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Basic nutritional investigation

Azuki bean polyphenols intake during lactation upregulate AMPK in male rat offspring exposed to fetal malnutrition

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ABSTRACT

Objective: Fetal malnutrition is an early-life inducer of dyslipidemia and glucose intolerance. The aim of this study was to examine whether maternal azuki bean (*Vigna angularis*) polyphenol (AP) intake during lactation affects the adenosine monophosphate–activated protein kinase (AMPK) pathway and lipid metabolism in offspring exposed to fetal malnutrition.
Methods: Pregnant Wistar rats were divided into three groups: a control diet offered during gestation and lactation (CC), a low-protein diet during gestation and a control diet during lactation (LPC); and a low-protein diet during gestation and a 1.0% AP-containing control diet during lactation (LPAP). Male pups were randomly selected for the study; half the pups were sacrificed at 3 wk of age and the other half were fed a standard diet and sacrificed at 23 wk. Hepatic triacylglycerol levels, phosphorylation levels of AMPK and acetyl-coenzyme A carboxylase (ACC), and mRNA levels of sterol regulatory element-binding protein-1c (SREBP-1c) were evaluated.
Results: Significant decreases in body weights and hepatic triacylglycerol levels were found in the LPAP compared with the LPC group. Plasma adiponectin levels in the LPAP group were higher than those in the LPC group. AMPK phosphorylation was upregulated in the livers and skeletal muscles in young and adult LPAP compared with LPC rats. ACC phosphorylation was upregulated in skeletal muscles of LPAP rats. SREBP-1c mRNA expression was decreased in the livers of LPAP rats.
Conclusion: Our results suggest that maternal AP intake during lactation upregulates AMPK phosphorylation not only in young but also in adult offspring exposed to fetal malnutrition and may lead to decreased hepatic lipid accumulation by ACC phosphorylation and downregulation of SREBP-1c expression.

Introduction

The azuki bean (*Vigna angularis*) is indigenous to the tropical regions of Asia; currently, it is one of the most important crops in Japan, China, and South Korea. The seed coats of the azuki bean are rich in dietary polyphenols such as proanthocyanidins, rutin, catechin, and quercetin derivatives [1,2]. Proanthocyanidins are natural antioxidants that exert antioxidant effects in pathologic conditions such as obesity and diabetes [3,4]. Rutin, quercetin, and epigallocatechin gallate have been demonstrated to suppress dyslipidemia and oxidative stress in food-induced obese animals [5–7]. We previously showed that azuki bean seed coats mixed with diets attenuated vascular oxidative stress and inflammation and decreased plasma glucose in hypertensive rats [1,8,9].

Recently, many epidemiologic and experimental studies have indicated that fetal and neonatal environments are strongly associated with the subsequent development of diseases in adult life. For instance, fetal malnutrition is an early-life inducer of glucose intolerance and obesity [10–12]. Maternal protein restriction during pregnancy and lactation has been shown to program impaired lipid metabolism, resulting in visceral adiposity in adult rat offspring [13].

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a major regulator of lipid metabolism in the liver and skeletal muscle. AMPK activation elicits a variety of beneficial metabolic effects with the potential to ameliorate the defects associated with metabolic syndrome [14]. For example, AMPK in the liver and skeletal muscle is activated by decreases in the

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cellular ratio of adenosine triphosphate to AMP, and, once activated, it switches on catabolic pathways. Briefly, activated AMPK phosphorylates acetyl-coenzyme A (CoA) carboxylase (ACC), rendering this enzyme inactive, and because ACC synthesizes malonyl-CoA, the malonyl-CoA–induced inhibition of carnitine palmitoyl transferase I, the rate-limiting enzyme of mitochondrial fatty acid uptake, is decreased and fatty acid oxidation is increased [15–18]. In addition, hepatic AMPK phosphorylation mediates the suppression of lipogenic gene expression, such as fatty acid synthase and ACC, by decreasing the activity of the transcriptional factors sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate responsive element-binding protein [19,20]. Thus, the activation of AMPK signaling is an important target in studies on obesity and lipid metabolism.

To date, certain plant polyphenols have been reported to have an AMPK-activating effect in lipid and glucose metabolism. For example, quercetin stimulated the AMPK pathway and increased glucose uptake in muscle cells [21]. In mouse liver steatosis, resveratrol led to decreased lipid synthesis and increased rates of fatty acid oxidation through the upregulation of an AMPK signaling system [22]. However, little is known about the effects of dietary polyphenols in azuki beans on the AMPK pathway and lipid metabolism in offspring exposed to fetal malnutrition.

The aim of this study was to investigate whether maternal azuki bean polyphenol (AP) intake during lactation affects AMPK phosphorylation in the liver and skeletal muscle of young and adult offspring exposed to fetal malnutrition. In addition, we determined the effect of AP ingested by the lactating mother on SREBP-1c mRNA expression and ACC phosphorylation in adult offspring.

Materials and methods

Azuki bean polyphenols

Azuki beans, harvested in Japan, were immersed overnight in distilled water at 25°C. The seed coats were removed, collected, dried, and ground in a Waring blender. Approximately 80 g in dry weight of seed coats was obtained from 1 kg of azuki beans. Seed coats contained 103 mg (*d*-catechin equivalent) of phenolic compounds (AP) per gram as measured by the Folin–Ciocalteu method [23].

