Ingestion of Green Tea Catechin and Caffeine Suppresses Brain Dysfunction in Mice Caused by High-Fat Diet Feeding

Keiko Unno[1], Tomokazu Konishi[2], Shiori Hagiwara[1], Ken-ichi Maeda[1], Hiroyuki Yamamoto[1], Atsushi Takeda[1], Minoru Hoshino[1], Akiko Takayanagi[3], and Keiji Wakabayashi[3]

1: Sch Pharmaceutical Sci, Univ Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka, 422-8526, Japan.

2: Basic Life Sci Res Gr, Akita Pref Univ, Shimoshinjyo Nakano, Akita, 010-0195, Japan.

3: Inst Environmental Sci, Univ Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka, 422-8526, Japan.

Correspondent email: unno@u-shizuoka-ken.ac.jp

Summary

Increased consumption of fat in a diet is an important risk factor for not only obesity and diabetes but also for aging and brain dysfunction. We investigated the effect of chronic consumption of a high-fat diet (HFD) on brain function. Moreover, the effect of green tea catechin (catechin) and caffeine on brain dysfunction in mice fed a HFD was investigated. Cerebral weight and working memory were lower in mice fed a HFD than the control and mice fed a HFD with catechin and caffeine in aged (>12 month-old) mice. A decrease in mitochondrial content was observed in the cerebral cortex of mice fed a HFD. However, that was suppressed in mice fed a HFD with catechin and caffeine. These results suggest that the ingestion of catechin and caffeine suppresses brain dysfunction with aging, which was accelerated by chronic consumption of a HFD.

Introduction

Alterations in our life styles have resulted in the consumption of more high-fat meals. A high-fat diet (HFD) can induce obesity and metabolic disorders in rodents, suggesting that it is also a factor affecting obesity and lifestyle diseases in humans. Such diets might induce changes in not only energy metabolism but also aging and brain function. Indeed, rats fed a HFD have been reported to show decreased brain function during learning and memory (Molteni et al, 2002; Winocur et al, 2005). Feeding rats a diet rich in fat decreased hippocampal neurogenesis (Lindqvist et al, 2006). In both Alzheimer's disease and mild cognitive impairment groups, higher body mass index was associated with brain volume deficits in frontal, temporal, parietal, and occipital lobes (Ho et al, 2010). Although increased consumption of fat in a diet is an important risk factor for not only obesity and diabetes but also aging and brain dysfunction, more work is needed to identify the mechanisms underlying the impairment by a HFD.

The consumption of green tea catechin (catechin) has been reported to suppress obesity (Murase et al, 2002; Zheng et al, 2004; Klaus et al, 2005). Catechin inhibits intestinal absorption of dietary lipid and stimulates lipid catabolism in the liver. We found another effect of catechin, namely that its consumption suppressed decreased brain function with aging (Unno et al, 2004). Caffeine has also been reported to modulate lipid metabolism and glucose homeostasis (Kobayashi-Hattori et al, 2005; Johansson et al, 2007; Park et al, 2007). These results suggest that a beneficial effect of catechin and caffeine consumption is expected in obesity and cognitive dysfunction under high-fat feeding. We investigated the effect of chronic consumption of a HFD on both pancreas and brain functions. Moreover, as green tea catechin has been found to improve brain atrophy, brain dysfunction and obesity (Unno et al, 2009), the effects of catechin and caffeine on brain and pancreas in mice fed a HFD were investigated.

Materials and methods

Animals and feeding: All experimental protocols were performed in accordance with the guidelines for the care and use of laboratory animals of the University of Shizuoka. C57BL/6 mice were purchased from Japan SLC Co., Ltd. (Shizuoka, Japan) and ICR mice were purchased from Charles River Laboratories (Tokyo, Japan). MIP-GFP mice are transgenic in which pancreatic β-cells are labeled with green fluorescence protein

(GFP) under the control of the mouse insulin 1 promoter (MIP) (Hara et al, 2003). MIP-GFP mice, donated by Dr. Toyoda (Kyoto Univ.), were used to observe morphological changes in the pancreas after feeding a HFD. The experimental mice had free access to a normal diet (CE-2; Clea Co., Ltd., Tokyo, Japan) from between 1 to 2 months of age, after which mice were fed a HFD (Quick Fat, Clea Japan Inc.) from 3 to 21 months. At the same time, mice drank water containing catechin (0.5 mg/ml, Sunphenon BG, Taiyo Kagaku Co. Ltd., Yokkaichi, Japan) and caffeine (0.08 mg/mg, Wako Pure Chemical industries, Ltd., Osaka, Japan), at concentrations similar to those in available green tea. Control mice drank water. The HFD consisted of 13.6% fat which is about three times higher than that in CE-2 (4.8% fat). Sunphenon BG had the following catechin composition: 46.5% (-)-epigallocatechin gallate, 9.6% (-)-epicatechin gallate, 7.5% (-)-epigallocatechin, 5.7% (-)-epicatechin, 4.6% (-)-gallocatechin gallate, and 1.6% (-)-gallocatechin.

Brain and pancreas samples: The whole brain and the cerebrum region were weighed. The pancreas was used to isolate islets of Langerhans – to measure insulin release – and to investigate morphological changes. The level of insulin was measured with competitive radioimmunoassay using ^{125}I-labeled insulin and anti-insulin antibody. The radioactivity of the supernatant was measured using a gamma-counter (Aloka Co., Tokyo, Japan).

Working memory: The searching behavior of mice was observed in a Y-maze (MYM-01M; Muromachi Kikai Co., Ltd., Tokyo, Japan) assessed by the number of times each Y arm was entered over 8 min. When a mouse entered three different arms successively, the alternation behavior was thought to reflect the capacity of working memory. The times of spontaneous alternation behavior was counted and the ratio of alternation was calculated as below: Ratio of alternation = (times of spontaneous alternation) / (total times of arm entries – 2).

