HOKKAIDO UNIVERSITY

Title	Studies on bioavailability of quercetin by combined feeding of -glycosyl-isoquercitrin and soybean fiber, and the protective role against glucose intolerance in rats
Author(s)	Trakooncharoenvit, Aphichat
Citation	. ( ) 14656
Issue Date	2021-09-24
DOI	10.14943/doctoral.k14656
Doc URL	http://hdl.handle.net/2115/83196
Type	theses (doctoral)
File Information	aphichat_trakooncharoenvit.pdf

Instructions for use

Studies on bioavailability of quercetin by combined feeding of

α-glycosyl-isoquercitrin and soybean fiber, and the protective

role against glucose intolerance in rats

(ラットにおける α-グルコシルイソクエルシトリンと大

豆繊維の共摂取によるケルセチンの生物学的利用能向上

とそれによる耐糖能障害の予防に関する研究)

Hokkaido University, Graduate School of Agriculture,

Division of Applied Bioscience, Doctor Course

Aphichat Trakooncharoenvit

Abstract

Quercetin is a naturally occurring flavonol found abundantly in vegetables and fruits as single- or multi-glycosylated forms. Dietary quercetin and its glycosides reportedly have a promising anti-diabetic effect. Conversely, this beneficial effect was limited due to the poor intestinal absorbability and degradation by commensal bacteria. Improvement of the bioavailability could be valuable to further promote beneficial effects of quercetin on the body. A previous study reported that a non-digestible oligosaccharide promoted intestinal absorption a flavonoid. This raises the possibility that some kinds of dietary fibers improve quercetin bioavailability. Thus, the objective of the study was to investigate the ability of dietary fibers in promotion of quercetin bioavailability, and whether certain combination of quercetin and a dietary fiber improves quercetin bioavailability and glucose tolerance in rats fed with an obesogenic diet.

The first experiment aimed to examine the effects of several dietary fibers on the bioavailability of the water-soluble quercetin glycosides, α-glycosyl-isoquercetin (AGIQ). Male Wistar/ST rats were fed a test diet containing 0.7% of AGIQ with or without 5% of each dietary fiber (pectin, soybean fiber, and guar gum) for 9 weeks. The quercetin bioavailability and degradation were evaluated every two weeks by collecting plasma, urine, and feces. At weeks 6 and 8, the soybean fiber supplemented group showed higher concentrations of total quercetin derivatives in plasma and urine, than control. Intestinal degradation of quercetin, calculated from differences between ingestion of aglycone and the sum of excretion in urine and feces, was suppressed by dietary fiber supplementations. From this experiment, soybean fiber showed the best potential to enhance the quercetin bioavailability after a long-term feeding, which may promote the beneficial effects of quercetin.

The second experiment aimed to clarify whether the combined feeding of soybean fiber and AGIQ could enhance quercetin bioavailability, subsequently affecting glucose metabolism in a rat model of diet-induced obesity. Male Wistar-ST rats were individually fed an AIN-93G diet as the control, a high-fat high-sucrose (30% fat and 40% sucrose; H) diet, H diet with soybean fiber (HS), H diet with AGIQ (HQ), or H diet with both soybean fiber and AGIQ (HSQ) for 9 weeks. Plasma, urine, feces, and tissue samples were similarly collected as the first experiment. Meal tolerance tests (MTTs) were conducted every 4 weeks in the feeding period to assess postprandial responses of glucose, insulin, and a gastrointestinal hormone glucagon-like peptide-1 (GLP-1). The HSQ group had higher levels of total quercetin derivatives in plasma, urine, feces, and cecal contents than the HQ group at the late period of

the experiment. These results demonstrated that the ability on enhancing quercetin bioavailability of soybean fiber under an obesogenic condition, possibly via promotion of cecal fermentation. In addition, the HSQ group had lower postprandial glycemia than the H group, indicating the improvement of glucose tolerance by the combination of quercetin and soybean fiber. Furthermore, a significantly positive correlations were observed between plasma GLP-1 concentrations and plasma quercetin derivative concentrations, suggesting promotive effect of quercetin on GLP-1 secretion.

In conclusion, contentious ingestion of soybean fiber can increase quercetin bioavailability in rats, possibly through enhancing intestinal fermentation. The combination of soybean fiber and AGIQ could be beneficial for reducing the risk of glucose intolerance, possibly via enhanced quercetin bioavailability and GLP-1 secretion. These findings might help to establish a new diet composition that combines functional flavonoids with dietary fiber to minimize the risk of diabetes and other related diseases.

Abbreviations

AGIQ	α-glycosyl isoquercitrin
AIN-93G	American Institute of Nutrition-93G
ANOVA	Analysis of variance
AUC	Area under the curve
BW	Body weight
DIO	Diet-induced obesity
GLP-1	Glucagon-like peptide-1
HOMA-IR	Homeostasis model assessment-insulin resistance
HPLC	High-performance liquid chromatography
IS	Internal standard
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LPH	Lactase-phlorizin hydrolase
MTT	Meal tolerance test
OGTT	Oral glucose tolerance tests
Q3G	Isoquercetin; quercetin-3-O-β-d-glucoside
SCFA(s)	Short-chain fatty acid(s)
SEM	Standard error of the mean
T2DM	Type 2 diabetes mellitus
TG	Triacylglycerol

List of figures

List of tables and figures (Cont.)

List of tables and figures (Cont.)

Contents

Contents (Cont.)

Chapter 1: General introduction

Flavonoid and quercetin

Flavonoids are a group of naturally occurring polyphenolic compounds, which backbone structure is shown in Figure 1.1. The structure consists of a common C6–C3–C6 structure as two aromatic rings (diphenylpropane; A and B rings) with a three-carbon bridge (heterocyclic ring with oxygen, the C ring). Flavonoids can be divided mainly into six subclasses according to the position of substituted group on the backbone structure [Cosme, 2020];

1) **Flavones** have the nearest structure to the flavonoid backbone with a ketone group at position 4 (4-one), but lack the hydroxyl group in the 3-position on the C ring (e.g., apigenin, tricetin, luteolin, sinensetin);

2) **Flavonols** are a flavone backbone with a hydroxyl group at the 3-position (e.g., kaempferol, myricetin, quercetin);

3) **Flavanones** have an unsaturated carbon-carbon bond in the C ring of the flavonoid skeleton (e.g., eriodictyol, hesperidin, naringenin);

4) **Flavanols** are compounds lacking both a unsaturated carbon-carbon bond in the C ring and a 4-one in the C ring (e.g., catechin, theaflavin, theaflavins);
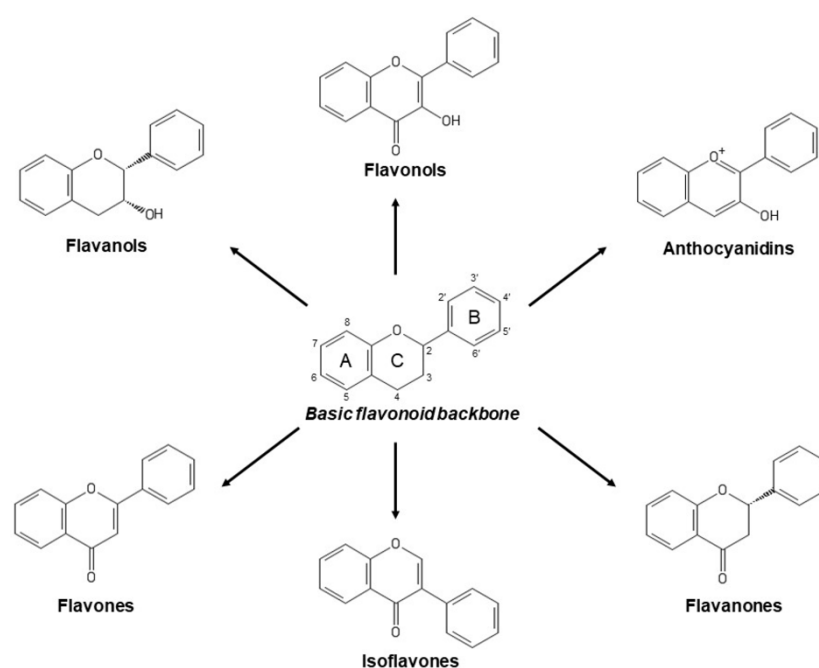

Figure 1.1 Basic flavonoid structure and main types of flavonoids [Cosme et al., 2020].

5) **Anthocyanins** are characterized by the presence of an oxygen cation, oxonioum, on the C ring. (e.g., cyanidin, maldivian, pelargonidin);

6) **Isoflavones** are distinguished by the B ring, which is attached to the C ring in the 3-position rather than the 2-position as in other flavonoids (e.g., daidzein, genistein, glycitein).

The biological functions of flavonoids, such as potent antioxidants, are determined by their structural properties. More than a thousand compounds with flavonoid structures have identified [Tungmunnithum, 2018]. In plant, these compounds are an important factor for protecting from UV radiation, pathogens, and herbivores [Mierziak, 2014].

Among discovered flavonol subclasses, quercetin is one of the most common and well-studied compound. The structure of quercetin is consisting of a pentahydroxyflavone with five hydroxy groups placed at the 3-, 3'-, 4'-, 5- and 7-positions as an aglycone form (Figure 1.2a). In fact, quercetin is commonly presented in glycosylated forms with one or more sugar groups linked to the aglycone (Figure 1.2b). This glycoside of quercetin can be found mainly in onions, broccoli, apples, and berries [Anand, 2016]. The advance of research contributed to developing an enzymatically modified form of quercetin, well-known as α-glycosyl isoquercitrin (AGIQ; Figure 1.2c), which is used in various quercetin supplement products. According to the abundance and variety of its structures, quercetin and its glycosides urge an interest of scientists to investigate their advantageous usages for functional food having beneficial effects in the health aspects [Ezati, 2021; Haohai, 2020; López, 2021; Ulusoy, 2020]. It has been reported that a low dose of quercetin (1-10 μM) exerted an anti-cancer ability to suppress the growth and number of cancer cells in human breast cancer cell lines [Jeong, 2009]. The mechanism on neuroprotective effects in *in vitro* and *in vivo* studies from quercetin was summarized by Suganthy and colleagues, this protection is also related to anti-inflammatory effect of quercetin [Suganthy, 2016]. The antioxidant abilities of quercetin play a vital role to maintain oxidative balance against various environmental and toxicological factors. Xu and colleagues also reviewed these useful effects in the medical application [Xu, 2019]. The meta-analysis on animal studies treated with quercetin at doses of 10, 25, and 50 mg/kg revealed a decrement in serum glucose level, which suggested anti-diabetic effects of quercetin [Bule, 2019]. In women with type 2 diabetes, 500 mg/day of quercetin significantly reduced tumor necrosis alpha and interleukin-6 markers which are risk factors for cardiovascular disease [Zahedi, 2013].
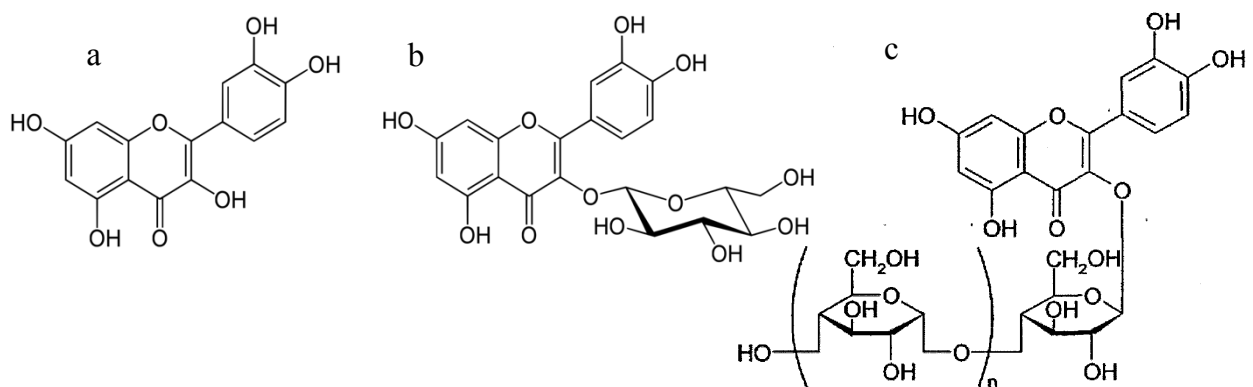
The number of glucose units may vary from 1 to 8 (n= 0-7)

Figure 1.2 The structure of a) quercetin aglycone; b) isoquercetin (Q3G); c) α-glycosyl-isoquercitrin (AGIQ), in another name: Enzymatically modified isoquercitrin or EMIQ.

Quercetin absorption and its degradation

The bioavailability of quercetin can be influenced by various factors, the most important factors are physicochemical properties and gut microbial degradation [Cai, 2013]. Quercetin with a glycoside was considered as a low hydrophilic molecule that is unable to instantly absorbed by passive diffusion in the small intestinal epithelial cells [Crespy, 2002]. To absorb this glycoside, the intestinal brush border enzyme, lactase-phlorizin hydrolase (LPH), hydrolyzes and liberates an aglycone. The aglycone up taken into the small intestinal epithelial cells are either directly transported into the hepatic portal vein, or metabolized intracellularly before transported. For this reason, the small intestine is thought to be the main site of quercetin absorption into the body [Walle, 2004]. The absorbed aglycones are transformed (methylation, sulfation, and/or glucuronidation) into a variety of metabolites, mainly in the liver, before distribution to other organs or tissues [Thilakarathna, 2013]. Conjugated metabolites that reach the kidney are finally excreted into the urine. Conversely, unabsorbed aglycone and re-secreted metabolites through the bile in the small intestinal lumen, flow into the large intestine, which is the main site of quercetin degradation by the commensal bacteria [Mullen, 2008; Zhang, 2014]. Behind this degradation, the commensal bacteria possess various enzymes to activate single or multiple of following reactions: heterocyclic ring fission (especially cleavage of C-ring), demethylation, dehydroxylation, ester bond cleavage, carbon double bonds cleavage, and decarboxylation [Braune, 2001; Braune 2016; Schneider, 1999; Schneider, 2000]. Several products that resulted from the breakdown of quercetin are including 3,4-dihydroxyphenylacetic acid, taxifolin, alphitonin, phloroglucinol, acetate, and butyrate

(Figure1.3) [Almeida, 2018]. *Clostridium perfringens* and *Bacteroides fragilis* were found to be the most capable of degrading quercetin [Zhang, 2014]. A portion of the undegraded quercetin metabolites is excreted into feces, or probably reabsorbed in the large intestine and circulated back to the liver via the hepatic portal vein [Thilakarathna, 2013].

The degradation in the digestive tract makes quercetin bioavailability less than 10% [Clifford, 2004; Hollman, 2009; Scalbert, 2000]. In human studies, the dosage of quercetin ranges from 1 to 1200 mg/day were tested [Patel, 2018]. Some of these studies failed to take advantage of quercetin. According to this statement, the absorption of quercetin has been determined by using ligated loops of rat small intestine perfused with [2-^{14}C] Quercetin-4′-glucoside, a radiolabeled quercetin glycoside. The result showed that only 1.5% of quercetin perfused radioactivity can cross the small intestinal wall [Carbonaro, 2005]. A similar experiment that monitoring radiolabeled products in body tissues, plasma, feces, and urine of rats was performed [Mullen, 2008]. The trace amount (0.5%) of radioactivity was detected in tail vein plasma 1 h after oral gavage with [2-^{14}C] Quercetin-4′-glucoside. Interestingly, the ingested radioactivity was not detected in plasma while 96% of the radioactivity was recovered as degraded product forms of quercetin in feces, urine, and the cage washes after 72 h of oral gavage. These studies revealed that as it passes through the gastrointestinal tract, ingested quercetin was absorbed only a small portion while almost of the unabsorbed quercetin was rapidly degraded and excreted into urine or feces. Thus, the degradation of quercetin by the commensal bacteria in the large intestine conceivably impacts its bioavailability. For this reason, the quercetin bioavailability is a critical factor to determine their biological activity because the degree of absorption has a positive correlation to the bioactivities of quercetin on target responsive cells or tissues [Dabeek, 2019].
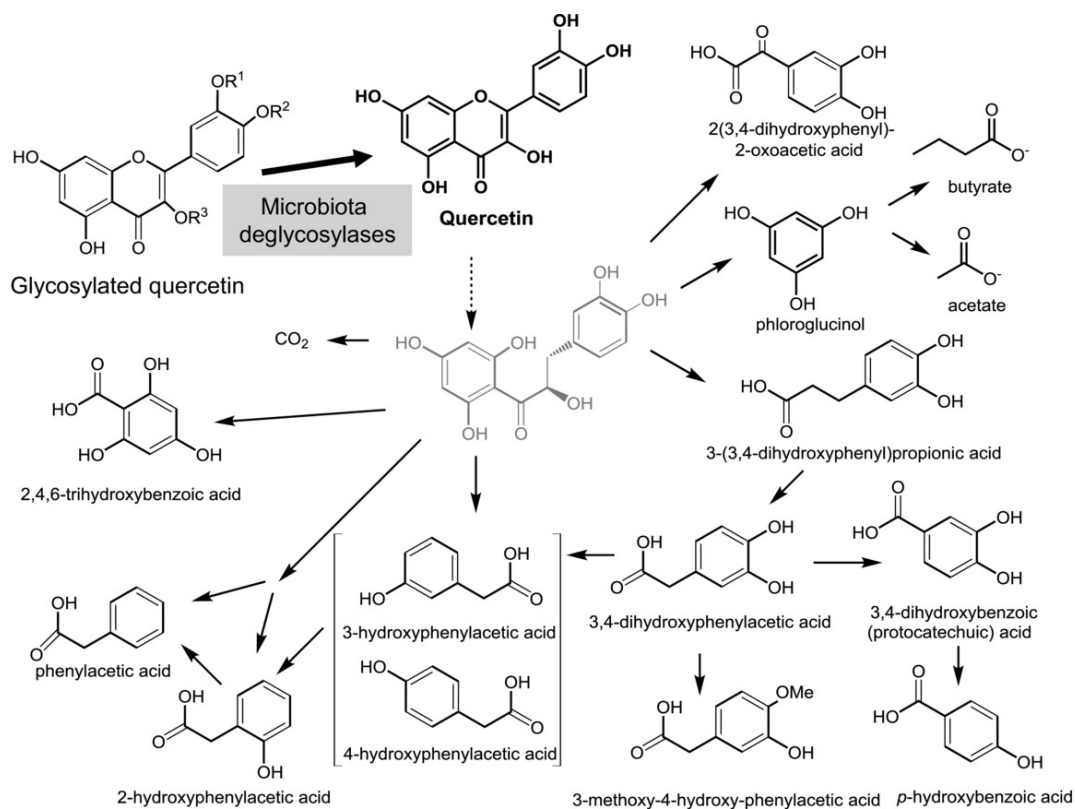
Figure 1.3 Schematic representation of quercetin degradation and its degraded products (Almeida et al., 2018).

Dietary fiber and cecal fermentation

The CODEX Alimentarius Commission describes the word "dietary fiber" as edible carbohydrate polymers or oligomers that are not digested or absorbed by endogenous enzymes in the human digestive tract [Jones, 2014]. Dietary fibers are roughly classified into fermentable and non-fermentable fibers. Fermentable fibers are easily fermented by bacteria in the colon (e.g. pectin, soybean fiber, guar gum), while non-fermentable fibers are resistant to bacterial degradation (e.g. cellulose, lignin, and some of hemicelluloses) [Berer, 2018]. The potential health benefits of dietary fiber have received considerable attention in the last several decades. Two health claims for dietary fiber have been approved by the Food and Drug Administration (FDA). The first claim states that the increment of dietary fiber along with a decreased consumption of fats (<30% of calories), may reduce some types of cancer [FDA, 2008]. This claim is supported by several studies that report the inverse relationship between dietary fiber and the growth of a variety of cancers, including colorectal, small intestine, dental, laryngeal, and breast cancer [Nomura, 2007; Park, 2009; Schatzkin, 2008]. However, their responsible mechanisms are still unclear. The second claim of the FDA states that diets high in

fermentable fiber components have a decreased risk of coronary heart disease [FDA, 2008]. Although the FDA has only approved and supported two claims, several other potential claims have been thoroughly investigated and reported [Dahl, 2017; Ogden, 2006; Swann, 2020; Singh, 2021; Weickert, 2008]. In addition, when dietary fiber reaches the large intestine, it is fermented by the colonic microflora followed by productions of short-chain fatty acids (SCFAs; primarily acetate, propionate, and butyrate), gases (methane, hydrogen, carbon dioxide), and biomass [Patterson, 2014]. The SCFAs are then absorbed through the epithelium and contribute to its energy generation [Inoue, 2014; LeBlanc, 2017]. The sufficient amount of SCFAs affect the health and wellbeing of the host [Bugaut, 1987].

Type 2 diabetes mellitus and glucagon-like peptide 1 (GLP-1)

Nowadays, type 2 diabetes mellitus (T2DM) is an increasingly prevalent disease that causes serious public health issues on a global scale [Lin, 2020]. The symptoms of T2DM presented as hyperglycemia, hyperinsulinemia, and insulin resistance [Cersosimo, 2018]. In addition, diabetic patients have an increased risk of other complications such as stroke, myocardial infarction, and coronary artery disease [Tang, 2008]. The impaired glucose represents that the body unable to control the blood glucose level [David, 2007]. Advancement in pharmaceutical research has developed many synthetic reagents (such as metformin, sitagliptin, saxagliptin, etc.) for the treatment of this chronic disease [Dowarah, 2020]. However, these synthetic compounds may have some side effects such as diarrhea, dizziness, headache, and vomiting [Chaudhury, 2017]. Researchers are looking for therapeutic methods to lessen these side effects. Thus, the search for effective and safer anti-diabetic strategies draw attentions in this recent decade.

GLP-1 is an incretin hormone released from intestinal L-cells, mainly in the distal ileum, in response to nutrient ingestion [Tolhurst, 2009]. This secreted hormone has a wide range of physiological actions, including inhibition of glucagon release, gastric emptying, and food intake, and activation of glucose-stimulated insulin secretion, enhancement of β-cell growth, and survival [Holst, 2007]. From these advantages, GLP-1 was considered as one of the targets on incretin-based diabetes therapies. Two major anti-diabetic drug classes, which are augmented in this therapy, are agonists of the GLP-1 receptor and inhibitors of the GLP-1 degrading enzyme dipeptidyl peptidase 4. Some naturally occurring flavonoid compounds have also been reported to potentiate incretin actions and acted as an agonist to the GLP-1 receptor [Rehman, 2019; Wang, 2020; Wootten, 2011]. A recent study reported that the flavone

hispidulin could stimulate the secretion of GLP-1 in GLP-1-producing cell models and the mouse ileum crypts. In addition, daily oral gavage of this flavone in streptozotocin-induced diabetic mice improved insulin release, and β-cell survival [Wang, 2020]. These results highlight the potential impacts of dietary flavonoids on incretin hormones.

Aim of studies

The bioavailability of quercetin primarily determines its beneficial effects. Since quercetin has a poor absorption rate in the intestine, increasing its bioavailability could be valuable to promote its beneficial effects with long-lasting changes in the health of the host body. A previous study reported that non-digestible oligosaccharides, such as fructooligosaccharide and difructose anhydride III, promote the absorption of flavonoids [Matsukawa, 2009]. This improvement of flavonoid bioavailability by oligosaccharide treatment raises the possibility of improvement of flavonoid bioavailability by dietary fibers.

