KOREAN J. FOOD SCI. TECHNOL. Vol. 52, No. 2, pp. 144~148 (2020)
https://doi.org/10.9721/KJFST.2020.52.2.144

KOREAN JOURNAL OF
한국식품과학회지
FOOD SCIENCE AND TECHNOLOGY

Corn silk extracts did not alter the adiposity and underlying substrate utilization in high-fat diet-induced obese C57BL/6 mice

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Abstract Corn silk (Okmi-su) was anciently adopted as a material for tea or beverage. Corn silk extracts (CSE) contain bioactive phytochemicals such as phenolic acid, flavonoids, ascorbic acid, tannins, and glycosides. Under the impact of these functional components, CSE has benefits for antioxidation, diuresis, anti-diabetes, and dyslipidemia recovery. Nonetheless, its role in whole-body adiposity was not investigated; therefore, the effects of CSE on obesity were evaluated in high-fat diet-induced obese mice. Mice were assigned to either group (n=12); 1) normal diet (18% kcal from fat), 2) high-fat diet (45% kcal from fat, the control), 3) high-fat diet with CSE (800 mg/kg diet), and 4) high-fat diet with orlistat (500 mg/kg diet, a comparable control for weight loss). Our results showed that body weight, adiposity, and energy expenditure in obese mice were not altered by CSE. Lean body mass tended to decrease by CSE, which can be explained by stimulation of diuresis (p=0.06). In conclusion, our results suggest that dietary consumption of CSE does not influence the adiposity and underlying substrate utilization in high-fat diet-induced obese mice.

Keywords: corn silk extracts, adiposity, energy expenditure, respiratory quotient, lean body mass, dual-energy X-ray absorptiometry

Introduction

Obesity is defined by undesirable fat accumulation in the body. Due to unhealthy diets and physical inactivity, obesity is now a main health issue worldwide. In Korea, nearly half of the adult population is overweight and 32% is obese (Korean Society for the Study of Obesity, 2016). The prevalence of many medical comorbidities and complications including cardiovascular diseases, type 2 diabetes, hypertension, chronic kidney disease, and osteoarthritis, are approximately 1.4-4.2 folds higher in individuals with abdominal obesity compared to lean people (Korean Society for the Study of Obesity, 2014). The prevention and treatment of obesity using natural functional foods have shown considerable effects. Reflecting on this situation, the market for health-functioning foods is growing around the world, and materials and products that help reduce body fat are particularly becoming popular (Trigueros et al., 2013). According to The Ministry of Food and Drug Safety in Korea, the greatest number of functional foods categories were body fat reduction with 91 cases as of 2018 (MFDS, 2020).

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Received February 21, 2020; revised April 3, 2020;
accepted April 3, 2020

Corn silk (also known as Okmi-su) has been anciently adopted by Koreans, Native Americans, and Chinese as a material of tea or beverage. Koreans practiced corn silk to acquire diuretic, circulatory, and hypoglycemic effects, which treated kidney infection, high blood pressure, and diabetes (BRIS, 2020). As if to prove the efficacy of traditional positive effects, previous studies demonstrated that corn silk extracts (CSE) include a variety of bioactive phytochemicals, including phenolic acid, flavonoids, ascorbic acid, tannins, and glycosides (Hasanudin et al., 2012). Researches have emerged the potential use of CSE as extraordinary functional foods for antioxidation, diuresis, anti-diabetes, and dyslipidemia rescue. There was a linear correlation between antioxidant capacity and tannin and proanthocyanidin, meaning the antioxidant power of corn silk was performed by its polyphenol contents (Maksimoviæ et al., 2005). CSE improved diuresis and kaliuresis, the discharge of urine in a large amount and the secretion of potassium in a large amount in urine, respectively (Velazquez et al., 2005). An aqueous extract of corn silk reduced the fasting glucose level, identifying corn silk as a hypoglycemic agent (Guo et al., 2009). Furthermore, CSE containing flavonoids rescued hypercholesterolemia induced by a high-cholesterol/cholic acid diet (Cha et al., 2016; Saheed et al., 2015).

