〈Brief Note〉

Effect of *Corchorus olitorius* on glucose metabolism, lipid metabolism, and bone strength in a rat model of obesity with hyperphagia

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Summary The aim of the present study was to examine the effect of the regular consumption of *Corchorus olitorius* powder on glucose metabolism, lipid metabolism, and bone strength in a rat model of obesity with hyperphagia. Otsuka Long-Evans Tokushima Fatty (OLETF) rats were used as model of obesity with hyperphagia, and Long-Evans Tokushima Otsuka (LETO) rats without obesity or hyperphagia were used as controls. At 20 weeks old, OLETF rats were divided into two groups: rats receiving a normal diet (O group, n = 8) and those with a diet supplemented with *C. olitorius* powder (O-C group, n = 8); all LETO rats were fed a normal diet (L group, n = 5). The experimental period continued until rats were 28 weeks old. Although OLETF rats reportedly have higher plasma glucose and higher serum insulin than that of LETO rats, no significant differences were found between the groups for these parameters. Plasma glucose and insulin in the O-C group trended lower than those in the O group, but without significance. Liver triglycerides in the O and O-C groups were significantly higher than those of the L group, but no difference arose between the O and O-C groups. In addition, no significant differences were found among the three groups in serum triglyceride, total serum cholesterol, total liver cholesterol, or total liver fat. In histopathology, no notable improvement was observed in pancreas and liver sections of O-C group rats. In addition, no significant differences were found in the length, breaking maximum load, breaking deformation, or breaking energy of femurs among the groups. Our research suggests that the regular consumption of *C. olitorius* powder was unable to improve abnormal glucose and lipid metabolism and low bone strength in obesity with hyperphagia.

Key words: Corchorus olitorius, Molokheiya, OLETF rats, Diabetes, Dyslipidemia

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1. Introduction

Obesity is risk factor of diabetes[1], dyslipidemia[2], and osteoporosis[3,4], all of which decrease quality of life and, in the worse cases, can lead to death. Thus, secondary Healthy Japan 21 aims to decrease the rate of obesity, diabetes, dyslipidemia, and osteoporosis among the elderly[5].

Consuming the leaves of the shrub *Corchorus olitorius* (molokheiya) may be expected to prevent to diabetes, dyslipidemia, or osteoporosis. *C. olitorius* shrub has long been recognized for its benefits and is still widely cultivated in Japan today[6]. Previous studies have reported that a single oral administration of the powder of *C. olitorius* prevented a rise in blood glucose as assessed by oral glucose tolerance test in humans[7]. Regular consumption of the powder or extract of *C. olitorius* improved hyperglycemia[8,9] and dyslipidemia[8-10] in a high-fat-fed or LDL receptor-deficient rodents. However, it is unclear whether the ability of *C. olitorius* to prevent hyperglycemia dyslipidemia persists in humans or rodents with hyperphagia. Additionally, *C. olitorius* may help to prevent osteoporosis caused by hyperglycemia because it also contains a high content of calcium and might have effect to prevent hyperglycemia.

The aim of the present study was to examine the effect of the regular consumption of *C. olitorius* powder on glucose metabolism, lipid metabolism, and bone strength in a rat model of obesity with hyperphagia. We hypothesized that *C. olitorius* would improve abnormal glucose and lipid metabolism and low bone strength.

2. Materials and Methods

Animals and experimental design

Long-Evans Tokushima Otsuka (LETO) rats were used as controls without obesity or hyperphagia, and Otsuka Long-Evans Tokushima Fatty (OLETF) rats were used as model of obesity with hyperphagia. The OLETF rat is a model of type 2 diabetes caused by obesity with hyperphagia that was selectively bred for null expression of the cholecystokinin-1 receptor. Noguchi et al. have reported that OLETF rats have higher serum glucose after five weeks of age and higher serum insulin, triglycerides, and cholesterol after eight weeks than that of LETO rats[11]. Hinton et al. have shown that OLETF rats have higher plasma glucose at 13 weeks of age and higher plasma insulin at 20 weeks old[12]. With reference to these previous studies, we decided to provide OLETF rats with *C. olitorius* after 20 weeks of age because there is a strong possibility that abnormal glucose and lipid metabolism appear in these rats at that age.

