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A diet containing grape powder ameliorates the cognitive decline in aged rats with a long-term high-fructose-high-fat dietary pattern☆

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Abstract

Research has suggested that the consumption of foods rich in polyphenols is beneficial to the cognitive functions of the elderly. We investigated the effects of grape consumption on spatial learning, memory performance and neurodegeneration-related protein expression in aged rats fed a high-fructose-high-fat (HFHF) diet. Six-week-old Wistar rats were fed an HFHF diet to 66 weeks of age to establish a model of an HFHF dietary pattern, before receiving intervention diets containing different amounts of grape powder for another 12 weeks in the second part of the experiment. Spatial learning, memory performance and cortical and hippocampal protein expression levels were assessed. After consuming the HFHF diet for a year, results showed that the rats fed a high grape powder-containing diet had significantly better spatial learning and memory performance, lower expression of β-amyloid and β-secretase and higher expression of α-secretase than the rats fed a low grape powder-containing diet. Therefore, long-term consumption of an HFHF diet caused a decline in cognitive functions and increased the risk factors for neurodegeneration, which could subsequently be ameliorated by the consumption of a polyphenol-rich diet.

Keywords: Polyphenols; Amyloid beta; Beta-secretase; Neurodegenerations; Grape; Cognitive functions

1. Introduction

Neurodegeneration (*e.g.*, dementia) is a serious problem in senior populations. Alzheimer's disease (AD) is the most common form of dementia and is categorized by a continual decline in cognitive and memory functions [1]. The prevalence of AD is approximately 1% in the population of 50–70 year olds and increases to 50% in those age >70 years [2]. The major pathological characteristics of AD are senile plaques (SPs), neurofibrillary tangles [3] and the accumulation of β-amyloid (Aβ) in the brain [1]. Aβ is derived from sequential cleavage of the amyloid precursor protein (APP) by the β-site APP cleaving enzyme (BACE). The BACE cleaves the ectodomain of APP, producing an insoluble APP C-terminal fragment [4]. However, if the APP is cleaved by disintegrin and metalloproteinase domain-containing protein 10 (ADAM 10, α-secretase), SPs are not formed [5].

Obesity and hyperglycemia are two of the risk factors of AD [6]. AD and diabetes are both age-associated diseases [7]. Diets high in fat and fructose have been indicated to cause obesity and diabetes [8,9]. Moreover, impaired glucose metabolism in the brain occurs in prediabetic and type 2 diabetes mellitus patients [10]. An impaired glucose metabolism may cause a reduction in the transport of insulin to the brain, which results in increased glucose autoxidation and oxidative stress in the brain [11]. Accordingly, glucose malmetabolism in the brain may be an early event that precedes the initial stage of cognitive impairment in relation to neurodegeneration.

Diets rich in antioxidants have beneficial effects on neurodegenerative onset and symptoms [12]. Grapes are a low glycemic index (GI) fruit, with a GI value of 50 [13]. They are rich in antioxidative flavonoids, including catechins, quercetins, anthocyanidins, proanthocyanidins [14], phenolic acids and resveratrol [15]. Dietary polyphenols can cross the blood–brain barrier (BBB) and provide neuroprotective effects in the central nervous system [16]. In this study, we investigated the ameliorative effects of dietary intervention on the early stage of neurodegeneration caused by a high-fructose-high-fat (HFHF) dietary pattern. We first fed rats an HFHF diet to establish an animal model of long-term consumption of a metabolic syndrome-causing dietary pattern. We then investigated the effects of flavonoid-rich grape (*Vitis vinifera*) consumption on cognitive functions using aged rats that had been consuming an HFHF diet for over a year.

2. Materials and methods

2.1. Preparation of grape powder

A single batch of fresh mature grapes (*V. vinifera*) was purchased from a traditional market in Taipei, Taiwan. The grapes (whole) were carefully washed and blended to juice them. The grape juice was diluted threefold to freeze dry with ice from deionized

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water and then packed in bags. The diluted grape juice was lyophilized and ground into grape powder.

2.2. Extraction of polyphenols

Polyphenols in the grape powder were extracted using a modified ultrasound-assisted method [17]. In brief, a mixture of 15 g of lyophilized grape powder and 80 ml of 80% aqueous methanol with 0.01% (v/v) HCl was sonicated for 50 min and then centrifuged (4000*g*, 25°C, 5 min). Extraction of the residues was repeated using the same conditions until the solution was colorless. The solvents were removed using a rotary evaporator at 40°C. The remaining phenolic concentrate was dissolved in 10 ml of 80% aqueous methanol with 0.01% (v/v) HCl and then stored at −30°C until it was analyzed.