Thus, the objective of the first study was to investigate the ability of selected dietary fibers on promoting the bioavailability of quercetin, AGIQ form. It was expected that different physicochemical properties, such as fermentability, of each selected dietary fiber may give a diverse degree on the absorption of quercetin. Along the feeding period for 9 weeks, quercetin and its derivatives were monitored every two weeks in plasma, urine, and feces. Results from this study may shed some light on the characteristics of ideal dietary fiber on the enhancement of flavonoid bioavailability.

In the next phase, it was speculated that the promotion of quercetin bioavailability gives health-beneficial effects. The second study was aimed to clarify whether the combination of AGIQ and the best candidate dietary fiber from the first experiment is able to enhance quercetin bioavailability, consequently affecting glucose metabolism accompanied with increased GLP-1 secretion in diet-induced obese (DIO) model rats. The DIO rats were employed because this model is closely related to the pathophysiology of human metabolic syndrome, rather than genetically obese models [Pinyo, 2019; Preguiça, 2020]. In this experiment, quercetin bioavailability and degradation were quantified in plasma, urine, feces, and cecal content samples. To observe the changes in postprandial glycemic-, insulin-, and GLP-1-responses, meal tolerance tests were performed every four weeks of the feeding period (totally for 9 weeks). Correlations between various parameters were evaluated to identify specific relationships between experimental endpoints (quercetin bioavailability, glucose tolerance, GLP-1 levels) and parameters measured.

Chapter 2: Water-soluble dietary fibers enhance bioavailability of quercetin and a fiber derived from soybean is most effective after long-term feeding in rats

Abstract

To investigate the effects of water-soluble dietary fibers (pectin, soybean fiber, and guar gum) on the bioavailability of quercetin from the α-Glycosyl isoquercitrin (AGIQ) ingestion. Male Wistar/ST rats were fed a test diet containing 0.7% AGIQ with or without 5% of each dietary fiber for 9 weeks. Total quercetin derivatives were evaluated with liquid chromatography-tandem mass spectrometry (LC-MS/MS) as total quercetin derivatives after enzymatic deconjugation in plasma, urine, and fecal samples on weeks 2, 4, 6, and 8. The excreted quercetin derivatives in feces were also measured. Results showed that fiber feeding elevated the whole cecal weight and reduced the cecal pH, indicative of cecal fermentation promotion. Changes in plasma and urinary quercetin levels revealed three phases of quercetin metabolism, including cumulative, transient, and stable phases. At weeks 2, total quercetin derivatives were higher in plasma samples from three fiber-fed groups than those control groups; however, urinary excretion increased in fiber-fed groups at weeks 4. Soybean fiber upregulated plasma and urinary quercetin levels on weeks 6 and 8. Intestinal degradation of quercetin by bacteria, calculated from differences between aglycone ingestion and the sum of urinary and fecal excretion, was suppressed after dietary fiber supplementation especially in pectin fiber, which may partly contribute to the increase in quercetin bioavailability. Fecal quercetin glucuronide excretion was high in soybean fiber-fed rats, suggestive of the reduction of β-glucuronidase in the colon. In conclusion, water-soluble dietary fibers, especially soybean fiber, enhanced quercetin bioavailability after chronic feeding and may promote beneficial effects of quercetin on disease prevention.

Introduction

Quercetin is an abundant natural flavonoid belonging to the class of flavonol. It is usually present in fruits and vegetables in glycosylated forms (sugar-bound) such as quercetin-3-O-β-*d*-glucoside (isoquercetin; Q3G). Several studies have demonstrated the biological activities of quercetin on the prevention of diseases such as colon cancer, cardiovascular diseases, and diabetes [Chen, 2016; Gee, 2002; Li, 2016; Vessal, 2003]. The bioavailability of quercetin and its glycosides is low, limiting their beneficial effects [Guo, 2015]. Furthermore, the bioavailability of quercetin varies depending on coexisting food ingredients.

There are two mechanisms related to quercetin glucoside absorption in the intestine. In the first mechanism, lactase-phlorizin hydrolase (LPH) cleaves sugar moieties from quercetin glucosides. [Day, 2003] The resulting aglycone was absorbed into intestinal epithelial cells by simple diffusion. In the second absorptive pathway, quercetin glucosides are transported via a glucose transporter [Chabane, 2009; Wolffram, 2002] and the absorbed quercetins are conjugated as glucuronides and sulfates, or methylated in the epithelial cells. Quercetin derivatives can further be conjugated and methylated at the liver. These quercetin conjugates in the blood are excreted into the urine and bile. The excreted quercetin conjugates into the bile are reabsorbed in the intestine. Urinary excretion under stable condition reflects the intestinal absorption rate of quercetin [Vrie, 1998]. The unabsorbed quercetin glycosides and aglycones in the small intestine flow into the cecum and colon. In these sites, the quercetin can be absorbed into the epithelium or broken down by the intestinal bacteria [Keppler, 2006; Manach, 1997; Marin, 2015]. The degradation of quercetin aglycone in the large intestine is a reason for the low bioavailability of quercetin [Rich, 2017; Thilakarathna, 2013]. Conjugated forms of quercetin excreted into the bile also reach into the large intestine, wherein these molecules are usually degraded by the bacterial β-glucuronidase. The high activity of this enzyme is known to increase the risk of colon cancer [Maruti 2008; Shiau, 1983].

Dietary fibers are non-digestible polysaccharides that prevent the onset of several metabolic diseases through physicochemical properties and intestinal fermentation. Being resistant to the digestive enzymes in the small intestine, dietary fibers increase the fermentation and production of organic acids, mainly short-chain fatty acids (SCFAs), in the large intestine [Andoh, 2003; Koh 2016]. Clinical studies have demonstrated that the consumption of dietary fibers attenuates severe increases in blood glucose, cholesterol, and triglyceride after a meal and lowers the risk of disease development [Lattimer, 2010; Threapleton 2013].

In recent studies, the bioavailability of quercetin glycosides was shown to be increased by co-ingested saccharides, including non-digestible oligosaccharides, depending on the intestinal bacterial metabolism [Matsukawa, 2009; Shinoki, 2013; Tanaka, 2016]. In the preliminary study, I performed two sets of feeding experiments by using male Wistar/ST rats to compare the effect of water soluble dietary fiber on Q3G absorption by the hypothesis that dietary fiber can enhance quercetin absorption. Fiber sources in these experiments were composed of acacia gum and pectin for the first experiment and partially hydrolyzed guar gum and soybean fiber for the second experiment. By these two cohort studies, the result suggested that pectin and soybean fiber can be used as supplement ingredients to promote quercetin absorption. These studies suggest that water-soluble dietary fibers can promote the bioavailability of dietary flavonoids. However, the effects of chronic feeding of water-soluble dietary fibers on quercetin bioavailability have not been studied.

For this reason, pectin was used in this investigation as representative vicious and low fermentable dietary fibers. Soybean fiber, a highly fermentable fiber with low viscosity was included as aforementioned on its ability. In addition, guar gum is another candidate dietary fiber from the different physicochemical property from the other two fibers. The experimenter hypothesized that the continuous ingestion of dietary fibers may increase quercetin bioavailability by promoting intestinal fermentation and/or the difference in physicochemical property of fibers may affect the intestinal absorption of quercetin.

Materials and methods

Materials

The α-Glycosyl isoquercitrin (AGIQ, kindly given by San-Ei Gen F.F.I., Inc., Osaka, Japan) consisted primarily of quercetin-3-O-glucoside (Q3G, 31.8%) and mono (23.3%) and di (20.3%) glucose adducts of Q3G with α-1,4-linkages. AGIQ was used as a quercetin source because the absorption of this quercetin glucosides is higher than Q3G (natural compound) [Matsukawa, 2009] and already used as a food additive with granted generally recognized as safe (GRAS) status in Japan. WSSF, a highly fermentable fiber with low viscosity, was kindly donated by Fuji Oil Co., Ltd (Osaka, Japan). Soybean fiber, a highly fermentable fiber with low viscosity, was kindly supported by Fuji Oil Co., Ltd (Osaka, Japan). Pectin from citrus and guar gum were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan) and Tokyo chemical industry Co., Ltd (Tokyo, Japan), respectively. As deconjugating enzymes, β-

glucuronidase ($\geq$ 100,000 units/mL) and sulfatase ($\geq$ 2,000 units/mL) from *Helix pomatia* extract (Cat. No. S9751) and sulfatase (10-20 units/mL) from *Aerobacter aerogenes* extract (Cat. No. S1629) were purchased from Sigma Aldrich (Saint Louis, USA). Two sources of sulfatases were applied because insufficient hydrolysis of sulfate conjugates by the only *Helix pometia* enzyme. Other reagents were provided by Wako unless specified.

Animals and diets

Male Wistar/ST rats, weighing about 120-140 g (5 weeks old) purchased from Japan SLC, Inc. (Hamamatsu, Japan), were housed in an individual stainless-steel cage with a wire mesh bottom. Rats were maintained in a controlled environment (12 h light/dark period, relative humidity 40-60%, and temperature 22°C ± 2°C) with ad libitum access to diet and water. Rats were assigned to five groups (n = 7 rats/group) after 1-week acclimation with a sucrose-based diet based on AIN-93G formulation [Reeves, 1993]. For 9 weeks, five groups of rats were fed with different diets as follows; sucrose-based AIN-93G diet for control (C) group, sucrose-based AIN-93G diet mixed with 0.7% AGIQ (Q) as a basal diet. The basal diet mixed with 5% of each test fiber, pectin (as QP), or soybean fiber (as QS), or guar gum (as QG). In the diet component, sucrose is replaced by supplemented AGIQ and cellulose is replaced by the test fibers for fiber treatment groups. The percentage (0.7%) of AGIQ used in the present study was equivalent to 0.5% of quercetin aglycone. The experimental diet composition was shown in Table. 2.1. The bodyweight and food consumption of rats were measured daily. Tail vein blood was collected at 10.00 - 11.30 a.m. on weeks 2, 4, 6, and 8 after feeding test diets. Urine and feces were collected for 2 and 3 days, respectively, in the same week before blood collection day to evaluate quercetin bioavailability. For urine collection, 0.05% sodium azide was added to prevent the degradation of quercetin metabolites by bacterial growth. Collected urine samples were filled up to 100 mL, and 15 mL of samples were stored at −40°C. Fecal samples were stored at −80°C. At the end of the feeding period, rats were anesthetized with sodium pentobarbital injection (Somnopentyl, Kyoritsu Seiyaku Corporation, Tokyo, Japan) and killed by the withdrawal of the aortic blood followed by exsanguination after overnight fasting. The liver and epididymal fat pad were collected and weighed. The cecum and its contents were collected for measurements of pH, SCFAs, and other organic acids.

This study was approved by the Hokkaido University Animal Committee, and the animals were maintained under the Guide for the Care and Use of Laboratory Animals of Hokkaido University.

Table 2.1 Experimental Diets Composition

Ingredient	Control (C)	AGIQ (Q)	Q + Pectin (QP)	Q + Soybean (QW)	Q + Guar gum (QG)
	g/kg of diet				
Sucrose	609.5	602.5	602.5	602.5	602.5
Casein [a]	200	200	200	200	200
Soybean oil	70	70	70	70	70
Cellulose [b]	70	70	20	20	20
Mineral (AIN-93G-MX) [c]	35	35	35	35	35
Vitamin (AIN-93-VX) [c]	10	10	10	10	10
AGIQ [d]	-	7	7	7	7
Pectin [e]	-	-	50	-	-
Soybean fiber [f]	-	-	-	50	-
Guar gum [g]	-	-	-	-	50
L-cystine	3	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5	2.5

[a] Acid casein (Fonterra, Ltd., Tokyo, Japan)
[b] Avicel PH102 (Asahi Kasei Chemicals Corporation, Tokyo, Japan)
[c] Mineral and vitamin mixtures were prepared according to the AIN-93G formulation
[d] α-Glycosyl isoquercitrin (AGIQ; kindly supplied by San-Ei Gen F.F.I., Inc., Osaka, Japan)
[e] Pectin from citrus (Wako Pure Chemical Industries, Ltd., Osaka, Japan)
[f] Soybean fiber (kindly supplied by Fuji Oil Co., Ltd., Osaka, Japan)
[g] Guar gum (Tokyo chemical industry Co., Ltd., Tokyo, Japan)

Sample treatments

Plasma or diluted urine sample (100 µL) was acidified with 10 µL of 0.58 mol/L acetic acid and treated with 7.5 µL of each deconjugation enzyme, β-glucuronidase and sulfatase [Tanaka, 2018]. Both enzymes treated- and untreated-samples were incubated at 35°C for 1 h and naringenin (2 µmol/L as an internal standard; IS) was added to all samples. The mixture was centrifuged (9,300g at 4°C for 5 min) and the supernatant was collected; the precipitate was re-extracted with methanol. The supernatant was loaded onto a C18 cartridge (Oasis HLB, Waters Co. LTD, Milford, MA, USA) and eluted with 1 mL methanol. After drying and reconstitution in a 50% methanol solution, quercetin and methylquercetin with enzyme-treated (aglycone form) and quercetin and methylquercetin metabolites without enzyme treatment

(conjugated form) were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Fecal samples were freeze-dried and milled and their dry weights measured. Powdered fecal samples (0.1 g) were suspended in 1 mL of 80% methanol solution and treated with 100 µL of 10 µmol/L naringenin as IS [Tanaka, 2016]. Mixtures were homogenized using an ultrasonic homogenizer (VP-050, Saitama, Japan) and incubated at 60°C for 1 h before centrifugation (2,300g for 10 min at 4°C). Methanol (1 mL) was added to the precipitate and homogenized with Teflon homogenizer (42W, ULTRAS homogenizer, Taitec Co. Ltd., Nagoya, Japan). The mixture was centrifuged to precipitate insoluble materials, including proteins. The entire procedure was repeated twice. The obtained supernatant was evaporated and the concentrations of total quercetin derivatives were measured as the same as plasma and urine extracts.

Quercetin measurement by LC-MS/MS

Quercetin and its metabolites were determined [Tanaka, 2018] using LC-MS/MS system (TSQ Quantum Access Max with Accela High-Speed LC System, Thermo Fisher Scientific Inc., MA, USA) with electrospray ionization (ESI) source with SRM mode. The analytical column was a 1.9 µm C18 column (Inertsustain Swift C18, 2.1 mm × 100 mm GL Sciences Inc., Tokyo, Japan) set at 40°C. The mobile phase was water, methanol, and formic acid (70:30:0.1, solvent A), and methanol with formic acid (100:0.1, solvent B). The ratio of solvent A and B was delivered through a linear gradient (90:10 for 1 min; 90:10-20:80 for 8 min; 90:10 for 1 min) at a flow rate of 0.2 mL/min. The quercetin aglycone and its derivatives were identified according to their m/z (mass to charge ratio) value (Table 2.2). Structures of quercetin aglycone and its derivatives are shown in Table 2.3. Concentrations of total quercetin derivatives and their metabolites were calculated from the calibration curve of standard compounds.

Table 2.2 The Chemical Formulation and Mass to Charge Ratio of Quercetin and Its Metabolites

No.	Compound	Chemical formula	Mass to Charge (m/z)		Ion
			Parent	Product	
1	Naringenin	$C_{15}H_{12}O_5$	273.069	273.069	+
2	Quercetin	$C_{15}H_{10}O_7$	303.043	303.043	+
3	Quercetin sulfate	$C_{15}H_{10}O_{10}S$	381.000	301.043	-
4	Quercetin disulfate	$C_{15}H_{10}O_{13}S_2$	460.956	301.043	-
5	Quercetin glucuronide sulfate	$C_{21}H_{18}O_{16}S$	557.032	301.043	-
6	Methyl quercetin sulfate	$C_{16}H_{12}O_{10}S$	395.015	315.058	-
7	Methyl quercetin glucuronide sulfate	$C_{22}H_{20}O_{16}S$	571.047	315.058	-
8	Quercetin diglucuronide sulfate	$C_{27}H_{26}O_{22}S$	733.064	301.043	-
9	Q3G (isoquercetin)	$C_{21}H_{20}O_{12}$	465.096	303.043	+
10	Q3G2	$C_{27}H_{30}O_{17}$	627.148	303.043	+
11	Q3G3	$C_{33}H_{40}O_{22}$	789.201	303.043	+
12	Q3G4	$C_{39}H_{50}O_{27}$	951.254	303.043	+
13	Q3G5	$C_{45}H_{60}O_{32}$	1113.307	303.043	+
14	Q3G6	$C_{51}H_{70}O_{37}$	1275.36	303.043	+
15	Q3G7	$C_{57}H_{80}O_{42}$	1437.412	303.043	+
16	Q3G8	$C_{63}H_{90}O_{47}$	1599.465	303.043	+
17	Methyl quercetin	$C_{16}H_{12}O_7$	317.058	317.058	+
18	Quercetin glucuronide	$C_{21}H_{18}O_{13}$	479.075	303.043	+
19	Quercetin diglucuronide	$C_{27}H_{26}O_{19}$	655.107	303.043	+
20	Methyl quercetin glucuronide	$C_{22}H_{20}O_{13}$	493.09	317.058	+
21	Methyl quercetin diglucuronide	$C_{28}H_{28}O_{19}$	669.123	317.058	+

Cecum treatment for pH and SCFAs measurement

Cecal contents and tissues were isolated and separately measured after frozen cecum samples were thawed. The contents of the cecum were diluted with four times the weight of deionized water, and the pH was determined using a pH meter (B-211 twin waterproof pH, Horiba, Ltd., Kyoto, Japan). Diluted samples (700 μL) were mixed with 200 μL of IS and a mixture of 25 mM crotonic acid in 50 mM sodium hydroxide (NaOH) and centrifuged. The supernatants were mixed with the same volume of chloroform and centrifuged again. The supernatant was filtrated through a 0.2 μm membrane, and concentrations of organic acids, including SCFAs, were measured with high-performance liquid chromatography (HPLC) constructed with two shim-pack SCR-102H (8 mm I.D. × 300 mm L Shimadzu Corp.) and conductivity detector CCD-6A (polarity: +, response: slow, temperature: 45°C; Shimadzu Corp.). The flow rate of the mobile phase (5 mM aqueous *p*-toluenesulfonic acid) and buffer (5 mM *p*-toluenesulfonic acid solution containing 100 μM ethylenediaminetetraacetic acid

Table 2.3 The Chemical Structure of Quercetin and Its Metabolites

No.	Compound	Substitution group				
		R1	R2	R3	R4	R5
1	Naringenin	H	OH	H	OH	OH
2	Quercetin	OH	OH	OH	OH	OH
3	Quercetin sulfate	OH	OH	Sulfate	OH	OH
4	Quercetin disulfate	OH	Sulfate	OH	OH	Sulfate
5	Quercetin glucuronide sulfate (Quercetin 3-glucuronide-3'-sulfate)	OH	OH	Glucuronide	OH	Sulfate
	Quercetin glucuronide sulfate (Quercetin 3-glucuronide-7-sulfate)	Sulfate	OH	Glucuronide	OH	OH
6	Methyl quercetin sulfate	OH	OH	Sulfate	OH	O-Methyl
7	Methyl quercetin glucuronide sulfate	Sulfate	OH	Glucuronide	OH	O-Methyl
8	Quercetin diglucuronide sulfate	OH	Glucuronide	Sulfate	OH	Glucuronide
9	Q3G (isoquercetin)	OH	OH	Monoglucoside	OH	OH
10	Q3G2	OH	OH	Diglucoside	OH	OH
11	Q3G3	OH	OH	Triglucoside	OH	OH
12	Q3G4	OH	OH	Tetraglucoside	OH	OH
13	Q3G5	OH	OH	Pentaglucoside	OH	OH
14	Q3G6	OH	OH	Hexaglucoside	OH	OH
15	Q3G7	OH	OH	Heptaglucoside	OH	OH
16	Q3G8	OH	OH	Octaglucoside	OH	OH
17	Methyl quercetin (Tamarixetin)	OH	O-Methyl	OH	OH	OH
	Methyl quercetin (Rhamnetin)	OH	OH	OH	OH	O-Methyl
	Methyl quercetin (Isorhamnetin)	O-Methyl	OH	OH	OH	OH
18	Quercetin glucuronide	OH	OH	Glucuronide	OH	OH
19	Quercetin diglucuronide	OH	OH	Glucuronide	OH	Glucuronide
20	Methyl quercetin glucuronide	OH	OH	O-Methyl	OH	Glucuronide
21	Methyl quercetin diglucuronide	O-Methyl	Glucuronide	OH	OH	Glucuronide
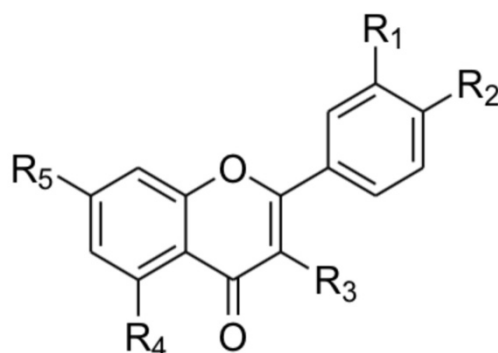
[EDTA] and 20 mM Bis-Tris) was 0.8 mL/min. The concentration of each organic acid was calculated from the peak area of a chromatogram and the calibration curves of standard compounds.

Statistics

Data are presented as means with standard errors of the mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA) with Tukey-Kramer's test. Two-way ANOVA was carried out to verify the effect of the duration of feeding test (week i.e., weeks 2, 4, 6, and 8) and diet treatment (group i.e., Q, QP, QS, and QG) on values of plasma, urine, feces quercetin aglycone as well as quercetin aglycone degradation. $P < 0.05$ was considered as significant. Statistic calculation was performed by JMP version 13.0 (SAS Institute Inc., Cary, NC).

Results

Food intake, body weight, and organ and dry fecal weights

In Table 2.4, the final body weight in the QP group tended to be slightly lower than the other groups. The lower amount of daily food intake appeared in dietary fiber-fed groups, however, there was no difference between the groups. A relative weight (grams per 100 g body weight) of the livers showed no change following dietary fiber treatment, but that of the epididymal fat pad was lower in the QS group than the control group.

Table 2.4 Initial and Final Body Weight, Average Food Intake, and Organ Weights of Rats Fed a Sucrose-Based AIN-93G Diet (C) with 0.7 % AGIQ (Q) with 5% Pectin (QP) or Soybean Fiber (QS) or Guar Gum (QG).

Group	initial BW (g)	final BW (g)	food intake (g/day)	liver (g/100g BW)	epididymal fat pad (g/100g BW)
C	162 ± 3	426 ± 11	19.46 ± 0.58	0.79 ± 0.02	1.51 ± 0.16[a]
Q	162 ± 2	432 ± 14	19.53 ± 0.66	0.80 ± 0.01	1.31 ± 0.03[ab]
QP	162 ± 2	405 ± 15	18.31 ± 0.38	0.71 ± 0.01	1.34 ± 0.07[ab]
QS	162 ± 2	425 ± 13	18.52 ± 0.56	0.73 ± 0.02	1.12 ± 0.07[b]
QG	162 ± 2	432 ± 14	18.87 ± 0.53	0.78 ± 0.02	1.34 ± 0.05[ab]

Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.