Measurement of PDS-95 and insulin receptor: A postsynaptic protein, PSD95, and insulin receptor were measured by immunoblotting. The brain sample (cerebral cortex) was homogenized with 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA and a protease inhibitor cocktail (Sigma-Aldrich Co., St. Louis, USA). The protein content of homogenates was determined with a Bio-Rad protein assay kit (Bio-Rad laboratories, Richmond, USA). Protein samples of 20 μg were loaded on a 10% SDS-polyacrylamide gel and electrophoresed. The bands were detected with anti-PSD-95 monoclonal antibody (Affinity BioReagents, CO, USA) or anti-insulin receptor (Thermo Fisher Scientific Anatomical Pathology, CA, USA) using the ECL™ detection system (GE Healthcare UK Ltd., Buckinghamshire, England). The densities of PSD-95 or insulin receptor bands were normalized to the appropriate tubulin bands.

DNA microarray analysis: Total RNA was extracted from the hippocampus of mice that ingested control or HFD with catechin and caffeine. RNA samples were measured with a mouse genome gene chip (Affimetrix, Santa Clara, CA, USA) with three biological repeats per group. The analyses focused on genes identified as positive by two-way analysis of variance (ANOVA).

Citrate synthase activity: The cerebral cortex was homogenized with a buffer containing 175 mM KCl, 10 mM glutathione and 2 mM EDTA (pH 7.4). The supernatant following centrifugation at 700 *g* was used as the enzyme solution. The reaction medium contained 0.1 mM dithionitrobenzoic acid, 0.1 mM oxaloacetate, 0.3 mM acetyl coenzyme A and 0.1 M Tris–HCl. Subsequently, 10 μl of enzyme solution were added to the reaction medium and citrate synthase activity was assessed spectrophotometrically at 412 nm.

Results and discussion

Effect of HFD on body weight, pancreas and brain: At 7-months of age, body weight tended to be high in HFD mice. Insulin release from isolated islets was high in mice fed a HFD but was suppressed by catechin and caffeine consumption. Working memory using the Y-maze test did not change.

At 12-months of age, large islets were observed in HFD mice. Total β-cell area in the pancreas was significantly higher in HFD mice but not in mice fed a HFD with catechin and caffeine. Working memory and insulin receptor level in the hippocampus was lower in HFD mice but not in mice fed a HFD with catechin and caffeine.

At 21-months of age, cerebral weight and PSD-95, a marker of post-synapse, were lower in HFD mice but not in mice fed a HFD with catechin and caffeine. These results indicate that chronic feeding of a HFD resulted in brain dysfunction in adult/aged mice.

These results also indicate that the pancreas was altered in young/adult (7 and 12 months) mice by feeding of a HFD and that brain function was lower in adult/aged (12 and 21 months) mice fed a HFD. Changes in the pancreas and brain were suppressed by ingestion of catechin and caffeine.

DNA microarray analysis: The effect of feeding a HFD on the brain was investigated using DNA microarray analysis in 7-month-old HFD mice. Working memory, one of the brain's functions, was not altered in young/adult mice but gene expression in the hippocampus was significantly altered in 7-months-old mice fed a HFD. Principal component (PC) analysis of microarray data indicated that feeding a HFD had a clear effect on gene expression and that ingestion of catechin and caffeine restored it to control levels (Fig. 1). These results suggest that brain function begins to be altered by chronic HFD feeding from a young/adult age but that changes are prevented by the ingestion of catechin and caffeine. Annotation keywords, which are genes down-regulated and up-regulated genes by a HFD and restored by catechin and caffeine, are shown in Table 1. Many genes in mitochondria showed low levels of expression in HFD mice. In addition, citrate synthase activity, a marker for the presence of intact mitochondria, was low in mice fed a HFD but not in mice fed a HFD with catechin and caffeine.

In conclusion, HFD feeding results in decreased mitochondrial gene expression in the brain. Ingestion of catechin and caffeine suppresses neuronal dysfunction and insulin resistance caused by chronic consumption of a HFD. Decrease of mitochondrial gene expression might be important for brain dysfunction by a HFD feeding.

Tabel 1 Annotation keywords for genes restored by catechin and caffeine

Down-regulation	Up-regulation
translation	regulation of transcription
ribosomal protein	multicellular organismal development
RNA processing	cell-cell signaling
electron transport chain	calcium channel
TCA cycle	growth factor

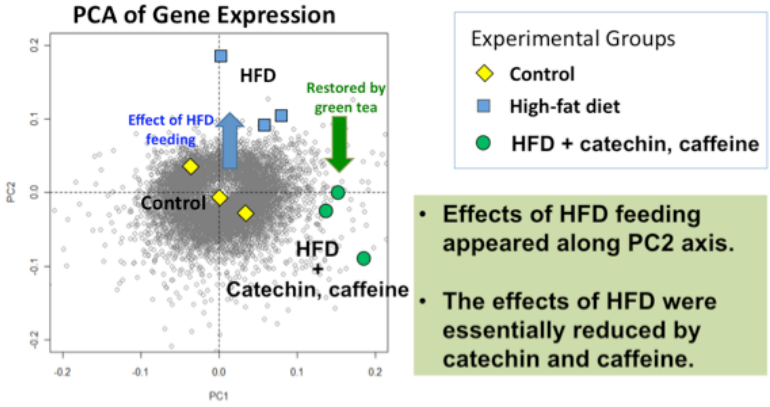

Fig. 1 Effect of HFD feeding on gene expression

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