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β-Cyclodextrin Reduces Obesity in C57BL/6J Mice Induced by High Fat Diet

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Abstract Anti-obesity effects of β-cyclodextrin in obese C57BL/6J mice induced by a high fat diet (HD) were observed. The administration of β-cyclodextrin reduced the gain of body weight, abdominal fat, liver weight, the lipid deposits of hepatocytes and the size of adipocytes in the HD group. In serum analysis, the total and low-density lipoprotein-cholesterols were significantly decreased in the β-cyclodextrin-supplemented HD group than in the HD group. However, high-density lipoprotein-cholesterol was not changed in these groups. In hypothalamic homogenates, the decrease of neuropeptide Y and increase of α-melanocyte stimulating hormone were detected in the β-cyclodextrin-supplemented HD group compared to that in the HD group. These effects of β-cyclodextrin were similar to those of *Garcinia cambogia*, which is widely used as a natural anti-obesity product. These results suggest that β-cyclodextrin has anti-obesity effects through the lowering of the abdominal fat pad and inhibits the central effects of hunger.

Keywords: β-cyclodextrin, C57BL/6J mice, leptin, cholesterol, body weight

Introduction

Obesity is a complex condition, one with serious social and psychological dimensions, that affects virtually all age and socioeconomic groups and threatens to overwhelm both developed and developing countries. There are many chronic health conditions associated with obesity, including type II diabetes, cardiovascular disease, and hypertension (1). Obesity, defined by body mass index (BMI) greater than 30 kg/m^2, has become a worldwide epidemic. It is estimated that, in the United States, it is a continuously growing epidemic, and that increases in body weight are continuing in men and in children (2). Obesity is the result of a positive energy balance; a sedentary lifestyle and/or over-consumption of food contribute to this positive energy balance. In this respect, the C57BL/6J mouse strain provides an excellent model with which to study the evaluation and assessment of anti-obesity substances because of its susceptibility to obesity and related disorders that are highly analogous to those conditions in humans (3,4).

Recently, there has been considerable concern regarding the dietary fibers and crude extracts that help to control body weight (5-8). Among these dietary fibers, natural cyclodextrins are produced from starch through the action of cyclodextrin glycosyltransferase, an enzyme produced by several organisms, including *Bacillus macerans*. β-Cyclodextrin is a cyclic heptamer that is composed of 7 glucose units held together by α-1,4 links via an enzymatic modification of starch that increases the aqueous solubility of a variety of compounds. Because the interior cavity is hydrophobic and the outside of the molecule is hydrophilic, it easily forms inclusion complexes with hydrophobic molecules such as steroids, cholesterol, and vitamins (9).

Although many studies have shown that β-cyclodextrin has high affinity to bind cholesterol and lower cholesterol levels, few studies have reported comprehensive studies of β-cyclodextrin in the peripheral organs and brain. Therefore, in this study, the anti-obesity effect of β-cyclodextrin in plasma, liver, fat, and brain induced by a high-fat diet in C57BL/6J mice was investigatied.

Materials and Methods

Animals and diets Twenty-one male C57BL/6J mice (7-week-old age) were purchased from Jackson Laboratory Co., Ltd. (Bar Harbor, ME, USA). They were housed in a conventional state under adequate temperature (23°C) and humidity (60%) control with a 12-hr light/12-hr dark cycle, and free access to food and water. The animals were adapted to a chow diet for 1 week, and then divided into 3 groups of 7 each using a randomized block design; a high fat diet (HD) group for negative control, β-cyclodextrin (InterHealth, Benicia, CA, USA; 3.3%, w/w, instead of cellulose)-supplemented high fat diet (CD-HD) group, and *Garcinia cambogia* (InterHealth, London, UK, 3.3%, w/w, instead of cellulose)-supplemented high fat diet (GC-HD) group for positive control. Each group was fed its respective

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Table 1. Composition of high fat diets (HFD) for each group

