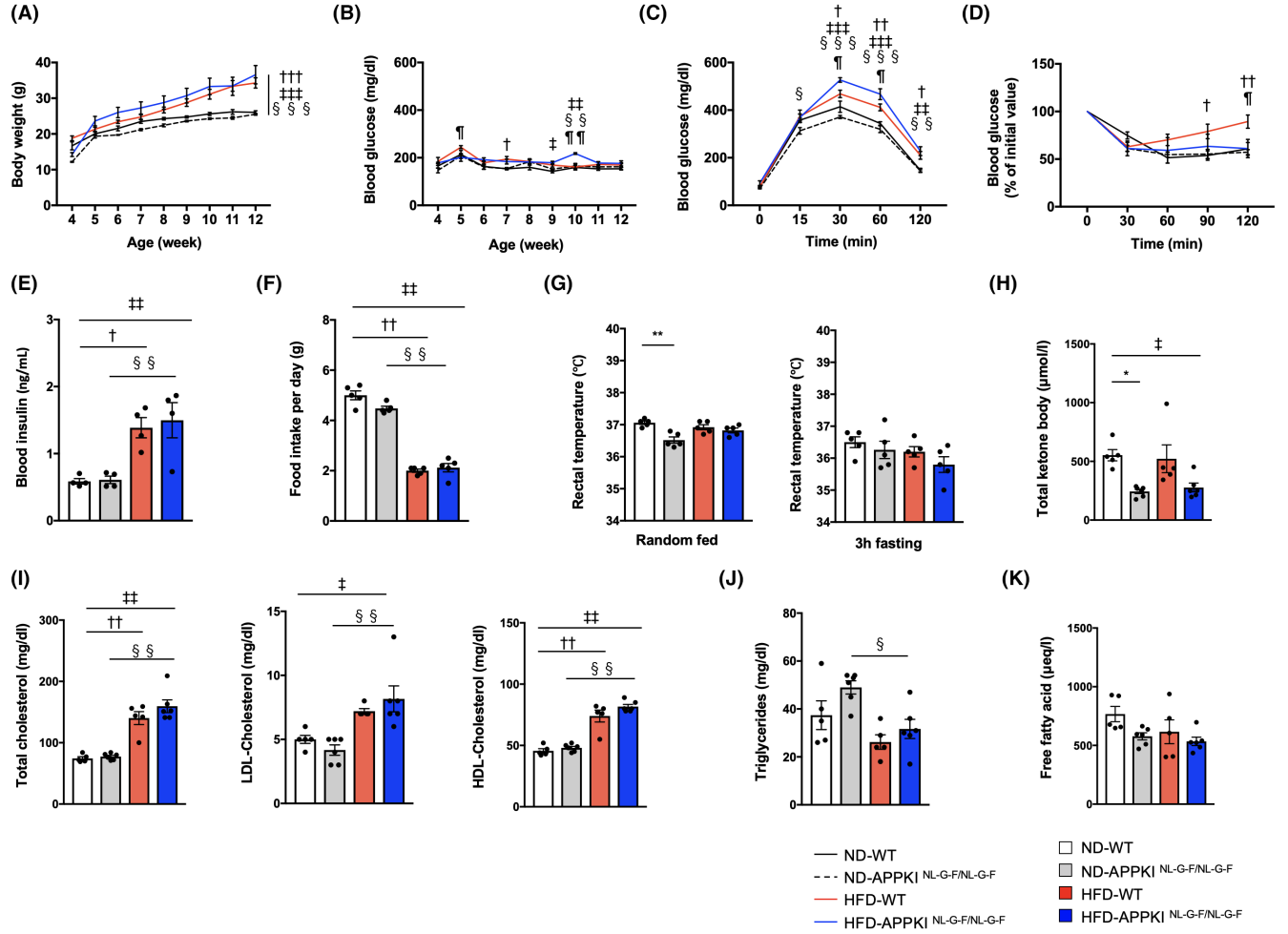

FIGURE 1 Changes in metabolic parameters in high-fat diet (HFD)-induced young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice. (A) Body weight and (B) randomly fed blood glucose levels in WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on normal diet (ND) and HFD (n = 11-13 per group). (C) The glucose tolerance test and (D) the insulin tolerance test (ITT) {12 weeks, n = 5-6 per group). (E) Fasting blood insulin levels (12 weeks, n = 4 per group). (F) Food intake and (G) rectal temperature in random-fed and 3-hour-fasted conditions (12 weeks, n = 5 per group). The blood levels of (H) total ketone body, (I) cholesterol (total, low-density lipoprotein [LDL], and high-density lipoprotein [HDL]), (J) triglyceride, and (K) free fatty acid under fasting condition (12 weeks, n = 5-6 per group). Results are presented as mean ± standard error of the mean (SEM). A post hoc Bonferroni's (if n in each group was the same) or Tukey-Kramer's (if n in each group was not the same) multiple comparison tests showed the following results: ND-WT vs ND-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$, $^{*}P<0.05$, $^{**}P<0.01$; ND-WT vs HFD-WT, †$P<0.05$, ††$P<0.01$, †††$P<0.001$; ND-WT vs HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$, ‡$P<0.05$, ‡‡$P<0.01$, ‡‡‡$P<0.001$; ND-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ vs HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$, §$P<0.05$, §§$P<0.01$, §§§$P<0.001$; HFD-WT vs HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$, ¶$P<0.05$, ¶¶$P<0.01$

These data suggest that HFD-induced metabolic disorders are insufficient to exert a negative impact on cognitive functions and Aβ levels in APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ and WT mice during young adulthood.

