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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Nutritional and physiological effects of soy isoflavone and the mechanisms
(大豆イソフラボンの栄養・生理効果とその機構)

Title of Dissertation

学位論文要約：

Dissertation Summary

Obesity is due to the chronic imbalance of energy homeostasis where the energy intake exceeds than the expenditure caused by high-caloric diet and luxury lifestyle. Obesity has become a major worldwide health problem. The occurrence of obesity is rising continuously and associated with morbidity, mortality and both medical and economical costs are expected to increase even in the developing country. Dyslipidemia, the main cause of obesity is a widely accepted risk factor for cardiovascular disease and is an important feature of metabolic syndrome. It has been suggested that the intake of soy and soy-products can control the obesity and hyperlipidemia. Compared to the Western countries, the Eastern societies have low rates of obesity which may be due to the consumption of legumes based products such as soybeans and products.

Dietary soy contains proteins, lipids, dietary fiber and bioactive compounds including soy isoflavones. The predominant isoflavones in the soy are, genistein, daidzein and glycitein. Numerous studies in animal models have investigated the hypolipidemic effects of isoflavones (Legette et al., 2011; Cassidy et al., 1995; Kirk et al., 1998; Pilšáková et al., 2010) or soy protein, (Anthony et al., 1997; Kritchevsky, 1995; Lucas et al., 2001) containing intact isoflavones with other contaminants like saponin and dietary fiber. FDA approved a health claim stating that consumption of 25g or more of soy protein containing isoflavones may reduce the risk of heart disease (F.l.h.c.s.p.a.c.h, 1999). Although, many researchers have reported that soy isoflavones have the physiological effect on obesity and dyslipidemia, potential mechanisms of action are still missing on explaining the molecular mechanism.

It has been claimed that soy isoflavones are known to be estrogenic agents and the existence of the biological effect of isoflavones could be through the estrogenic-dependent pathways because the daidzein and genistein have known to be analogous to 17β-estradiol. The increase in 17β-estradiol level causes a decrease in food intake and consequently a decrease in body weight gain (Gong et al., 1989; Parker et al., 2001). It has been suggested that estrus cycle is known to affect eating behavior: a decrease in food intake during the preovulatory phase has been reported in many female animals, including humans and rats (Lyons et al., 1989). When estrogen production is disturbed and in case of menopause there might have a higher risk of developing numerous diseases, e.g., cardiovascular disease, neurogenerative diseases, cancer, osteoporosis, and obesity (Deroo and Korach, 2006; Louet et al., 2004; Toth et al., 2000). In the recent year, estrogens has been frequently screening for its energy homeostasis maintenances as the intake of energy continuously exceed than expenditure in obesity.

Key findings of the presented work

The aim of the present work was to obtain more detailed insights into the action of isoflavones on energy intake, body weight, metabolism of cholesterol in liver and small intestine. We hypothesized that the contribution of estrogenic property of soy isoflavone in anorectic and hypocholesterolemic effect which act similar to estrogen and play a role in therapy of estrogen-associated diseases. We initially carried out the experiments (Fujitani et al., 2015) in anorectic action of daidzein and estradiol in CCK or leptin receptor deficiency rats to ensure that the daidzein follow the same anorectic mechanism as the estrogen do. However, in this study, we observed the daidzein have no or very weak estrogenic effect so we designed the next study (Fujitani et al., 2015) in order to elucidate the mechanism of anorectic effect of daidzein by studying the appetite-mediated gene expression in the hypothalamus and small intestine. To the best of our knowledge, no one has examined so far the anorectic effect of daidzein in appetite-mediated gene expression. Finally we studied (Bhattarai et al., 2017) the hypocholesterolemic effect of daidzein and confirmed the anorectic effect of daidzein is not associated with this hypocholesterolemic effect. The key finding of the presented work are listed below.

The anorectic effect of daidzein could be due to the suppressing expression of NPY and galanin and increasing expression of CRH in the hypothalamus.