Formula	AIN-93G		HFD 45% kcal	
	g%	kcal%	g%	kcal%
Protein	20	20	24	20
Carbohydrate	64	64	40	35
Fat	7	16	24	45
Total		100		100
kcal/g	4.0		4.8	
Ingredient	g	kcal	g	kcal
Casein, lactic	200	800	200	800
L-Cystine	3	12	3	12
Corn starch	397.486	1,590	165.9	664
Sucrose	100	400	73.5	294
Dextrose	132	528	97.6	390
Cellulose	50	0	50	0
Soybean oil	70	630	70	630
Lard	0	0	130	1170
t-Butylhydroquinone	0.014	0	0.014	0
AIN-93G Mineral mix	35	0	35	0
AIN-93 Vitamin mix	10	40	10	40
Choline bitartrate	2.5	0	2.5	0
Total	1,000	4,000	837.45	4,000

AIN-93 modified HD (Hanlive R&D, Korea) for 14 weeks (Table 1). The body weight in each group was measured at 09:00 twice a week. The procedures for handling and caring for the animals adhered to the guidelines that are in compliance with the current international laws and policies (10). All of the experiments were conducted to minimize the number of animals used and the suffering caused by the procedures used in the present study.

Blood and tissue sampling For the blood and tissue sampling, 7 animals in each group were anesthetized with sodium pentobarbital and the blood samples were collected from each mouse by cardiac puncture. Serum was separated from the blood by centrifugation at 1,100×g for 15 min at 4℃ and kept at −80℃ until analyzed. The abdominal fat and liver were removed, quickly weighed, and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4).

Analysis of lipids and leptin The total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels were measured with Konelab 20XT (Thermo Fisher Scientific, Waltham, MA, USA). Serum leptin levels were determined by radioimmunoassay (RIA). The serum leptin was measured using a mouse leptin RIA kit from Linco Research (St. Charles, MO, USA). Radioactivity of the samples was determined in a gamma scintillation counter.

Tissue processing for histology The fat and liver were dehydrated by immersing for 2 hr in 50, 70, 80, 90, 95, and 100% ethanol baths in succession at room temperature. Then, the tissues were placed 2 times, each time for 1 hr, in fresh pure xylene and then in molds containing melted paraffin (Histowax; Leica, Wetzlar, Germany). Sections of 7 μm thickness were cut, and the sections were mounted on gelatin-coated microscopy slides. Hematoxylin and Eosin (H&E) staining was conducted with commonly used method. After dehydration the sections were mounted with Canada balsam (Kanto, Tokyo, Japan).

Western blot analysis To confirm changes in neuropeptide Y (NPY) or alpha-melanocyte stimulating hormone (α-MSH) levels in the paraventricular nucleus of each groups, 7 animals used in the blood sampling were sacrificed and used for Wstern blot analysis. After sacrificing them and removing the brain, it was serially and transversely cut into a thickness of 400 μm on a vibratome (Leica), and the paraventricular nucleus was then dissected with a surgical blade. The tissues were homogenized in 50 mM phosphate buffer saline (PBS, pH 7.4) containing 0.1 mM ethylene glycol bis (2-aminoethyl ether)-*N,N,N',N'* tetraacetic acid (EGTA) (pH 8.0), 0.2% Nonidet P-40, 10 mM ethylendiamine tetraacetic acid (EDTA) (pH 8.0), 15 mM sodium pyrophosphate, 100 mM β-glycerophosphate, 50 mM NaF, 150 mM NaCl, 2 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mM dithiothreitol (DTT). After centrifugation, protein levels were determined in the supernatants using a Micro BCA protein assay kit with bovine serum albumin as the standard (Pierce Chemical, Rockford, IL, USA). Aliquots containing 20 μg of total protein were boiled in loading buffer containing 150 mM Tris (pH 6.8), 3 mM DTT, 6% sodium dodecylsulfate (SDS), 0.3% bromophenol blue, and 30% glycerol. The aliquots were then loaded onto a 7.5% polyacrylamide gel. After electrophoresis, the gels were transferred to nitrocellulose transfer membranes (Pall Crop, East Hills, NY, USA). To reduce background staining, the membranes were incubated with 5% non-fat dry milk in PBS containing 0.1% Tween 20 for 45 min, followed by incubation with rabbit anti-NPY (1:1,000, Chemicon, Temecula, CA, USA) or rabbit anti-α-MSH (1:2,000, Chemicon), peroxidase-conjugated horse anti-rabbit IgG (Sigma-Aldrich, St. Louis, MO, USA) and an ECL kit (Pierce Chemical).