Male LETO rats (n = 8) and OLETF rats (n = 16) at four weeks old were purchased from Japan SLC (Shizuoka, Japan). At 20 weeks old, OLETF rats were divided into two groups: those receiving a normal diet (O group, n = 8) and those receiving a normal diet supplemented with *C. olitorius* (O-C group, n = 8). All LETO rats were fed a normal diet (L group, n = 5). The experimental period was 28 weeks old. The number of rats in the LETO group was reduced from 8 to 5 because it was determined that some of the LETO rats contained the OLETF gene from the vendor. Thus, the integrity of the L group became uncertain. Of the eight original LETO rats, only five were not obese; we therefore determined that these five LETO rats could act as controls without obesity or hyperphagia.

The CE-7 diet (containing 336 kcal energy, 8.6 g moisture, 18.1 g protein, 3.8 g fat, 5.8 g dietary fiber, 6.3 g ash (1.06 g calcium) in each 100 g of chow) as a normal diet was purchased from CLEA Japan (Tokyo, Japan). *C. olitorius* diet contained 97% CE-7 and 3% dry powder of *C. olitorius* (IGAMACHI MOROHEIYA producer's association, Mie, Japan). Analysis of the *C. olitorius* powder by Tsukuba Analysis Center Co., Ltd. (Ibaraki, Japan) revealed that the powder contained 351 kcal energy, 5.6 g moisture, 25.8 g protein, 4.5 g fat, 48.9 g carbohydrate (13.7 g soluble dietary fiber and 30.3 g insoluble dietary fiber) 12.2 g ash (1.8 g calcium), 1.33 mg vitamin A, and 13 mg vitamin C per 100 g.

All rats were individually housed in cages, and the room was maintained at 22 ± 2°C under a

constant 12:12 h light–dark cycle (light 9:00–21:00). Animal care and experimental procedures were approved by the ethics committee of Suzuka University of Medical Science. All experimental protocols were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals (approval number: 262).

Biochemical assay for glucose metabolism

Three days before the end of the study, blood samples were collected from the tail vein into heparinized capillary tubes from fasted rats (overnight 15 h). The samples were immediately transferred into microcentrifuge tubes on ice and centrifuged at 2500 rpm (4°C) for 10 min. Glucose concentration was measured using a spectrophotometric assay kit (LabAssay Glucose, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Insulin concentration was measured using an enzyme-linked immunosorbent assay kit (Rat insulin kit, Morinaga Institute of Biological Science, Inc., Yokohama, Japan).

Tissue collection

On the day prior to euthanasia and dissection, all rats fasted for 15 h. Whole blood samples were collected from the abdominal aorta using syringes under chloral hydrate anesthesia (40 mg/mL chloral hydrate in normal saline; dose, 0.1 mL/100 g body weight). Serum samples were separated by centrifugation at 2500 rpm for 10 min at 4°C. The abdominal fat, kidney, liver, pancreas, plantaris muscle, soleus muscle, gastrocnemius muscle, and femur were collected from each rat after euthanasia. Serum and a part of each liver were then stored at −80°C until biochemical assays were performed. Pancreases and another part of each liver were fixed in 10% formalin neutral buffer solution until histological assay. Femurs were collected, adhering connective tissues were removed, wet femur weight and size were measured, and bone mineral density (BMD) and bone strength were immediately measured.

Biochemical assay for lipid metabolism

Liver fat was measured using the Folch method[13]. One-gram liver samples were homogenized in 4 mL of saline. Fifteen milliliters of chloroform/methanol at a relative volume of 2:1 was added to the homogenates and stirred. The samples were filtered and centrifuged at 1000 rpm for 5 min. After removing the aqueous phase, 3 mL of the chloroform phase was collected and evaporated by drying. Three milliliters of the lower phase samples in the test tubes were dried in a hot water bath and weighed to calculate the total fat in the liver. The obtained fats were weighed to calculate the total fat in the liver and dissolved in isopropanol to measure the triglycerides and total cholesterol using spectrophotometric assay kits. Triglyceride and total cholesterol levels in the serum and liver were measured using spectrophotometric assay kits (LabAssay Cholesterol and LabAssay Triglyceride Wako, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

Measurement of BMD and bone strength

Dual-energy X-ray absorptiometry (DCS-600, ALOKA, Tokyo, Japan) was used to measure the bone mineral content (BMC), bone area, and BMD values of femurs as previously described[14]. A three-point bending test was used to assess the strength of the femoral mid-diaphysis as described[15]. Maximum load, deformation, and total energy were determined by the load-deformation curve. Total energy was the integral of the load until the breaking point.