2.3. Determination of total polyphenols

Total polyphenols in the grape powder were determined using a modified microplate-adapted colorimetric method with the Folin–Ciocalteu reagent [18]. In brief, 100 μl of each diluted extract, a gallic acid standard solution (0–0.8 mg/ml) and a blank were added to 1.5-ml microtubes. The Folin–Ciocalteu phenol reagent at 200 μl (10%, v/v) was added to each mixture and thoroughly mixed. Subsequently, 800 μl of 700 mM Na_2CO_3 was added to each tube, mixed and incubated at room temperature for 2 h. The absorbance was read against a prepared blank in a 96-well microplate at 765 nm. Total polyphenols in the grape powder are expressed as milligrams of gallic acid equivalents (GAE) per gram of grape powder and per 100 g of the fresh edible portion.

2.4. Analysis of polyphenolic compounds in grape powder

Several polyphenols in the grape powder were modified using reversed-phase high-performance liquid chromatography (HPLC) [19]. In brief, 4 mg of precisely weighed gallic acid (Sigma, St. Louis, MO, USA), catechin, procyanidin B2 (Sigma), chlorogenic acid (Sigma), (−)-epigallocatechin (Sigma), caffeic acid (Sigma), syringic acid (Sigma), *p*-coumaric acid (Sigma), ferulic acid (Sigma), rutin (Sigma), resveratrol (Sigma), malvidin (Sigma), quercetin (Wako, Osaka, Japan) and kaempferol (Sigma) were dissolved in 80% aqueous methanol with 0.01% (v/v) HCl and quantified to 1 ml to a concentration of 4000.0 μg/ml as the stock standard solution. This solution was stored at −30°C before further analysis. All stock standard solutions were diluted with 80% aqueous methanol with 0.01% (v/v) HCl in serial concentrations. The grape powder extract and the standard solution were filtered through a syringe filter (0.45 μm) and injected directly into the HPLC apparatus.

The apparatus and conditions of HPLC analysis were as follows: a C18 reversed-phase column (Luna 5 μm C18 250 mm×4.6 mm; Phenomenex, Torrance, CA, USA), a temperature of 30°C, a flow rate of 1.0 ml/min, an analytical volume of 20 μl, a wavelength of 280 nm and an analytical system (Thermo Fisher Scientific, Waltham, MA, USA) composed of a pump (P-1000), a detector (UV-6000LP detector, Spectra-System) and software (Chrom Quest 4.X). The mobile phase of the gradient mobile system is illustrated as follows: solvent A, 0.5% aqueous phosphoric acid; solvent B, methanol; from 0 min to 52 min, A: 85% to 5%.

2.5. Animals

A total of 60 six-week-old male Wistar rats (180–200 g) were obtained from BioLASCO Taiwan (Taipei, Taiwan). All animal experiments were conducted according to the ethical guidelines outlined in the *Guide for Care and Use of Laboratory Animals*. The animal facilities and protocol were approved by the Laboratory Animal Care and Use Committee at Taipei Medical University (Taipei, Taiwan). The rats were housed in individual cages in a room maintained at 23±2°C and 50–60% relative humidity on a 12:12 h light–dark cycle. Food and water were available *ad libitum*, and the rats were starved overnight before the experiments.

2.6. Diets

As shown in Table 1, the diet (20 g/rat/day) was isocalorically prepared every 6–7 days by adding each macronutrient and micronutrient into the diet as modified from previous studies [20,21]. The control group received the AIN-93M diet containing 76%, 15% and 9% calories from carbohydrates, protein and fat, respectively. The HFHF group received the AIN-93M-based HFHF diet in which total carbohydrates (*i.e.*, corn starch, sucrose and cellulose) were replaced by 61.5% (w/w) fructose and 16% (w/w) soybean oil to obtain a final concentration of 50% and 38% calories from carbohydrate and fat, respectively. The MT group received the HFHF diet supplemented with 0.05% metformin. The LGP and HGP groups received the HFHF diet supplemented with 3% (w/w) and 6% (w/w) grape powder, respectively. The amounts of fructose in the HFHF diet were adjusted accordingly when the grape powder was added for preparing the LGP and HGP diets. The grape powder was mixed into the diet immediately before feeding.