Quantification of data and statistical analysis All measurements were performed in order to ensure objectivity in blind conditions, by 2 observers for each experiment, carrying out the measures of experimental samples under the same conditions.

The result of the Western blot analysis was scanned, and the quantification of the Western blotting was done using Scion Image software (Scion Corp., Frederick, MD, USA), which was used to count the optical density.

To elucidate the effects of β-cyclodextrin and *G. cambogia* against the high fat diet-induced increase of adipocytes, the measurement of adipocytes size was performed using an image analyzing system equipped (software: Optimas 6.5, CyberMetrics, Scottsdale, AZ, USA). The size of H&E stained adipocytes was measured in a 250×250 μm of abdominal fat tissue in 20 sections per animal. The size of H & E stained adipocytes was compared to that of the HD group.

The data shown here represent the means of experiments performed for each experimental area. Differences among the means were statistically analyzed by 2-tailed Student's

t-test using SPSS 10.0 program in order to elucidate differences between HD and CD-HD/GC-HD groups. Statistical significance was considered at $p<0.05$.

Results and Discussion

In this study, feeding consumptions of the groups did not significantly differ. However, weight gains were found to be significantly decreased in the CD-HD and GC-HD groups compared to that in the HD group, respectively (Fig. 1). The weight gains were reduced by 47.9 and 33.9% in the CD-HD and GC-HD groups compared to that in the HD group, respectively. In our knowledge, we first observed the lowering effects of β-cyclodextrin on body weight. In this study, we used the *G. cambogia* for the positive control because it has been generally accepted that this material has anti-obesity effects in the HD group (11,12).

Next, we investigated the effect of β-cyclodextrin or *G. cambogia* on serum lipid levels. The total and LDL cholesterol levels were significantly decreased in the CD-HD and GC-HD groups compared to those in the HD group. However, the HDL cholesterol levels were not significantly different between HD and CD-HD/GC-HD groups (Fig. 2). The serum leptin level in the HD group was 29.7 ng/mL. The administration of β-cyclodextrin or *G. cambogia* resulted in a significant reduction of serum leptin concentrations, 69.4 or 65.7% compared to the HD group, respectively (Fig. 2).

The abdominal fat pad weight in the HD group was higher than in the CD-HD and GC-HD groups. The effects of β-cyclodextrin or *G. cambogia* administration on abdominal fat pad weight corresponded with change in weight gain in

