



Title	Combination of alpha-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
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1 **Title**

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

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16 **Abstract**

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

29

30 **Keywords: Diabetes, Diet-induced obesity, Fermentable fiber, Glucose tolerance**

31 **Introduction**

32 Quercetin (3,3',4',5,7-pentahydroxyflavone) is a flavonoid belonging to the flavonol
33 class and is widely distributed in various kinds of vegetables and fruits, mainly as in a
34 glycosylated form.¹ Reportedly, quercetin possesses protective effects against numerous
35 diseases such as; cancer in *in vitro* and animal studies;² cardiovascular diseases in animal
36 and human studies;³ obesity in *in vitro*, high-fat diet fed animal and obese human studies;^{4,5}
37 and diabetes in db/db mice and streptozotocin induced rat studies.⁶ Numbers of *in vitro* and
38 animal studies suggest the beneficial potential of quercetin, but studies demonstrating its
39 efficacy in human subjects are limited.^{5,7}

40 After ingestion, hydrolysis of glycoside into aglycone by the intestinal enzyme is
41 thought to be an essential step for its absorption into intestinal epithelial cells. After
42 absorbed, the conjugation for quercetin metabolites mainly occurred in the liver. However,
43 intestinal absorbability of quercetin is known to be low.²⁻⁷ Concomitantly, unabsorbed
44 quercetin aglycone and some metabolites were transported to the large intestine, the main
45 site of gut fermentation.⁸ In here, the intestinal bacteria with various enzymatic activities
46 can deconjugate (transform) quercetin metabolites and further degrade the quercetin
47 aglycone, which results in loss of biological activities from the original compound.⁹ An *in*
48 *vitro* fermentation study revealed that quercetin can be degraded by the anaerobic pathogen.
49 Along with this degradation, a reduction in antioxidant activities and increment in a
50 degraded product of quercetin, 3,4-dihydroxyphenylacetic acid were demonstrated.¹⁰ Thus,
51 the degradation by large intestinal bacteria and poor intestinal absorption of quercetin limit
52 quercetin's bioavailability, and health beneficial effects of this flavonol.

53 Recently, we have reported that soybean fiber is the best candidate, among other
54 tested water-soluble dietary fibers such as pectin and guar gum, to improve quercetin

55 bioavailability in a long-term feeding study performed in rats.¹¹ This study has revealed
56 that cecal fermentation promoted by feeding the dietary fiber is linked with the
57 bioavailability of quercetin. Accordingly, it was expected that the increased quercetin
58 bioavailability would prevent the impairment of glucose homeostasis induced by chronic
59 excessive energy intake.

60 Currently, type 2 diabetes is turning into an epidemic worldwide.¹² This disease
61 gradually develops owing to impaired glucose homeostasis, comprised of glucose
62 intolerance and insulin resistance.¹³ For the treatment of type 2 diabetes, incretin-based
63 therapy has been developed and successfully used.¹⁴ Glucagon-like peptide-1 (GLP-1) is
64 an incretin hormone released by enteroendocrine L cells in response to luminal nutrients.¹⁵
65 GLP-1 plays a crucial role in glucose homeostasis by enhancing insulin secretion in a
66 glucose dependent manner and possibly promoting the beta-cell mass.¹⁶ Furthermore, we
67 have demonstrated the protective role of enhanced GLP-1 secretion during the development
68 of glucose intolerance in diet-induced obese rats.¹⁷ Recently, manipulating GLP-1 secretion
69 by various strategies has been gaining momentum as a promising strategy for preventing
70 glucose intolerance.^{18,19} In our previous study using rats, the combination of a fructo-
71 oligosaccharide and quercetin-3-O- β -d-glucoside (isoquercitrin) demonstrated an
72 improvement in glucose tolerance and insulin sensitivity, with increased GLP-1 secretion
73 and plasma quercetin concentration.²⁰

74 Thus, in the present study, we examined whether the combined ingestion of α -
75 glycosyl-isoquercitrin (AGIQ) and soybean fiber promotes quercetin bioavailability,
76 affects GLP-1 secretion, and prevents glucose intolerance in rats continuously fed an
77 obesogenic high-fat high-sucrose diet.

