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Dietary resistant starch improves selected brain and behavioral functions in adult and aged rodents

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Abstract

Resistant starch (RS) is a dietary fiber that exerts multiple beneficial effects. The current study explored the effects of dietary RS on selected brain and behavioral functions in adult and aged rodents. Because glucokinase (GK) expression in hypothalamic arcuate nucleus and area postrema of the brainstem is important for brain glucose sensing, GK mRNA was measured by brain nuclei microdissection and PCR. Adult RS-fed rats had a higher GK mRNA than controls in both brain nuclei, an indicator of improved brain glucose sensing. Next, we tested whether dietary RS improve selected behaviors in aged mice. RS-fed aged mice exhibited (1) an increased eating responses to fasting, a behavioral indicator of improvement in aged brain glucose sensing; (2) a longer latency to fall from an accelerating rotarod, a behavioral indicator of improved motor coordination; and (3) a higher serum active GLP-1. Third, GLP-1 receptor null (GLP-1RKO) mice were used to test the role of GLP-1 in brain glucose sensing, and they exhibited impaired eating responses to fasting. We conclude that in rodents (1) dietary RS improves two important indicators of brain function: glucose sensing and motor coordination, and that (2) GLP-1 is important in the optimal feeding response to a fast.

Keywords

Aging; Brain function; GK; Glucose sensing; GLP-1; Resistant Starch

Resistant starch (RS) is a dietary fiber that exerts multiple beneficial effects on energy homeostasis peripherally, such as enhancing glucose tolerance and insulin sensitivity, and decreasing body fat [1–5]. Several mechanisms have been investigated to elucidate these benefits, including gastrointestinal modulation of microbiota and hormone production in RS-fed rodents [1, 6, 7]. However, there is a limited knowledge as to whether dietary RS improves brain function [8].

To ensure peripheral energy homeostasis, certain brain areas can “sense” body energy status and adjust physiological responses accordingly. The hypothalamic arcuate nucleus (ARC) and area postrema (AP) in the brainstem are two important brain areas for sensing glucose status and consequently adjusting eating behavior [9, 10]. Although brain glucose sensing involves a complex cascade, glucokinase (GK), which converts glucose into glucose-6-phosphate in the initial step of glycolysis, is an important participant in this process [11, 12]. Because of its high K_m (6 mM), GK responds to a wide dynamic range of fluctuations in glucose concentrations. For example, fasted animals had lower levels of blood glucose and higher GK mRNA expressions in hypothalamus and brainstem, compared to the animals that had the same fasting regime, but re-fed for only one hour [13]. Thus, brain can “sense” energy demands in fasted and re-fed animals by adjusting GK mRNA expression in the aforementioned brain areas, and a higher GK expression indicates a better sensing ability in these brain areas.

To determine the effects of dietary RS on brain glucose sensing, we first examined GK expression in the ARC and AP in RS-fed rats. Adult Sprague-Dawley (SD) rats (n=15 per group) were fed 20% RS or control diet for 6 weeks and killed by decapitation. Their whole brains were collected for nuclei microdissection of ARC and AP (supporting information for housing, diet, and nuclei microdissection). Total RNA was extracted (Stratagene RNA Microprep kit) from dissected ARC and AP nuclei. Real-time quantitative RT-PCR was used to measure GK mRNA expression in these nuclei as previously described [14]. Cyclophilin was used as the housekeeping gene to normalize GK expression levels. Protein level of GK in these brain nuclei could not be measured due to small sample volume. Dietary RS increased GK expression in ARC and AP ($P<0.01$ and 0.05 respectively, Fig. 1A), but did not significantly affect expression of any other genes (Glut-2, GLP-1R, SUR1, NPY and AGRP) (supplemental data). The increased GK expression in ARC and AP regions suggests that the RS-fed animals were more sensitive to blood glucose fluctuations, which should lead to a better eating response to fasting. Therefore, we next examined the eating response to fasting in a mouse model that has a deficiency in brain glucose sensing.

Aging is associated with a decreased brain glucose sensing ability and a decreased eating response to fasting. Aged animals and humans fail to increase food intake after fasting and are slow to regain lost body weight on re-feeding [15–17]. This age-related reduction in food intake and nutrient sensing has been termed “the anorexia of aging” [18]. Thus, we used aged mice to test if dietary RS can improve brain glucose sensing by measuring their eating response to fasting. Fifty healthy male C57BL/6J mice (18–20 months analogous to 56–65 years of age in humans [19]) were fed 0%, 18% or 36% RS diet for 10-weeks (supporting information for housing and diets). Their eating responses to fasting were examined at the 9th week by measuring their food intake during re-feeding period after fasting. Because of the advanced age of mice used in this experiment, we only fasted them for 13 hours (9:00pm to 10:00am). Compared to control diet fed mice, RS-fed mice significantly increased their 1-, 2- and 4-hour food intakes during the re-feeding period following a mild fasting (Fig. 1B). Combining these data with the data presented in Figure 1A, our results suggest that dietary RS could be beneficial for reducing anorexia of aging in rodents by increasing GK expression in the brain nuclei that are important for glucose sensing.