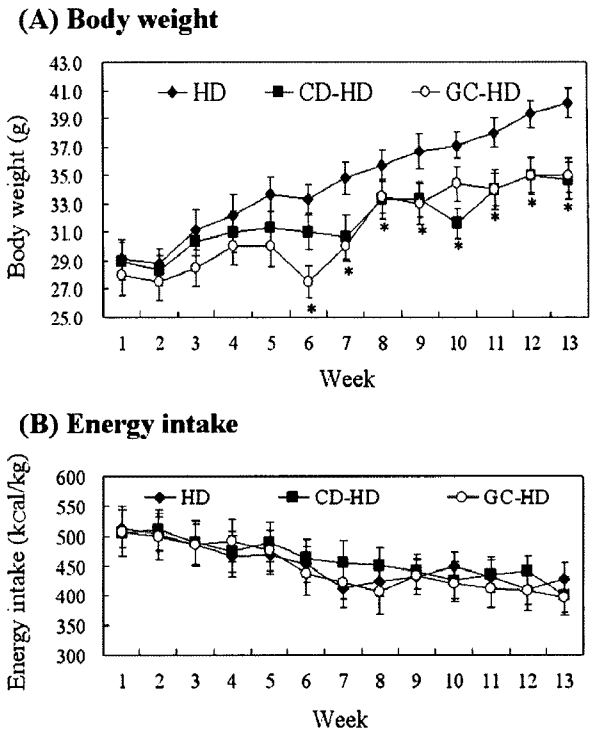

Fig. 1. Effects of β-cyclodextrin and *Garcinia cambogia* administration on body weight (A) and energy intake (B) in high fat diet-fed C57BL/6J mice. Differences among the means are statistically analyzed by 2-tailed Student's *t*-test ($n=7$ per group; $*p<0.05$, significantly different from the high fat diet (HD) group). CD-HD, β-cyclodextrin-supplemented high fat diet; GC-HD, *G. cambogia*-supplemented high fat diet. Bars indicate means±SEM.

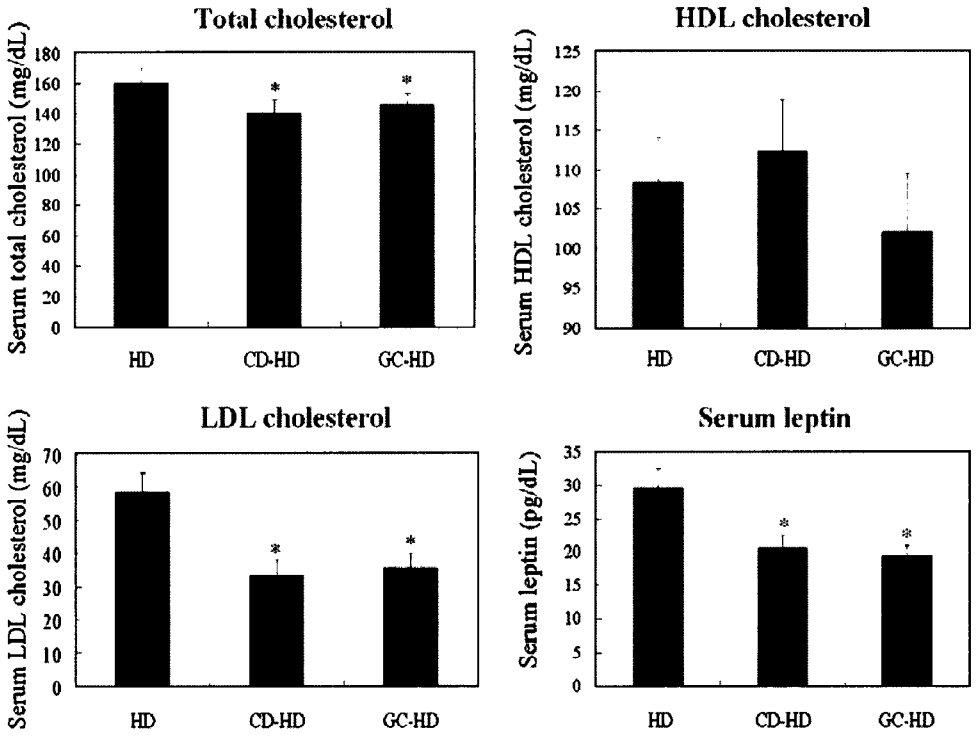

Fig. 2. Effects of β-cyclodextrin and *Garcinia cambogia* administration on cholesterols and leptin levels in high fat diet-fed C57BL/6J mice. Differences among the means are statistically analyzed by 2-tailed Student's *t*-test ($n=7$ per group; $*p<0.05$, significantly different from the high fat diet (HD) group). CD-HD, β-cyclodextrin-supplemented high fat diet; GC-HD, *G. cambogia*-supplemented high fat diet. Bars indicate means±SEM.