In fact, the market for tea and beverage products using corn silk has been steadily rising and maintained (Food Information Statistics System, 2017). Nevertheless, there were limited studies to identify CSE as an anti-obesity agent. We, therefore, investigated the adiposity-regulatory effect of CSE using high-fat diet-induced obese mice.

Materials and Methods

Animals and diets

All animal experiments were approved by the Institutional Animal Care and Use Committee of Kookmin University (KMU-2016-1). Six to eight-week-old C57BL/6 mice (all males) were purchased from Dae-Han Biolink (Eumsung-si, Korea). The mice have housed in individually ventilated cages under a 12 h light/dark cycle, the humidity of 50±10%, and a temperature of 22±1°C. After 1 week of climatization, mice were allocated into one of the following treatment groups: normal chow (18 kcal% from fat); high-fat diet (HFD, 45 kcal% fat, the control; D12451, Research Diets, New Brunswick, USA); HFD+CSE (800 mg/kg diet), and HFD+Orlistat (Roche Korea, Seoul, Korea; 500 mg/kg diet). All groups had 12 mice (n=12). A gastrointestinal lipase inhibitor, Orlistat, was applied as a positive control for weight loss. Feed and water were provided ad libitum during the experiment (8 weeks).

Corn silk extracts were provided by Kwangdong Co. LTD. (Seoul, Korea). The corn silk (harvested from Jilin Province in China) was extracted with water approximately 98-100°C for one hour. The crude extract was filtered through microfilter paper followed by concentrating under reduced pressure at 50°C or lower (concentrated Brix: 44-48 Brix). Final sterilization was followed at 95-98°C for 20 min.

Growth performance and body composition

Body weight and feed intake were measured every week. Total energy intake (kcal) was calculated using the feed intake. We examined body composition, including fat in tissue, lean mass, and bone mineral density, using a dual-energy X-ray absorptiometry (DEXA, Medikors, Seongnam-si, Korea). To this end, mice were first anesthetized with Ketamine (100 mg/kg bw; Yuhan Co., Seoul, Korea) and Xylazine (10 mg/kg bw; Bayer Korea, Seoul, Korea).

Energy expenditure and respiratory quotient

Energy expenditure and respiratory quotient were analyzed to assess metabolic phenotypes using an indirect calorimetry system (Oxylet, Panlab, Barcelona, Spain). The indirect calorimetry was calibrated using mixed gases, each containing 50% O_2/1.5% CO_2/48.5% N_2 (high point) and 20% O_2/0% CO_2/80%N_2 (low point). A single animal was housed in a separate cage, where the amount of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was observed for 24 h. Mice were provided feed and water *ad libitum* during the experiment. The respiratory exchange ratio was calculated, and the data were presented as the amount of O_2 or CO_2/h/animal. Energy expenditure and respiratory quotient were determined from the respiratory gas analysis with the Metabolism® software (Panlab).

Statistical analysis

All data are expressed as the mean±standard deviation. The difference between normal diet vs. a high-fat diet (negative control) was analyzed by unpaired *t*-test. The difference between samples vs. negative control were analyzed by one-way ANOVA, followed by Tukey's multiple comparisons. All analyses were carried out with Prism 6.0 (GraphPad Software, San Diego, USA).

Results and Discussion

Growth performance

Male C57BL/6J mice were fed a high-fat diet (45 kcal% fat) with or without CSE (800 mg/kg bw). A standard chow diet (18 kcal% fat) was supplied to secure non-obese mice. Orlistat, a lipase inhibitor, was adopted as a control for weight loss. Body weight and feed intake were measured every week to observe weight loss. There was no difference in body weight between the control and the CSE-treated group at week 8. Only mice provided orlistat, as expected, showed a decrease in body weight compared to the control at week 8 (Fig. 1A) ($p<0.05$). A previous study reported that the ethanol extraction of corn sigma (100 mg/kg bw) decreased body weight by approximately 9% in a high-fat diet (47% kcal) fed obese mice after the 4^{th} week (Lee et al., 2016). We speculate that this discrepancy might be due to the difference in the solvent because ethanol extracts contained higher bioactive compounds such as maysin and other flavonoids compared to hot water extracts.