78

79 **Materials and methods**80 **Animals and diets**

81 Thirty-five male Wistar-ST rats 5 weeks old, weighing about 130-150 g (purchased from
82 Japan SLC, Inc., Shizuoka, Japan) were housed in individual stainless-steel cages with wire mesh
83 bottoms. Rats were maintained in a temperature-controlled room ($22 \pm 2^\circ\text{C}$) with a 12 h light-dark
84 cycle (light period 08.00–20.00) and fed using a modified American Institute of Nutrition-93G
85 (AIN-93G) diet,²¹ by changing the cellulose content from 5% to 7% (2% of cellulose substituted
86 with corn starch, Table 1). In the test diet, α -glycosyl isoquercitrin (AGIQ, kindly provided by
87 San-Ei Gen F.F.I., Inc., Osaka, Japan), mainly consisting of quercetin-3-O- β -d-glucoside (Q3G,
88 31.8%), mono (23.3%), and di (20.3%) D-glucose adducts with an α -1,4-linkage, was used as a
89 quercetin source. Soybean fiber (kindly provided by Fuji Oil Co., Ltd, Osaka, Japan), a low
90 viscosity highly fermentable fiber, was used as supplemental fiber. After one week of acclimation,
91 the rats were divided into five groups (n=7) based on body weight and basal plasma glucose
92 concentration. For 9 weeks, each group of rats was respectively fed the modified AIN-93G as a
93 control (C) diet, a high-fat high-sucrose (H) diet containing 30% fat and 40% sucrose,^{17,22} a high-
94 fat high-sucrose diet with either soybean fiber (HS) or AGIQ (HQ), and high-fat high-sucrose with
95 both soybean fiber and AGIQ (HSQ), ad libitum. The supplementary doses of AGIQ and soybean
96 fiber were selected based on a previous study.¹¹ In addition, the usage of higher concentrations
97 (0.7%) of quercetin in both mice and human studies had been reported.^{23,24} Body weights and food
98 intakes were measured every 1 or 2 days. Nine weeks after the start of test feeding, rats were fasted
99 overnight and euthanized by whole blood withdrawal. Furthermore, the visceral adipose tissue
100 (mesenteric, retroperitoneal, and epididymal adipose tissue) was carefully dissected and weighed.

101 The whole cecum was collected and stored at -80°C until measurement. The experiments were
102 conducted according to the guidelines of the Hokkaido University for the care and use of laboratory
103 animals, and the study was approved by the Hokkaido University Animal Committee (approval
104 reference number: 19-0072).

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

109

110 **Cecal content treatment for pH and short-chain fatty acid measurement**

111 The frozen cecum was separated into cecal tissue and contents. The contents were diluted
112 with four times weight of deionized water, and the pH was measured using a pH meter (B-211
113 twin waterproof pH, Horiba, Ltd., Kyoto, Japan). The remaining diluted sample (700 μ l) was
114 mixed with 200 μ L of the internal standard (25 mM crotonic acid in 50 mM sodium hydroxide)
115 and centrifuged at 15600g for 10 min at 4°C. The supernatant (600 μ L) was mixed with the same
116 volume of chloroform and centrifuged again. The supernatant was filtrated through a 0.2 μ m
117 membrane, followed by short-chain fatty acid measurement using high-performance liquid
118 chromatography (HPLC)^{25,26} with two shim-pack SCR-102H (8 mm I.D.×300 mm L, Shimadzu
119 Corp., Kyoto, Japan) and a conductivity detector CCD-6A (polarity: +, response: slow,
120 temperature: 45°C; Shimadzu Corp., Kyoto, Japan).

