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Original Research Article

Melinjo (*Gnetum gnemon*) extract intake during lactation stimulates hepatic AMP-activated protein kinase in offspring of excessive fructose-fed pregnant rats

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ABSTRACT

Excessive maternal fructose intake during pregnancy and in early postnatal life has metabolic consequences for the offspring. We investigated the effects of melinjo (*Gnetum gnemon*) extract (MeE) intake during lactation on the expression and phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) in the liver of offspring from excessive fructose-fed pregnant dams. Pregnant Wistar rats received a normal diet and 100 g/L fructose solution during gestation *ad libitum*. At delivery, dams were divided into two groups: a control diet (FC) or a 0.1% MeE-containing diet (FM) fed during lactation. The dams that were not treated with fructose were fed a control diet (CC). At postnatal week 3, some pups were sacrificed, while the remaining continued to receive a normal diet and were sacrificed at week 17. Blood chemistry and phosphorylation levels of AMPK and acetyl-coenzyme A carboxylase (ACC) were evaluated. Plasma glucose levels in FC female offspring increased compared to that receiving CC at weeks 3 and 17; however, the levels in FM female offspring decreased at week 17. The insulin levels in FM female offspring decreased significantly compared to that in FC female offspring at week 3. Hepatic AMPK phosphorylation was upregulated in FM offspring at week 3 and in female, but not male, offspring at week 17. ACC phosphorylation in FM female offspring was upregulated at week 17. Our results suggest that maternal MeE intake during lactation may modulate the hepatic AMPK pathways in female offspring.

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1. Introduction

Fructose, a simple monosaccharide, is used as a sweetener in food and drinks. An increase in sugar consumption, particularly of fructose, is paralleled by an increased risk of type 2 diabetes and obesity worldwide [1]. In animals, fructose overload produces increased oxidative stress, insulin resistance, hyperinsulinaemia, hypertriglyceridaemia, impaired glucose tolerance, and increased blood pressure [2–4]. In addition, maternal dietary fructose ingestion during gestation and lactation has metabolic consequences for the offspring [5]. Maternal dietary fructose ingestion during both gestation and lactation caused hyperinsulinaemia and changes in glucose metabolism in rat dams and in their offspring [6–8]. Maternal high fat and fructose feeding during prenatal and postnatal development increases blood glucose levels and glucose intolerance in offspring [9].

Melinjo (*Gnetum gnemon*) is a widely cultivated plant in Southeast Asia and is a common ingredient in Indonesian food. Ethanol extracts from the endosperm of melinjo contains various stilbenoids including *trans*-resveratrol, gnetin C, gnetin L, gnemonoside A, gnemonoside C, and gnemonoside D [10]. For instance, the extract of melinjo seeds contained 5.26 μmol/g, which was equivalent to approximately 1200 mg/kg, of *trans*-resveratrol [11]. In contrast, the content of *trans*-resveratrol in grape berries such as Chardonnay and Saint Laurent was between 0.3 and 2.3 mg/kg [12], and a lyophilized grape powder contained 7 μmol/kg *trans*-resveratrol [13]. Moreover, the concentration of *trans*-resveratrol obtained from the grape juice of the cultivars was 0.62 mg/L [14]. Recent evidence suggests that melinjo and its constituents may have beneficial effects in certain pathological conditions. For example, the resveratrol derivative-rich melinjo seed extract was reported to suppress angiogenesis-related endothelial cell dysfunction and tumour angiogenesis in vitro [15]. In animal experiments, melinjo resveratrol intake relieved alveolar bone resorption, activated sirtuin 1/adenosine monophosphate (AMP)-activated protein kinase (AMPK) in inflamed gingival tissues, and improved systemic oxidative stress [16]. In a human randomized controlled study, melinjo seed extract decreased serum uric acid level and improved lipid metabolism by increasing HDL cholesterol levels [17]. In addition, *trans*-resveratrol is beneficial in the management of patients with type 2 diabetes [18].

AMPK plays a major role in regulating glucose and lipid metabolism in various tissues and organs, including the liver and skeletal muscle [19]. For example, hepatic AMPK leads to the stimulation of fatty acid oxidation and inhibition of lipogenesis [20]. When activated, AMPK phosphorylates acetyl-coenzyme A carboxylase (ACC), rendering it inactive and increasing fatty acid oxidation. In addition, hepatic AMPK inactivation increases the expression of gluconeogenic genes and endogenous glucose production, resulting in hyperglycaemia [21,22]. In recent years, natural polyphenolic compounds such as resveratrol, epigallocatechin gallate, and quercetin have been reported to be involved in the modulation of AMPK, thereby mimicking the beneficial effects of exercise or caloric restriction on metabolic diseases [23,24]. Little is known about the effects of melinjo extract (MeE) on the AMPK pathway or on the glucose metabolism of offspring exposed to excessive fructose.

The aim of this study was to investigate whether MeE intake during lactation could modulate the expression and activity of AMPK in the liver of young and adult offspring from excessive fructose-fed pregnant dams. In addition, whether or not MeE intake affected glucose metabolism via AMPK activation was assessed.

2. Material and methods

2.1. Melinjo extract

Endosperm of melinjo seeds was collected in Bogor City, West Java province, Indonesia, in September 2012. The dried endosperm of melinjo was powdered and soaked in 50% (v/v) ethanol at room temperature for 1 h. After centrifugation at 2000 × *g* for 20 min at room temperature, the supernatant was concentrated with a rotary evaporator and then freeze-dried to obtain MeE. The total polyphenol content in the MeE, as determined by the Folin-Ciocalteu method [25], was 9.3 g of (+)-catechin hydrate equivalent per 100 g of MeE.

2.2. Animals and experimental procedure

This study was approved by the Animal Research Committee, Aomori University of Health and Welfare, and all experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Aomori University of Health and Welfare (Permission number; 12006). Seven-week-old virgin female and male Wistar rats were obtained from Clea Japan (Tokyo, Japan). Rats were maintained at a constant temperature of 23 ± 1 °C under a 12-h light-dark cycle with ad libitum access to a standard commercial laboratory normal diet (MF diet; supplied by Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. At 12–13 weeks of age, the oestrous stage of female rats was determined using a vaginal impedance reader (Model MK-10C; Muromachi Kikai, Osaka, Japan). This procedure was routinely performed in the afternoon and a reading of >3kΩ indicated that the females were in pro-oestrous and presumably in oestrous. One female rat was mated overnight with one male rat. The next morning, the presence of a vaginal plug indicated successful mating and was recorded as Day 0 of gestation. Pregnant rats were randomly divided into two groups: untreated ($n = 4$) or fructose treated ($n = 7$). The fructose group received 100 g/L of D(−)-fructose solution (Wako Pure Chemical Industries, Osaka, Japan) via drinking water during gestation. After the pups were born, the drinking water of the fructose group was replaced with normal water. Dams, weighing 245–282 g, treated with fructose during gestation were randomly divided into two groups: a control diet (FC) or a 0.1% MeE-containing diet (FM) fed during lactation. Fructose-untreated dams were fed a control diet (CC) during gestation and lactation. The diets were isocaloric, and the composition of the melinjo extract-containing diet was as follows: casein; 20,000 g/100 g diet, L-cystine; 0.300 g/100 g, cornsrarch; 39,749 g/100 g, α-cornstarch; 13,200 g/100 g, sucrose; 10,000 g/100 g, soybean oil; 7000 g/100 g, cellulose; 4900 g/100 g, mineral