We evaluated the effect of CSE on total energy consumption throughout the experiment (Fig. 1B). A high-fat diet increased ($p<0.05$) total energy intake (712.90±40.02 kcal) compared to the normal chow diet (579.50±21.83 kcal). Mice provided orlistat further increased total energy intake ($p<0.01$) compared to the control diet group, which can be explained by the occurrence of fat absorption impairment in the small intestine by orlistat (Lucas et al., 2020). High-fat diet-induced adiposity and its rescue by the drug orlistat were successfully established. However, CSE did not modulate body weight and total calorie intake during the experiment. Similarly, an oral administration of corn silk ethanol extract did not alter feed intake in obese mice (Cha et al., 2016). Thus, it is concluded that the CSE does not affect the amount of food intake or the total calories consumed.

Body composition

We analyzed body composition, including fat mass, lean mass, and bone mineral density, even if there was no difference in growth performance (Fig. 2). As expected, a high-fat diet substantially increased fat accumulation compared to the standard diet ($p<0.01$) (Fig. 2A). Body fat tended to decrease among the mice given orlistat compared to the control ($p=0.10$). CSE had no effect on adiposity compared to the control. DEXA scan images also showed no differences in the fat of CSE-treated mice. In contrast to our findings, some of the previous studies reported a reduction of adiposity when treated with CSE. For instance, Chaiittianan et al. (2016) reported that ethanol extracts of corn silk suppressed lipid accumulation in 3T3-L1 adipocytes. Lee et al. (2016) also reported that the administration of corn silk ethanol extracts in obese mice reduced body weight as it inhibited adipocyte differentiation, fatty acid synthesis, and beta-oxidation. Both studies used inorganic solvents to produce the corn silk extracts, which might have

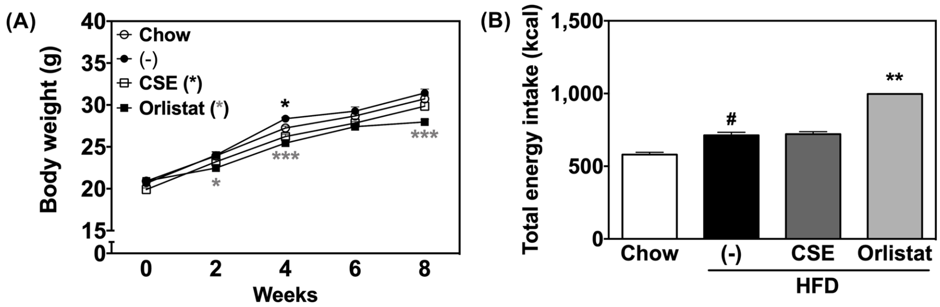

Fig. 1. Growth performance in mice given different diets for eight weeks. (A) Body weight and (B) total energy intake. Data is expressed as mean±standard error (n=12). The difference between normal diet vs. a high-fat diet (negative control) was analyzed by unpaired *t*-test (#p<0.05). The difference between samples vs. negative control were analyzed by one-way ANOVA, followed by Tukey's multiple comparisons (*p<0.05, **p<0.01, and ***p< 0.001).