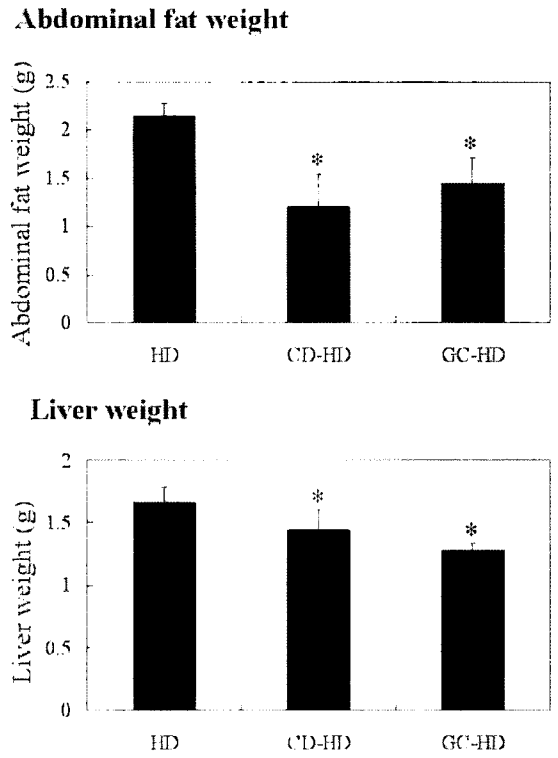

Fig. 3. Effects of β-cyclodextrin and *Garcinia cambogia* administration on abdominal fat pad weight and liver weight in high fat diet-fed C57BL/6J mice. Differences among the means are statistically analyzed by 2-tailed Student's *t*-test (n=7 per group; *p<0.05, significantly different from the high fat diet (HD) group). CD-HD, β-cyclodextrin-supplemented high fat diet; GC-HD, *G. cambogia*-supplemented high fat diet. Bars indicate means±SEM.

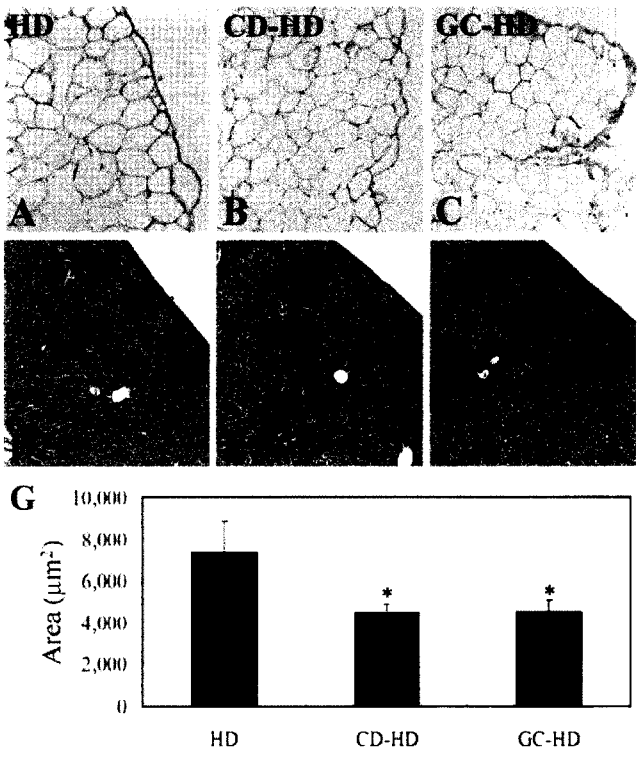

Fig. 4. Effects of β-cyclodextrin and *Garcinia cambogia* administration indicated by microscopic findings using H & E staining on adipocytes (A-C) and hepatocytes (D-F) and area analysis using Optimas software on adipocytes (G) in high fat diet-fed C57BL/6J mice. The administration of high fat diet (HD) increases the size of adipocytes (A) and hepatocytes (D). β-Cyclodextrin and *G. cambogia* reduce the size of adipocytes (B, C, and G) and the presence of lipid droplets in the liver (E and F). CD-HD, β-cyclodextrin-supplemented high fat diet; GC-HD, *G. cambogia*-supplemented high fat diet. Bar=100 μm.

the HD group (Fig. 3). The increase of liver weight in the HD group is in good agreement with previous study (13). However, in the CD-HD and GC-HD groups, the liver weight was decreased compared to that in the HD group.