Weights of cecum tissues and cecum contents were significantly higher in QS and QG groups, but not in the QP group, than the non-dietary fiber supplemented groups (C and Q). Maximum weight was reported for the samples from the QS group (Table 2.5). The pH of cecal contents for samples from dietary fiber supplemented (QP, QS, and QG) groups was significantly lower than the non-dietary fiber supplemented groups (C and Q) groups. These results suggested that cecal fermentation was promoted by these dietary fibers. The amounts of total organic acids, which are the sum of succinic, lactic, acetic, propionic, iso-butyric, and n-butyric acids. According to this result, the acetic and propionic acids were higher in the QS group than other groups (Figure 2.1).

Table 2.5 Whole Cecum, Cecum Tissue, and Content Weight and pH Value of the Cecal's Rats Fed a Sucrose-based AIN-93G Diet (C) with 0.7 % AGIQ (Q) with 5% Pectin (QP) or Soybean Fiber (QS) or Guar Gum (QG).

Group	Whole cecum weight (g/100 g BW)	Cecum tissue weight (g/100 g BW)	Content weight (g/100 g BW)	pH
C	0.77 ± 0.01^{c}	0.23 ± 0.01^{d}	0.54 ± 0.07^{c}	6.7 ± 0.1^{a}
Q	0.82 ± 0.04^{c}	0.27 ± 0.01^{c}	0.55 ± 0.05^{c}	6.6 ± 0.1^{a}
QP	0.97 ± 0.07^{bc}	0.30 ± 0.01^{bc}	0.67 ± 0.12^{c}	6.1 ± 0.0^{b}
QS	3.35 ± 0.45^{a}	0.41 ± 0.01^{a}	2.94 ± 0.25^{a}	6.1 ± 0.1^{b}
QG	1.88 ± 0.15^{b}	0.35 ± 0.02^{b}	1.53 ± 0.12^{b}	6.1 ± 0.0^{b}

Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.
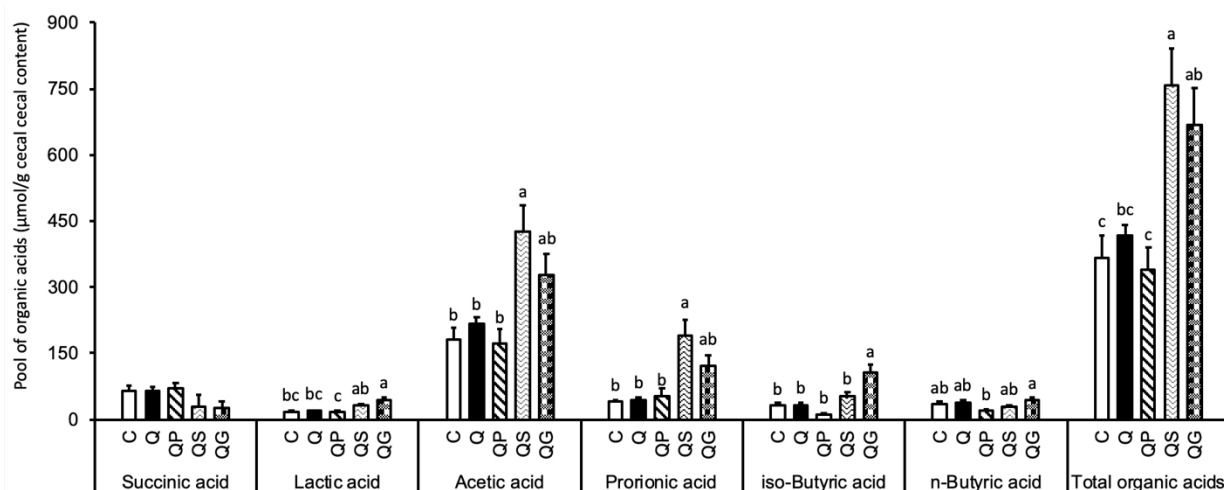
Figure 2.4 Pools of organic acids in cecal contents of rats fed a sucrose-based AIN-93G (C) diet with 0.7 % AGIQ (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Total organic acids (sum of succinic, lactic, acetic, propionic, iso-butyric and n-butyric acids). Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly (*P* < 0.05) by Tukey-Kramer's post hoc test.

Dry weights of feces (Table 2.6) collected on weeks 2, 4, 6, and 8 showed lower values in QP, QS, and QG groups than C and Q groups, suggesting that dietary fibers added to diets were similarly degraded by intestinal bacterial fermentation.

Table 2.6 Total Dry Fecal Weight of Rats Fed a Sucrose-based AIN-93G Diet (C) with 0.7 % AGIQ (Q) with 5% Pectin (QP) or Soybean fiber (QW) or Guar Gum (QG).

Group	Total dry fecal weight (g/3days)			
	Week 2	Week 4	Week 6	Week 8
C	5.22 ± 0.36[a]	5.46 ± 0.35[a]	5.38 ± 0.25[a]	5.51 ± 0.15[a]
Q	4.93 ± 0.19[a]	5.49 ± 0.30[a]	5.64 ± 0.29[a]	5.87 ± 0.24[a]
QP	2.59 ± 0.26[b]	3.23 ± 0.16[b]	3.10 ± 0.24[b]	3.53 ± 0.20[b]
QS	2.00 ± 0.18[b]	2.28 ± 0.15[b]	3.31 ± 0.20[b]	3.02 ± 0.19[b]
QG	2.23 ± 0.14[b]	3.23 ± 0.28[b]	3.11 ± 0.39[b]	3.42 ± 0.29[b]

Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly (*P* < 0.05) by Tukey-Kramer's post hoc test.

Total quercetin derivatives in plasma

In plasma, the concentrations of methylquercetin derivatives were higher than those of quercetin derivatives (Figure 2.2). On week 2, a combination of AGIQ and each dietary fiber elevated the concentrations of the plasma quercetin derivatives. From weeks 2 to 6, plasma total quercetin derivatives gradually decreased in all groups. On weeks 6 and 8, all groups of quercetin-fed rats showed steady levels of total quercetin derivatives, with the highest value reported for the QS group. Two-way ANOVA results revealed that both duration of feeding the test diets and each diet treatment influenced the plasma concentrations of total quercetin derivatives and showed significant interaction (Week × Group: $P < 0.0001$).
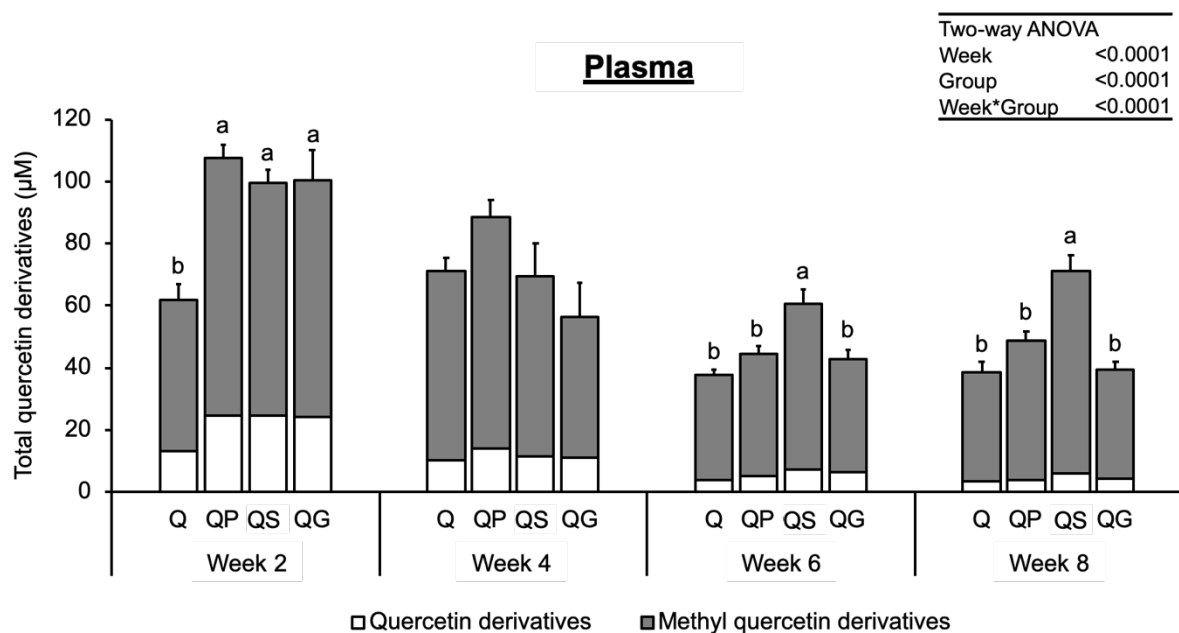

Figure 2.5 Plasma concentration of quercetin and methyl quercetin derivatives in the tail blood of rats fed the sucrose-based AIN 93G diet with 0.7 % AGIQ (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.

Total quercetin derivatives in urine

In the urine samples collected on week 2, the level of total quercetin derivatives was significantly higher in the QG group than the Q group. Both QS and QG groups had significantly higher levels of total derivatives in urine than the Q group on week 4 (Figure 2.3). From weeks 4 to 8, urinary levels of total quercetin gradually decreased in all groups. Urinary quercetin level in the QS group was higher than that in all other groups on weeks 6 and 8. The fluctuation in total quercetin excretion was influenced by both feeding period and dietary treatment, as per two-way ANOVA results, and showed significant interactions (Week × Group: $P = 0.0066$).
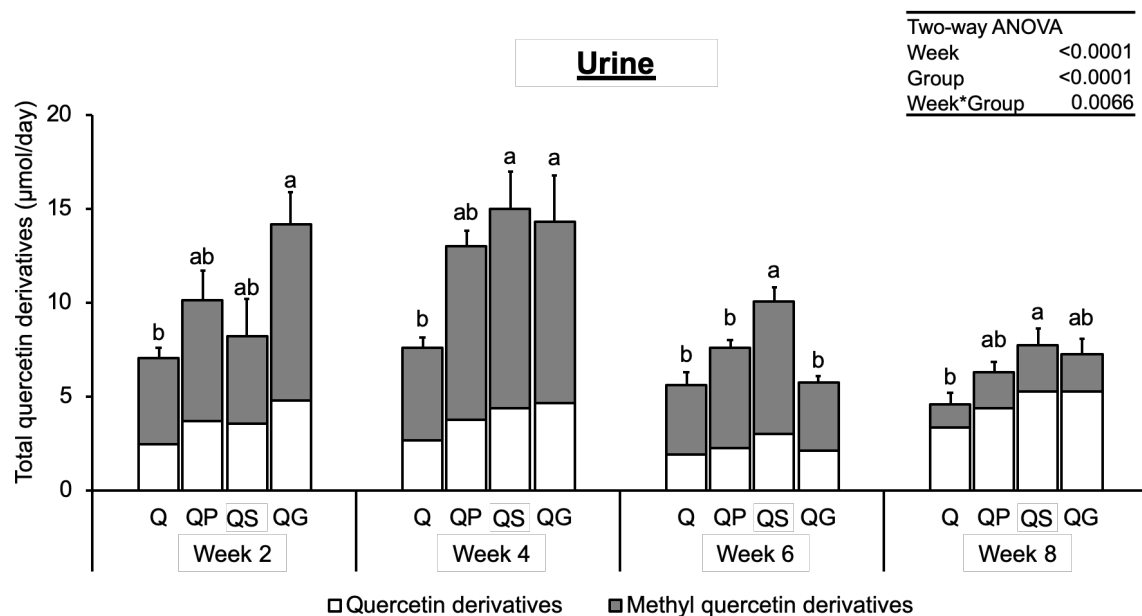

Figure 2.6 Urinary excretion of quercetin and methyl quercetin derivatives of rats fed the sucrose-based AIN-93G diet with 0.7 % AGIQ (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.

Total quercetin derivatives in feces

During an 8-week feeding period, significantly higher total quercetin derivatives excretion than the Q group was observed in the QP group, while ingestion of soybean fiber (QS group) improved total quercetin excretion from week 4 to week 8 (Figure 2.4). QG group showed an increasing tendency of fecal total quercetin throughout the experiment. According to two-way ANOVA results, dietary treatment, but not the feeding period, influenced the fecal total quercetin derivatives.
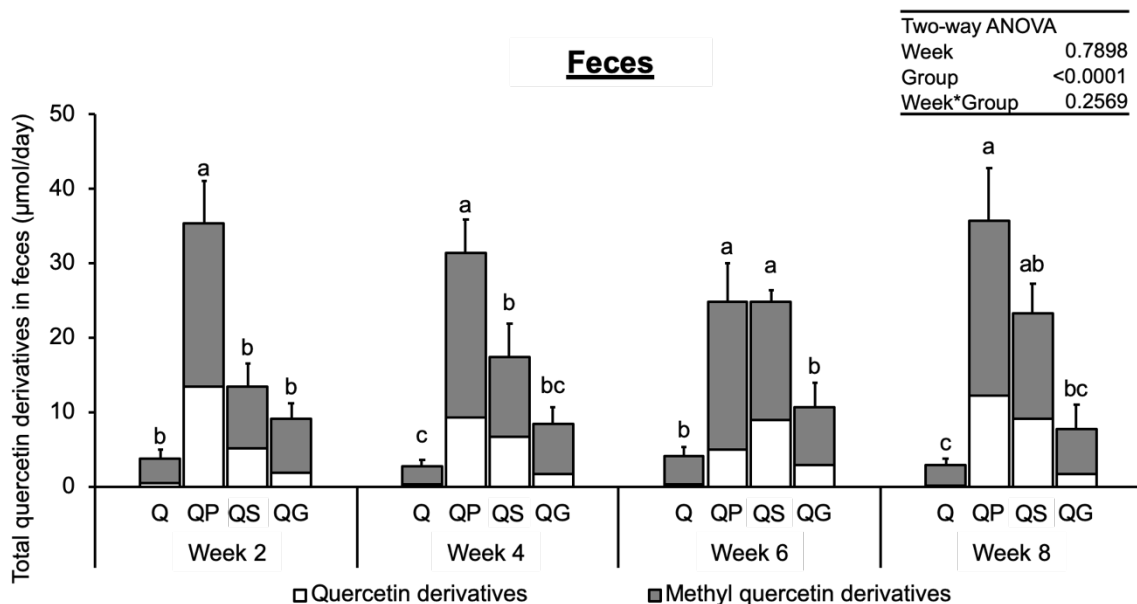

Figure 2.4 Fecal excretion of quercetin and methyl quercetin derivatives of rats fed the sucrose-based AIN-93G diet with 0.7 % AGIQ (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.

In addition, quercetin glucuronides in feces were almost undetectable in the Q group, but were detectable in QP and QS groups (Figure 2.5). In particular, total glucuronide levels (quercetin glucuronides and methylquercetin glucuronides) gradually increased in the QS group and were higher than in the other groups.
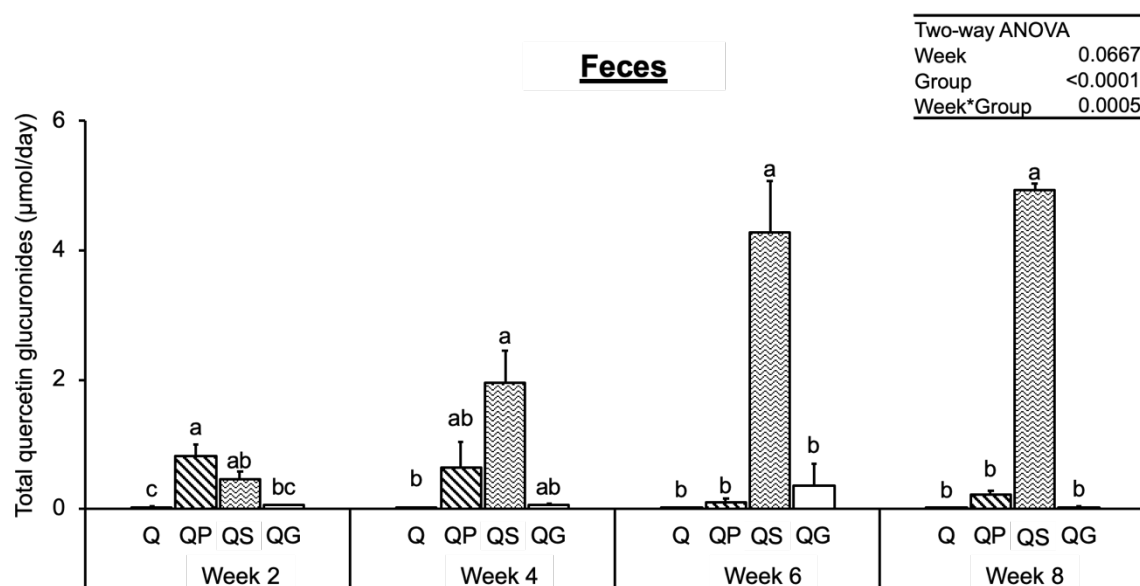

Figure 2.5 Fecal excretion of quercetin and methyl quercetin monoglucuronide metabolites of rats fed the sucrose-based AIN-93G diet with 0.7 % AGIQ (Q) with 5% pectin (QP) or soybean fiber (QS) or guar gum (QG). Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.

Degradation of quercetin and methylquercetin derivatives

The amounts of quercetin and methylquercetin derivatives excreted in urine and feces samples were lower than the levels of ingested quercetin, suggestive the large amounts of quercetin aglycones were degraded by intestinal bacteria (Table 2.7). The amount of quercetin and methylquercetin derivatives degraded was calculated by subtracting the sum of aglycone excreted in urine and feces from the total ingested aglycone. The difference between ingested and degraded aglycone is excreted aglycone that survived from intestinal bacteria degradation. The dietary fiber-fed groups (QP, QS, and QG) had, at least, nearly two times of excreted aglycone percentage as the individually feeding AGIQ group. The values of the QP group were the highest in the experiment (approximately five times higher than the Q group). Notably, the degradation ratio was suppressed in all dietary fiber supplemented groups and the lowest value was reported for the QG group. These differences were maintained during the 8-week feeding period, which was supported by the absence of any significant interaction between period and dietary treatment as analyzed with two-way ANOVA.

Table 2.7 Amount of Quercetin Aglycone Degraded per Day and Rate (%) of Excreted Aglycone in Rats Fed Test Diet Containing a Sucrose-based AIN-93G diet with 0.7 % AGIQ (Q) with 5% Pectin (QP) or Soybean Fiber (QS) or Guar Gum (QG).

Group	Ingested aglycone (μmol/day)	Excreted aglycone* (μmol/day)	Excreted aglycone** (%)	Degraded aglycone*** (%)
Week2				
Q	195.91 ± 5.44^a	6.69 ± 1.67^b	3.42 ± 0.85^b	96.58 ± 0.85^a
QP	177.42 ± 7.46^b	33.65 ± 4.97^a	18.97 ± 2.82^a	81.03 ± 2.82^b
QS	171.72 ± 5.07^b	11.37 ± 2.64^b	6.65 ± 1.60^b	93.35 ± 1.60^a
QG	169.73 ± 3.60^b	12.77 ± 1.66^b	7.59 ± 1.07^b	92.41 ± 1.07^a
Week4				
Q	202.77 ± 7.75	6.22 ± 0.99^c	3.12 ± 0.53^c	96.88 ± 0.53^a
QP	187.39 ± 4.57	32.76 ± 3.43^a	17.63 ± 1.98^a	82.37 ± 1.98^c
QS	188.23 ± 5.54	17.10 ± 2.72^b	9.20 ± 1.57^b	90.80 ± 1.57^b
QG	189.62 ± 4.74	13.59 ± 2.88^{bc}	7.13 ± 1.52^{bc}	92.87 ± 1.52^{bc}
Week6				
Q	206.87 ± 7.51	6.23 ± 1.20^c	2.97 ± 0.53^c	97.03 ± 0.53^a
QP	193.36 ± 5.18	26.14 ± 3.59^a	13.52 ± 1.72^a	86.48 ± 1.72^c
QS	193.75 ± 5.71	19.40 ± 1.30^{ab}	10.08 ± 0.78^{ab}	89.92 ± 0.78^{bc}
QG	197.56 ± 5.22	11.38 ± 2.99^{bc}	5.84 ± 1.57^{bc}	94.16 ± 1.57^{ab}
Week8				
Q	209.19 ± 7.54	5.40 ± 1.02^b	2.55 ± 0.43^b	97.45 ± 0.43^a
QP	195.79 ± 4.80	34.28 ± 5.91^a	17.33 ± 2.86^a	82.67 ± 2.86^b
QS	197.60 ± 6.05	17.75 ± 2.29^b	9.13 ± 1.31^b	90.87 ± 1.31^b
QG	201.56 ± 5.02	10.03 ± 3.45^b	4.98 ± 1.71^b	95.02 ± 1.71^b
Two-way ANOVA				
Week	<0.0001	0.8679	<0.0001	0.6599
Group	0.0004	<0.0001	<0.0001	<0.0001
Week×Group	0.9299	0.4457	0.8832	0.4099

Values are mean ± SEM (n = 7) and not sharing a common letter differ significantly ($P < 0.05$) by Tukey-Kramer's post hoc test.

* Excreted aglycone (μmol/day) = Total aglycone in urine + Total aglycone in feces

** Excreted aglycone (%) = (Excreted aglycone/ Ingested aglycone) x 100

*** Degraded aglycone (%) = [100 - (Excreted aglycone / Ingested aglycone)] × 100

Discussion

The effects of three water-soluble dietary fibers on the bioavailability of a quercetin glucoside in rats were determined by monitoring improvements in quercetin derivative concentrations in plasma, urine, and feces samples. The results of plasma and urine quercetin levels suggest three phases in quercetin metabolism, including cumulative (~week 2), transient (week 4), and stable (week 6-8) phases. At chronic feeding of test diets, soybean fiber showed the greatest improvement in plasma quercetin derivatives among all three supplemented dietary fibers. This increase was due to the reduction in quercetin degradation. It was expected that cecal fermentation is an important factor behind this change. In addition, fecal quercetin glucuronide excretion was high in soybean fiber-fed rats, suggestive of the reduction of β-glucuronidase in the colon. These results may construct the new strategy on promoting the quercetin health beneficial effects.

In this experiment, the daily intake of quercetin glucoside (AGIQ) with individual dietary fibers for 2 weeks increased the plasma concentrations of total quercetin derivatives, suggestive of the enhanced intestinal absorption of the ingested quercetin. However, urinary excretion of total quercetin derivatives, which usually reflects the bioavailability of ingested flavonoids, failed to increase in the QP and the QS groups. On week 2, quercetin absorption may increase after water-soluble fiber feeding and the absorbed quercetin accumulated in blood and tissues in these groups. Mullen *et al*. have reported the accumulation of radio-labeled quercetin in tissues [Mullen, 2008]. In contrast, urinary excretion of quercetin derivatives in QG-fed rats largely increased on week 2. Quercetin in the body was probably saturated in the QS group earlier than other dietary fiber groups due to higher absorption of quercetin. The greater effect of guar gum on the absorption of quercetin glycosides is not known, however, the fermentable rate of guar gum was expected to be faster than pectin and soybean fiber on at the early phase (week 2). On week 4, urinary excretion of quercetin in the supplemented dietary fiber (QP, QS, and QG) groups tended to be or was largely increased, but the increased plasma quercetin concentrations disappeared on week 6 except in the QS group. These results suggest that week 4 is a transient phase of increased total quercetin derivatives. From week 6 to 8, levels of plasma quercetin and urinary excretion reduced but stabilized, with significantly higher levels observed in the QS group than the Q group. On week 6 of all groups, the reduction of quercetin derivatives in both plasma and urinary levels suggests the reduction of quercetin bioavailability that possibly be associated with the increasing degradation of quercetin aglycone. However, the increment in fecal excretion of quercetin from week 4 to week 6 in the QS group was found.