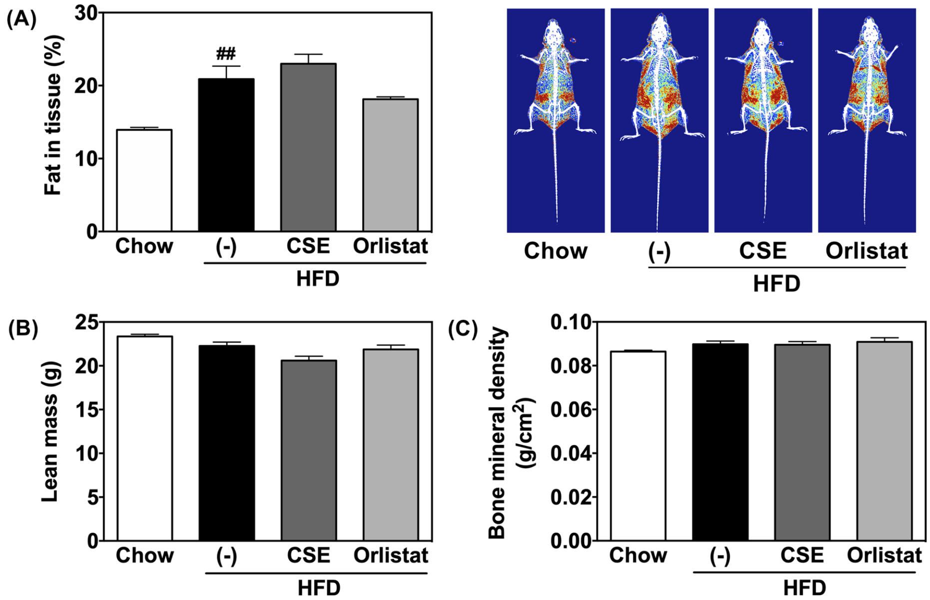

Fig. 2. Body composition in mice given different diets for eight weeks. (A) fat in tissue, (B) lean mass, and (C) bone mineral density. Data is expressed as mean±standard error (n=12). The difference between normal diet vs. a high-fat diet (negative control) was analyzed by unpaired *t*-test (##p<0.01).

yielded anti-obese phenolic compounds, including quercetin, an inhibitor of fat absorption in the small intestine (Rodrìiguez-Peìrez et al., 2019). On the other hand, in this study, CSE were extracted with hot distilled water, which has lower phenolic contents compared to alcohol extracts (Ali and El-Anany, 2017). Thus, we believe this might explain why the CSE used in this study had no effect on adiposity. However, our results have been important to understand that CSE effects on adiposity are apparently associated with the type of solvent used for the extraction.

Given that CSE had no effect on body weight and adiposity, we examined if there were any changes in leanness. Lean mass, also known as fat-free mass, is calculated by subtracting fat mass from body weight. Thus, lean mass is mainly water, muscle mass, and mineral contents, meaning that a decrease in lean mass indicates either muscle loss or water loss. Interestingly, lean body mass marginally decreased in CSE-treated group (p=0.06) compared to the control (Fig. 2B). If the loss of lean body mass was caused by a decrease in muscle mass, this is a very fatal risk. However, previous studies on CSE have provided important evidence to explain this change. For instance, corn silk supplements have been reported to promote urine excretion, which causes a decrease in lean mass (Sepehri et al., 2011; Velazquez et al., 2005). There is so far no evidence of muscle wasting and a negative impact on muscle physiology by CSE. Thus, a reduction in lean body mass by CSE can be observed due to diuretic stimulation, not by muscle wasting. Further research needs to examine the role of CSE in skeletal muscle physiology and kidney functions.

Additionally, our results demonstrated that dietary supplementation with corn silk did not modify the bone mineral density in obese mice (Fig. 2C). Similar observations have recently been explored in a prior study using ovariectomized rats, which exhibited ethanol extracts of corn silk had no impact on bone density (Triutomo et al., 2016). There is limited evidence of a regulatory role of CSE in bone health and further study needs to assess.

Energy expenditure and respiratory quotient

One of the most state-of-the-art techniques to investigate metabolic assimilation is an indirect calorific analysis, providing