121

122 **Sample treatment for quercetin measurement**

123 At week 2, 4, 6, and 8 of the feeding periods, urine and feces were collected for 2 and 3
124 days, respectively. On the day after feces collection, tail vein blood samples were collected into a
125 tube containing heparin (final concentration of 50 IU/mL blood; Ajinomoto Company, Inc., Tokyo,
126 Japan) and aprotinin (final concentration of 500 kallikrein inhibitor units (kIU)/ml blood; Wako
127 Pure Chemical Industries, Ltd., Osaka, Japan). The collected blood samples were centrifuged at
128 2300g for 10 min at 4°C to separate the plasma before storage at -80°C. During urine collection,
129 bacterial degradation of quercetin metabolites was prevented by adding 0.05% sodium azide
130 (Sigma Aldrich, Saint Louis) into a collection tray. After two days, the collected urine was filled
131 up to 100 mL, and 15 mL of diluted urine samples were stored at -40°C. Fecal samples were
132 collected by placing a steel sieve mesh under the rat cage for three days, followed by storage at -
133 80°C. After the urine and feces were collected, the rats were rested for one day before overnight
134 fasting, and the meal tolerance test (MTT) or plasma collection was performed on the next day as
135 mentioned above.

136 On extraction, plasma and urine samples (100 µL) were acidified with 10 µL of 0.58 mol/L
137 acetic acid. In both samples, quercetin and its metabolites have been detected in substantial
138 amounts as conjugate metabolites.²⁷ To obtain quercetin and methylquercetin aglycone derivatives,
139 7.5 µL of β-glucuronidase and sulfatase (from *Helix pomatia* extract; Sigma Aldrich, Saint Louis),
140 and 7.5 µL of sulfatase (from *Aerobacter aerogenes*; Sigma Aldrich, Saint Louis), were added and
141 incubated at 37°C for 1 h. The reaction mixture was mixed with 100 µL of the internal standard
142 (naringenin 10 µM at final), heated at 100°C for 1 min, and centrifuged at 9300g for 5 min at 4°C.
143 The supernatant was collected and the precipitate was twice re-extracted with methanol to recover
144 aglycone of quercetin derivatives in the precipitant. The collected supernatant was loaded into a
145 C18 cartridge (Oasis HLB; Waters Co. Ltd., Massachusetts) and eluted with 1 mL methanol.

146 For feces, frozen samples were dried and milled into a powder. The feces powder (0.1 g)
147 was mixed with 1 mL of 80% methanol and 100 μ L of internal standard (naringenin 50 μ M at
148 final), then homogenized using an ultrasonic homogenizer (VP-050, ULTRAS homogenizer;
149 Taitec Co. Ltd., Nagoya, Japan) with 30% power for 20 s. The homogenized sample was incubated
150 at 60°C for 1 h and centrifuged at 2300g for 10 min at 4°C. This extraction procedure was repeated
151 twice. In the case of cecal contents, one gram of the content was suspended in 5 mL of deionized
152 water. The suspended sample (100 μ L) was mixed with 100 μ L of internal standard (naringenin
153 40 μ M at final) and heated at 100°C for 1 min. The supernatant was collected by centrifugation at
154 9300g for 5 min at 4°C. This extraction procedure was repeated two times by adding methanol
155 instead of the internal standard as in the previous step. The accumulated supernatant, feces, or
156 cecal content, was loaded into a C18 cartridge similar to plasma and urine samples. As fecal and
157 cecal contents usually appear in the aglycone form, these samples were extracted without
158 deconjugation enzyme treatment. All eluted samples were dried by evaporation and then
159 reconstituted with 50% methanol on the measurement day.

160

161 **Quercetin measurement using HPLC**

162 The sum of quercetin aglycone and methylquercetin aglycone was considered as the total
163 quercetin derivative, which was quantified using HPLC²⁸ (Thermo Scientific Dionex UltiMate™
164 3000 HPLC system; Thermo Fisher Scientific Inc., Massachusetts). The analyte was separated
165 using a ZORBAX RRHD Eclipse Plus Phenyl-Hexyl C18 (2.1×100 mm, 1.8 μ m; Agilent
166 Technologies Japan, Ltd., Tokyo, Japan) column with setting temperature at 50°C. The mobile
167 phases were methanol with formic acid (99.99:0.01 as solvent A) and water with formic acid
168 (99.99:0.01 as solvent B). For measurement, the linear gradient started with solvent A and B at a