The reduction of intestinal absorption of quercetin through prolonged feeding of AGIQ by a feedback mechanism was thought to be associated with the reduction of quercetin in plasma and urinary excretion on week 6.

In recently, some intestinal bacteria catabolize are able to convert quercetin aglycone to phenolic acids such as 3-(3,4-dihydroxyphenyl) propionic acid and 3,4-dihydroxy-phenylacetic acid through the ring scission process [Aura, 2002; Marin, 2015]. These degraded products are known to have strong anti-oxidative properties and also effective for the prevention of many diseases, which depend on anti-oxidative ability [El-Ansary, 2013; Raneva 2001]. However, quercetin has more various actions independent of anti-oxidative properties such as strong interaction with cell-signaling proteins [Suzuki, 2009]. Some researchers reported that phenolic acids possessed a lower ability to suppress carcinogenesis compared with quercetin aglycone [Kern 2007; Skrbek, 2009]. However, an adaptation of intestinal bacteria to long-term feeding of quercetin may increase the levels of the enzymes that decompose quercetin aglycone mainly in the cecum [Moco, 2012; Wu, 2011].

An increase in the fecal excretion of quercetin derivatives following pectin (QP) and soybean fiber (QS) diet feeding was found, this probably due to the suppression of quercetin degradation. The amount of excreted aglycone, the restoration rates of quercetin aglycone through the avoidance of bacteria degradation in rats, largely increased in the QP and the QS groups on weeks 6 and 8. A possible explanation for this observation is that dietary fibers were used as an energy source instead of quercetin by cecal bacteria [Hervert, 2011; Palafox, 2011]. *Bacteroidetes* spp. are known to upregulate the expression of several genes related to carbohydrate-metabolizing enzymes and may use ingested dietary fiber as substrate [Flint, 2012]. The suppression of bacterial degradation of quercetin aglycone may be responsible for the constant high levels of quercetin in plasma and urinary excretion in the QS group. In the pectin-fed group, no increase in quercetin levels in plasma and urine was observed for the maximum amount of available aglycone. The high viscosity property of pectin in the intestinal lumen possibly suppressed quercetin absorption in the small intestine, and this property might also contribute to the low bioavailability in guar gum-fed rats during the late phases.

Supplementation with fibers, especially soybean fiber, largely and gradually increased the fecal excretion of quercetin and methylquercetin glucuronides. This finding indicates that soybean fiber suppressed the deconjugation of quercetin glucuronides. Cermak et al. reported the transformation of the conjugated metabolites of quercetin to aglycone by certain strains of *Pediococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., and *Bacteroides* spp [Cermark, 2006]. Rowland et al. also found a significant increase in β-

glucuronidase activity in rats fed with a 5% pectin diet [Rowland, 1983], consistent with the results of the present study that glucuronide levels in feces reduced from week 4 to 6 in the pectin group (QP). In the QS group, fecal glucuronide levels were higher than those in other groups and further increased after long-term dietary fiber ingestion. Bacterial β-glucuronidase activity in the large intestine is one of the strong inducers of colon cancer [Kim, 2001]. Ingested soybean fiber may suppress the proliferation of β-glucuronidase-producing bacteria or inhibit β-glucuronidase activity in the large intestine. Therefore, feeding soybean fiber may serve as a strategy to prevent colon cancer.

In the present study, it was hypothesized that ideal dietary fibers may enhance quercetin bioavailability by increasing cecal fermentation and suppressing quercetin degradation. Pectin showed the highest ability to suppress bacterial degradation of quercetin aglycone and seemed to be the best candidate for enhancing blood levels of quercetin, but it was true only in the early phase of feeding (week 2). Among the three water-soluble and fermentable dietary fibers experimented herein for long-term feeding, soybean fiber is the most efficient at increasing quercetin bioavailability. Soybean fiber is known to increase mucosal surface area with the expansion of the cecal wall [Hara, 2010], consistent with the increase in cecal contents and tissue weight observed in the present study. Soybean fiber has relatively low viscosity as compared with pectin and guar gum [Rideout 2008; Takahashi, 1999] that may have contributed to the prolonged effects on quercetin bioavailability.

In summary, this study provides novel insights into the potential of water-soluble dietary fibers, especially soybean fiber, to enhance quercetin bioavailability following the long-term feeding of rats. Duration of feeding is an important factor to modulate these abilities of dietary fibers. The findings of the present study suggest that dietary fibers may promote the actions of quercetin on prevention of diseases.

Chapter 3: Combination of α-glycosyl-isoquercitrin and soybean fiber promotes quercetin bioavailability and glucagon-like peptide-1 secretion and improves glucose homeostasis in rats fed a high-fat high-sucrose diet

Abstract

The present study examined the effects of a combination of soybean fiber and α-glycosyl-isoquercitrin (AGIQ) on improving quercetin bioavailability and glucose metabolism in rats fed an obesogenic diet. For 9 weeks, rats were individually fed a control diet, a high-fat high-sucrose (H) diet, H with soybean fiber (HS), or with AGIQ (HQ), or with both (HSQ). Quercetin derivatives in plasma, feces, urine, and cecal content were quantified by high-performance liquid chromatography to assess the bioavailability of quercetin, and meal tolerance tests were performed to assess postprandial glycemia and glucagon-like peptide-1 (GLP-1) responses. The HSQ group had higher plasma quercetin levels than HQ. The postprandial glycemia was attenuated in the HSQ group when compared to the H group. The basal plasma GLP-1 concentrations positively correlated with plasma quercetin derivative concentrations. Hence, the combination of soybean fiber and AGIQ could be beneficial for reducing the risk of glucose intolerance, possibly involving enhanced quercetin bioavailability and GLP-1 secretion.

Introduction

Quercetin is a flavonoid belonging to the flavonol class and is widely distributed in various kinds of vegetables and fruits, mainly in glycosylated form [Wach, 2007]. Reportedly, quercetin possesses protective effects against numerous diseases such as cancer in *in vitro* and animal studies; [Srivastava, 2016] cardiovascular diseases in animal and human studies;[Patel, 2018] obesity in *in vitro* high-fat diet-fed animal and obese human studies; [Seo, 2015; Zhao, 2017] and diabetes in db/db mice and streptozotocin-induced rat studies [Shi, 2019]. Numbers of *in vitro* and animal studies suggest the beneficial potential of quercetin, but studies demonstrating its efficacy in human subjects are limited [Gao, 2011; Zhao, 2017].

After ingestion, hydrolysis of glycoside into aglycone by the intestinal enzyme is thought to be an essential step for its absorption into intestinal epithelial cells. After absorption, the conjugation for quercetin metabolites mainly occurred in the liver. However, intestinal absorbability of quercetin is known to be low [Gao, 2011; Patel, 2018; Seo, 2015; Shi, 2019; Srivastava, 2016; Zhao, 2017]. Concomitantly, unabsorbed quercetin aglycone and some metabolites were transported to the large intestine, the main site of gut fermentation [Mullen, 2008]. In here, the intestinal bacteria with various enzymatic activities can deconjugate (transform) quercetin metabolites and further degrade the quercetin aglycone, which results in loss of biological activities from the original compound [Almeida, 2018]. An *in vitro* fermentation study revealed that quercetin can be degraded by anaerobic pathogens. Along with this degradation, a reduction in antioxidant activities and an increment in a degraded product of quercetin, 3,4-dihydroxyphenylacetic acid, were demonstrated [Peng, 2014]. Thus, the degradation by large intestinal bacteria and poor intestinal absorption of quercetin limit the bioavailability of quercetin and the health beneficial effects of this flavonol.

From previous chapter, it was found that soybean fiber is the best candidate, among other tested water-soluble dietary fibers such as pectin and guar gum, to improve quercetin bioavailability in a long-term feeding study performed in rats [Trakooncharoenvit, 2020]. This study has revealed that cecal fermentation promoted by feeding the dietary fiber is linked with the bioavailability of quercetin. Accordingly, it was expected that the increased quercetin bioavailability would prevent the impairment of glucose homeostasis induced by chronic excessive energy intake.

Currently, type 2 diabetes is turning into an epidemic worldwide. [Murakami, 2020] This disease gradually develops owing to impaired glucose homeostasis, composed of glucose intolerance and insulin resistance [Cavaghan, 2000]. For the treatment of type 2 diabetes, incretin-based therapy has been developed and successfully used [Drucker, 2010]. Glucagon-like peptide-1 (GLP-1) is an incretin hormone released by enteroendocrine L-cells in response to luminal nutrients [Holst, 2007]. GLP-1 plays a crucial role in glucose homeostasis by enhancing insulin secretion in a glucose-dependent manner and possibly promoting the beta-cell mass [Drucker, 2018]. Furthermore, we have demonstrated the protective role of enhanced GLP-1 secretion during the development of glucose intolerance in diet-induced obese rats [Pinyo, 2019a]. Recently, manipulating GLP-1 secretion by various strategies has been gaining momentum as a promising strategy for preventing glucose intolerance [Tsuda, 2015; Tolhurst, 2009]. In previous study using rats, the combination of a fructo-oligosaccharide and quercetin-3-O-β-d-glucoside (isoquercitrin) demonstrated an improvement in glucose tolerance and insulin sensitivity, with increased GLP-1 secretion and plasma quercetin concentration [Phuwamongkolwiwat, 2014].

Thus, this study aimed to clarify whether the combined ingestion of α-glycosyl-isoquercitrin (AGIQ) and soybean fiber promotes quercetin bioavailability, affects GLP-1 secretion, and prevents glucose intolerance in rats continuously fed an obesogenic high-fat high-sucrose diet.

Materials and Methods

Animals and Diets

Thirty-five male Wistar-ST rats 5 weeks old, weighing about 130–150 g (purchased from Japan SLC, Inc., Shizuoka, Japan), were housed in individual stainless-steel cages with wire mesh bottoms. Rats were maintained in a temperature-controlled room (22 ± 2 °C) with a 12 h light–dark cycle (light period 08.00–20.00) and fed using a modified American Institute of Nutrition-93G (AIN-93G) diet [Reeves, 1993] by changing the cellulose content from 5 to 7% (2% of cellulose substituted with corn starch, Table 3.1). In the test diet, α-glycosyl isoquercitrin (AGIQ, kindly provided by San-Ei Gen F.F.I., Inc., Osaka, Japan), mainly consisting of quercetin-3-O-β-d-glucoside (Q3G, 31.8%), mono (23.3%), and di (20.3%) D-glucose adducts with an α-1,4-linkage, was used as a quercetin source. Soybean fiber (kindly provided by Fuji Oil Co., Ltd., Osaka, Japan), a low viscosity highly fermentable fiber, was used as supplemental fiber. After 1

week of acclimation, the rats were divided into five groups ($n = 7$) based on body weight and basal plasma glucose concentration. For 9 weeks, each group of rats was, respectively, fed the modified AIN-93G as a control (C) diet, a high-fat high-sucrose (H) diet containing 30% fat and 40% sucrose, [Nakajima 2015; Pinyo, 2019a] a high-fat high-sucrose diet with either soybean fiber (HS) or AGIQ (HQ), and high-fat high-sucrose with both soybean fiber and AGIQ (HSQ) ad libitum. The supplementary doses of AGIQ and soybean fiber were selected based on a previous study [Trakooncharoenvit, 2020]. In addition, the usage of higher concentrations (0.7%) of quercetin in both mice and human studies had been reported [Stewart, 2008; Williamson, 2005]. Body weights and food intakes were measured every 1 or 2 days. Nine weeks after the start of test feeding, rats were fasted overnight and euthanized by whole blood withdrawal. Furthermore, the visceral adipose tissue (mesenteric, retroperitoneal, and epidydimal adipose tissue) was carefully dissected and weighed. The whole cecum was collected and stored at −80 °C until measurement. The experiments were conducted according to the guidelines of the Hokkaido University for the care and use of laboratory animals, and the study was approved by the Hokkaido University Animal Committee (approval reference number: 19-0072).

This study was designed to elucidate the impact of soybean fiber on quercetin bioavailability and glycemic response in a diet-induced obesity rat model, together with the role of GLP-1 in insulin secretion that is well established under normal conditions. Therefore, the effect of soybean fiber and/or AGIQ was examined only in a high-fat high-sucrose diet (not in the control diet-fed rats).

Table 3.1 Experimental Diets Composition

Ingredients (g/kg)	Normal diet	High-fat high-sucrose diets			
	C	H	HS	HQ	HSQ
Corn starch [a]	377.5	-	-	-	-
Casein [b]	200	200	200	200	200
Dextrinized corn starch [c]	132	-	-	-	-
Soybean oil	70	70	70	70	70
Sucrose	100	379.5	379.5	372.5	372.5
Lard oil	-	230	230	230	230
Cellulose [d]	70	70	20	70	20
Mineral (AIN-93G-MX) [e]	35	35	35	35	35
Vitamin (AIN-93-VX) [e]	10	10	10	10	10
L-Cystine	3	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
Tertiary butylhydroquinone	0.014	0.014	0.014	0.014	0.014
AGIQ (Q) [f]	-	-	-	7	7
Soybean fiber (S) [g]	-	-	50	-	50
Total	1000	1000	1000	1000	1000
Energy per gram diet (kcal/g)	4.04	5.19	5.19	5.16	5.16

[a] Amylalpha (Chuo Foods Co., Ltd., Aichi, Japan)
[b] Acid casein (Fonterra, Ltd., Tokyo, Japan)
[c] TK-16 (kindly supplied by Matsutani Chemical Industry Co., Ltd., Hyogo, Japan)
[d] Avicel PH102 (Asahi Kasei Chemicals Corporation, Tokyo, Japan)
[e] Mineral and vitamin mixtures were prepared according to the AIN-93G formulation.
[f] α-Glycosyl isoquercitrin (AGIQ; Q, kindly supplied by San-Ei Gen F.F.I., Inc., Osaka, Japan)
[g] Soybean fiber (kindly supplied by Fuji Oil Co., Ltd., Osaka, Japan)

Cecal Content Treatment for pH and Short-Chain Fatty Acid Measurement

The frozen cecum was separated into cecal tissue and contents. The contents were diluted with four times weight of deionized water, and the pH was measured using a pH meter (B-211 twin waterproof pH, Horiba, Ltd., Kyoto, Japan). The remaining diluted sample (700 μL) was mixed with 200 μL of the internal standard (25 mM crotonic acid in 50 mM sodium hydroxide) and centrifuged at 15600g for 10 min at 4 °C. The supernatant (600 μL) was mixed with the same volume of chloroform and centrifuged again. The supernatant was filtrated through a 0.2 μm

membrane followed by short-chain fatty acid measurement using high-performance liquid chromatography (HPLC) [Hara, 1994; Hira, 2018] with two shim-pack SCR-102H (8 mm I.D. × 300 mm L, Shimadzu Corp., Kyoto, Japan) and a conductivity detector CCD-6A (polarity: +, response: slow, temperature: 45 °C; Shimadzu Corp., Kyoto, Japan).

Sample Treatment for Quercetin Measurement

At weeks 2, 4, 6, and 8 of the feeding periods, urine and feces were collected for 2 and 3 days, respectively. On the day after feces collection, tail vein blood samples were collected into a tube containing heparin (final concentration of 50 IU/mL blood; Ajinomoto Company, Inc., Tokyo, Japan) and aprotinin (final concentration of 500 kallikrein inhibitor units (kIU)/ml blood; Wako Pure Chemical Industries, Ltd., Osaka, Japan). The collected blood samples were centrifuged at 2300g for 10 min at 4 °C to separate the plasma before storage at −80 °C. During urine collection, bacterial degradation of quercetin metabolites was prevented by adding 0.05% sodium azide (Sigma Aldrich, Saint Louis) into a collection tray. After 2 days, the collected urine was filled up to 100 mL, and 15 mL of diluted urine samples was stored at −40 °C. Fecal samples were collected by placing a steel sieve mesh under the rat cage for 3 days followed by storage at −80 °C. After the urine and feces were collected, the rats were rested for 1 day before overnight fasting, and the meal tolerance test (MTT) or plasma collection was performed on the next day as mentioned above.

On extraction, plasma and urine samples (100 μL) were acidified with 10 μL of 0.58 mol/L acetic acid. In both samples, quercetin and its metabolites have been detected in substantial amounts as conjugate metabolites [Manach, 1997]. To obtain quercetin and methylquercetin aglycone derivatives, 7.5 μL of β-glucuronidase and sulfatase (from *Helix pomatia* extract; Sigma Aldrich, Saint Louis) and 7.5 μL of sulfatase (from *Aerobacter aerogenes*; Sigma Aldrich, Saint Louis), were added and incubated at 37 °C for 1 h. The reaction mixture was mixed with 100 μL of the internal standard (naringenin, 10 μM at final), heated at 100 °C for 1 min, and centrifuged at 9300g for 5 min at 4 °C. The supernatant was collected, and the precipitate was twice re-extracted with methanol to recover aglycone of quercetin derivatives in the precipitant. The collected supernatant was loaded into a C18 cartridge (Oasis HLB; Waters Co. Ltd., Massachusetts) and eluted with 1 mL of methanol.

For feces, frozen samples were dried and milled into a powder. The feces powder (0.1 g) was mixed with 1 mL of 80% methanol and 100 μL of internal standard (naringenin, 50 μM at

final) and then homogenized using an ultrasonic homogenizer (VP-050, ULTRAS homogenizer; Taitec Co. Ltd., Nagoya, Japan) with 30% power for 20 s. The homogenized sample was incubated at 60 °C for 1 h and centrifuged at 2300g for 10 min at 4 °C. This extraction procedure was repeated twice. In the case of cecal contents, 1 g of the content was suspended in 5 mL of deionized water. The suspended sample (100 μL) was mixed with 100 μL of internal standard (naringenin, 40 μM at final) and heated at 100 °C for 1 min. The supernatant was collected by centrifugation at 9300g for 5 min at 4 °C. This extraction procedure was repeated two times by adding methanol instead of the internal standard as in the previous step. The accumulated supernatant, feces, or cecal content was loaded into a C18 cartridge similar to plasma and urine samples. As fecal and cecal contents usually appear in aglycone form, these samples were extracted without deconjugation enzyme treatment. All eluted samples were dried by evaporation and then reconstituted with 50% methanol on the measurement day.

Quercetin Measurement by HPLC

The sum of quercetin aglycone and methylquercetin aglycone was considered as the total quercetin derivative, which was quantified by HPLC [Stefova, 2001] (Thermo Scientific Dionex UltiMate 3000 HPLC system; Thermo Fisher Scientific Inc., Massachusetts). The analyte was separated using a ZORBAX RRHD Eclipse Plus Phenyl-Hexyl C18 (2.1 × 100 mm, 1.8 μm; Agilent Technologies Japan, Ltd., Tokyo, Japan) column with a setting temperature at 50 °C. The mobile phases were methanol with formic acid (99.99:0.01 as solvent A) and water with formic acid (99.99:0.01 as solvent B). For measurement, the linear gradient started with solvents A and B at a ratio of 70:30, steadily changed to solvent B 100% in 5 min (0–5 min), isocratically held for 1 min (5–6 min), and then decreased back to 70:30 within 1 min (6–7 min) followed by re-equilibration for 1 min (7–8 min). Naringenin and quercetin (quercetin aglycone and methylquercetin aglycone) were detected at 280 and 370 nm, respectively. The concentrations of quercetin derivatives were calculated from the peak area based on the calibration curve of standard compounds.

Meal Tolerance Test

An MTT was performed instead of an oral glucose tolerance test because nutrients from a mixed meal can provide relevant postprandial responses regarding glycemia, GLP-1, and insulin secretions [Pinyo , 2019a; Pinyo, 2019b; Pinyo, 2020].

During the feeding period, the MTT was performed after overnight fasting (16 h) every 4 weeks (at weeks 4 and 8). Here, tubes containing anticoagulants, as described in plasma collection for quercetin measurement, were used. Blood samples from the tail vein of rats were collected before (0 min) and after (15, 30, 60, 90, and 120 min) oral gavage administration of the liquid meal (15 kcal/10 mL per kg body weight; Ensure H, Abbott, Japan). The collected blood samples were centrifuged, and then the plasma was separated and stored at −80 °C. The collected plasma was used for glucose, insulin, and total GLP-1 measurements using the Glucose CII Test Kit (Wako Pure Chemical Industries, Osaka, Japan), Insulin ELISA Kit (U-E type; Shibayagi Company Limited, Gunma, Japan), and Total GLP-1 ELISA Kit (Wako Pure Chemical Industries, Osaka, Japan), respectively. All analytical procedures, using the respective test kits, were performed in accordance with the instructions provided with the assay kit.

GLP-1 Measurement in Cecal Tissue

GLP-1 concentrations in cecal tissue were measured as described before [Pinyo, 2020]. Briefly, a segment of cecal tissue was homogenized in a mixture of ethanol:water:12 M HCl (74:25:1, 5 mL/g tissue) and extracted at 4 °C for 24 h. The homogenized sample was centrifuged at 2000g for 20 min at 4 °C. Then, the supernatants were collected and diluted (500-fold) with saline to measure the total GLP-1 concentrations by Multi Species GLP-1 Total ELISA kit (EZGLP1T-36K; Merck Millipore, Darmstadt, Germany).

Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM). Before analyses with statistical tests, the normal distribution of variables and the homogeneity of variances were checked. Differences among treatment groups were analyzed with the Tukey–Kramer's test or Student's t test (described in the legend of each figure) after one-way ANOVA. A P value of less than 0.05 was considered significant. Pearson's correlation coefficients (r) and the significance of

correlations were analyzed between various parameters. The area under the curve (AUC) for postprandial glucose, insulin, and GLP-1 was calculated using the trapezoidal rule. Additionally, the AUC of HOMA-IR [Cacho, 2008; Pinyo, 2019b] was calculated to compute the degree of insulin resistance resulting from the feeding diet as follows:

HOMA-IR AUC = (AUC postprandial glucose (mg/dL×2h) × AUC postprandial insulin (μU/mL×2h))/2430

where 1 mg insulin = 26 IU.

All statistical analyses were performed using the JMP Pro version 13.0 software (SAS Institute, Inc., North Carolina).

Results

Body and Tissue Weights, Energy Intake, and pH of Cecal Content

Although no difference was observed in the energy intake and the final body weight among groups (Figure 3.1a,b), the highest visceral fat pad weight was observed in the H group (Figure 3.1c). Rats fed with a soybean fiber supplemented high-fat high-sucrose diet demonstrated a markedly decreased accumulation of visceral fat when compared with the H group ($P < 0.05$; Tukey–Kramer's test).