Animals and experimental procedure

All experimental procedures were performed in accordance with the guidelines for animal experimentation provided by the Aomori University of Health and Welfare. Eight-week-old virgin female Wistar rats were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan). The rats were accommodated at a constant temperature of 23 ± 1°C under a 12-h light/dark cycle with access to food and tap water ad libitum. A vaginal impedance reader (MK-10C; Muromachi Kikai Co. Ltd., Osaka, Japan) was used to determine whether the female rats were in the appropriate stage of the estrus cycle for mating. This procedure was routinely performed in the afternoon, and a reading higher than 3 kΩ indicated that the female rats were in proestrus and presumably in estrus. One female rat was mated overnight with one male rat. The next morning, the presence of a vaginal plug indicated successful mating and was documented as day 0 of gestation. Pregnant rats weighing 258 to 319 g were randomly divided into three groups: a control diet during gestation and lactation (CC); a low-protein diet during gestation and a control diet during lactation (LPL); and a low-protein diet during gestation and a 1.0% AP-containing control diet during lactation (LPAP). The diets were isocaloric, as detailed in Table 1. Because our previous report showed that plasma glucose in 1.0% AP-treated hypertensive model rats was significantly lower than that in untreated rats [1], a 1.0% AP-containing diet was selected to supply the same quantity of phenolic compounds. Dams had access to each food and tap water ad libitum. The pups were weighed at postnatal day 4, and six male pups per dam were randomly selected to ensure adequate nutrition during lactation. At weaning (week 3), half the pups were separated from each group (n = 5–12) and weighed. Then, pentobarbital anesthesia was induced and the liver, kidneys, and heart were removed immediately and weighed. The other half (n = 5–11) continued to receive a standard commercial laboratory diet (MF Diet; Oriental Yeast, Tokyo, Japan) and were weighed once every 2 wk. Before sacrifice at week 23, the animals were fasted overnight. Under ether anesthesia, blood samples were collected and then the liver, kidney, and heart were removed and weighed. The livers and removed parts of the skeletal muscles were stored at −80°C for the evaluation of mRNA and protein expressions.

Table 1
Compositionof diets

Ingredient	Control diet (g/100 g diet)	Low-protein diet (g/100 g diet)	AP-containing control diet (g/100 g diet)
Casein	20.000	8.000	20.000
L-Cystine	0.300	0.123	0.300
Cornstarch	39.749	48.826	39.749
α-Corn starch	13.200	16.300	13.200
Sucrose	10.000	10.000	10.000
Soybean oil	7.000	7.000	7.000
Cellulose	5.000	5.000	4.000
Mineral mixture*	3.500	3.500	3.500
Vitamin mixture†	1.000	1.000	1.000
Choline chlorhydrate	0.250	0.250	0.250
tert-Butyl-hydroquinone	0.001	0.001	0.001
AP	—	—	1.000

AP, azuki bean polyphenol
* AIN-93G mineral mixture (Oriental Yeast, Tokyo, Japan).
† AIN-93G vitamin mixture (Oriental Yeast, Tokyo, Japan).

Blood chemistry

Plasma samples were separated by centrifugation at 800 × *g* for 10 min at 4°C and glucose, triacylglycerol, total cholesterol, adiponectin, and leptin levels were determined using the following commercial kits: glucose CII-test, triacylglycerol E-test, and cholesterol E-test (Wako Pure Chemical Industries, Ltd., Osaka, Japan), a mouse/rat high-molecular-weight adiponectin enzyme-linked immunosorbent assay kit (Shibayagi, Gunma, Japan), and a rat leptin enzyme-linked immunosorbent assay kit (Morinaga Institute of Biological Sciences, Yokohama, Japan).

Hepatic triacylglycerol measurement

Hepatic lipids were extracted by the method of Folch et al. [24] using 40 vol of a chloroform–methanol mix (2:1 vol/vol) followed by centrifugation at 1500 × *g* for 5 min. The lipid-containing chloroform layer was isolated and evaporated to dryness. Triacylglycerol was measured spectrophotometrically using enzymatic assay kits from Wako Pure Chemical Industries.

Western blotting

The livers and skeletal muscles were homogenized in homogenizing buffer (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid [HEPES] 50 mmol/L, NaCl 150 mmol/L, dithiothreitol 1 mmol/L, and 0.5% (v/v) Tween-20; pH 7.4) containing protease inhibitor cocktail tablets (Roche Applied Science, Indianapolis, IN, USA). The homogenates were centrifuged at 5000 × *g* for 45 min at 4°C. Supernatants were collected, and the protein concentration was determined using a BCA™ protein assay kit (Pierce, Rockford, IL, USA). Proteins were resolved by 10% sodium dodecylsulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (GE Healthcare UK Ltd., Buckinghamshire, UK). The membranes were probed with rabbit AMPKα, phospho-AMPKα-Thr172 polyclonal antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA) or rabbit phospho-ACC-Ser79 (1:500; Millipore Corp., Billerica, MA, USA). The specifically bound primary antibodies were detected with horseradish peroxidase–conjugated secondary antibodies and enhanced with ECL western blotting detection reagents (GE Healthcare UK) on Hyperfilm (GE Healthcare UK). Quantitative analysis of the specific band density was performed using ATTO densitometry software (ATTO Corp., Tokyo, Japan). Protein levels were normalized to β-actin expression from the same sample and reported as fold values of the levels obtained for control rats.

Real-time polymerase chain reaction

Total RNA was extracted from the livers using an SV Total RNA Isolation System (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions, and cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The SREBP-1c mRNA levels were analyzed using the following primers and probes: 5′-GGAGCCATGGATTGCA CATT-3′ and 5′-AGGAAGGCTTCCAGAGAGGA-3′ with 5′-ACATGCTTCAGCTCA