169 ratio of 70:30, steadily change to solvent B 100% in 5 min (0-5 min), isocratically hold for 1 min
170 (5-6 min), then decreased back to 70:30 within 1 min (6-7 min), followed by re-equilibration for
171 1 min (7-8 min). Naringenin and quercetin (quercetin aglycone and methylquercetin aglycone)
172 were detected at 280 nm and 370 nm, respectively. The concentrations of quercetin derivatives
173 were calculated from the peak area based on the calibration curve of standard compounds.

174

175 **Meal tolerance test**

176 MTT was performed instead of an oral glucose tolerance test because nutrients from a
177 mixed meal can provide relevant postprandial responses regarding glycemia, GLP-1, and insulin
178 secretions.^{17,29,30}

179 During the feeding period, MTT was performed after overnight fasting (16 h) every 4
180 weeks (at week 4 and 8). Here, tubes containing anti-coagulant, as described in plasma collection
181 for quercetin measurement, were used. Blood samples from the tail vein of rats were collected
182 before (0 min) and after (15, 30, 60, 90, and 120 min) oral gavage administration of the liquid meal
183 (15 kcal/ 10 ml per kg body weight; Ensure H, Abbott, Japan). The collected blood samples were
184 centrifuged, then the plasma was separated and stored at -80°C. The collected plasma was used for
185 glucose, insulin, and total GLP-1 measurements using the Glucose CII Test Kit (Wako Pure
186 Chemical Industries, Osaka, Japan), Insulin ELISA (U-E type; Shibayagi Company Limited,
187 Gunma, Japan), and Total GLP-1 ELISA Kit (Wako Pure Chemical Industries, Osaka, Japan),
188 respectively. All analytical procedures, using the respective test kits, were performed in
189 accordance with the instructions provided with the assay kit.

190

191 **GLP-1 measurement in cecal tissue**

192 GLP-1 concentrations in cecal tissue were measured as described before.³⁰ Briefly, A
193 segment of cecal tissue was homogenized in a mixture of ethanol:water:12 M HCl (74:25:1, 5
194 mL/g tissue), and extracted at 4°C for 24 hr. The homogenized sample was centrifuged at 2000g
195 for 20 min at 4°C. Then, the supernatants were collected and diluted (500-fold) with saline to
196 measure total GLP-1 concentrations by ELISA.

197

198 **Statistical analysis**

199 All data are expressed as mean ± standard error of the mean (SEM). Before analyses with
200 statistical test, normal distribution of the variables and there was homogeneity of variances were
201 checked. Differences among treatment groups were analyzed with the Tukey–Kramer’s test or
202 Student’s t-test (described in the legend of each figure) after one-way ANOVA. A *P* value of less
203 than 0.05 was considered significant. Pearson’s correlation coefficient (*r*) and the significance of
204 correlations were analyzed between various parameters. The area under the curve (AUC) for
205 postprandial glucose, insulin, and GLP-1 was calculated using the trapezoidal rule. Additionally,
206 the AUC of HOMA-IR^{29,31} was calculated to compute the degree of insulin resistance resulting
207 from the feeding diet as follows:

208
$$\text{HOMA-IR AUC} = (\text{AUC postprandial glucose (mg/dL} \times 2\text{hr)}) \times \text{AUC postprandial insulin}$$

209
$$(\mu\text{U/mL} \times 2\text{hr}) / 2430$$

210 where 1 mg insulin = 26 IU.

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

213

214 **Results**

215 **Body and tissue weights, energy intake, and pH of cecal content**

216 Although no difference was observed in the energy intake and the final body weight among
217 groups (Fig. 1a, b), the highest visceral fat pad weight was observed in the H group (Fig. 1c). Rats
218 fed with a soybean fiber supplemented high-fat high-sucrose diet demonstrated a markedly
219 decreased accumulation of visceral fat when compared with H group ($P < 0.05$; Tukey–Kramer’s
220 test).