Soybean fiber-fed groups (HS and HSQ) had higher cecal tissue and cecal content weights as well as reduced pH of the cecal content (Figure 3.1d–f) when compared to groups without soybean fiber (C, H, and HQ). These results suggested that cecal fermentation was promoted by soybean fiber supplementation.
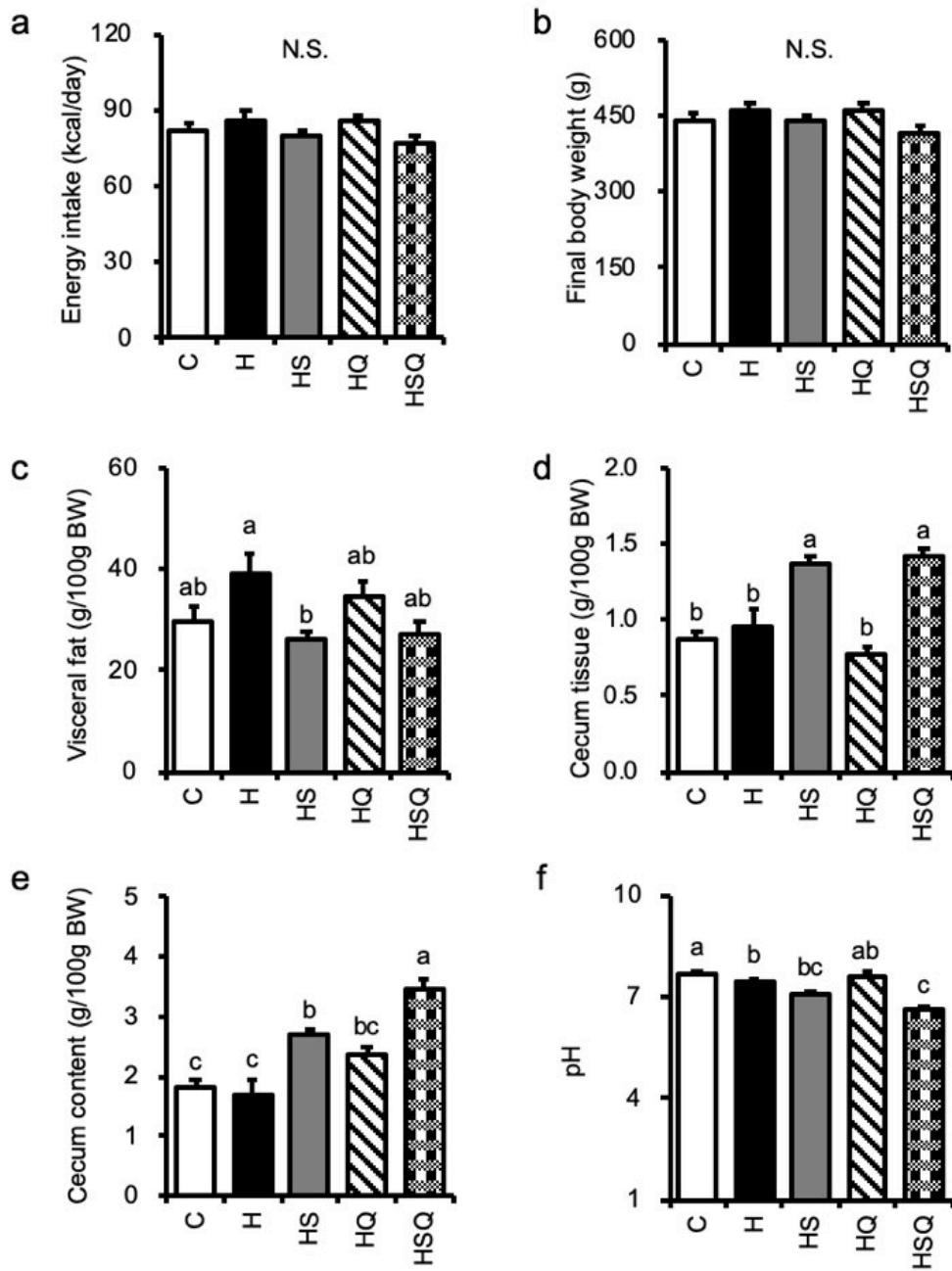
Figure 3.7 (a) Energy intake, (b) final body weight, (c) visceral fat pad weight, (d) cecal tissue weight, (e) cecal content weight, and (f) pH of cecal content after feeding test diets for 9 weeks. Values represent means with standard errors indicated by vertical bars ($n = 7$). Mean values not sharing a common alphabetical letter differ significantly ($P < 0.05$, Tukey-Kramer's post hoc test). N.S. indicates the absence of a significant difference among treatments.

Short-Chain Fatty Acids (SCFAs) in Cecal Content

The amount of SCFAs (Figure 3.2) represents the degree of cecal fermentation and metabolic activity of the intestinal microbiota [Holota, 2019]. A diet with soybean fiber increased the amount of both acetic and propionic acids, with the highest amounts of acetic acid (53.4 ± 2.3 µmol/total cecum) and propionic acid (16.6 ± 2.6 µmol/total cecum) observed in the HSQ group. Correspondingly, an extensive elevation of total SCFAs was also observed in both soybean fiber-fed groups (HS and HSQ).
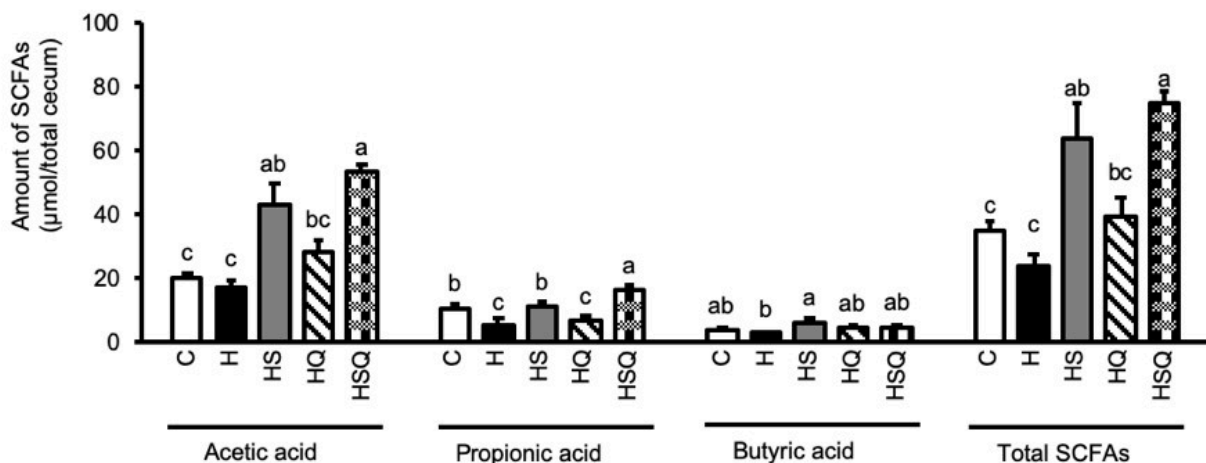
Figure 3.8 Amount of individual/total SCFA(s) of cecal content after feeding test diets for 9 weeks. Values represent means with standard errors indicated by vertical bars (n = 7). Mean values not sharing a common alphabetical letter differ significantly ($P < 0.05$, Tukey-Kramer's post hoc test).

Total Quercetin Derivatives in Plasma, Urine, Feces, and Cecal Content

Quercetin and its derivatives were not detected in C, H, and HS groups (data not shown). The total quercetin derivatives represent the sum of quercetin aglycone and methylquercetin aglycone (isorhamnetin and tamarixetin) derivatives. In rats fed the diet containing AGIQ, almost all the quercetin derivatives detected in the plasma were in methylated form. From week 2 to 8 of the feeding period, the HSQ group showed higher total quercetin derivative levels than the HQ group (Figure 3.3a).

a Plasma

b Urine

c Feces

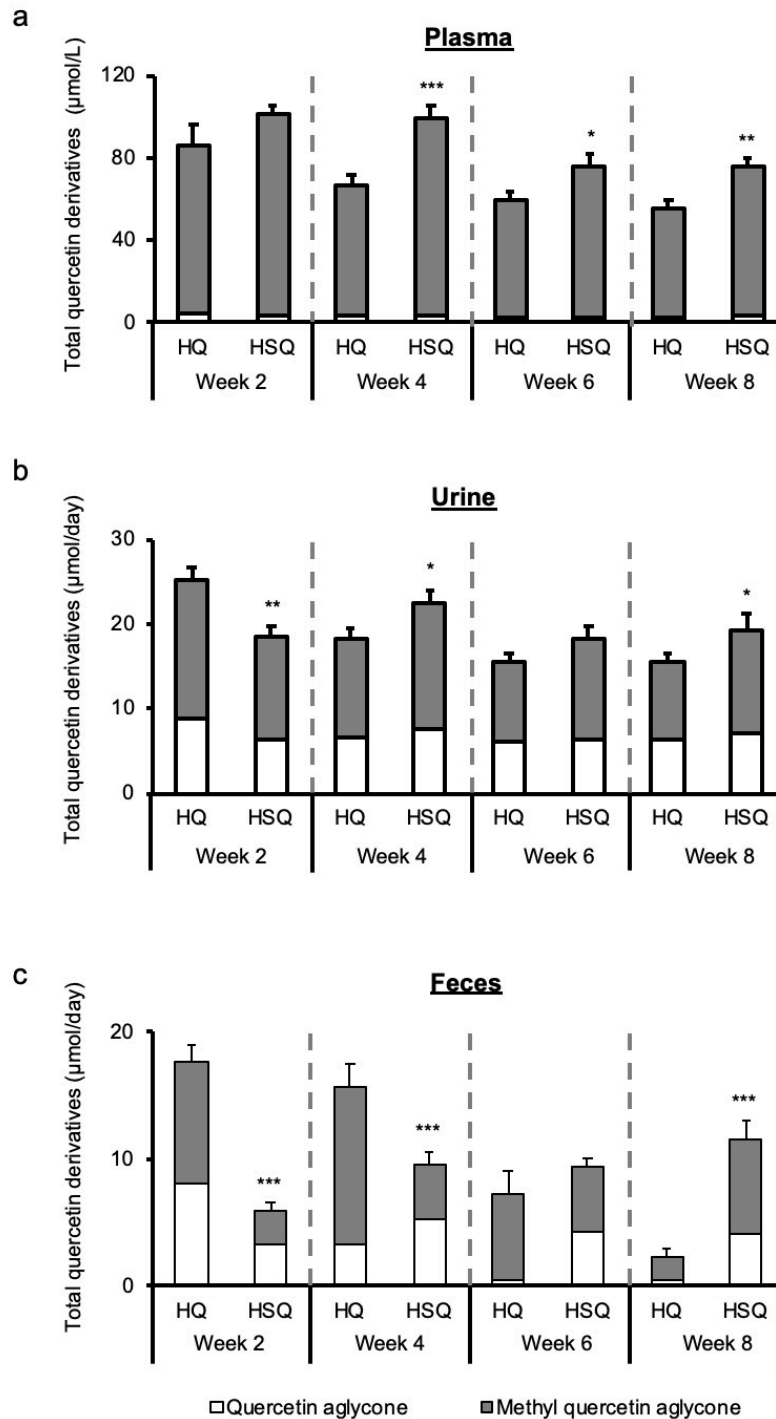☐ Quercetin aglycone   ■ Methyl quercetin aglycone

Figure 3.9 Concentrations or amounts of total quercetin derivatives (quercetin aglycone and methylquercetin aglycone) in the (a) plasma, (b) urine, and (c) feces of rats fed with AGIQ-containing diets (HQ and HSQ) after 2, 4, 6, and 8 weeks. Values represent means with standard errors indicated by vertical bars (n = 7). Asterisks indicate a significant difference between mean values of HQ vs HSQ (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$, Student's t test).

Rats fed with HSQ demonstrated a higher urinal quercetin level from week 4 to 8 (Figure 3.3b) than the HQ group. Regarding the fecal sample, higher quercetin levels were observed in the HQ group from week 2 to 4 than in the HSQ group. The decreased tendency in fecal quercetin derivatives of the HQ group was observed in a later period, while the HSQ group demonstrated a reciprocal trend with a five times greater level than the HQ group at week 8 of the feeding period (Figure 3.3c).

The quercetin content of cecum in HSQ-fed rats was greater (Figure 3.4) than that in the HQ group at the end of the experimental period. In HQ-fed rats, quercetin aglycone levels (0.34 ± 0.18 µmol/cecum) were considerably lower than those observed in HSQ-fed rats (6.5 ± 0.75 µmol/cecum).
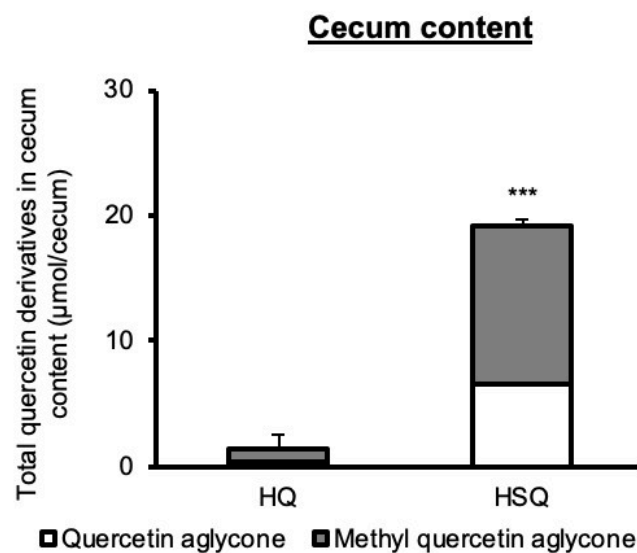

Figure 3.10 Concentration of total quercetin derivatives (quercetin aglycone and methylquercetin aglycone) in the cecal content of rats fed AGIQ-containing diets (HQ and HSQ) after 9 weeks. Values represent means with standard errors indicated by vertical bars (n = 7). Asterisks indicate a significant difference between mean values of HQ vs HSQ (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$, Student's t test).

Degradation of Quercetin and Methylquercetin Derivatives

The effect of test diets on quercetin degradation was calculated as described in Table 3.2 [Matsukawa, 2009]. To determine the precise aglycone levels ingested, we averaged the diet intake data from the day before urine and feces collection until the day after plasma collection (complete week period) for the calculation. In addition, the ingested aglycone weight is calculated by subtracting glycoside weight from ingested quercetin glycoside weight. The sum of excreted

aglycone (quercetin aglycone + methylquercetin aglycone of urine and feces) can be considered as quercetin that escaped from degradation by intestinal bacteria [Hollman, 2004].

In the present study, the HQ group had a higher ingested aglycone weight than the HSQ group. At week 2, the HQ group showed a lower percentage of degraded aglycone, but it gradually rose from week 4 to 8. Markedly, two-way repeated measures ANOVA for degraded aglycone revealed a significant interaction between the duration of feeding period and the diet ($P = 0.0034$).

In contrast, the fluctuations in the degraded percentage in the HSQ group (80–86%) were observed. In addition, the HSQ group demonstrated significantly lower percentage of degraded aglycone compared to the HQ group at week 8 of feeding period. These results indicate that soybean fiber supplementation in long-term feeding could potentially suppress quercetin degradation induced by intestinal bacteria.

Table 3.2 The Amount of Quercetin Aglycone Degraded per Day, and the Rate (%) of Excreted Aglycone Derivatives in Rats Fed with AGIQ-containing Diets (HQ, and HSQ) After 2, 4, 6, and 8 weeks.

Group		Ingested aglycone (μmol/day)	Excreted aglycone [a] (μmol/day)	Degraded aglycone [b] (μmol/day)	Excreted aglycone [c] (%)	Degraded aglycone [d] (%)
Week 2	HQ	188.51± 5.33	41.64± 2.20	146.87± 5.59	22.18± 1.29	77.82± 1.29
	HSQ	167.33± 6.91 **	23.84± 1.69 ***	143.50± 6.17	14.24± 0.82 ***	85.76± 0.82 ***
Week 4	HQ	181.38± 5.36	32.61± 1.98	148.77± 5.33	18.04± 1.12	81.96± 1.12
	HSQ	167.20± 6.86 *	31.23± 1.55	135.98± 6.29 *	18.76± 0.91	81.24± 0.91
Week 6	HQ	177.82± 4.84	21.68± 2.35	156.14± 5.51	12.25± 1.37	87.75± 1.37
	HSQ	165.49± 6.31 *	26.09± 1.67	139.39± 6.50 **	15.91± 1.10 *	84.09± 1.10 *
Week 8	HQ	186.22± 3.50	17.40± 0.70	168.82± 3.44	9.36± 0.38	90.64± 0.38
	HSQ	164.83± 6.39 *	29.83± 2.95 **	135.00± 5.29 ***	18.04± 1.48 ***	81.96± 1.48 ***

Values represent mean ± SEM (n = 7). Asterisk indicate a significant difference between mean values of HQ vs. HSQ (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.005$, Student's t test).
[a] Excreted aglycone (μmol/day) = Total aglycone in urine + Total aglycone in feces
[b] Degraded aglycone (μmol/day) = Ingested aglycone - Excreted aglycone
[c] Excreted aglycone (%) = (Excreted aglycone / Ingested aglycone) × 100
[d] Degraded aglycone (%) = [100 - (Excreted aglycone / Ingested aglycone)] × 100

Glycemic, Insulin, and GLP-1 Responses under the MTT

Because statistically significant differences were not detectable in time course changes of glucose, insulin, and GLP-1 (Figures 3.5a, c, e and 3.6a, c, e), results are explained based on AUC values (Figures 3.5b, d, f, g and 3.6b, d, f, g). At week 4 of the feeding period, the postprandial glycemic response in the H group was significantly higher than that observed in the C group, while the HSQ group showed a markedly lower glycemic response than the H group (Figure 3.5b). No significant differences were seen in insulin responses between groups (Figure 3.5d). The highest levels of basal and postprandial GLP-1 (Figures 3.5e, f) were seen in the HSQ group among experimental groups.

Regarding the MTT at week 8, the HSQ group showed a reduction in the postprandial glycemic responses when compared to the H group (Figures 3.6b). A significant elevation was observed in the AUC of postprandial insulin in the H group compared to the C group (Figure 3.6d). Among all treatments, the basal GLP-1 concentration in the HSQ group was the greatest (16.07 ± 1.00 pM), while that in the H group was the lowest (11.24 ± 0.58 pM) (Figure 3.6e). The HSQ group also maintained substantially higher postprandial GLP-1 AUC (Figure 3.6f). Rats fed the H diet demonstrated significantly higher HOMA-IR AUC when compared with the C group, indicating the development of insulin resistance after continuous feeding with a high-fat high-sucrose diet. Markedly, the HSQ group had a HOMA-IR AUC value almost similar to that observed for the C group (Figure 3.6).
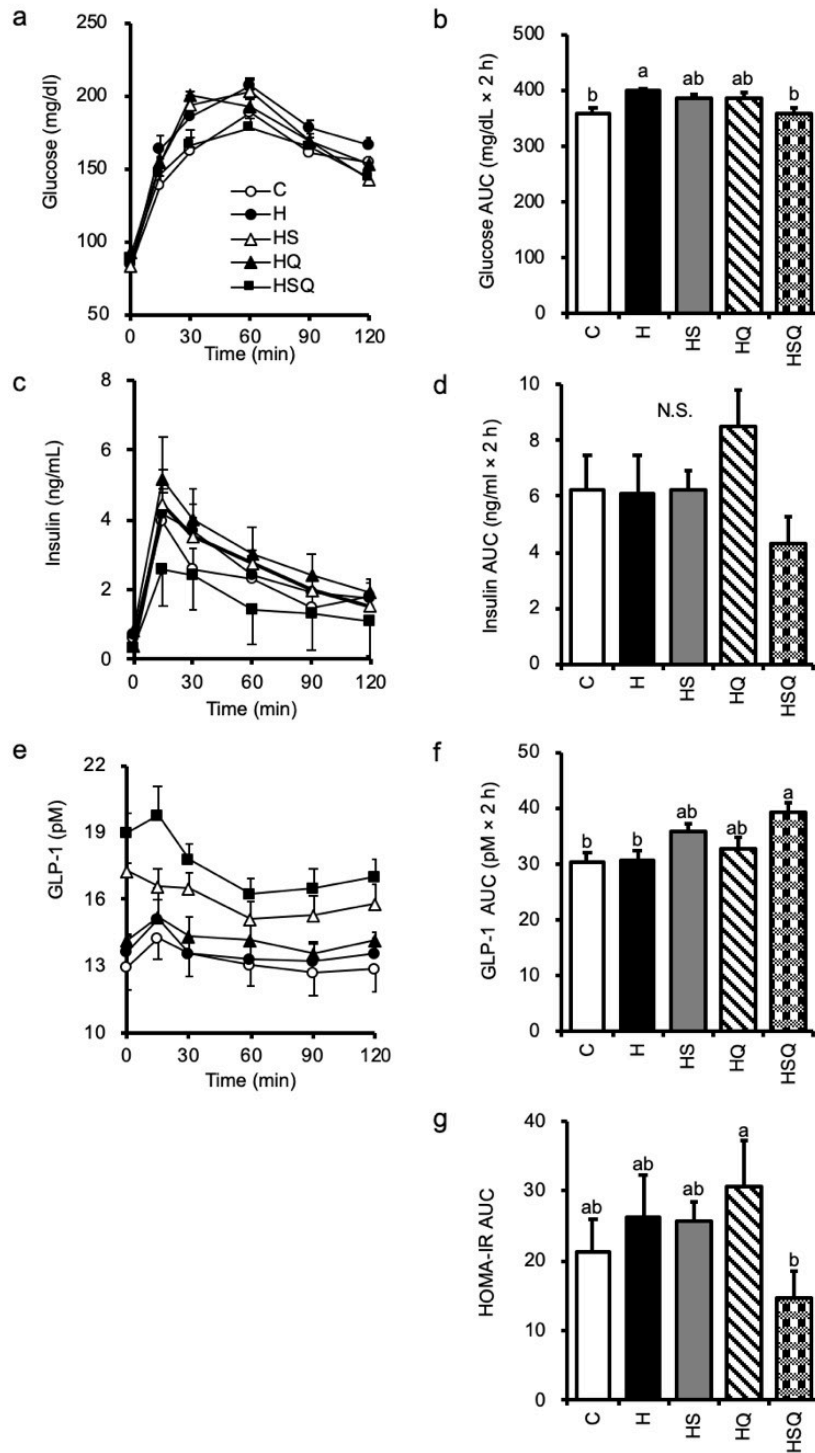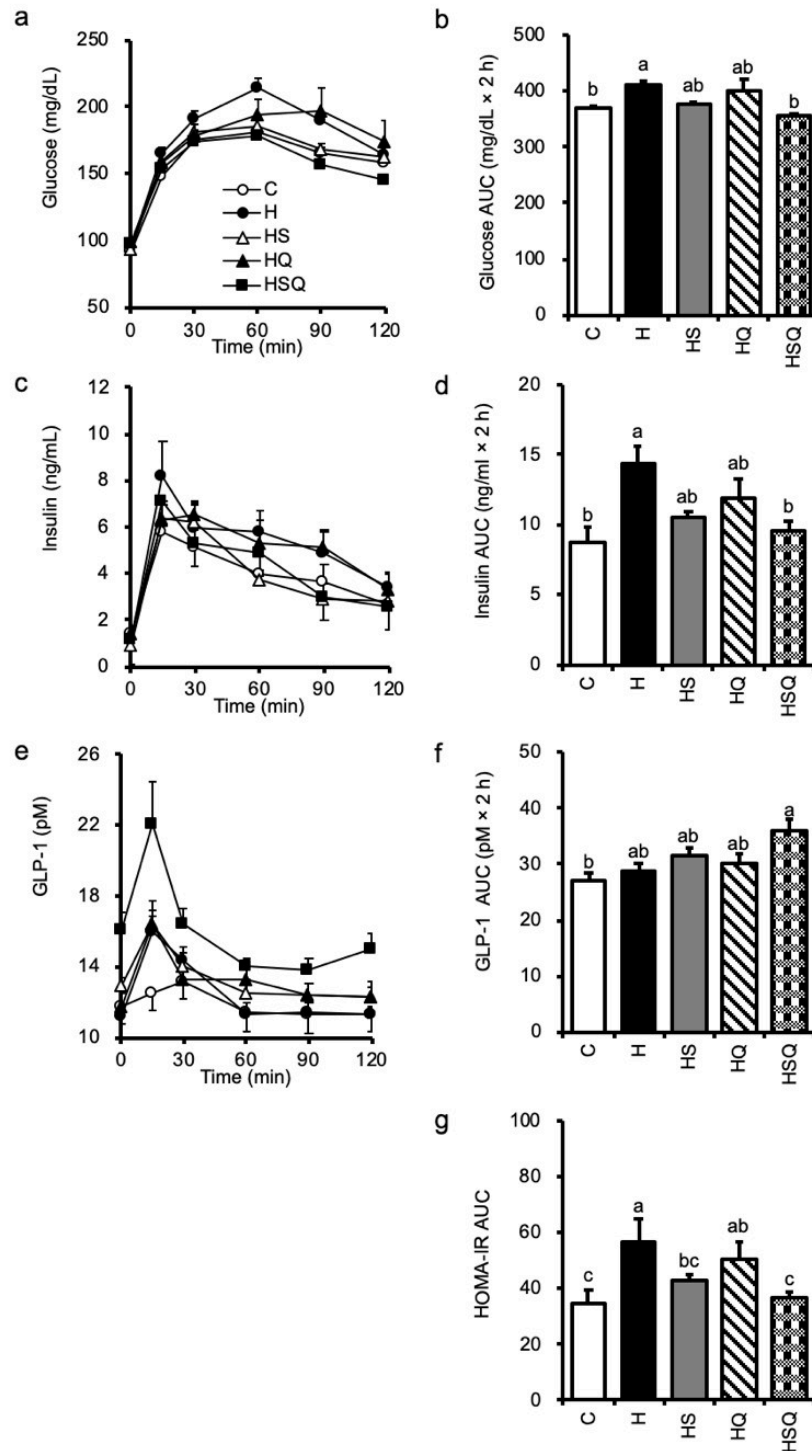
Figure 3.11. (a–g) Postprandial glucose, insulin, glucagon-like peptide-1 level, and homeostatic model assessment of insulin resistance during the meal tolerance test after 4 weeks of feeding the test diet. Values represent means with standard errors indicated by vertical bars (n = 7). Mean values not sharing a common alphabetical letter differ significantly ($P < 0.05$, Tukey-Kramer's post hoc test) among all treatments (panels b, d, f, and g). N.S. indicates the absence of a significant difference among treatments.

