（様式５）(Style5)

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

氏名：
Name Adhikari Sudhashree

学位論文題目：
Title of Dissertation Effects of daidzein and its metabolite on lipid and glucose metabolism
（ダイゼインおよびその代謝物が脂質・糖代謝に与える影響）

学位論文要約：
Dissertation Summary

The aim of this research was to obtain detail information of isoflavones mainly daidzein on energy intake, body weight, cholesterol and glucose metabolism in control and high-fat high sucrose fed rats with the detailed study on biodistribution of daidzein in bile, serum, urine and feces with its anorectic effects related to gastric contents. Our previous lab study found that dietary daidzein but not the genestein lowered the food intake and body weight in ovx rats by lowering the serum cholesterol levels. The cholesterol-lowering phenomenon was explained by HMGCoAR and ApoB gene expression. In first study, we choose the six week old non-ovariectomized (non-ovx) and ovariectomized (ovx) SD rats which were fed daidzein (D, 150 mg/kg diet), genistein (G, 150 mg/kg diet), daidzein:genistein (D+G, 1:1, 300 mg/kg diet) or casein-based control AIN 76 cholesterol-free diets for four weeks. Here, dietary daidzein downregulates the hepatic and intestinal HMGCoAR gene expression which suppresses the cholesterol metabolism with changing the serum cholesterol precursors levels like lathosterol, desmosterol, squalene and other sterols. The reduction of cholesterol and lipid concentrations is associated with the stimulation of LDLR and LXR gene expression in the liver. Similarly, dietary daidzein upregulated the LDLR and SREBP 1a gene expression, which may be

associated with the lowering of serum and hepatic lipid concentration with changing the concentration of sterols in serum. Furthermore, genestein did not show any physiological effects on lipid concentration but affect some genes related to cholesterol mechanism. Serum isoflavones concentration shows the higher concentration of equol rather than daidzein by dietary daidzein which showed the link that equol has higher bioavailability compared to daidzein itself. To elucidate the hypocholesterolemic effect and anorectic action of dietary daidzein, it was necessary to determine the distributions of daidzein and S-equol, a metabolite of intestinal bacterial conversion from daidzein in the body.

In the second experiment, we studied the bio-distribution of daidzein and its metabolite equol to verify its role in the enterohepatic circulation. The sham-operated and ovariectomized female rats were fed with a diet containing 150 mg/kg daidzein or 150 mg/kg equol. Dietary daidzein lowered the food intake after day 5 in OVX and day 7 in sham groups suggested that continuous intake of daidzein for at least several days was required to exert its anorectic effect, regardless of ovariectomy. Dietary daidzein significantly increased serum and bile concentrations of S-equol in a time-dependent manner but the daily changes in daidzein concentrations in serum and bile are negligible. Urinary excretions of daidzein and S-equol was not changed by dietary daidzein indicates that the amounts of daidzein and S-equol enters the systemic circulation, while fecal excretions may indicate the amounts of daidzein and S-equol that undergo enterohepatic and enteric circulations. Overall, we found that continuous intake of daidzein at the nutritional level induced accumulation of S-equol in enterohepatic circulation to far higher levels than that of daidzein itself.

The effect of dadizein and genistein on lipid metabolism provides some evidence on the hypolipidemic properties of isoflavones. Hypoglycemic effect of daidzein might be related to the improved blood lipid profile and fat accumulation in the liver and abdomen, however, the mechanism of daidzein in hypoglycemia is still debatable. High intake of energy-dense foods is considered to be a major cause of obesity and associated insulin resistance in the recent century. In third study, we aimed to investigate the effects of dietary daidzein on fat accumulation in the abdominal area and liver and insulin resistance and glucose-lowering effect impaired by a high-fat/high-sucrose (HF/HS) diet-fed female OVX SD rats. In this study six week old SD rats were chosen and fed with control and HF/HS diet along with daidzein (300mg/kg) for 5 weeks. The HF/HS diet increased the adipose tissue mass with increasing the blood glucose and insulin levels compared to control in 5 weeks of the study period. These indicate that continuous feeding of HF/HS diet induces prediabetes conditions accompanied by insulin resistance. After 2 and 4 weeks of feeding, dietary daidzein significantly decreased the fasting blood glucose levels in rats fed with the HF/HS diet but not in rats fed with the normal diet. Dietary daidzein significantly lowered the caloric intake and white adipose tissue weight by lowering the fasting blood glucose level in the HF/HS diet by increasing the PPG gene expression in intestinal mucosa and PGC1α in the liver. The upregulation of the PGC1α was associated with the regulation of mitochondrial function with upregulating insulin sensitivity and glucose metabolism. Further, dietary daidzein also tended to lower the IRS-2 gene expression in the liver by lowering the serum glucose and insulin concentrations. In addition, dietary daidzein increased hepatic ChREBP and intestinal preproglucagon gene expression. These findings indicated that dietary daidzein decreased caloric

intake through increased preproglucagon gene expression, which can prevent obesity and associated insulin resistance caused by a high intake of energy-dense foods.

GLP-1 is the products of the proglucagon peptide encoded by the preproglucagon gene which suppresses the food intake via pancreatic effects. Daidzein improves the fat accumulation, lowered the body weight, food and caloric intake and glucose tolerance through anorexigenic effect by upregulating the PPG gene expression in the intestinal mucosa. GLP-1, a cleavage product of PPG; is known to decrease the feeding activity and body weight via the inhibition of gastric emptying and glucagon secretion. Therefore, the last experiment was designed to study the effects of daidzein on gastric content and its association on anorectic effect. Seven-week-old female ovariectomized (OVX) Sprague Dawley rats were chosen which are gone through the ovariectomy operation. Different meal feeding method was used to find the gastric content during restriction feeding method. Dietary daidzein decreases food intake after 3 or 4 days of feeding regardless of time restriction feeding and delays in gastric emptying were observed in 3h meal feeding groups. This experiment also shows that daidzein lowered the food intake in second feeding compared to first feeding in 2 times meal feeding method via delaying the gastric emptying. Further, we analyzed whether the gastric emptying was responsible for the anorectic effect that cannot accumulate food in their stomach by sleeve gastrectomy (SG) operation. Dietary daidzein was replaced by equol in this study where equol lowered the food intake and gastric content in non-SG rats compared to SG operated rats. It is suggested that the capacity to accumulate food in the stomach is required for the anorectic effect. This shows that dietary daidzein has an anorectic effect with residual gastric contents, but not without gastric contents via delaying

gastric emptying. Overall our study showed that dietary daidzein has a beneficial effect on lowering cholesterol, lipid and glucose metabolism with the enterohepatic circulation into its metabolite form by gut microbiota. The food lowering effect of daidzein was also elucidated by anorectic effect via gastric emptying and intestinal PPG gene expression.