221 Soybean fiber-fed groups (HS and HSQ) had higher cecal tissue and cecal content weights,
222 as well as reduced pH of the cecal content (Fig. 1d-f), when compared to groups without soybean
223 fiber (C, H, and HQ). These results suggested that cecal fermentation was promoted by soybean
224 fiber supplementation.

225

226 **Short-chain fatty acids (SCFAs) in cecal content**

227 The amount of SCFAs (Fig. 2) represents the degree of cecal fermentation and metabolic activity
228 of the intestinal microbiota.³² A diet with soybean fiber increased the amount of both acetic and
229 propionic acids, with the highest amounts of acetic acid ($53.4 \pm 2.3 \mu\text{mol}/\text{total cecum}$) and
230 propionic acid ($16.6 \pm 2.6 \mu\text{mol}/\text{total cecum}$) observed in the HSQ group. Correspondingly, an
231 extensive elevation of total SCFAs was also observed in both soybean fiber fed groups (HS and
232 HSQ).

233

234 **Total quercetin derivatives in plasma, urine, feces, and cecal content**

235 Quercetin and its derivatives were not detected in C, H, and HS groups (data not shown).
236 The total quercetin derivatives represent the sum of quercetin aglycone and methylquercetin
237 aglycone (isorhamnetin and tamarixetin) derivatives. In rats fed the diet containing AGIQ, almost

238 all the quercetin derivatives detected in the plasma were methylated form. From week 2 to 8 of the
239 feeding period, the HSQ group showed higher total quercetin derivative levels than the HQ group.
240 (Fig. 3a).

241 Rats fed with HSQ demonstrated a higher urinal quercetin level from week 4 to 8 (Fig. 3b)
242 than HQ group. Regarding the fecal sample, higher quercetin levels were observed in HQ group
243 from week 2 to 4, than in HSQ group. The decreased tendency in fecal quercetin derivatives of
244 HQ group was observed in a later period, while HSQ group demonstrated a reciprocal trend with
245 a five-times greater level than HQ group at week 8 of the feeding period. (Fig. 3c).

246 The quercetin content of cecum in HSQ fed rats was greater (Fig. 4) than that in HQ group
247 at the end of the experimental period. In HQ fed rats, quercetin aglycone levels (0.34 ± 0.18
248 $\mu\text{mol}/\text{cecum}$) were considerably lower than those observed in HSQ fed rats (6.5 ± 0.75
249 $\mu\text{mol}/\text{cecum}$).

250

251 **Degradation of quercetin and methylquercetin derivatives**

252 The effect of test diets on quercetin degradation was calculated as described in Table 2.³³
253 To determine the precise aglycone levels ingested, we averaged the diet intake data from the day
254 before urine and feces collection until the day after plasma collection (complete week period) for
255 the calculation. In addition, the ingested aglycone weight is calculated by subtracting glycoside
256 weight from ingested quercetin glycoside weight. The sum of excreted aglycone (quercetin
257 aglycone + methylquercetin aglycone of urine and feces) can be considered as quercetin that
258 escaped from degradation by intestinal bacteria.³⁴

259 In the present study, HQ group had a higher ingested aglycone weight than HSQ group. At
260 week 2, HQ group showed a lower percentage of degraded aglycone, but it gradually rose from

261 week 4 to 8. Markedly, two-way repeated measures ANOVA for degraded aglycone revealed a
262 significant interaction between the duration of feeding period and the diet ($P = 0.0034$).

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

268

269 **Glycemic, insulin, and GLP-1 responses under MTT**

270 Because statistically significant differences were not detectable in time course changes of
271 glucose, insulin, and GLP-1 (Fig. 5a, 5c, 5e and 6a, 6c, 6e), results are explained based on AUC
272 values (Fig. 5b, 5d, 5f, 5g and 6b, 6d, 6f, 6g). At week 4 of the feeding period, the postprandial
273 glycemic response in the H group was significantly higher than that observed in the C group, while
274 the HSQ group showed a markedly lower glycemic response than the H group (Fig. 5b). No
275 significant differences were seen in insulin responses between groups (Fig. 5d). The highest levels
276 of basal and postprandial GLP-1 (Fig. 5e, f) were seen in the HSQ group among experimental
277 groups.