Figure 3.12. (a–g) Postprandial glucose, insulin, glucagon-like peptide-1 level, and homeostatic model assessment of insulin resistance during the meal tolerance test after 8 weeks of feeding the test diet. Values represent means with standard errors indicated by vertical bars ($n = 7$). Mean values not sharing a common alphabetical letter differ significantly ($P < 0.05$, Tukey-Kramer's post hoc test) among all treatments (panels b, d, f, and g). N.S. indicates the absence of a significant difference among treatments.

Cecal Tissue GLP-1 Content

No significant differences were observed in intestinal GLP-1 concentrations (Figure 3.7a). The total amount of GLP-1 in the cecal tissue was the highest in the HSQ group than HQ and other groups, while the HS group had almost the same level as the H group (Figure 3.7b).
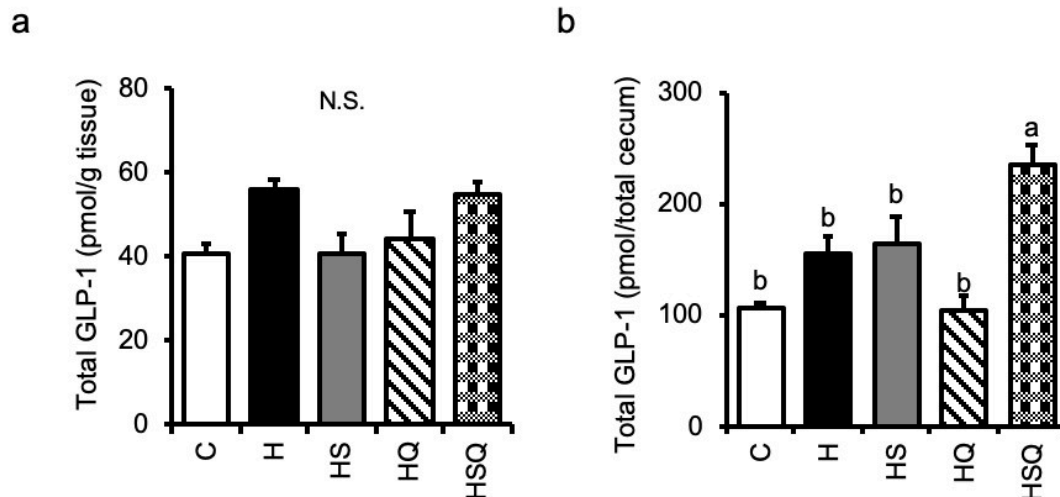

Figure 3.13 (a) Concentrations and (b) amounts of GLP-1 contents in cecal tissue of rats after feeding test diets for 9 weeks. Values represent means with standard errors indicated by vertical bars (n = 7). Mean values not sharing a common alphabetical letter differ significantly ($P < 0.05$, Tukey–Kramer's test). N.S. indicates the absence of a significant difference among treatments.

Correlation between the Concentrations of Basal GLP-1 and Total Quercetin Derivatives in Plasma or Cecal Tissue GLP-1 Content

Correlations between plasma quercetin concentrations and various parameters, including basal and postprandial glucose/insulin/GLP-1, were analyzed. Basal GLP-1 concentrations (Figure 3.8a,b) were positively and significantly associated with the total plasma quercetin derivatives at weeks 4 ($r = 0.60$, $P = 0.0289$) and 8 ($r = 0.77$, $P = 0.0081$).

Correlation analyses were performed between basal plasma GLP-1 at week 8 and tissue GLP-1 content (total amount in the cecum), separatory in Q-fed groups (HQ and HSQ), and non-Q-fed groups (H and HS). A significant and positive correlation was observed for data from Q-fed groups, but not for data from non-Q-fed groups (Figure 3.8c,d).
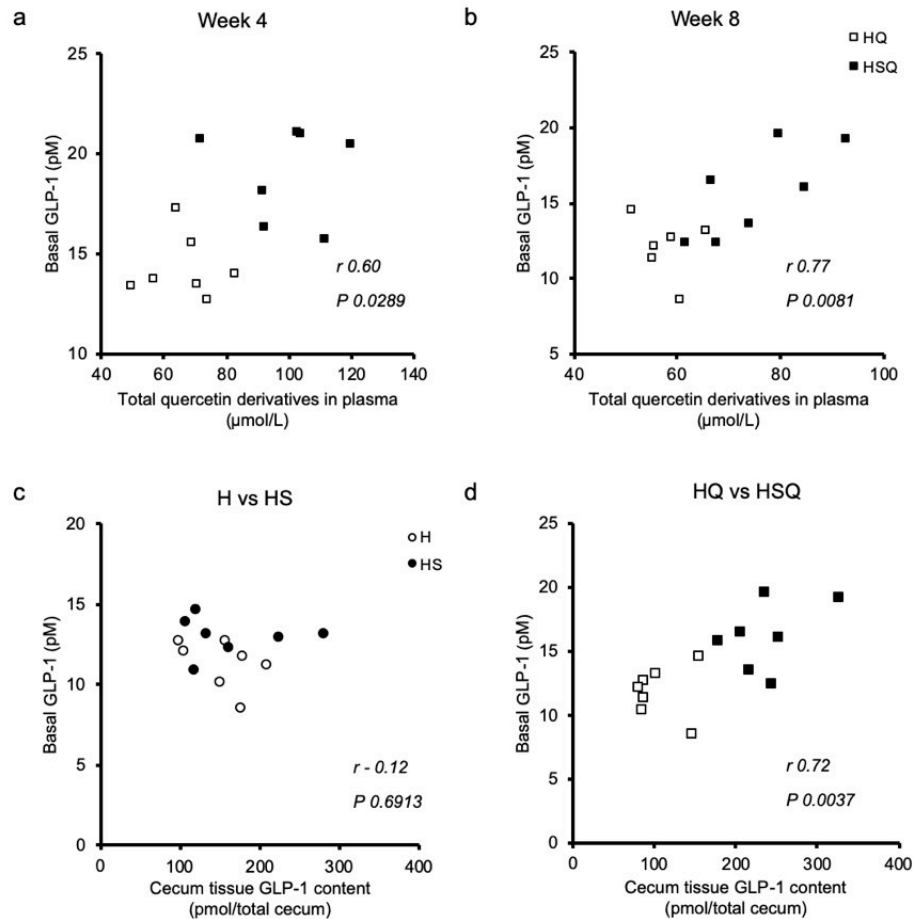
Figure 3.14 Correlation between basal GLP-1 concentrations and total quercetin derivative concentrations in the plasma at (a) week 4 and (b) week 8 in rats fed AGIQ-containing diets (HQ and HSQ). Correlation between basal GLP-1 at week 8 and amount of GLP-1 contents in cecal tissue of rats fed (c) non-AGIQ-containing diets (H and HQ) and (d) AGIQ-containing diets (HQ and HSQ).

Discussion

From previous chapter, a substantial elevation in the total quercetin derivatives were detected in the plasma of rats fed a diet supplemented with a combination of soybean fiber and AGIQ was discovered [Trakooncharoenvit, 2020]. This study was conducted to determine the impact of combined soybean fiber and AGIQ supplementation on quercetin bioavailability in rats fed an obesogenic high-fat high-sucrose diet, as well as whether this supplementation exerts a preventive effect against glucose intolerance. At the end of this study, the combination of soybean fiber and AGIQ in a high-fat high-sucrose (HSQ) diet gave the highest concentration of total quercetin derivatives in plasma and promoted cecal fermentation. The combined supplementation of soybean fiber and AGIQ also attenuated the glucose intolerance induced by the continuous feeding of the high-fat high-sucrose diet. Furthermore, plasma quercetin concentrations were positively associated with plasma GLP-1 concentrations. These

results indicated that the co-ingestion of soybean fiber promotes quercetin bioavailability followed by enhanced GLP-1 secretion even under an obesogenic dietary status to prevent the development of glucose intolerance.

Although apparent effects of experimental diets on body weight were not observed in the present study, visceral fat accumulation caused by high-fat high-sucrose diet has been mitigated by soybean fiber or AGIQ or both components. Consistent with these findings, there is downregulation of leptin and TNF-alpha genes in mice fed a high-fat diet containing soybean fiber, resulting in reduced epididymal adipose tissue weight [Matsumoto, 2007]. Another study has demonstrated that quercetin improves energy expenditure and reduces fat accumulation in mice fed a high-fat diet by promoting white adipocyte browning [Choi, 2018].

Elevated cecal fermentation from soybean fiber ingestion was confirmed by an increase in SCFAs and a decrease in pH [Farmer, 2014; Taciak, 2015]. As mentioned in the previous chapter, soybean fiber supplementation can increase quercetin bioavailability, which resulted from enhances cecal fermentation in normal rats. This modification in the large intestine can reduce the degradation of luminal quercetin, increasing the available quercetin that can be absorbed in the large intestine.

In the present study, total quercetin derivatives in fecal and cecal samples were higher in the HSQ group (Figures 3.3 and 3.4), which correlated with the percentage of quercetin degradation that was lower than the HQ group in the later experimental period (Table 3.2). These results revealed that the proposed mechanism by which soybean fiber enhances quercetin bioavailability is maintained even under an obesogenic dietary condition. Notably, the proposed mechanism is not related to the absorption rate but possibly depends on the absorptive capacity with the expansion of the cecal tissues and the available amount of quercetin and its derivatives in the intestine.

Previous studies demonstrated that a diet containing a high percentage of lard oil (17%) increased quercetin bioavailability [Lesser, 2004] and that quercetin can be transported into lymph circulation along with fat [Chen, 2010; Makino 2018]. One possible mechanism underlying these findings was the increased formation of chylomicrons that incorporates quercetin together with fat in the diet [Murota, 2013]. In the present study, quercetin degradation gradually increased over time in the HQ group, which may be linked with increased quercetin-degrading bacteria such as *Bacteroides fragilis* [Zhang, 2014]. In lard-fed mice, increases in *Bacteroides* and *Bilo-phila* bacteria have been reported [Singh, 2017]. Enhancement of quercetin bioavailability by soybean fiber could relate to the modification of intestinal microflora. The promotive effect of dietary fiber on the growth of intestinal bacteria

such as *Bifidobacteria* and *Lactobacilli* has been shown [Gibson, 1995]. This, in turn, controls or reduces the growth of harmful bacteria such as *Clostridium perfringens* and *Escherichia coli* that are known as quercetin-degrading bacteria [Zhang, 2014].

At the beginning of the experimental period (week 2), the higher percentage of aglycon degradation in the HSQ group than in the HQ group seems inconsistent with the proposed mechanism for increasing plasma quercetin (quercetin bioavailability). However, amounts of degraded aglycon are very similar in these groups, indicating that both of groups had almost equivalent "degradation capacity". Therefore, in this case, higher percentage of degradation in HSQ than the HQ group is owing to the lower intake of aglycon in HSQ than in the HQ group. After 4 weeks, amounts of degraded aglycon gradually increased in the HQ group while decreased in the HSQ group accompanied with significant differences between two groups ($P < 0.05$ at week 4, $P < 0.01$ at weeks 6 and 8) and resulted in a significant interaction between the duration and the diet ($P = 0.0034$ by two-way repeated measures ANOVA). The former is likely a chronic effect of a high-fat diet, and the latter is a chronic effect of soybean fiber supplementation. Thus, the supplementation of soybean fiber is an alternatively effective approach to enhance quercetin bioavailability even under a chronic feeding of a high-fat diet.

Significant differences in energy consumption were not detected by the Tukey–Kramer's test (Figure 3.1a), but the HSQ group had approximately 10% lower energy intake than the HQ group, which resulted in significant differences in aglycon ingestion between the HQ and HSQ groups, when calculated by Student's *t* test (Table 3.2). Such a small difference in energy intake might also contribute improved glucose tolerance in the HSQ group compared to the HQ group. Lack of comparison under iso-energetic conditions such as pair-fed design may be one of the limitations of the present study. However, it is a valuable finding that the quercetin bioavailability is higher in HSQ than the HQ group, even though HSQ had slightly lower quercetin intake than the HQ group.

The long-term feeding of an HSQ diet can increase GLP-1 secretion, especially at the basal level, but the lower concentration of glucose in plasma may not be sufficient to activate the function of GLP-1 to enhance insulin secretion in a glucose-dependent manner. According to this assumption, the combination of AGIQ and soybean fiber also improved glucose tolerance and insulin sensitivity in rats fed a high-fat high-sucrose diet (Figures 3.5 and 3.6). Relatively, but insignificantly, lower energy consumption and visceral fat accumulation cannot be used for explanation because both HS and HSQ groups have shown similar results for these parameters (Figure 3.1). Soybean fiber supplementation has been shown to mitigate plasma glucose and triglyceride levels without body weight changes in both male random-bred albino

control and streptozotocin-induced diabetic rats [Madar, 1983]. Reportedly, quercetin acts on 5′ AMP-activated protein kinase stimulation and glucose transporter type 4 translocation, promoting glucose uptake in skeletal muscle and liver [Eid, 2015]. These data partly explain the preventive effects of combined soybean fiber and AGIQ against metabolic impairments induced by excessive energy intake. Possibly, an extension on the feeding period may show clearer effects; therefore, a longer chronic feeding of this combination diet is needed in the future.

There was a strong positive association between basal GLP-1 levels and total quercetin derivatives in plasma (Figure 3.8) during the experiment. The basal GLP-1 level was possibly promoted by increased methylquercetin metabolites because a high concentration of these metabolites was detected in the plasma, cecum, urine, and feces of AGIQ-fed rats (Figures 3.3 and 3.4). It is well-known that methylquercetin metabolites are resistant to degradation by intestinal bacteria, [Matsukawa, 2009; Spencer, 2003] and long-term quercetin consumption can extend the methylation of quercetin [Manach, 1999]. The stimulatory effect of AGIQ on GLP-1 secretion has been demonstrated following an acute infusion into the rat small intestine as well as following acute exposure in a murine GLP-1-producing cell line (GLUTag cells) [Phuwamongkolwiwat, 2014]. Luminal amounts (Figure 3.4) and concentrations (0.62 ± 0.17 mM in HQ, 5.57 ± 0.67 mM in HSQ) of quercetin in the cecum were much higher in the HSQ group than the HQ group, but significant correlations between plasma GLP-1 concentrations were not observed ($r = 0.36$, $P = 0.2080$ for the amount of luminal quercetin; $r = 0.37$, $P = 0.1869$ for the concentration of luminal quercetin). Although, the luminal quercetin in the small intestine were not measured, it was speculated that the luminal quercetin in the ileum might be involved in the increment of basal GLP-1 secretion. Another possible explanation is the involvement of increased mass of the cecal tissue for the increment of GLP-1 secretion. There were no differences in tissue GLP-1 concentrations among treatments (Figure 3.7a); however, the amount of GLP-1 in the whole cecum was higher in the HSQ group than in the HQ group (Figure 3.7b). This suggests that the number of GLP-1-producing cells was simultaneously increased together with the increased mass of cecal tissue (Figure 3.1d). The correlation analysis (Figures 3.8c,d) revealed the significant and positive correlation between plasma GLP-1 concentrations and total amount of cecal tissue GLP-1 ($r = 0.72$, $P = 0.0037$) in data from Q-fed groups (HQ vs HSQ) but not in the data from non-Q-fed groups (H vs HQ). Therefore, not only tissue expansion by soybean fiber but also the presence of quercetin (in the intestinal lumen and/or plasma) could be responsible for the increased basal GLP-1 secretion. Further

studies are needed to clarify the molecular mechanisms by which quercetin and its metabolites promote GLP-1 secretion.

In this work, the supplementation with soybean fiber promoted quercetin bioavailability in rats fed a high-fat high-sucrose diet by enhancing cecal fermentation. The combination of soybean fiber and AGIQ prevented the high-fat high-sucrose diet-induced glucose intolerance, accompanied by an increase in plasma GLP-1 levels. Furthermore, plasma GLP-1 levels positively correlated with plasma quercetin concentrations. These findings reveal the effects of soybean fiber and quercetin (AGIQ) on reducing the risk of glucose intolerance and subsequent diabetes.

Chapter 4: General discussion

The results throughout the study clearly showed that quercetin bioavailability can be augmented by chronic feeding with a combination of the AGIQ and the soybean fiber. In addition, this combination maintained the ability even under an obesogenic status induced by the high-fat high-sucrose diet. From this discovery, it can be suggested that the high amounts of digestible carbohydrate and fat in the diet are unable to deteriorate the capability of the combination of AGIQ and soybean fiber to promote quercetin bioavailability. According to Neilson et al. [Neilson, 2009], sucrose in chocolate can increase the bioavailability of catechin and epicatechin in humans. Another research using Sprague-Dawley rats found that plasma concentrations of total flavan-3-ol were highest in chocolate containing high-sucrose as compared to reference dark chocolate with low-sucrose which yielded intermediate plasma concentrations of the total flavan-3-ol [Neilson, 2010]. With regard to dietary fat, when overweight men and postmenopausal women consumed quercetin with a fat-free (0.5 g), low-fat (4.0 g), or high-fat (15.4 g) diet, plasma quercetin concentrations increased more than 40% by the high-fat diet compared to the fat-free diet [Guo, 2013]. In agreement with the results in the third chapter, methylated metabolites of quercetin were mainly increased during the high-fat diet treatment. Behind this increment, it has been suggested that dietary fat may increase the quercetin micelle formation in the upper small intestine, so that promoted its intestinal absorption [Guo, 2013]. Protein is another macronutrient that was not examined in this thesis. In a recent study, the effect of dairy products on the bioavailability of cherry flavonoids was investigated in *in vitro*, the gastrointestinal digestion. The researchers found that the bioavailability of rutin and cyanidin-3-O-glucoside was suppressed when combined with protein-based food matrices including cowmilk, soymilk, and yogurt [Oksuz, 2019]. The reduction from protein-based food raised a further question that whether the combination of quercetin and soybean fiber still be able to promote bioavailability under protein-rich diets.

Under the same macronutrients composition, it is no exaggeration to claim that dietary fibers, particularly soybean fiber, are an important factor in promoting quercetin bioavailability. As revealed in the second chapter, differences in supplemented dietary fibers can cause a diverse promotive effect. Dietary fibers employed in this thesis are water-soluble dietary fibers. Advantages from these dietary fibers were thought to be dependent on viscosity, and fermentability. A diet containing water-soluble polysaccharide increases the viscosity of the intestinal contents. Among all three chosen dietary fibers, pectin and guar gum represent viscous dietary fiber, by the latter has a higher viscosity [Saha, 2010], while soybean fiber

represents low viscous dietary fiber [Mitamura, 2003]. Reportedly, absorption rates of carbohydrate, protein, and fat linearly declined as concentrations of guar gum or the chyme viscosity increased, in humans [Brenelli, 1997]. Continuous (6 weeks) feeding with a diet containing pectin (from apple skin) showed an increase in absorption rate of orally given quercetin and rutin, in rats [Nishijima, 2009]. The data in the second chapter partially agree with this statement. Pectin supplementation showed the highest excreted percentage of quercetin aglycone during the feeding period, but the plasma concentrations appeared no significant difference from weeks 4 to 8 of the study compared to no supplemented fiber group. Daily feeding the combination of pectin and AGIQ may attribute to the protective effect of pectin against the degradation of quercetin but did not promote absorption.

The results in this thesis implied three possibilities in the promotion of quercetin bioavailability by the cecal fermentation of dietary fiber.

1) The enlargement in cecal tissue weight increases the surface area of the cecum as well as absorptive capacity;

2) The extension in an available bolus in the cecal lumen can prolong the duration for absorption;

3) The high amount of quercetin in bolus may reduce the quercetin degradation by suppressing the growth or degraded activity of commensal bacteria.

Difructose anhydride III, and fructooligosac-charides are reported to modify a cecal fermentation in a dose-dependent manner. This modification resulted in suppressing the degradation of rutinoside of quercetin in the large intestine [Matsukawa, 2009]. Ohta and colleagues discovered that dietary short-chain fructooligosaccharides enhanced recovery from iron deficiency in rats. In that analysis, they also discovered increased net iron absorption and soluble iron in cecal contents [Ohta, 1995]. A similar study by treatment with potato fiber in rats showed an increase in cecal fermentation and mineral absorption [Pastuszewska, 2010]. In both studies in this thesis, it may possible that quercetin degraded products led to the formation of organic acids. However, quercetin catabolites are not the majority of the cecal organic acids when compared to organic acids produced from the fermentation of dietary fiber [Dhingra, 2012; Pastuszewska, 2010]. This statement is confirmed by the cecal organic acids result. In both studies, organic acids or SCFAs of the quercetin group were lower than that of the soybean fiber-treated group. This suggests that cecal organic acids were mainly caused by cecal fermentation of dietary fibers and not quercetin degradation. In addition, SCFAs at higher concentrations in the large intestine have been demonstrated to increase the weight of cecal tissue by stimulating the proliferation of epithelial cells and augmented the absorptive surface

area [Busserolles, 2003; Inagaki, 2005; Sakata, 1987]. These studies supported the results in this thesis, and they raise another question on the enhancement of the bioavailability for other flavonoids by the combination with certain dietary fiber.