Histological assay

Pancreases and livers were fixed in formalin buffer and embedded in paraffin wax. Two-micrometer vertical serial slices were prepared using a microtome (SM2010R, Leica Biosystems, Germany), and the sections were stained with hematoxylin and eosin (Muto Pure Chemicals, Tokyo, Japan).

Statistical analysis

All data are expressed as mean ± standard error (SE). Statistical analysis was conducted using one-way analysis of variance with Tukey's post-hoc comparison test to determine specific differences.

The significance level was set at $p < 0.05$. The SPSS Statistical Package (Ver. 25; IBM Inc., Chicago, IL, USA) was used to perform all statistical analyses.

3. Results

Food intake, body weight, internal organ weight, and muscle weight

The food intake, body weight, abdominal fat weight, and kidney weight in the O and O-C groups were significantly higher than those in the L group, whereas these parameters were not significantly different between the O and O-C groups (Table 1). The liver weight in the O group was significantly higher than that in the L group, but that in O-C group was not significantly different from that in the L or O groups. There were no significant differences in plantaris weight, soleus weight, or gastrocnemius weight among the groups.

Effect of oral consumption of *C. olitorius* on glucose metabolism

OLETF rats have higher plasma glucose and higher serum insulin than LETO rats, but we found no significant differences in this study (Fig. 1). The plasma glucose and insulin in the O-C groups were lower than those in the O group, albeit not significantly.

In histopathology, the pancreas contained normal acinar cells and islets of Langerhans in the L group (Fig. 1C); normal acinar cells and islets of Langerhans, but fat depositions in O group (Fig. 1D); fibrositic, abnormal cell polarity (Fig. 1E), and slight hyperplasia of islets of Langerhans (Fig. 1F), and fat depositions (Fig. 1G) in the O-C group.

Effect of oral consumption of *C. olitorius* on lipid metabolism

Liver triglycerides in the O and O-C groups were significantly higher than those in the L group, but they were not significantly different between the O and O-C groups (Fig. 2). Meanwhile, there were no significant differences in serum triglyceride, total serum cholesterol, total liver cholesterol, and total liver fat among the three groups.

In histopathology, the liver contained normal hepatocytes and sinusoids in the L group (Fig. 2F), many fat depositions and Kupffer cells in the O group (Fig. 2G), and many fat depositions in the O-C group (Fig. 2H).

Effect of oral consumption of *C. olitorius* on bone strength

The wet weight, BMC, and BMD of femurs in the O and O-C groups were significantly higher those in the L group, and none of these was

Table 1 Food intake, body weight, internal organ weight, and muscle weight.

	L	O	O-S	ANOVA
Food intake (g/day)	26.3 ± 0.5^{b}	31.3 ± 0.4^{a}	30.4 ± 0.5^{a}	$p < 0.001$
Body weight (g)	477 ± 10^{b}	580 ± 10^{a}	556 ± 13^{a}	$p < 0.001$
Abdominal fat weight (g)	32.1 ± 4.3^{b}	61.5 ± 3.9^{a}	55.3 ± 4.5^{a}	$p < 0.01$
Liver weight (g)	13.0 ± 0.8^{b}	16.1 ± 0.5^{a}	14.9 ± 0.4^{ab}	$p < 0.01$
Kidney weight (g)	1.37 ± 0.03^{b}	1.71 ± 0.02^{a}	1.66 ± 0.05^{a}	$p < 0.01$
Plantaris weight (g)	0.421 ± 0.02	0.421 ± 0.01	0.420 ± 0.02	ns
Soleus weight(g)	0.166 ± 0.01	0.172 ± 0.01	0.170 ± 0.01	ns
Gastrocnemius weight (mg)	2.31 ± 0.07	2.20 ± 0.04	2.26 ± 0.09	ns

Values are means ± SE. Means with unlike alphabet are significantly different. “a” was significantly higher than “b” ($p < 0.05$). “ab” was not significantly different from “a” or “b”.