Aging affects multiple physiological functions that lead to other functional declines. Therefore, we also assessed effects of dietary RS on motor coordination in aged mice. After being fed RS diets for 6 weeks, aged mice were placed on an accelerating rotarod and the time before falling from the rotarod was recorded [20]. Increased length of stay on the rotarod indicated improved motor coordination. RS-fed aged mice stayed longer on the accelerating rotarod, calculated as mean falling time from three trials (Fig. 2A). Notably, only mice fed 36% RS showed improved motor coordination, which suggests that a higher

dose of RS or a longer feeding time may be required to demonstrate this improvement in aged mice. The better motor coordination could also contribute to the increased eating response in RS-fed aged mice as they might simply act better physically. This beneficial effect on motor coordination might result from the action(s) of GLP-1, as GLP-1 has been shown to protect motor neurons from glucotoxicity [21].

One distinguishing feature of RS fed animals is increased expression of GLP-1 in all young and adult animal models tested to date in our laboratory, including mice and rats of both genders [1, 3, 4, 19]. To evaluate whether aged mice also showed the same increase in GLP-1 expression, we measured active GLP-1 levels in these aged mice at the end of study. Serum active GLP-1, measured by ELISA (active GLP-1 kit, Millipore Corporation, Billerica, MA), was increased in RS-fed aged mice by 44% and 87% for 18% and 36% RS, respectively, compared with control mice ($P<0.05$ and 0.01) (Fig. 2B).

GLP-1 is a gut secreted hormone that has multiple biological functions. The primary clinical functions of GLP-1 and its analogs are to stimulate pancreatic insulin secretion and improve peripheral tissue insulin sensitivity. However, the role of GLP-1 on brain function has recently attracted more attention [22–24]. GLP-1 has been shown to prevent the degenerative process and to ameliorate neurodegenerative changes in cellular and animal models of Alzheimer's disease [25–28]. Also, GLP-1 is known to increase GK expression in beta cells of the pancreas [29], evidence that it improves pancreatic glucose sensing. In addition, GLP-1 enhances GK activity in neurons in a glucose-dependent manner via GLP-1 receptor (GLP-1R) [30]. Thus, we speculate that GLP-1 might be important in the cascade of delaying brain aging, and that blocking GLP-1 signaling should accelerate brain aging and consequently, impair the eating response to fasting. We therefore used GLP-1R null mice to compare their eating response to fasting with that of wild type mice.

Adult GLP-1R null and wild type mice ($n=8$ per group) were used to compare eating responses to fasting (support information for housing, diet, and fasting conditions). After a 13-hour fast (9:00pm-10:00am), all mice were re-fed, and their food intake at 1, 2, and 4 hours was recorded. Food intake in GLP-1R null mice was significantly lower than that in wild type mice at all times measured (Fig. 3). These results suggest that impaired GLP-1 signaling in mice decreases eating response to fasting; consistent with the notion of GLP-1 is involved in brain glucose sensing. Because of much smaller size in mice brain compared to rat brain, we could not obtain GK mRNA expression data that are accurately measured in ARC and AP in mice brain.

The current study used several different rodent models to provide strong support that dietary RS improves selected brain and behavioral functions in adult and aged rodents. In aged mice, dietary RS improves two important behavioral indicators of brain function: eating response to mild fasting and motor coordination. Additionally, dietary RS increased serum concentrations of GLP-1. In contrast, blockade of GLP-1 signaling impaired the eating response to fasting, a sign of impaired brain glucose sensing. Although we observed increased GK mRNA expression in critical brain nuclei for glucose sensing only in adult, but not aged rodents, our findings provide support for further investigation of whether dietary RS improves brain glucose sensing in aged rodents via stimulating gut GLP-1 expression and GK expression in brain areas associated with nutrient sensing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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A list of abbreviations

RS	Resistant starch
ARC	arcuate nucleus
AP	area postrema
GK	glucokinase
GLP-1	Glucagon-like peptide-1

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Fig. 1.

A: Glucokinase mRNA (GK) expression in ARC and AP in SD rats fed control or 20% RS diet for 6 weeks, measured by RT-PCR (n=9–14 per group). ** and * indicate $P<0.01$ or $P<0.05$ compared to the controls. **B:** Feeding response to 13-hour fasting in aged mice (n=13–15 per group) fed control, 18% or 36% RS diet for nine weeks. Values with different letters over the column indicate they are statistically different ($P<0.05$). ab: not statistically different from either a or b. Data are Mean $\pm$ SE.

Fig. 2.

Effects of dietary RS on motor coordination (A) and serum active GLP-1 (B) in aged C57BL/6J mice fed control, 18%, or 36% RS diet. Motor coordination was evaluated on accelerating rotarod after feeding the diets for 6 weeks (n=15–17 per group). Serum active GLP-1 was measured at the end of study (pooled sample, n=7 per group). Data are Mean $\pm$ SE. * and ** indicate $P < 0.05$ and $P < 0.01$ compared to the mice fed control diet.

Fig. 3. Feeding response to 13-hour fasting in wild type (□) and GLP-1R null mice (■). ** indicate $P < 0.01$ compared to the wild type mice (Data are Mean $\pm$ SE, $n=8$ per group)