Studies on the antidiabetic ability of flavonoids have been variously reported. Some of the flavonoids' anti-diabetic effects are due in part to their antioxidative properties and ability to modulate several cellular signaling. The study done by rutin treatment in streptozotocin-induced diabetic rats showed a reduction of plasma glucose level by suppression of NF-κB and activation Nrf-2 expression in sciatic nerves and dorsal root ganglions (DRG, L4-L6) [Tian, 2016]. This evidence suggests the beneficial effect of rutin on diabetic neuropathy. Several flavonoids, including tiliroside, quercetin, epigallocatechin gallate, and epicatechin gallate, are reportedly able to inhibit carbohydrate digestion and absorption in the small intestine [Goto, 2012]. Regulation of glucose production in the liver is another function that can be affected by flavonoids. In a human HepG2 cell line and the isolated hepatocytes from obese mice, a reduction in hepatic gluconeogenesis with suppressed hepatic pyruvate carboxylase activity was found by kaempferol treatment [Alkhalidy, 2018] In addition, the positive effects of flavonoids on preserving pancreatic islet and increasing beta-cell mass against oxidative pancreatitis induction have been elucidated [Prasath, 2013]. In an *in vitro* study using rat L6 myotubes, quercetin treatment activated the JAK2/STAT pathways through IRS1/PI3K/Akt signaling. The activation of the mentioned pathway has resulted in the translocation of the glucose transporter 4 (GLUT4) to promote glucose uptake in the myocyte [Eid, 2015]. This study suggested the promotion of glucose uptake in the skeletal muscle by flavonoids. Furthermore, the secretion of several gastrointestinal hormones, such as GLP-1 and GIP (Glucose-dependent insulinotropic polypeptide), can be augmented by flavonoids supplementation [Kwon, 2019].

The current results and previous studies mentioned above may hint at some beneficial effects provided from the combination of AGIQ and soybean fiber on the ability to prevent T2DM in humans. Although, it has been described in the individual effect of quercetin in cells, animal, and human studies [Arias, 2014; Salehi, 2020; Seiva, 2012; Shi, 2019; Peng, 2017] or soybean fiber [Chang, 2008; Hosokawa, 2016; Nam, 2012; Nguyen, 2019; Xu, 2001] in animal and human studies on diabetes prevention. The combination of AGIQ and soybean fiber revealed the synergistic effect on improving glucose homeostasis as seen in the third chapter. This modification was accounted for the increase in GLP-1 secretion. Furthermore, the correlations between total quercetin derivatives and the basal level of GLP-1 in plasma have

appeared positively. This may shed some light on the next step of flavonoids and the regulation of incretin hormones which will be useful for disease prevention.

To the best of my knowledge, this thesis provides the first evidence for the benefit of the combination of quercetin and soybean fiber on the promotion of quercetin bioavailability and prevention of glucose intolerance in rat models. Future studies should investigate the underlying molecular mechanism of quercetin on the enhancement of basal GLP-1 secretion.

Conclusion

Results from this study provide evidences that quercetin bioavailability can be promoted by long-term feeding of the combination with AGIQ and dietary fibers, particularly with soybean fiber. Furthermore, this combination also provided the promotive effects on basal GLP-1 secretion in a positive correlation to the total quercetin derivatives in plasma. These findings would contribute to the development of a novel diet composition employing the combination of functional flavonoids and dietary fibers to reduce the risk of metabolic diseases including T2DM.

References

Alkhalidy H, Moore W, Wang A, Luo J, McMillan RP, Wang Y, Zhen W, Hulver MW, Liu D. Kaempferol ameliorates hyperglycemia through suppressing hepatic gluconeogenesis and enhancing hepatic insulin sensitivity in diet-induced obese mice. J Nutr Biochem. 2018;58:90-101. doi: 10.1016/j.jnutbio.2018.04.014.

Almeida AF, Borge GIA, Piskula M, Tudose A, Tudoreanu L, Valentová K, Williamson G, Santos CN. Bioavailability of quercetin in humans with a focus on interindividual variation. Compr Rev Food Sci Food Saf. 2018;17(3):714-731. doi: 10.1111/1541-4337.12342.

Anand David AV, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: a bioactive flavonoid. Pharmacogn Rev. 2016;10(20):84-89. doi: 10.4103/0973-7847.194044.

Andoh A, Tsujikawa T, Fujiyama Y. Role of dietary fiber and short-chain fatty acids in the colon. Curr Pharm Des. 2003;9(4):347-58. doi: 10.2174/1381612033391973.

Arias N, Macarulla MT, Aguirre L, Martínez-Castaño MG, Portillo MP. Quercetin can reduce insulin resistance without decreasing adipose tissue and skeletal muscle fat accumulation. Genes Nutr. 2014;9(1):361. doi: 10.1007/s12263-013-0361-7.

Aura AM, O'Leary KA, Williamson G, Ojala M, Bailey M, Puupponen-Pimiä R, Nuutila AM, Oksman-Caldentey KM, Poutanen K. Quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids but not methylated by human fecal flora *in vitro*. J Agric Food Chem. 2002;50(6):1725-30. doi: 10.1021/jf0108056.

Berer K, Martínez I, Walker A, Kunkel B, Schmitt-Kopplin P, Walter J, Krishnamoorthy G. Dietary non-fermentable fiber prevents autoimmune neurological disease by changing gut metabolic and immune status. Sci Rep. 2018;8(1):10431. doi: 10.1038/s41598-018-28839-3.

Braune A, Blaut M. Bacterial species involved in the conversion of dietary flavonoids in the human gut. Gut Microbes. 2016;7(3):216-34. doi: 10.1080/19490976.2016.1158395.

Braune A, Gütschow M, Engst W, Blaut M. Degradation of quercetin and luteolin by *Eubacterium ramulus*. Appl Environ Microbiol. 2001;67(12):5558-67. doi: 10.1128/AEM.67.12.5558-5567.2001.

Brenelli SL, Campos SD, Saad MJ. Viscosity of gums *in vitro* and their ability to reduce postprandial hyperglycemia in normal subjects. Braz J Med Biol Res. 1997;30(12):1437-40. doi: 10.1590/s0100-879x1997001200009.

Bugaut M. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. Comp Biochem Physiol B. 1987;86:439–72. doi: 10.1016/0305-0491(87)90433-0

Bule M, Abdurahman A, Nikfar S, Abdollahi M, Amini M. Antidiabetic effect of quercetin: A systematic review and meta-analysis of animal studies. Food Chem Toxicol. 2019;125:494-502. doi: 10.1016/j.fct.2019.01.037.

Busserolles J, Gueux E, Rock E, Demigné C, Mazur A, Rayssiguier Y. Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats. J Nutr. 2003;133(6):1903-8. doi: 10.1093/jn/133.6.1903.

Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. Am J Physiol Endocrinol Metab. 2008;295(5):E1269-76. doi: 10.1152/ajpendo.90207.2008.

Cai X, Fang Z, Dou J, Yu A, Zhai G. Bioavailability of quercetin: problems and promises. Curr Med Chem. 2013;20(20):2572-82. doi: 10.2174/09298673113209990120.

Carbonaro M, Grant G. Absorption of quercetin and rutin in rat small intestine. Ann Nutr Metab. 2005;49(3):178-82. doi: 10.1159/000086882.

Cavaghan MK, Ehrmann DA, Polonsky KS. Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. J Clin Invest. 2000;106(3):329-33. doi: 10.1172/JCI10761.

Cermak R, Breves GM. *In vitro* degradation of the flavonol quercetin and of quercetin glycosides in the porcine hindgut. Arch Anim Nutr. 2006;60(2):180-9. doi: 10.1080/17450390500467695.

Cersosimo E, Triplitt C, Solis-Herrera C, et al. Pathogenesis of Type 2 Diabetes Mellitus. [Updated 2018 Feb 27]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279115/

Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, Marco A, Shekhawat NS, Montales MT, Kuriakose K, Sasapu A, Beebe A, Patil N, Musham CK, Lohani GP, Mirza W. Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. Front Endocrinol (Lausanne). 2017;8:6. doi: 10.3389/fendo.2017.00006.

Chen IL, Tsai YJ, Huang CM, Tsai TH. Lymphatic absorption of quercetin and rutin in rat and their pharmacokinetics in systemic plasma. J Agric Food Chem. 2010;58(1):546-51. doi: 10.1021/jf9026124.

Chen S, Jiang H, Wu X, Fang J. Therapeutic effects of quercetin on inflammation, obesity, and type 2 diabetes. Mediators Inflamm. 2016;2016:9340637. doi: 10.1155/2016/9340637.

Choi H, Kim CS, Yu R. Quercetin upregulates uncoupling protein 1 in white/brown adipose tissues through sympathetic stimulation. J Obes Metab Syndr. 2018;27(2):102-109. doi: 10.7570/jomes.2018.27.2.102.

Clifford M.N. Diet-derived phenols in plasma and tissues and their implications for health. Planta Med. 2004;70:1103–1114. doi: 10.1055/s-2004-835835.

Cosme P, Rodríguez AB, Espino J, Garrido M. Plant phenolics: Bioavailability as a key determinant of their potential health-promoting applications. Antioxidants (Basel). 2020;9(12):1263. doi:10.3390/antiox9121263

Crespy V, Morand C, Besson C, Manach C, Demigne C, Remesy C. Quercetin, but not its glycosides, is absorbed from the rat stomach. J Agric Food Chem. 2002;50(3):618-21. doi: 10.1021/jf010919h.

Hervert-Hernandez D, Goñi I. Dietary polyphenols and human gut microbiota: A review. Food Reviews International. 2011;27(2):154-69. doi:10.1080/87559129.2010.535233

Dabeek WM, Marra MV. Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans. Nutrients. 2019;11(10):2288. doi: 10.3390/nu11102288.

Dahl WJ, Agro NC, Eliasson ÅM, Mialki KL, Olivera JD, Rusch CT, Young CN. Health benefits of fiber fermentation. J Am Coll Nutr. 2017;36(2):127-136. doi: 10.1080/07315724.2016.1188737.

Day AJ, Gee JM, DuPont MS, Johnson IT, Williamson G. Absorption of quercetin-3-glucoside and quercetin-4'-glucoside in the rat small intestine: The role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. Biochem Pharmacol. 2003;65(7):1199-206. doi: 10.1016/s0006-2952(03)00039-x.

De Vries JH, Hollman PC, Meyboom S, Buysman MN, Zock PL, van Staveren WA, Katan MB. Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. Am J Clin Nutr. 1998;68(1):60-5. doi: 10.1093/ajcn/68.1.60.

Dhingra D, Michael M, Rajput H, Patil RT. Dietary fibre in foods: A review. J Food Sci Technol. 2012;49(3):255-66. doi: 10.1007/s13197-011-0365-5.

Dowarah J, Singh VP. Anti-diabetic drugs recent approaches and advancements. Bioorg Med Chem. 2020;28(5):115263. doi: 10.1016/j.bmc.2019.115263.

Drucker DJ, Sherman SI, Gorelick FS, Bergenstal RM, Sherwin RS, Buse JB. Incretin-based therapies for the treatment of type 2 diabetes: Evaluation of the risks and benefits. Diabetes Care. 2010;33(2):428-33. doi: 10.2337/dc09-1499.

Drucker DJ. Mechanisms of action and therapeutic application of glucagon-like peptide-1. Cell Metab. 2018;27(4):740-756. doi: 10.1016/j.cmet.2018.03.001.

Eid HM, Nachar A, Thong F, Sweeney G, Haddad PS. The molecular basis of the antidiabetic action of quercetin in cultured skeletal muscle cells and hepatocytes. Pharmacogn Mag. 2015;11(41):74-81. doi: 10.4103/0973-1296.149708.

El-Ansary A, Shaker G, Siddiqi NJ, Al-Ayadhi LY. Possible ameliorative effects of antioxidants on propionic acid / clindamycin - induced neurotoxicity in Syrian hamsters. Gut Pathog. 2013;5(1):32. doi: 10.1186/1757-4749-5-32.

Ezati P, Rhim JW. Fabrication of quercetin-loaded biopolymer films as functional packaging materials. ACS Appl. Polym. Mater. 2021;3(4):2131–2137. doi: 10.1021/acsapm.1c00177

Farmer AD, Mohammed SD, Dukes GE, Scott SM, Hobson AR. Caecal pH is a biomarker of excessive colonic fermentation. World J Gastroenterol. 2014;20(17):5000-7. doi: 10.3748/wjg.v20.i17.5000.

FDA. Health claims: Fiber-contaning grain products, fruits and vegetables and cancer. In Code of Federal Regulations; Food and Drug Administration: Silver Spring, MD, USA, 2008; Volume 2.

FDA. Health claims: Fruits, vegetables, and grain products that contain fiber, particularly soluble fiber, and risk of coronary heart disease. In Code of Federal Regulations; Food and Drug Administration: Silver Spring, MD, USA, 2008; Volume 2.

Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut Microbes. 2012;3(4):289-306. doi: 10.4161/gmic.19897.

Gao L, Liu G, Wang X, Liu F, Xu Y, Ma J. Preparation of a chemically stable quercetin formulation using nanosuspension technology. Int J Pharm. 2011;404(1-2):231-7. doi: 10.1016/j.ijpharm.2010.11.009.

Gee JM, Hara H, Johnson IT. Suppression of intestinal crypt cell proliferation and aberrant crypt foci by dietary quercetin in rats. Nutr Cancer. 2002;43(2):193-201. doi: 10.1207/S15327914NC432_10.

Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125(6):1401-12. doi: 10.1093/jn/125.6.1401.

Goto T, Horita M, Nagai H, Nagatomo A, Nishida N, Matsuura Y, Nagaoka S. Tiliroside, a glycosidic flavonoid, inhibits carbohydrate digestion and glucose absorption in the gastrointestinal tract. Mol Nutr Food Res. 2012;56(3):435-45. doi: 10.1002/mnfr.201100458.

Guo Y, Bruno RS. Endogenous and exogenous mediators of quercetin bioavailability. J Nutr Biochem. 2015;26(3):201-10. doi: 10.1016/j.jnutbio.2014.10.008.

Guo Y, Mah E, Davis CG, Jalili T, Ferruzzi MG, Chun OK, Bruno RS. Dietary fat increases quercetin bioavailability in overweight adults. Mol Nutr Food Res. 2013;57(5):896-905. doi: 10.1002/mnfr.201200619.

Haohai H, Dan L, Yong D, Rong P. Effect of quercetin supplementation on plasma lipid profiles, blood pressure, and glucose levels: A systematic review and meta-analysis, Nutr. Rev. 2020;78(8):615–626. doi: 10.1093/nutrit/nuz071

Hara H, Onoshima S, Nakagawa C. Difructose anhydride III promotes iron absorption in the rat large intestine. Nutrition. 2010;26(1):120-7. doi: 10.1016/j.nut.2009.05.024.

Hara H, Saito Y, Idei A, Kiriyama S. Evaluation of short-chain fatty acid production in the cecum of fiber fed rats. Food Hydrocoll. 1994;485– 490. doi: 10.1007/978-1-4615-2486-1_74

Hira T, Suto R, Kishimoto Y, Kanahori S, Hara H. Resistant maltodextrin or fructooligosaccharides promotes GLP-1 production in male rats fed a high-fat and high-sucrose diet, and partially reduces energy intake and adiposity. Eur J Nutr. 2018;57(3):965-979. doi: 10.1007/s00394-017-1381-7.

Hollman P.C.H. Absorption, bioavailability, and metabolism of flavonoids. Pharm. Biol. 2009;42:74–83. doi: 10.3109/13880200490893492.

Holota Y, Dovbynchuk T, Kaji I, Vareniuk I, Dzyubenko N, Chervinska T, Zakordonets L, Stetska V, Ostapchenko L, Serhiychuk T, Tolstanova G. The long-term consequences of antibiotic therapy: Role of colonic short-chain fatty acids (SCFA) system and intestinal barrier integrity. PLoS One. 2019;14(8):e0220642. doi: 10.1371/journal.pone.0220642.

Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87(4):1409-39. doi: 10.1152/physrev.00034.2006.

Hosokawa M, Katsukawa M, Tanaka H, Fukuda H, Okuno S, Tsuda K, Iritani N. Okara ameliorates glucose tolerance in GK rats. J Clin Biochem Nutr. 2016;58(3):216-22. doi: 10.3164/jcbn.15-44.

Inagaki A, Sakata T. Dose-dependent stimulatory and inhibitory effects of luminal and serosal n-butyric acid on epithelial cell proliferation of pig distal colonic mucosa. J Nutr Sci Vitaminol (Tokyo). 2005;51(3):156-60. doi: 10.3177/jnsv.51.156.

Inoue D, Tsujimoto G, Kimura I. Regulation of energy homeostasis by GPR41. Front Endocrinol (Lausanne). 2014;5:81. doi: 10.3389/fendo.2014.00081

Jeong JH, An JY, Kwon YT, Rhee JG, Lee YJ. Effects of low dose quercetin: Cancer cell-specific inhibition of cell cycle progression. J Cell Biochem. 2009;106(1):73-82. doi: 10.1002/jcb.21977.

Jones JM. CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. Nutr J. 2014;13:34. doi: 10.1186/1475-2891-13-34.

Keppler K, Hein EM, Humpf HU. Metabolism of quercetin and rutin by the pig caecal microflora prepared by freeze-preservation. Mol Nutr Food Res. 2006;50(8):686-95. doi: 10.1002/mnfr.200600016.

Kern M, Fridrich D, Reichert J, Skrbek S, Nussher A, Hofem S, Vatter S, Pahlke G, Rüfer C, Marko D. Limited stability in cell culture medium and hydrogen peroxide formation affect the growth inhibitory properties of delphinidin and its degradation product gallic acid. Mol Nutr Food Res. 2007;51(9):1163-72. doi: 10.1002/mnfr.200700004.

Kim DH, Jin YH. Intestinal bacterial beta-glucuronidase activity of patients with colon cancer. Arch Pharm Res. 2001;24(6):564-7. doi: 10.1007/BF02975166.

Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. Cell. 2016;165(6):1332-1345. doi: 10.1016/j.cell.2016.05.041.

Kwon EY, Choi MS. Dietary Eriodictyol Alleviates adiposity, hepatic steatosis, insulin resistance, and inflammation in diet-induced obese mice. Int J Mol Sci. 2019;20(5):1227. doi: 10.3390/ijms20051227.

Lattimer JM, Haub MD. Effects of dietary fiber and its components on metabolic health. Nutrients. 2010;2(12):1266-89. doi: 10.3390/nu2121266.

LeBlanc JG, Chain F, Martín R, Bermúdez-Humarán LG, Courau S, Langella P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. Microb Cell Fact. 2017;16(1):79. doi: 10.1186/s12934-017-0691-z.

Lesser S, Cermak R, Wolffram S. Bioavailability of quercetin in pigs is influenced by the dietary fat content. J Nutr. 2004;134(6):1508-11. doi: 10.1093/jn/134.6.1508.

Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, Liu H, Yin Y. Quercetin, inflammation and immunity. Nutrients. 2016;8(3):167. doi: 10.3390/nu8030167.

Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, Song X, Ren Y, Shan PF. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. Sci Rep. 2020;10(1):14790. doi: 10.1038/s41598-020-71908-9.

López de Dicastillo C, Garrido L, Velásquez E, Rojas A, Gavara R. Designing biodegradable and active multilayer system by assembling an electrospun polycaprolactone mat containing quercetin and nanocellulose between polylactic acid films. Polymers (Basel). 2021;13(8):1288. doi: 10.3390/polym13081288.

Madar Z. Effect of brown rice and soybean dietary fiber on the control of glucose and lipid metabolism in diabetic rats. Am J Clin Nutr. 1983;38(3):388-93. doi: 10.1093/ajcn/38.3.388.

Makino R, Takano K, Kita K, Nishimukai M. Influence of long-term feeding of high-fat diet on quercetin and fat absorption from the small intestine in lymph duct-cannulated rats. Biosci Biotechnol Biochem. 2018;82(11):2007-2011. doi: 10.1080/09168451.2018.1498726.

Manach C, Morand C, Demigné C, Texier O, Régérat F, Rémésy C. Bioavailability of rutin and quercetin in rats. FEBS Lett. 1997;409(1):12-6. doi: 10.1016/s0014-5793(97)00467-5.

Manach C, Texier O, Morand C, Crespy V, Régérat F, Demigné C, Rémésy C. Comparison of the bioavailability of quercetin and catechin in rats. Free Radic Biol Med. 1999;27(11-12):1259-66. doi: 10.1016/s0891-5849(99)00159-8.

Marín L, Miguélez EM, Villar CJ, Lombó F. Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. Biomed Res Int. 2015;2015:905215. doi: 10.1155/2015/905215.

Maruti SS, Chang JL, Prunty JA, Bigler J, Schwarz Y, Li SS, Li L, King IB, Potter JD, Lampe JW. Serum beta-glucuronidase activity in response to fruit and vegetable supplementation: A controlled feeding study. Cancer Epidemiol Biomarkers Prev. 2008;17(7):1808-12. doi: 10.1158/1055-9965. EPI-07-2660.

Matsukawa N, Matsumoto M, Chiji H, Hara H. Oligosaccharide promotes bioavailability of a water-soluble flavonoid glycoside, alpha G-rutin, in rats. J Agric Food Chem. 2009;57(4):1498-505. doi: 10.1021/jf802390v.

Matsukawa N, Matsumoto M, Shinoki A, Hagio M, Inoue R, Hara H. Nondigestible saccharides suppress the bacterial degradation of quercetin aglycone in the large intestine

and enhance the bioavailability of quercetin glucoside in rats. J Agric Food Chem. 2009;57(20):9462-8. doi: 10.1021/jf9024079.

Matsumoto K, Watanabe Y, Yokoyama S. Okara, soybean residue, prevents obesity in a diet-induced murine obesity model. Biosci Biotechnol Biochem. 2007;71(3):720-7. doi: 10.1271/bbb.60563.

Mierziak J, Kostyn K, Kulma A. Flavonoids as important molecules of plant interactions with the environment. Molecules. 2014;19(10):16240-65. doi: 10.3390/molecules191016240.

Mitamura R, Hara H, Aoyama Y, Takahashi T, Furuta H. Ingestion of water-soluble soybean fiber prevents osteopenia and hypercholesterolemia induced by ovariectomy in rats. J Agric Food Chem. 2003;51(4):1085-9. doi: 10.1021/jf025999j.

Moco S, Martin FP, Rezzi S. Metabolomics view on gut microbiome modulation by polyphenol-rich foods. J Proteome Res. 2012;11(10):4781-90. doi: 10.1021/pr300581s.

Mullen W, Rouanet JM, Auger C, Teissèdre PL, Caldwell ST, Hartley RC, Lean ME, Edwards CA, Crozier A. Bioavailability of [2-(14)C]quercetin-4'-glucoside in rats. J Agric Food Chem. 2008;56(24):12127-37. doi: 10.1021/jf802754s.

Murakami H, Yasui-Furukori N, Otaka H, Nakayama H, Murabayashi M, Mizushiri S, Matsumura K, Tanabe J, Matsuhashi Y, Yanagimachi M, Sugawara N, Shimoda K, Daimon M. Coping styles associated with glucose control in individuals with type 2 diabetes mellitus. J Diabetes Investig. 2020;11(5):1215-1221. doi: 10.1111/jdi.13225.