We conducted H & E staining to identify morphological change in the fat and liver with the administration of β-cyclodextrin or *G. cambogia*. The sizes of adiopocytes were significantly decreased by 39.5 and 39.7% in the CD-HD and GC-HD groups compared to that in the HD group, respectively (Fig. 4A-C and 4G). In the HD group, lipid deposition was detected in the hepatocytes. However, the administration of β-cyclodextrin or *G. cambogia* significantly reduced the deposition of lipids in the liver (Fig. 4D-F).

This study was supported by a previous study which indicated that β-cyclodextrin accelerated body cholesterol turnover by reducing cholesterol absorption (14) and increasing cholesterol synthesis; suggested mechanisms for the hypolipidemic effect of β-cyclodextrin include the greater excretion of fecal bile acids and up-regulation of bile acid biosynthesis (15-18).

In this study, we also observed the central role of β-cyclodextrin by measuring the NPY, and α-MSH levels in hypothalamus homogenates, because leptins exert their anorexigenic actions, in part, by suppressing NPY and increasing α-MSH (19,20). In addition, we found that leptin stimulated α-MSH secretion and inhibited NPY/AgRP secretion. In contrast, leptin failed to modulate the secretion of melanocortin peptides in obese mice (21). The protein levels of NPY were significantly decreased in the CD-HD and GC-HD groups compared to that in the HD group; whereas α-MSH levels were increased in the CD-HD and GC-HD groups (Fig. 5). In the present study, the energy intake (kcal/kg) was not changed among the groups. However, the total energy intake per animals was significantly high in the CD-HD and GC-HD group compared to that in the CD group. This result supported by the previous study that the ablation of NPY attenuated the hyperphagic and thermoregulatory responses to fasting and diet-induced obesity in C57BL/6J mice (22). In addition, in our previous study, we observed that NPY immunoreactivity was higher in obese rats than in lean Zucker diabetic fat rats in both males and females in a type II diabetic stage (23).

In conclusion, we observed that β-cyclodextrin had anti-obesity and anti-cholesterol effects in the HD C57BL/6J mice. The reduction of NPY and increase of α-MSH in the CD-HD group compared to that in the HD group may be associated with the anti-obesity effect of β-cyclodextrin. The potency of β-cyclodextrin in relation to the anti-obesity effects is similar to that of *G. cambogia*, which is a natural substance used to suppress appetite and enhance fat-burning.

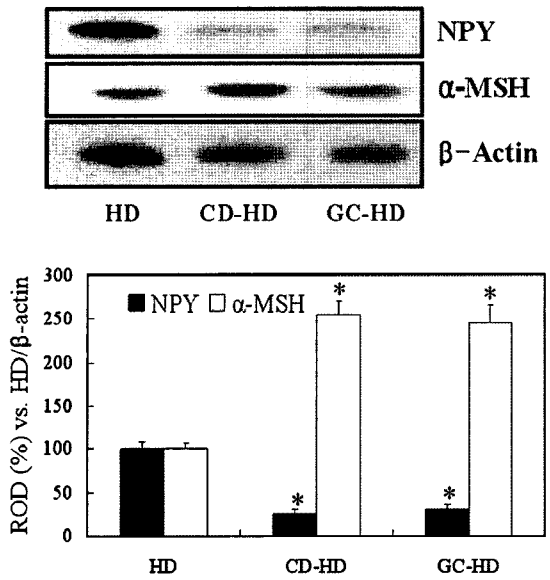

Fig. 5. Western blot analysis of NPY and α-MSH in the hypothalamus of high fat diet-fed C57BL/6J mice to elucidate the effects of β-cyclodextrin and *Garcinia cambogia* administration. Differences among the means are statistically analyzed by 2-tailed Student's *t*-test (n=7 per group; *p<0.05, significantly different from the high fat diet (HD) group). CD-HD, β-cyclodextrin-supplemented high fat diet; GC-HD, *G. cambogia*-supplemented high fat diet. Bars indicate means±SEM.

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