Table 1
Ingredients of the diets (%) [a]

Group	C group	HFHF group	LGP group	HGP group	MT group
Ingredient	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)
Fructose [b]		61.1	59.5	58.0	61.1
Corn starch	46.6				
Dextrin	15.5				
Sucrose	10				
Cellulose	5				
Casein	14	14	14	14	14
Soybean oil	4	20	20	20	20
Minerals [c]	3.5	3.5	3.5	3.5	3.5
Vitamins [d]	1	1	1	1	1
Choline	0.25	0.25	0.25	0.25	0.25
Cysteine	0.18	0.18	0.18	0.18	0.18
TBHQ	0.0008	0.0008	0.0008	0.0008	0.0008
Grape powder			3.0	6.0	
Metformin [e]					0.05
Energy (%)					
Carbohydrate	76	50	50	50	50
Protein	15	12	12	12	12
Fat	9	38	38	38	38

C, control; MT, metformin; LGP, low-dose grape powder; HGP, high-dose grape powder; TBHQ, *tert*-butylhydroquinone. The diets were formulated based on the AIN-93M diet.

[a] The amount of food provided for the rats was 20 g/rat/day.

[b] The amount of fructose was adjusted accordingly when the grape powder was added.

[c] AIN-93M-MX.

[d] AIN-93-VX.

[e] Metformin hydrochloride.

2.7. Experimental procedures

We first fed the rats an HFHF diet for 52 weeks to establish an animal model of long-term consumption of a metabolic syndrome-causing dietary pattern. The body weight and food intake of the rats were monitored weekly. After 2 weeks of adaptation, the rats were randomly divided into two groups: a control group ($n=10$) and an HFHF group ($n=50$). They were given the experimental diets until they were 52 weeks old. At 52 weeks old, 10 rats randomly chosen from the HFHF group and the control group ($n=10$) were given spatial learning and memory performance examinations using the Morris water maze (MWM) test. After the MWM test, the rats were anesthetically (intraperitoneal injection, 0.1 ml/kg body weight) euthanized with the mixture (1:1) of Zoletil 50 (containing 25 mg/ml Tiletamine and Zolazepam, Virbac, France) and Rompun (2% in water, containing 20 mg/ml Xylazine, Bayer, Germany), and their cortical and hippocampal tissues were obtained.

The remaining 40 rats in the HFHF group were kept and fed the HFHF diet until they were 66 weeks old, and the intervention diets were then initiated. They were randomly divided into four groups: an HFHF group ($n=10$), an HFHF + 0.05% metformin group (MT, $n=10$), an HFHF + 3% low-dose grape powder group (LGP, $n=10$) and an HFHF + 6% high-dose grape powder group (HGP, $n=10$). They were given the intervention diets until they were 78 weeks old. At 78 weeks of age, spatial learning and memory performance were evaluated by the MWM test. After the MWM test, the rats were euthanized, and their cortical and hippocampal tissues were obtained. Blood samples were drawn from the tail vein at 0, 52 and 78 weeks after overnight starvation, and samples were immediately centrifuged (4000*g*, 4°C). The serum was separated and frozen at −30°C until it was analyzed. The study schedule is shown in Fig. 1.

2.7.1. MWM task

The spatial learning and memory performance of the rats were assessed by the modified MWM task [22] at ages 52 and 78 weeks. The apparatus (TSE Systems, Bad Homburg vor der Höhe, Germany) consisted of a circular pool (150 cm in diameter, 100 cm high) filled with water (to a depth of 60 cm at 24±2°C), which was placed in a room with consistently located spatial cues. An escape platform (14 cm in diameter) was placed in the middle of one of the quadrants at 1–2 cm below the water surface, equidistant from the side wall and the middle of the pool. The platform provided the only escape from the water and was located in the same quadrant in every trial. Four different starting positions were equally spaced around the perimeter of the pool. On each training day of the acquisition trial, all four starting positions were used once in a random sequence (*i.e.*, four training trials per day). A trial began by placing an animal in the water facing the wall of the pool at one of the starting points. If the animal failed to escape within 120 s, it was gently moved to the platform by the experimenter. The rat was allowed to stay there for 15 s. The intertrial interval was 15 min. After each trial, the rat was dried and returned to its cage at the end of the session. The animals were trained for 3 days. At 24 h after the last training session, the rats were given a probe trial. Before this session, the submerged platform was removed. The retention test consisted of