278 Regarding the MTT at week 8, HSQ group showed a reduction in the postprandial glycemic
279 responses when compared to H group (Fig. 6b). A significant elevation was observed in the AUC
280 of postprandial insulin in the H group than the C group (Fig. 6d). Among all treatments, the basal
281 GLP-1 concentration in the HSQ group was the greatest (16.07 ± 1.00 pM), while that in the H
282 group was the lowest (11.24 ± 0.58 pM) (Fig. 6e). The HSQ group also maintained substantially
283 higher postprandial GLP-1 AUC (Fig. 6f). Rats fed the H diet demonstrated significantly higher

284 HOMA-IR AUC when compared with C group, indicating the development of insulin resistance
285 after continuous feeding with a high-fat high-sucrose diet. Markedly, the HSQ group had a
286 HOMA-IR AUC value almost similar to that observed for the C group (Fig. 6g).

287

288 **Cecal tissue GLP-1 content**

289 No significant differences were observed in intestinal GLP-1 concentrations (Fig. 7a).
290 Total amount of GLP-1 in the cecal tissue was highest in HSQ group than HQ and other groups,
291 while HS group had almost same level as H group (Fig. 7b).

292

293 **Correlation between concentrations of basal GLP-1 and total quercetin derivatives in plasma** 294 **or cecal tissue GLP-1 content**

295 Correlations between plasma quercetin concentrations and various parameters, including
296 basal and postprandial glucose/insulin/GLP-1, were analysed. Basal GLP-1 concentrations (Fig. 8
297 a and b) were positively and significantly associated with total plasma quercetin derivatives at
298 week 4 ($r = 0.60$, $P = 0.0289$) and 8 ($r = 0.77$, $P = 0.0081$).

299 Correlation analysis were performed between basal plasma GLP-1 at week 8 and tissue
300 GLP-1 content (total amount in the cecum), separatory in Q-fed groups (HQ and HSQ), and non-
301 Q-fed groups (H and HS). A significant and positive correlation was observed for data from Q-fed
302 groups, but not for data from non-Q-fed groups (Fig. 8c, d).

303

304 **Discussion**

305 Previously, we have shown a substantial elevation in the total quercetin derivatives
306 detected in the plasma of rats fed a diet supplemented with a combination of soybean fiber and

307 AGIQ.¹¹ Thus, we examined here whether the combined supplementation of soybean fiber and
308 AGIQ promotes quercetin bioavailability in rats fed an obesogenic high-fat high-sucrose diet and
309 whether this supplementation exerts a preventive effect against glucose intolerance. At the end of
310 the feeding test, the combination of soybean fiber and AGIQ in a high-fat high-sucrose (HSQ) diet
311 gave the highest concentration of total quercetin derivatives in plasma and promoted cecal
312 fermentation. The combined supplementation of soybean fiber and AGIQ also attenuated the
313 glucose intolerance induced by the continuous feeding of the high-fat high-sucrose diet.
314 Furthermore, plasma quercetin concentrations were positively associated with plasma GLP-1
315 concentrations. These results indicated that the co-ingestion of soybean fiber promotes quercetin
316 bioavailability, following by enhanced GLP-1 secretion even under an obesogenic dietary status
317 to prevent the development of glucose intolerance.

318 Although apparent effects of experimental diets on body weight were not observed in the
319 present study, visceral fat accumulation caused by high-fat high-sucrose diet has been mitigated
320 by soybean fiber or AGIQ or both components. Consistent with these findings, the downregulation
321 of leptin and TNF- α genes in mice fed a high-fat diet containing soybean fiber, resulting in
322 reduced epididymal adipose tissue weight.³⁵ Another study has demonstrated that quercetin
323 improves energy expenditure and reduces fat accumulation in mice fed a high-fat diet by promoting
324 white adipocyte browning.³⁶

325 Elevated cecal fermentation from soybean fiber ingestion was confirmed by an increase in
326 SCFAs and a decrease in pH.^{37,38} As previously reported, we proposed that quercetin
327 bioavailability can be promoted by soybean fiber supplementation, which in turn enhances cecal
328 fermentation in normal rats.¹¹ This modification in the large intestine can reduce the degradation
329 of luminal quercetin, increased the available quercetin which can be absorbed in the large intestine.