3.3 | HFD has no impact on specific Ser phosphorylation of hippocampal IRS1 in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice[7] was assessed. Consistent with the unchanged levels of Aβ42 between ND- and HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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: $F_{1,16} = 0.7947$, $P = 0.3859$; p-IRS1(S612)/IRS1: genotype: $F_{1,16} = 1.775$, $P = 0.2014$; diet: $F_{1,16} = 8.37$, $P<0.05$; interaction: $F_{1,16} = 0.1783$, $P = 0.6785$). 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,[6,7] whereas the IRS1 mSer1097 site in APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice was resistant to HFD, indicating the *monotonous level of IRS1* phosphorylation (Figure 3B: two-way ANOVA, p-IRS1(S1097)/

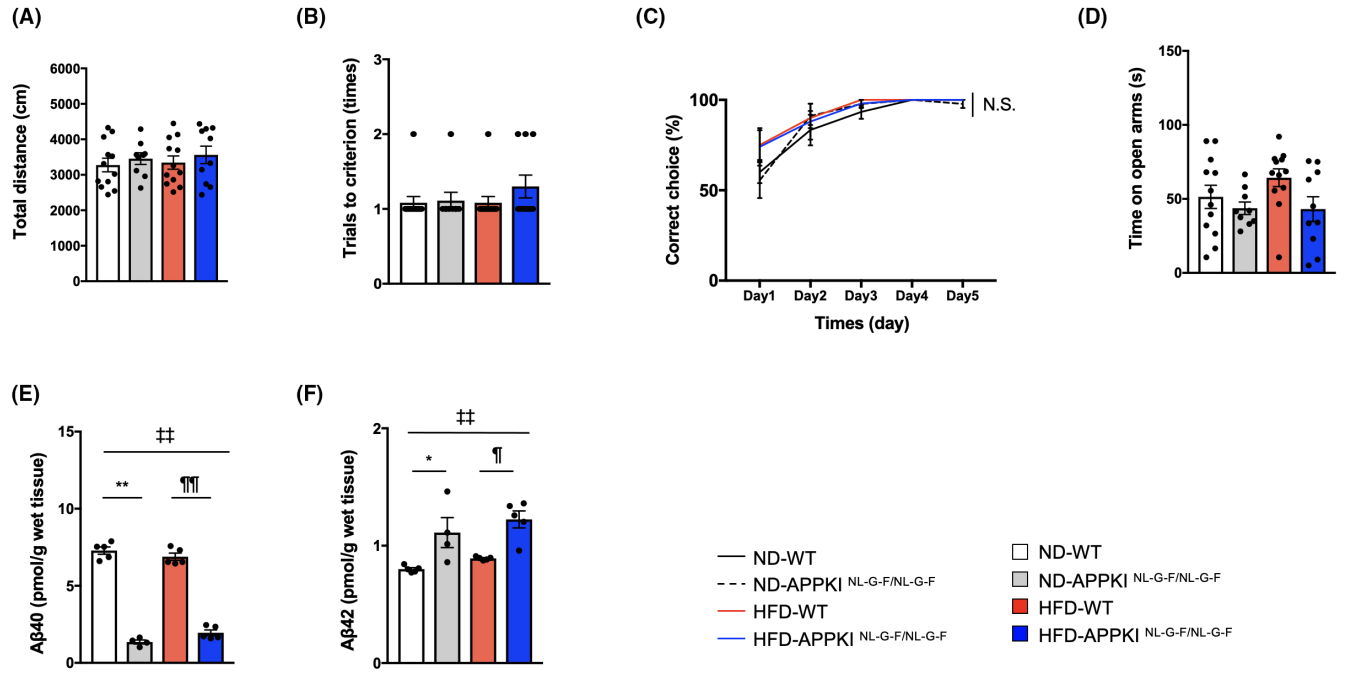

FIGURE 2 No effect of high-fat diet (HFD) on hippocampus-related behaviors in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice. (A) Open field, (B) passive avoidance test, (C) the water T maze test, and (D) elevated plus maze in WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$, $^{*}P<0.05$, $^{**}P<0.01$; ND-WT vs HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$, ‡‡$P<0.01$; HFD-WT vs HFD-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$, ¶$P<0.05$, ¶¶$P<0.01$. N.S., not significant