Murota K, Cermak R, Terao J, Wolffram S. Influence of fatty acid patterns on the intestinal absorption pathway of quercetin in thoracic lymph duct-cannulated rats. Br J Nutr. 2013;109(12):2147-53. doi: 10.1017/S0007114512004564.

Nait Chabane M, Al Ahmad A, Peluso J, Muller CD, Ubeaud G. Quercetin and naringenin transport across human intestinal Caco-2 cells. J Pharm Pharmacol. 2009;61(11):1473-83. doi: 10.1211/jpp/61.11.0006.

Nakajima S, Hira T, Hara H. Postprandial glucagon-like peptide-1 secretion is increased during the progression of glucose intolerance and obesity in high-fat/high-sucrose diet-fed rats. Br J Nutr. 2015;113(9):1477-88. doi: 10.1017/S0007114515000550.

Nam H, Jung H, Karuppasamy S, Park YS, Cho YS, Lee JY, Seong SI, Suh JG. Anti-diabetic effect of the soybean extract fermented by *Bacillus subtilis* MORI in *db/db* mice. Food Science and Biotechnology. 2012;21(6):1669-76. doi: 10.1007/s10068-012-0222-y.

Neilson AP, George JC, Janle EM, Mattes RD, Rudolph R, Matusheski NV, Ferruzzi MG. Influence of chocolate matrix composition on cocoa flavan-3-ol bioaccessibility *in vitro*

and bioavailability in humans. J Agric Food Chem. 2009;57(20):9418-26. doi: 10.1021/jf902919k.

Nguyen LT, Nguyen TH, Nguyen LT, Kamoshita S, Tran TP, LE HT, Shimura F, Yamamoto S. Okara Improved blood glucose level in Vietnamese with type 2 diabetes mellitus. J Nutr Sci Vitaminol (Tokyo). 2019;65(1):60-65. doi: 10.3177/jnsv.65.60.

Nishijima T, Iwai K, Saito Y, Takida Y, Matsue H. Chronic ingestion of apple pectin can enhance the absorption of quercetin. J Agric Food Chem. 2009;57(6):2583-7.

Nomura AM, Hankin JH, Henderson BE, Wilkens LR, Murphy SP, Pike MC, Le Marchand L, Stram DO, Monroe KR, Kolonel LN. Dietary fiber and colorectal cancer risk: The multiethnic cohort study. Cancer Causes Control. 2007;18(7):753-64. doi: 10.1007/s10552-007-9018-4.

Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA. 2006;295(13):1549-55. doi: 10.1001/jama.295.13.1549.

Ohta A, Ohtsuki M, Baba S, Takizawa T, Adachi T, Kimura S. Effects of fructooligosaccharides on the absorption of iron, calcium and magnesium in iron-deficient anemic rats. J Nutr Sci Vitaminol (Tokyo). 1995;41(3):281-91. doi: 10.3177/jnsv.41.281.

Oksuz T, Tacer-Caba Z, Nilufer-Erdil D, Boyacioglu D. Changes in bioavailability of sour cherry (*Prunus cerasus* L.) phenolics and anthocyanins when consumed with dairy food matrices. J Food Sci Technol. 2019;56(9):4177-4188. doi: 10.1007/s13197-019-03888-2.

Palafox-Carlos H, Ayala-Zavala JF, González-Aguilar GA. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. J Food Sci. 2011;76(1):R6-R15. doi: 10.1111/j.1750-3841.2010.01957.x.

Park Y, Brinton LA, Subar AF, Hollenbeck A, Schatzkin A. Dietary fiber intake and risk of breast cancer in postmenopausal women: The National Institutes of Health-AARP Diet and Health Study. Am J Clin Nutr. 2009;90(3):664-71. doi: 10.3945/ajcn.2009.27758.

Pastuszewska B, Taciak M, Tuśnio A, Misztal T, Ochtabińska A. Physiological effects of long-term feeding diets supplemented with potato fibre or cellulose to adult rats. Arch Anim Nutr. 2010;64(2):155-69. doi: 10.1080/17450390903478885.

Patel RV, Mistry BM, Shinde SK, Syed R, Singh V, Shin HS. Therapeutic potential of quercetin as a cardiovascular agent. Eur J Med Chem. 2018;155:889-904. doi: 10.1016/j.ejmech.2018.06.053.

Patterson E, Cryan JF, Fitzgerald GF, Ross RP, Dinan TG, Stanton C. Gut microbiota, the pharmabiotics they produce and host health. Proc Nutr Soc. 2014;73(4):477-89. doi: 10.1017/S0029665114001426.

Peng J, Li Q, Li K, Zhu L, Lin X, Lin X, Shen Q, Li G, Xie X. Quercetin improves glucose and lipid metabolism of diabetic rats: Involvement of Akt signaling and SIRT1. J Diabetes Res. 2017;2017:3417306. doi: 10.1155/2017/3417306.

Peng X, Zhang Z, Zhang N, Liu L, Li S, Wei H. *In vitro* catabolism of quercetin by human fecal bacteria and the antioxidant capacity of its catabolites. Food Nutr Res. 2014;58. doi: 10.3402/fnr.v58.23406.

Phuwamongkolwiwat P, Hira T, Hara H. A nondigestible saccharide, fructooligosaccharide, increases the promotive effect of a flavonoid, α-glucosyl-isoquercitrin, on glucagon-like peptide 1 (GLP-1) secretion in rat intestine and enteroendocrine cells. Mol Nutr Food Res. 2014;58(7):1581-4. doi: 10.1002/mnfr.201300871.

Phuwamongkolwiwat P, Suzuki T, Hira T, Hara H. Fructooligosaccharide augments benefits of quercetin-3-*O*-β-glucoside on insulin sensitivity and plasma total cholesterol with promotion of flavonoid absorption in sucrose-fed rats. Eur J Nutr. 2014;53(2):457-68. doi: 10.1007/s00394-013-0546-2.

Pinyo J, Hara H, Hira T. Diet-induced obesity enhances postprandial glucagon-like peptide-1 secretion in Wistar rats, but not in diabetic Goto-Kakizaki rats. Br J Nutr. 2020;1-13. doi: 10.1017/S000711452000433X.

Pinyo J, Hira T, Hara H. Continuous feeding of a combined high-fat and high-sucrose diet, rather than an individual high-fat or high-sucrose diet, rapidly enhances the glucagon-like peptide-1 secretory response to meal ingestion in diet-induced obese rats. Nutrition. 2019a;62:122-130. doi: 10.1016/j.nut.2019.01.004.

Pinyo J, Hira T, Hara H. Enhanced postprandial glucagon-like peptide-1 secretion during obesity development has a protective role against glucose intolerance induction in rats. Br J Nutr. 2019b;122(4):411-422. doi: 10.1017/S0007114519001223.

Prasath GS, Sundaram CS, Subramanian SP. Fisetin averts oxidative stress in pancreatic tissues of streptozotocin-induced diabetic rats. Endocrine. 2013;44(2):359-68. doi: 10.1007/s12020-012-9866-x.

Preguiça I, Alves A, Nunes S, Fernandes R, Gomes P, Viana SD, Reis F. Diet-induced rodent models of obesity-related metabolic disorders-A guide to a translational perspective. Obes Rev. 2020;21(12):e13081. doi: 10.1111/obr.13081.

Raneva V, Shimasaki H, Ishida Y, Ueta N, Niki E. Antioxidative activity of 3,4-dihydroxyphenylacetic acid and caffeic acid in rat plasma. Lipids. 2001;36(10):1111-6. doi: 10.1007/s11745-001-0821-6.

Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 1993;123(11):1939-51. doi: 10.1093/jn/123.11.1939.

Rehman K, Ali MB, Akash MSH. Genistein enhances the secretion of glucagon-like peptide-1 (GLP-1) via downregulation of inflammatory responses. Biomed Pharmacother. 2019;112:108670. doi: 10.1016/j.biopha.2019.108670.

Rich GT, Buchweitz M, Winterbone MS, Kroon PA, Wilde PJ. Towards an understanding of the low bioavailability of quercetin: A study of its interaction with intestinal lipids. Nutrients. 2017;9(2):111. doi: 10.3390/nu9020111.

Rideout TC, Harding SV, Jones PJ, Fan MZ. Guar gum and similar soluble fibers in the regulation of cholesterol metabolism: Current understandings and future research priorities. Vasc Health Risk Manag. 2008;4(5):1023-33. doi: 10.2147/vhrm.s3512.

Rowland IR, Mallett AK, Wise A. A comparison of the activity of five microbial enzymes in cecal content from rats, mice, and hamsters, and response to dietary pectin. Toxicol Appl Pharmacol. 1983;69(1):143-8. doi: 10.1016/0041-008x(83)90130-8.

Saha D, Bhattacharya S. Hydrocolloids as thickening and gelling agents in food: A critical review. J Food Sci Technol. 2010;47(6):587-97. doi: 10.1007/s13197-010-0162-6.

Sakata T. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. Br J Nutr. 1987;58(1):95-103. doi: 10.1079/bjn19870073.

Salehi B, Machin L, Monzote L, Sharifi-Rad J, Ezzat SM, Salem MA, Merghany RM, El Mahdy NM, Kılıç CS, Sytar O, Sharifi-Rad M, Sharopov F, Martins N, Martorell M, Cho WC. Therapeutic potential of quercetin: New insights and perspectives for human health. ACS Omega. 2020;5(20):11849-11872. doi: 10.1021/acsomega.0c01818.

Scalbert A., Williamson G. Dietary intake and bioavailability of polyphenols. J. Nutr. 2000;130:2073S–2085S. doi: 10.1093/jn/130.8.2073S.

Schatzkin A, Park Y, Leitzmann MF, Hollenbeck AR, Cross AJ. Prospective study of dietary fiber, whole grain foods, and small intestinal cancer. Gastroenterology. 2008;135(4):1163-7. doi: 10.1053/j.gastro.2008.07.015.

Schneider H, Blaut M. Anaerobic degradation of flavonoids by *Eubacterium ramulus*. Arch Microbiol. 2000;173(1):71-5. doi: 10.1007/s002030050010.

Schneider H, Schwiertz A, Collins MD, Blaut M. Anaerobic transformation of quercetin-3-glucoside by bacteria from the human intestinal tract. Arch Microbiol. 1999;171(2):81-91. doi: 10.1007/s002030050682.

Seiva FR, Chuffa LG, Braga CP, Amorim JP, Fernandes AA. Quercetin ameliorates glucose and lipid metabolism and improves antioxidant status in postnatally monosodium glutamate-induced metabolic alterations. Food Chem Toxicol. 2012;50(10):3556-61. doi: 10.1016/j.fct.2012.07.009.

Seo MJ, Lee YJ, Hwang JH, Kim KJ, Lee BY. The inhibitory effects of quercetin on obesity and obesity-induced inflammation by regulation of MAPK signaling. J Nutr Biochem. 2015;26(11):1308-16. doi: 10.1016/j.jnutbio.2015.06.005.

Shi GJ, Li Y, Cao QH, Wu HX, Tang XY, Gao XH, Yu JQ, Chen Z, Yang Y. *In vitro* and *in vivo* evidence that quercetin protects against diabetes and its complications: A systematic review of the literature. Biomed Pharmacother. 2019;109:1085-1099. doi: 10.1016/j.biopha.2018.10.130.

Shiau SY, Chang GW. Effects of dietary fiber on fecal mucinase and beta-glucuronidase activity in rats. J Nutr. 1983;113(1):138-44. doi: 10.1093/jn/113.1.138.

Shinoki A, Lang W, Thawornkuno C, Kang HK, Kumagai Y, Okuyama M, Mori H, Kimura A, Ishizuka S, Hara H. A novel mechanism for the promotion of quercetin glycoside absorption by megalo α-1,6-glucosaccharide in the rat small intestine. Food Chem. 2013;136(2):293-6. doi: 10.1016/j.foodchem.2012.08.028.

Singh AK, Kim WK. Effects of dietary fiber on nutrients utilization and gut health of poultry: A review of challenges and opportunities. Animals (Basel). 2021;11(1):181. doi: 10.3390/ani11010181.

Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu TH, Bhutani T, Liao W. Influence of diet on the gut microbiome and implications for human health. J Transl Med. 2017;15(1):73. doi: 10.1186/s12967-017-1175-y.

Skrbek S, Ruefer CE, Marko D, Esselen M. Quercetin and its microbial degradation product 3,4-dihydroxyphenylacetic acid generate hydrogen peroxide modulating their stability under *in vitro* conditions. J Food Nutr. 2009;48:129–140.

Spencer JP, Kuhnle GG, Williams RJ, Rice-Evans C. Intracellular metabolism and bioactivity of quercetin and its *in vivo* metabolites. Biochem J. 2003;372(Pt 1):173-81. doi: 10.1042/BJ20021972.

Srivastava S, Somasagara RR, Hegde M, Nishana M, Tadi SK, Srivastava M, Choudhary B, Raghavan SC. Quercetin, a natural flavonoid interacts with DNA, arrests cell cycle and causes tumor regression by activating mitochondrial pathway of apoptosis. Sci Rep. 2016;6:24049. doi: 10.1038/srep24049.

Stefova M, Kulevanova S, Stafilov T. Assay of flavonols and quantification of quercetin in medicinal plants by HPLC with UV-diode array detection. J. Liq. Chromatogr. Relat. Technol. 2001;24:2283– 2292. doi: 10.1081/JLC-100105140

Stewart LK, Soileau JL, Ribnicky D, Wang ZQ, Raskin I, Poulev A, Majewski M, Cefalu WT, Gettys TW. Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet. Metabolism. 2008;57(7 Suppl 1):S39-46. doi: 10.1016/j.metabol.2008.03.003.

Suganthy N, Devi KP, Nabavi SF, Braidy N, Nabavi SM. Bioactive effects of quercetin in the central nervous system: Focusing on the mechanisms of actions. Biomed Pharmacother. 2016;84:892-908. doi: 10.1016/j.biopha.2016.10.011.

Suzuki T, Hara H. Quercetin enhances intestinal barrier function through the assembly of zonula [corrected] occludens-2, occludin, and claudin-1 and the expression of claudin-4 in Caco-2 cells. J Nutr. 2009;139(5):965-74. doi: 10.3945/jn.108.100867.

Swann OG, Kilpatrick M, Breslin M, Oddy WH. Dietary fiber and its associations with depression and inflammation. Nutr Rev. 2020;78(5):394-411. doi: 10.1093/nutrit/nuz072.

Taciak M, Barszcz M, Tuśnio A, Pastuszewska B. Interactive effects of indigestible carbohydrates, protein type, and protein level on biomarkers of large intestine health in rats. PLoS One. 2015;10(11):e0142176. doi: 10.1371/journal.pone.0142176.

Takahashi T, Maeda H, Aoyama T, Yamamoto T, Takamatsu K. Physiological effects of water-soluble soybean fiber in rats. Biosci Biotechnol Biochem. 1999;63(8):1340-5. doi: 10.1271/bbb.63.1340.

Tanaka S, Oyama M, Nishikawa M, Ikushiro S, Hara H. Simultaneous collection of the portal and superior vena cava blood in conscious rats defined that intestinal epithelium is the major site of glucuronidation, but not sulfation and methylation, of quercetin. Biosci Biotechnol Biochem. 2018;82(12):2118-2129. doi: 10.1080/09168451.2018.1515615.

Tanaka S, Shinoki A, Hara H. Melibiose, a nondigestible disaccharide, promotes absorption of quercetin glycosides in rat small intestine. J Agric Food Chem. 2016;64(49):9335-9341. doi: 10.1021/acs.jafc.6b03714.

Tang YH, Pang SM, Chan MF, Yeung GS, Yeung VT. Health literacy, complication awareness, and diabetic control in patients with type 2 diabetes mellitus. J Adv Nurs. 2008;62(1):74-83. doi: 10.1111/j.1365-2648.2007.04526.x.

Thilakarathna SH, Rupasinghe HP. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients. 2013;5(9):3367-87. doi: 10.3390/nu5093367.

Thilakarathna, S.; Rupasinghe, H. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients. 2013, 5, 3367–3387. doi: 10.3390/nu5093367

Threapleton DE, Greenwood DC, Evans CE, Cleghorn CL, Nykjaer C, Woodhead C, Cade JE, Gale CP, Burley VJ. Dietary fibre intake and risk of cardiovascular disease: Systematic review and meta-analysis. BMJ. 2013;347:f6879. doi: 10.1136/bmj.f6879.

Tian R, Yang W, Xue Q, Gao L, Huo J, Ren D, Chen X. Rutin ameliorates diabetic neuropathy by lowering plasma glucose and decreasing oxidative stress via Nrf2 signaling pathway in rats. Eur J Pharmacol. 2016;771:84-92. doi: 10.1016/j.ejphar.2015.12.021.

Tolhurst G, Reimann F, Gribble FM. Nutritional regulation of glucagon-like peptide-1 secretion. J Physiol. 2009;587(1):27-32. doi: 10.1113/jphysiol.2008.164012.

Trakooncharoenvit A, Tanaka S, Mizuta E, Hira T, Hara H. Water-soluble dietary fibers enhance bioavailability of quercetin and a fiber derived from soybean is most effective after long-term feeding in rats. Eur J Nutr. 2020;59(4):1389-1398. doi: 10.1007/s00394-019-01992-9.

Tsuda T. Possible abilities of dietary factors to prevent and treat diabetes via the stimulation of glucagon-like peptide-1 secretion. Mol Nutr Food Res. 2015;59(7):1264-73. doi: 10.1002/mnfr.201400871.

Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines (Basel). 2018;5(3):93. doi:10.3390/medicines5030093

Ulusoy HG, Sanlier N. A minireview of quercetin: From its metabolism to possible mechanisms of its biological activities. Crit Rev Food Sci Nutr. 2020;60(19):3290-3303. doi: 10.1080/10408398.2019.1683810.

Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comp Biochem Physiol C Toxicol Pharmacol. 2003;135C(3):357-64. doi: 10.1016/s1532-0456(03)00140-6.

Wach, A.; Pyrzyńska, K.; Biesaga, M. Quercetin content in some food and herbal samples. Food Chem. 2007;100:699– 704. doi: 10.1016/j.foodchem.2005.10.028

Walle T. Absorption and metabolism of flavonoids. Free Radic Biol Med. 2004;36(7):829-37. doi: 10.1016/j.freeradbiomed.2004.01.002.

Wang Y, Wang A, Alkhalidy H, Luo J, Moomaw E, Neilson AP, Liu D. Flavone hispidulin stimulates glucagon-like peptide-1 secretion and ameliorates hyperglycemia in streptozotocin-induced diabetic mice. Mol Nutr Food Res. 2020;64(6):e1900978. doi: 10.1002/mnfr.201900978. Epub 2020 Feb 3. Erratum in: Mol Nutr Food Res. 2020;64(7):e2070015. doi: 10.1002/mnfr.201900978.

Weickert, M.O.; Pfeiffer, A.F.H. Metabolic effects of dietary and prevention of diabetes. J. Nutr. 2008;138(3):439-42. doi: 10.1093/jn/138.3.439

Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr. 2005;81(1 Suppl):243S-255S. doi: 10.1093/ajcn/81.1.243S.

Wolffram S, Blöck M, Ader P. Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. J Nutr. 2002;132(4):630-5. doi: 10.1093/jn/132.4.630.

Wootten D, Simms J, Koole C, Woodman OL, Summers RJ, Christopoulos A, Sexton PM. Modulation of the glucagon-like peptide-1 receptor signaling by naturally occurring and synthetic flavonoids. J Pharmacol Exp Ther. 2011;336(2):540-50. doi: 10.1124/jpet.110.176362.

Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;334(6052):105-8. doi: 10.1126/science.1208344.

Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019;24(6):1123. doi: 10.3390/molecules24061123.

Xu H, Tan SM, Li SQ. Effects of soybean fibers on blood sugar, lipid levels and hepatic-nephritic histomorphology in mice with diabetes mellitus. Biomed Environ Sci. 2001;14(3):256-61.

Zahedi M, Ghiasvand R, Feizi A, Asgari G, Darvish L. Does Quercetin improve cardiovascular risk factors and inflammatory biomarkers in women with type 2 diabetes: A Double-blind randomized controlled clinical trial. Int J Prev Med. 2013;4(7):777-85.

Zhang Z, Peng X, Li S, Zhang N, Wang Y, Wei H. Isolation and identification of quercetin degrading bacteria from human fecal microbes. PLoS One. 2014;9(3):e90531. doi: 10.1371/journal.pone.0090531.

Zhao Y, Chen B, Shen J, Wan L, Zhu Y, Yi T, Xiao Z. The beneficial effects of quercetin, curcumin, and resveratrol in obesity. Oxid Med Cell Longev. 2017;2017:1459497. doi: 10.1155/2017/1459497.

Academic conferences

2018   Oral & Poster presentation in the title of "Effect of water-soluble dietary fiber on a quercetin glucoside absorption in rats" in Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry, Nagoya, Japan (2018.3.15-18)

2018   Oral & Poster presentation in the title of "Water-soluble soybean fiber improves alpha- glycosyl-isoquercetin absorption with long term feeding in rats" in Annual Meeting of Japanese Society for Food Science and Technology, Tohoku University, Kawauchi-Kita Campus, Miyagi, Japan (2018.8.22-24)

2019   Oral & Poster presentation in the title of "Combined feeding of α-glycosyl-isoquercitrin and soluble soybean fiber prevents glucose intolerance in rats" in The 9th International Conference on Polyphenols and Health, Kobe, Japan (2019.11.28-12.01)

2020   Online presentation in the title of "The increment of quercetin bioavailability by feeding α-glycosyl-isoquercitrin with soybean fiber promotes the glucagon-like peptide-1 secretion, and alleviates glucose intolerance in diet-induced obesity rat model" in The 25th Annual Meeting of the Japanese Association for Dietary Fiber Research (2020.11.21-23)

Publications

<u>Trakooncharoenvit A</u>, Tanaka S, Mizuta E, Hira T, Hara H. Water-soluble dietary fibers enhance bioavailability of quercetin and a fiber derived from soybean is most effective after long-term feeding in rats. *Eur J Nutr*. 2020 Jun;59(4):1389-1398. doi: 10.1007/s00394-019-01992-9.

<u>Trakooncharoenvit A</u>, Tanaka S, Mizuta E, Hira T, Hara H. Reply to the Letter: Increased bioavailability of plasma polyphenols via the intestinal fermentation of soybean fibers: a role for gut microbiome? *Eur J Nutr*. 2019 Sep;58(6):2563. doi: 10.1007/s00394-019-02030-4.

Tanaka S, <u>Trakooncharoenvit A</u>, Nishikawa M, Ikushiro S, Hara H. Comprehensive Analyses of Quercetin Conjugates by LC/MS/MS Revealed That Isorhamnetin-7- O-glucuronide-4'- O-sulfate Is a Major Metabolite in Plasma of Rats Fed with Quercetin Glucosides. *J Agric Food Chem*. 2019 Apr 17;67(15):4240-4249. doi: 10.1021/acs.jafc.8b06929.

<u>Trakooncharoenvit A</u>, Hara H, Hira T. Combination of α-Glycosyl-Isoquercitrin and Soybean Fiber Promotes Quercetin Bioavailability and Glucagon-like Peptide-1 Secretion and Improves Glucose Homeostasis in Rats Fed a High-Fat High-Sucrose Diet. *J Agric Food Chem*. 2021 May 18. doi: 10.1021/acs.jafc.1c01388.

Acknowledgment