L: LETO rats fed normal diet group. O: OLETF rats fed normal diet group. O-C: OLETF rats fed *Corchorus olitorius* diet group.

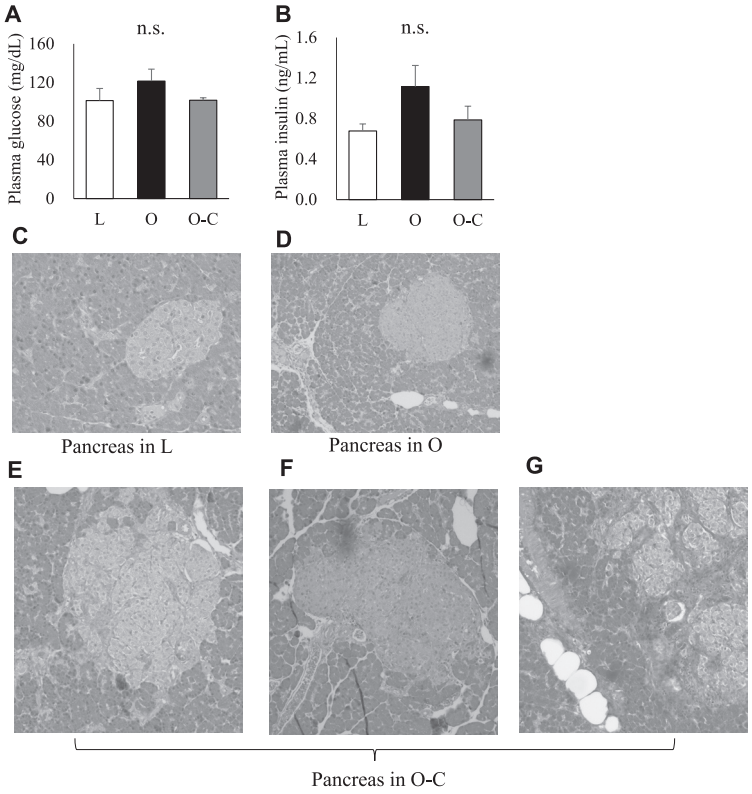

Fig. 1 Glucose metabolism and histopathology of pancreas. Plasma glucose (A) and Plasma insulin (B). Bars are means ± SE. L: LETO rats fed normal diet group. O: OLETF rats fed normal diet group. O-C: OLETF rats fed *Corchorus olitorius* diet group. In histopathology of pancreas; normal acinar cells and islets of Langerhans in L group (C); normal acinar cells and islets of Langerhans, but fat depositions in O group (D); fibrositic, abnormal cell polarity (E), and slight hyperplasia of islets of Langerhans (F), and fat depositions (G) in O-C group.

significantly different between the O and O-C groups (Fig. 3). The bone area of the femur in the O group was significantly higher than that of the L group, and that in O-C group was not significantly different from the L and O groups. There were no significant differences in length, breaking maximum load, breaking deformation, and breaking energy of femur among the groups.

4. Discussion

The present study aimed to examine the effect of the regular consumption of *C. olitorius* powder on glucose metabolism, lipid metabolism, and bone strength in a rat model of obesity with hyperphagia. Our study demonstrated that the regular consumption of *C. olitorius* powder induced lower plasma glucose and insulin levels in a rat model, but these changes were not significant. Other glucose metabolism, lipid metabolism, and bone parameters did not differ significantly between a normal diet and *C. olitorius* diet in a rat model of obesity with hyperphagia.