Energy balance is maintained via a homeostatic system involving both the brain and the periphery. A key component of this system is the hypothalamus (Schwartz et al., 2000). The hypothalamus is the

focus of many peripheral signals and neural pathways that control energy homeostasis and body weight. NPY are referred to as orexigenic hormones, because they stimulate food intake and decrease energy expenditure. α-MSH and CART in turn inhibit food intake and increase energy expenditure and referred to as anorexigenic hormones (Simpson et al., 2009). It has been hypothesized that decreasing effects of estradiol on food intake might be mediated by decreased NPY and increased the CRH in the hypothalamus (Bonavera et al., 1994; McEwen and Alves, 1999). However the mechanism in anorectic effect of isoflavones have not been elucidated yet, we confirmed the daidzein decreased a feeding-induced increase of NPY and galanin expression (Fig. 3.3) parallel with the suppression of food intake. We also confirmed the daidzein feeding increased the expression of CRH mRNA in the hypothalamus (Fujitani et al., 2015). Pages et al., 1990 claimed that the intraperitoneal injection of CCK reduces NPY expression in the rat hypothalamus. In our study, daidzein feeding significantly increased the expression of CCK mRNA in the upper small intestine before and after feeding during the second feeding session (Fujitani et al., 2015), indicating that the increased CCK might triggers the suppression of NPY expression. However we are not clear how the increased CCK levels suppressed the food intake behavior of daidzein because we have also demonstrated that CCK signaling via CCK1R is not essential for the anorectic effects of daidzein (Fujitani et al., 2015).

CCK1R and ObRb signalings were not essential for the daidzein- and estradiol-induced anorectic action.

CCK is a brain–gut peptide (called as mediators of satiation) is released immediately from the gastrointestinal tract after the response to the presence of food and binds to the CCK-1 receptor (CCK1R) distributed in the brain and gastrointestinal tract (Bi and Moran, 2002). As a “longer-term” signal, leptin is secreted by adipose cells bound to ObRb which inturn regulates energy intake and expenditure by acting on several neurons on the hypothalamus (Hamann and Matthaei, 1996). Some of the past studies has been claimed that the anoretic effect of estrogen is induced by leptin and cholecystokinin (CCK) signaling in hypothalamus (Geary, 2001) which in contradicting, of our current finding. In our study (Fujitani et al., 2015), the estradiol treatment and the daidzein both decreased the food intake and body weight gain even in CCK1R- and OVX ObRb− deficiency rats, indicated that CCK1R and ObRb signalings were not the key mechanism for the daidzein- and estradiol-induced anorectic action.

Dietary daidzein might have weak or no estrogenic effect.

Estrogen-like effect of soy isoflavones is associated with the possibility in decreasing the food intake in female rats. It is widely recognized that isoflavones have estrogenic effects, although such effects are weaker than endogenous estrogens (Setchell et al., 2001). Santell et al., 1997 reported that the decrease in uterine weight caused by ovariectomy was prevented by comparatively high amounts of dietary soy isoflavone (750 mg/kg) as well as by administration of physiological levels of 17β-estradiol in OVX rats. However, our findings are not consistent with the hypothesis that the estrogenic effects of soy isoflavones cause a decrease in food intake in female rats. Firstly, daidzein administration neither affect uterus weight in female rats with or without ovariectomy nor recovery the uterus weight reduced by ovariectomy (Fujitani et al., 2015). Secondly, in our lab, it has been shown that estradiol administration caused a decrease in food intake and body weight gain in male rats (Kishida et al., 2008). Estrogen acts through estrogen receptor (ER) α and β. The essential actions of estrogen such as its uterotropic property, requires activation of ERα. According to studies using ER knockout mice, the decreasing effect of estrogen on food intake may also be mediated by the ERα (Geary, 2004). Moreover, although the binding property of equol to ERs, and ERs activating efficacy of equol, is still controversial, many reports indicate equol binds to and activates ERβ more strongly than it does to ERα. Inconsistent responses in food intake and genital tissues in this study (Fujitani et al., 2015) supports the possibility that the decreasing effect of dietary daidzein was caused by other mechanisms rather than a simple estrogenic effect by ERα. It seems possible that estrogenic effects on feeding behavior and the reproductive system could require different doses at present. However, this hypothesis fails to explain why dietary daidzein did not decrease food intake in male rats which should be sensitive to estradiol.