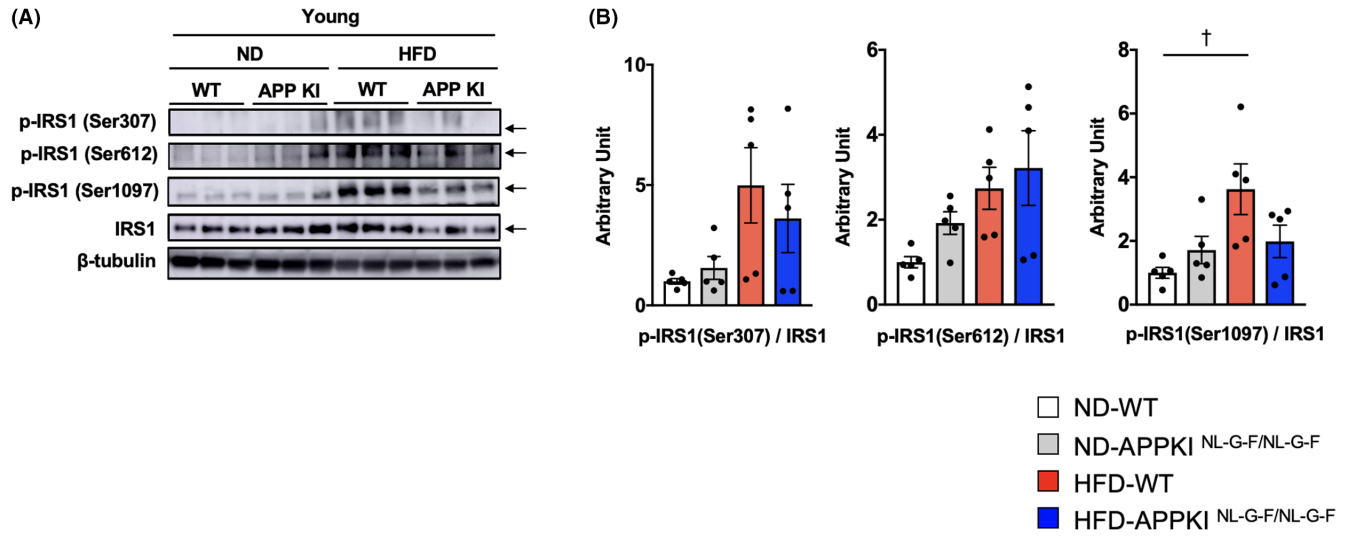

FIGURE 3 Young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on high-fat diet (HFD) display no alteration in IRS1 Ser phosphorylation in the hippocampus of young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice. Western blot analysis: (A) the phosphorylation levels of mouse Ser307 (mSer307), and mSer1097 sites on IRS1, the respective total protein levels, and β-tubulin in the hippocampi of 3-h-fasted WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, does not modify the hippocampal IRS1 via phosphorylation at 3 Ser sites in mice exhibiting normal cognitive functions.

4 | DISCUSSION

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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, while thermoregulatory disorder and impaired ketogenesis specifically occur only in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice. Nevertheless, both WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 on ND exhibit metabolic abnormalities. For example, ND-3xTgAD mice naturally gain increased body weight and elevated blood glucose and triglyceride.[13,14] By contrast, ND-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice display normal metabolic parameters even after middle age similar to APPKI$^{NL/N}$ and APPKI$^{NL\text{-}F/NL\text{-}F}$ mice on ND.[7,15,16] Nevertheless, young APPKI$^{NL/N}$ and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice respond to HFD, showing metabolic disorders similar to young WT mice (whether young APPKI$^{NL\text{-}F/NL\text{-}F}$ mice respond to HFD is not reported). Given that APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice carry 3 mutations at mouse APP locus than none in WT mice, 1 mutation in APPKI$^{NL/N}$ mice, or 2 mutations in APPKI$^{NL\text{-}F/NL\text{-}F}$ mice, APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on HFD, (ii) between young or middle-aged WT and APPKI$^{NL/N}$ mice on HFD, (iii) between middle-aged WT and APPKI$^{NL\text{-}F/NL\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, middle-aged APPKI$^{NL/N}$, and APPKI$^{NL\text{-}F/NL\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on HFD under fed conditions and on both ND and HFD under 3-hour-fasting conditions, ND-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice despite normal lipid metabolism (comparable levels of blood triglyceride and plasma free fatty acid between WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice on ND.[7,28,29] Hence, APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, need to be conducted.

Although long- or short-term HFD-induced metabolic dysfunctions that cause Aβ-unrelated behavioral alterations in WT mice[7,19,21] have no impact on hippocampus-associated behaviors in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice, a simple comparison in these different impacts of HFD on behaviors between WT and APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI mice groups.[15,16] Nevertheless, chronic HFD for 6 months or more that causes metabolic disorders induces hippocampus-associated behavioral abnormalities in middle-aged APPKI$^{NL/NL}$ and APPKI$^{NL\text{-}F/NL\text{-}F}$ mice regardless of alterations in the Aβ levels, differences in APPKI 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice compared with that in ND-APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice are resistant to HFD-induced activation.

In conclusion, in young APPKI$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ 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 the APPKI mice and interpreted the data. A.T. designed experiments, analyzed data, and wrote and edited 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.

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SUPPORTING INFORMATION

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