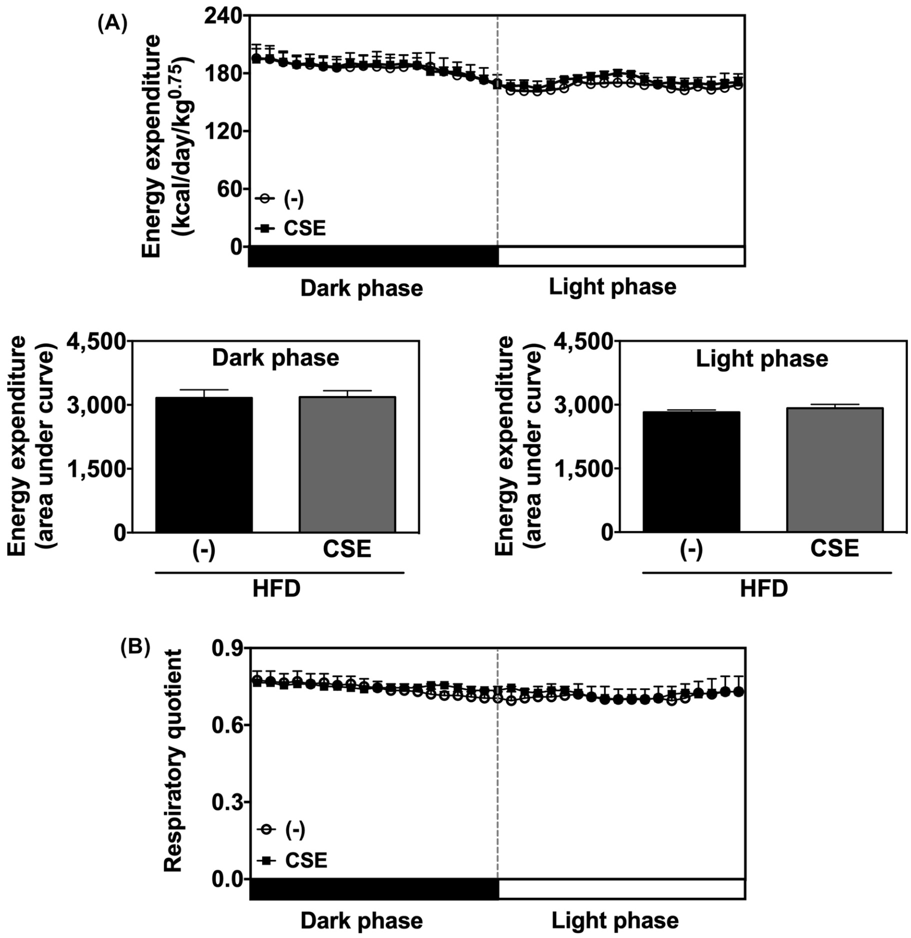

Fig. 3. The effects of corn silk extracts on energy metabolism and substrate utilization. (A) Energy expenditure and (B) respiratory quotient. Dark and light phases (9 hours each). Data is expressed as mean±standard error (n=12; p<0.05).

energy consumption and respiratory quotas. Respiratory quotient presents verified data about the type of fuel being metabolized (e.g., carbohydrates versus fat). To determine the energy catabolism of obese mice fed CSE, we evaluated their metabolic phenotypes (including total energy expenditure and respiratory quotient) using an indirect calorimetry (Fig. 3). Total energy expenditure did not differ among groups, indicating that CSE did not alter adiposity given it did not affect energy expenditure (Fig. 3A). The respiratory quotient did not differ between the control and the CSE-treated groups, with all groups exhibiting values ranging between 0.7-0.8 (Fig. 3B). Although the respiratory quotient increased slightly during the late stage of the dark phase and the early stage of the light phase, we did not observe any evidence to claim that CSE altered the type of fuel, mainly from carbohydrates to fatty acids. To our knowledge, the direct effect of CSE on respiratory quotient using indirect calorimetry has not been investigated. Instead, previous studies have reported that certain flavonoids (e.g., quercetin) were associated with non-shivering thermogenesis (Stewart et al., 2008; Zhang et al., 2019).

Conclusion

In conclusion, we investigated the effects of CSE on adiposity in mice fed a high-fat diet. CSE did not modify the markers of growth performance, such as body weight and total energy intake. According to DEXA analysis, fat mass and bone mineral density were not modulated by CSE supplementation. Interestingly, a reduction in lean body mass by CSE was observed. Previous studies explained this observation was due to diuretic stimulation, not by muscle wasting. Corn silk tea has been confirmed to be still valid in terms of traditional diuretic improvement. The current study first demonstrated the effect of CSE on energy expenditure and fuel type. As a result, total energy expenditure and utilized fuel types were not affected by CSE. Verification of the hypothesis of body fat reduction and energy consumption enhancement by hot water extract of corn silk has not been successful. Our findings were seldom inconsistent with previous studies using CSE; however, our results have been essential to understanding that bioactivity of CSE is associated with the type of solvent used for the extraction. Follow-up research is needed to clarify the physiological activity of the extraction method. Therefore, hot water extract of corn silk had no impact on the prevention of fat accumulation or acceleration of the use of stored energy in high-fat diet-induced obese mice.

Acknowledgments

This research was supported by Kwangdong Co. Ltd.

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