Daidzein induced suppression of feeding primarily during the second feeding while using the MF3 method.

The meal feeding method is effective at comparing levels of gene expression of before and after ingestion of test diet. We studied the anorectic effect of daidzein by examining the dynamics of hypothalamic and gastrointestinal appetite-mediated gene expression using the MF3 method (Fujitani et al., 2015). Primarily daidzein reduced the feeding behavior during the second feeding, while the anorectic effect was not observed clearly in first and third feeding. In parallel, we also observed significant differences in appetite-mediated gene expression during the second feeding session by daidzein. The

orexigenic neuropeptide NPY and galanin expression which was induced by feeding was suppressed by the daidzein during the second feeding. Thus our study suggested that daidzein attenuated the postprandial increase in NPY and galanin expression.

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The lowering effect of serum cholesterol by daidzein could be explained by the decreasing effect of ApoB mRNA expression.

In the next experiment (Bhattarai et al., 2017), we chose the dietary daidzein and genistein (150 mg/kg diet) in order to confirm that they are responsible for the hypocholesterolemic effect on intact and ovariectomized female SD rats. We also investigated gene expression of the key regulators of cholesterol and bile acid metabolism in the liver and small intestinal mucosa. In this study, daidzein decreased the body weight, food intake in Ovx rats only but significantly reduced serum and hepatic total cholesterol in rats, regardless of Ovx status. Although, many researchers have reported that soy isoflavones have the physiological effect on lowering the serum lipid levels, potential mechanisms of action are still missing on explaining how the isoflavones reduce the serum cholesterol concentration. However some of the suggested mechanisms of cholesterol lowering effect could be due to the stimulation of LDLR transcription, inhibition of bile acid and/or cholesterol absorption and suppression of cholesterol synthesis. At our present study (Bhattarai et al., 2017), the mechanisms by which the dietary daidzein reduces serum and hepatic cholesterol are not clear, but the potential mechanism of reducing serum and hepatic cholesterol would involve the decreasing ApoB mRNA expression in the liver. This time, we do not have any evidence to support the hypothesis that ApoB is the main factor in reducing the serum and hepatic cholesterol levels.

Anorectic effect of daidzein may not be associated with the lowering serum cholesterol effect.

Dietary daidzein decreased the food intake only in Ovx rats decreased the serum total cholesterol level in both intact female and Ovx rats. Our lab also previously reported that the serum cholesterol concentration of rats pair-fed a diet containing FSBE was not significantly different from that in rats fed the control diet. Thus we hypothesized that decreased food intake by daidzein might not simply be associated with the hypocholesterolemic effect.

Dietary daidzein, but not genistein, has a hypocholesterolemic effect.

When compared the effects of two major isoflavones, daidzein and genistein, on lipid metabolism in rats. Dietary daidzein, but not genistein, reduced serum and hepatic total cholesterol levels significantly relative to that by the control group, regardless of whether the rats had undergone Ovx. It has been argued that the dietary dose of genistein has adipogenic nature in a gender-specific manner (Penza et al., 2006). However, it has been also reported that more than 500-1500 mg/kg genistein produces antilipogenic effects in mice (Kim et al., 2006). Previous study claimed that genistein has a greater affinity for the estrogen compared with daidzein (Ricketts et al., 2005). However, the equol, the metabolic end product of enterobacteria, has highest binding affinity for ERα than genistein and daidzein and metabolic bioavailability may be an important determinant of bioactivity after soy intake (Gu et al., 2006). In our study (Bhattarai et al., 2017), genistein did not exhibit any physiological effects on lipid levels, but did affect genes involved in cholesterol metabolism. We also noticed that the effect of decreasing hepatic free cholesterol by increasing esterified cholesterol, without affecting the total hepatic cholesterol levels by the genistein could not contribute in lowering the serum total cholesterol.