In contrast with our present work, previous studies have reported that the regular consumption of *C. olitorius* powder or abstract ameliorated obesity[8,9]. Both our present study and Wang et al.'s study[9] used the same percentage of dry powder of Japanese *C. olitorius* (3%) in the diet for eight weeks. The differences between these studies may

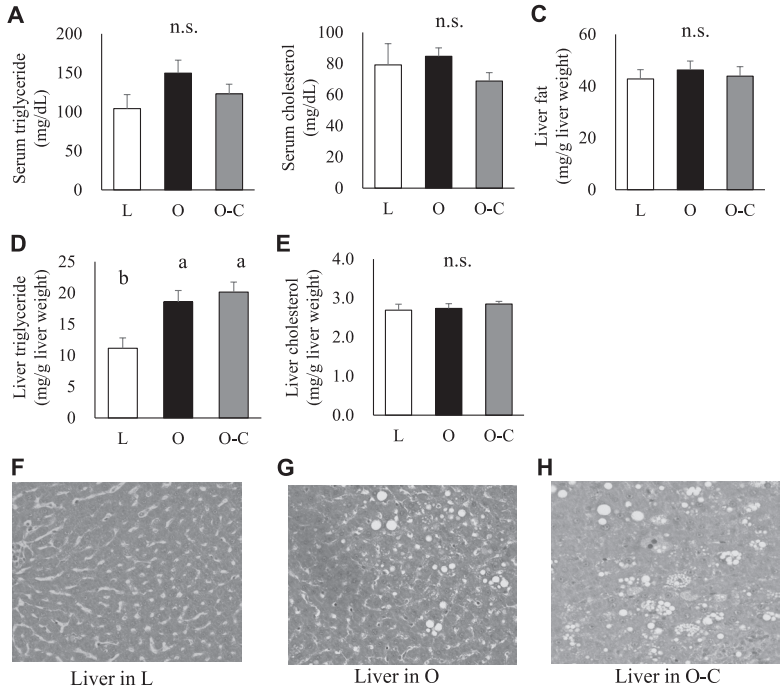

Fig. 2 Lipid metabolism and histopathology of livers. Serum triglyceride (A), total serum cholesterol (B), liver fat (C), liver triglyceride (D), and total liver cholesterol (E). Means with unlike alphabet are significantly different. "a" was significantly higher than "b" ($p < 0.05$). L: LETO rats fed normal diet group. O: OLETF rats fed normal diet group. O-C: OLETF rats fed *Corchorus olitorius* diet group. In histopathology of livers; normal hepatocyte and sinusoids in L group (F); many fat depositions, and Kupffer cells in O group (G); many fat depositions in O-C group (H).

be attributable to different methods of inducing obesity; the present study used a genetic rat model of hyperphagia, whereas previous studies used a high-fat diet[8] or LDL receptor-deficient[9] rodents. Wang et al. noted that the polyphenolic compounds in *C. olitorius* may have been responsible for the antioxidative activities that stimulated lipid metabolism in the liver, suppressed fat accumulation in adipose tissue and the liver, and reduced plasma and hepatic lipid levels, leading to the anti-obesity effect[9]. In cases when a high-fat diet is not consumed or the rodent is lipid receptor-deficient, it may take some time for the anti-obesity effect of *C. olitorius* to appear.

Previous studies have reported that OLETF rats have significantly higher plasma glucose and serum insulin levels than LETO rats prior to 28 weeks of age[11], which was assessed in our study. We consider the present data to be dissimilar from previous studies because of the small sample size of LETO rats.

Our results suggest that the oral consumption of *C. olitorius* powder is insufficient to improve abnormal glucose and lipid metabolism in cases of obesity with hyperphagia. In the obese state, the enlarged adipose tissue leads to the dysregulated secretion of adipokines and the increased release of free fatty acids, and these secretions cause abnormal glucose and lipid metabolism[16]. Therefore, improving obesity is important for improving glucose and lipid metabolism. Previous studies have reported that the regular consumption of *C. olitorius* improved hyperglycemia and dyslipidemia and also improved obesity[8,9]. *C. olitorius* powder may not

