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学位論文題名

Promotion of anti-diabetic effects of flavonoid glycosides by nondigestible saccharides

（難消化性糖質によるフラボノイド配糖体の抗糖尿病作用を高める研究）

Type 2 Diabetes Mellitus (T2DM), a common metabolic disorder, can be chronic and progressively destructive condition that is characterized by insulin resistance or decreased pancreatic β-cell production of insulin. In addition to implementing lifestyle modifications as a first-line therapy for early stage diabetic patients, management of blood glucose level is predominately done with exogenous insulin or prescription drugs. Many prescription drugs however, carry undesirable side effects and potential adverse drug interactions. To mitigate the limitations of diabetic medications, researchers have looked at flavonoids, which are in various fruits and vegetables.

An example of a flavonoid is quercetin 3-*O*-glucoside (Q3G), which is shown to possess anti-diabetic effects (i.e., decrease plasma glucose level). Q3G, however, have poor bioavailability due to poor absorption in the small intestine. Fructooligosaccharide (FOS) is a nondigestible oligosaccharides that reduces the risk of hyperglycemia and dyslipidemia, and enhances secretion of GLP-1. GLP-1 is an eneroendrocrine-derived peptide, which reduces blood glucose level by stimulating insulin secretion and regulating lipid metabolism (i.e., anti-diabetic effects).

The anti-diabetic effects of FOS, however, among human participants produced inconsistent findings (i.e., FOS increases or decreases serum blood glucose level) and endure some methodological limitations. These methodological limitations may include differing concentration of FOS per day (i.e., 5-25g of FOS/day) and the short duration of the study (i.e., 14-20 days). We have previously demonstrated that FOS promotes the bioavailability of Q3G by way of suppressing the degradation in the caecum. The inter-relationship between Q3G, FOS and GLP-1 suggests an important but not yet realized effects on insulin secretion, which in turn has implications for reducing the risk of T2DM as well as hyperglycemia and dyslipidemia.

Investigating the anti-diabetic effects of Q3G, FOS and GLP-1 were conducted via *in vivo*, *in situ* and *in vitro* experiments. Our *in vivo* rat experiment investigated both individual and synergistic effects of Q3G and FOS in diets on visceral (i.e., abdominal) fat deposition, HOMA-IR and oral glucose tolerance test

(OGTT). HOMA-IR and OGTT were chosen because they are standard diagnostic tests for insulin resistance. *In situ* rat experiment tested for the synergistic effects of Q3G and FOS on GLP-1 secretion in the distal part of ileum. *In vitro* experiment tested for direct effects of Q3G with- and without FOS on GLP-1 using a murine enteroendocrine cell line, GLUTag (i.e., L-cell model).

For the *in vivo* experiment, we hypothesized that supplementation of Q3G+FOS in a sucrose based AIN-93G diet would reduce a) visceral mass, b) OGTTs, c) fasting blood glucose, d) fasting insulin concentration, e) plasma total cholesterol, and f) HOMA-IR when compared to the S diet. We also hypothesized that Q3G+FOS supplementation would increase in the plasma concentration of Q3G. For the *in situ* experiment, we hypothesized that test solution of Q3G+FOS injected directly into distal ileum would increase the plasma GLP-1 signaling the beta cells to increase insulin secretion and consequently, reduction in hyperglycemia.

To conduct our *in vivo and in situ* experiments, four groups of rats were fed a dextrin-based (D) diet as the normal reference group, or sucrose-based (S) diets with 0.3% Q3G, 5% FOS, or 0.3% Q3G+5% FOS (Q3G+FOS) for 48 days. Oral Glucose Tolerance Tests (OGTTs) were conducted on day 0, 14, 28 and 45, and adipose tissue and aortic blood were collected on day 48. Effect of Q3G and FOS on portal GLP-1 secretion was separately examined using rats after ileal administration.

In vitro experiment was conducted using a murine enteroendocrine cell line, GLUTag (i.e., L-cell model). Baseline blood samples were obtained via jugular vein for *in vivo* but portal vein for *in situ* rat group with subsequent samples obtained at 15, 30, 60, 90 and 120 minutes to evaluate plasma GLP-1 levels.

Our *in vivo* and *in situ* experiment yielded significantly lower blood glucose level for the Q3G+FOS group at 60 min in OGTT than S group on day 14, 28 and 45. HOMA-IR value was significantly lower in the Q3G+FOS group than in S group throughout the experimental period (0.25 ± 0.03 vs 0.83 ± 0.12 on day 45, $P < 0.05$). The plasma quercetin derivatives increased for FOS diet group on day 48 (18.37 ± 1.20 with FOS, 2.02 ± 0.30 without FOS, $P < 0.05$). Plasma total cholesterol levels for the Q3G+FOS group (3.10 ± 0.12, $P < 0.05$ on day 45) was suppressed compared to the S (4.03 ± 0.18). GLP-1 secretion was enhanced in Q3G+FOS group than other diet groups.

From our *in situ* experiment, we found that ileal injection of Q3G with FOS significantly and prolonged increases in plasma GLP-1 concentrations, which was much higher than those after oral administration (*in vivo*). Application of Q3G on GLUTag cells stimulated GLP-1 secretion and FOS promote the effect of Q3G.

Findings of our study suggests that synergistic effects of Q3G with FOS has the potential for prevention as well as management of T2DM by mediating the GLP-1 secretion and prolongation the high plasma concentration of the antidiabetic hormone with direct stimulation of L-cell.