Anorectic and hypocholesterolemic effect of daidzein might be associated with its metabolite product equol level in the serum.

Our previous lab study in isoflavone aglycone-rich fermented soybean extract (FSBE) decreased the food intake and serum cholesterol concentration in both ovariectomized and non-ovariectomized female rats, but not in male rats (Kishida et al., 2006; Kishida et al., 2008). While conducting the experiments on the pure isoflavones, our study and other (Zhang et al., 2010) found that daidzein had both anorectic and hypolipodemic effects. We speculated that the anorectic and hypolipodemic effects of dietary daidzein are caused by equol. Equol is produced from the daidzein in the gastrointestinal tract by gut microflora, which is stronger than daidzein and genistein in its agonistic activity for the estrogen receptors (Ricketts et al., 2005). Our lab study also previously noticed that the continuous administration of daidzein for several days was required for its female-specific anorectic effect (unpublished data), although it has been showed that the time to reach maximum plasma concentration of equol was 20.67 ± 4.67 h by oral administration of daidzein (single oral doses of 10 and 2 mg/mL) in ovariectomized rats (Legette et al., 2014). In our second study the serum level of equol was much higher than that of daidzein in rats fed daidzein diet (Fujitani et al., 2015). Unexpectedly, equol concentrations in both before and

after second feeding were relatively lower than the first and third feedings. Thus it is not clear why consumption of the daidzein diet reduced equol during the second feeding only, or why the daidzein-induced anorectic effect was weaker during the first and third feeding.

In our first study we observed the lower serum equol level than daidzein in Zucker strain (ObRb+ and −). (Fujitani et al., 2015), the Zucker strain (ObRb+ and −) rats had lower serum equol level when compared with Long–Evans strain (CCK1R+ and −) rats and SD rats. These results suggest that the equol-producing activity was different even in the strains. Although we cannot explain clearly why the biotransformation capacities from daidzein to equol were different between Zucker, Long–Evans and SD rats. However, the elevated level of serum equol level result in the anorectic and hypolipodemic effects of daidzein.

Conclusion and outlook

Obesity increases the prevalence of increased adipose tissues, insulin resistance, increased serum VLDL and LDL cholesterol, decreased HDL cholesterol, elevated triglycerides, and fatty liver and ultimately lead to the metabolic syndrome. Evidence from past studies in humans and animals have emerged that dietary isoflavones play a beneficial role in obesity related diseased. Our study also clearly support the fact that the pure isoflavones have anorectic and hypocholesterolemic effect. We demonstrated that the daidzein has an anorectic effect although CCK1R and ObRb signalings were not essential for the daidzein-induced anorectic action. We also found that the anorectic effect of daidzein by suppressing expression of NPY and galanin and increasing expression of CRH in the hypothalamus. However, orexigenic neuropeptide NPY and galanin expression which was induced by feeding was suppressed by the daidzein during the second feeding.

We also found the daidzein has hypocholesterolemic effect. Dietary daidzein, but not genistein, reduced serum and hepatic total cholesterol levels regardless of whether the rats had undergone Ovx. The lowering of serum cholesterol by daidzein could be explained by the decreasing effect of ApoB mRNA expression. However, at present, we do not have evidence to support the hypothesis that ApoB is the main factor in reducing the serum cholesterol levels. Further investigation at the molecular level is required to understand the effects of dietary daidzein on cholesterol regulation and energy homeostasis.

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