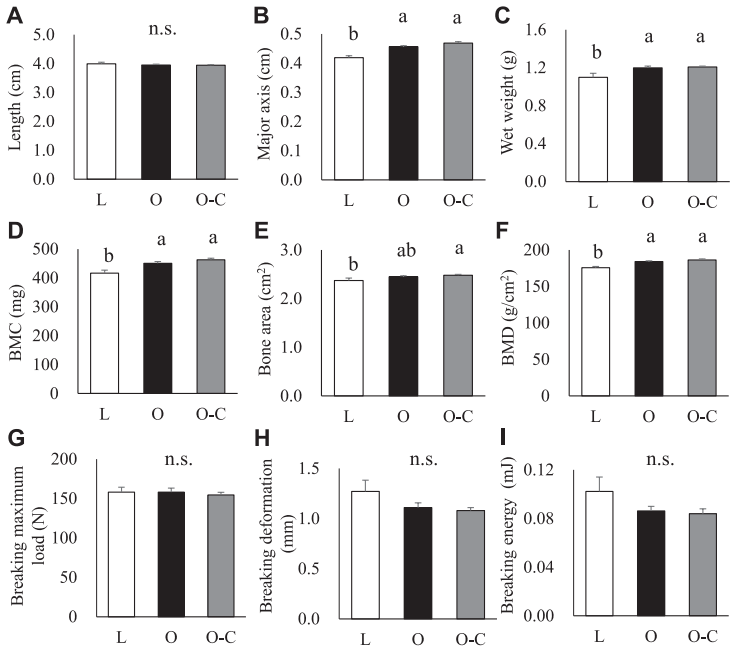

Fig. 3 Bone mass and strength of femur. Length (A), major axis (B), wet weight (C), BMC (D), bone area (E), BMD (F), breaking energy (G), breaking deformation (H), and breaking energy (I). Means with unlike alphabet are significantly different. "a" was significantly higher than "b" ($p < 0.05$). "ab" was not significantly different from "a" or "b". L: LETO rats fed normal diet group. O: OLETF rats fed normal diet group. O-C: OLETF rats fed *Corchorus olitorius* diet group.

have improved abnormal glucose and lipid metabolism enough in our study because obesity was not improved.

Our histopathology results suggest that *C. olitorius* may suppress immune responses in the liver. Kupffer cells are critical component of the mononuclear phagocytic system and critical mediators of both liver injury and repair[17]. Kupffer cells appear in the liver of obese rats fed a normal diet but do not appear in obese rats fed a *C. olitorius* diet. This result induces two possibilities: inflammation was suppressed or the repair of inflammation was suppressed. In the obese state, the secretion of inflammatory cytokines, e.g., tumor necrosis factor (TNF) and interleukin (IL), increases[16]. In Gomaa et al.'s study[8], the regular consumption of *C. olitorius* extract decreased TNF-α and IL-1β levels with the improvement of obesity in high-fat-fed rats. Meanwhile, in the present study, the *C. olitorius* diet did not improve obesity. In view of this point, the regular consumption of *C. olitorius* dry powder may suppress the repair of inflammation in a rat model of hyperphagia.

There were no effects of *C. olitorius* on bone in this rat model of obesity with hyperphagia. The *C. olitorius* powder used in the present study contained 1800 mg calcium/100 g total weight. However, the *C. olitorius* diet contained only 3% dry powder, so calcium in the diet increased only 1.05 times. Whether a high calcium intake induces high bone strength remains under debate, but previous studies reporting "high" calcium intake have used at least a twofold calcium intake[18,19]. We therefore conclude that the *C. olitorius* diet did not contain a sufficient amount of calcium to affect bone strength. Additionally, because a previous study has reported that improving glucose metabolism improved bone strength in OLETF rats[20], we reason that the *C.*

olitorius diet of the present study did not yield high bone strength because it was insufficient to improve glucose metabolism.

Limitations

The primary limitation of this study is the uncertainty that the L group was a sufficient control of no obesity without hyperphagia because the gene of OLETF rats had been crossed with the LETO rats. Therefore, the sample size of the rats in the control L group was small, which may have affected statistical power.

Conclusion

Our research suggests that the regular consumption of *C. olitorius* could not ameliorate abnormal glucose and lipid metabolism or low bone strength in a rat obesity model with hyperphagia.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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