330 In the present study, total quercetin derivatives in fecal and cecal samples were higher in
331 the HSQ group (Fig. 3 and 4), which correlated with the percentage of quercetin degradation that
332 was lower than the HQ group in the later experimental period (Table 2). These results revealed
333 that the proposed mechanism by which soybean fiber enhances quercetin bioavailability is
334 maintained even under an obesogenic dietary condition. Notably, the proposed mechanism is not
335 related to the absorption rate, but possibly depends on the absorptive capacity with the expansion
336 of the cecal tissues and the available amount of quercetin and its derivatives in the intestine.

337 Previous studies demonstrated that a diet containing a high percentage of lard oil (17%)
338 increased quercetin bioavailability,³⁹ and that quercetin can be transported into lymph circulation
339 along with fat.^{40,41} One possible mechanism underlying these findings was the increased formation
340 of chylomicrons, that incorporates quercetin together with fat in the diet.⁴² In the present study,
341 quercetin degradation gradually increased over time in HQ group, which may be linked with
342 increased quercetin degrading bacteria such as *Bacteroides fragilis*.⁴³ In lard fed mice, increases
343 in *Bacteroides* and *Bilophila* bacteria have been reported.⁴⁴ Enhancement of quercetin
344 bioavailability by soybean fiber could relate to the modification of intestinal microflora. Promotive
345 effect of dietary fiber on the growth of intestinal bacteria such as *Bifidobacteria* and *Lactobacilli*
346 has been shown.⁴⁵ This, in turn, controls or reduces the growth of harmful bacteria such as
347 *Clostridium perfringens* and *Escherichia coli* that are known as quercetin degrading bacteria.⁴³

348 At the beginning of the experimental period (week 2), the higher percentage of aglycon
349 degradation in HSQ group than in HQ group seems inconsistent with the proposed mechanism for
350 increasing plasma quercetin (quercetin bioavailability). However, amounts of degraded aglycon
351 are very similar in these groups, indicating that both of groups had almost equivalent “degradation
352 capacity”. Therefore, in this case, higher percentage of degradation in HSQ than HQ group is

353 owing to lower intake of aglycon in HSQ than in HQ group. After 4 weeks, amounts of degraded
354 aglycon gradually increased in HQ group while decreased in HSQ group accompanied with
355 significant differences between two groups ($P < 0.05$ at week 4, $P < 0.01$ at week 6 and 8), and
356 resulted in a significant interaction between the duration and the diet ($P = 0.0034$ by two-way
357 repeated measures ANOVA). The former is likely chronic effect of high fat diet, and the latter is
358 chronic effect of soybean fiber supplementation. Thus, the supplementation of soybean fiber is an
359 alternatively effective approach to enhance quercetin bioavailability even under a chronic feeding
360 of high-fat diet.

361 Significant differences in energy consumption were not detected by Tukey–Kramer’s test
362 (Fig. 1a), but HSQ group had approximately 10% lower energy intake than HQ group, which
363 resulted in significant differences in aglycon ingestion between HQ and HSQ group, when
364 calculated by Student’s t-test (Table 2). Such small difference in energy intake might also
365 contribute improved glucose tolerance in HSQ group compared to HQ group. Lack of comparison
366 under iso-energetic conditions such as pair-fed design may be one of limitations of the present
367 study. However, it is valuable finding that the quercetin bioavailability is higher in HSQ than HQ
368 group, even though HSQ had slightly lower quercetin intake than HQ group.

369 The long-term feeding of HSQ diet can increase GLP-1 secretion, especially at the basal
370 level, but the lower concentration of glucose in plasma may not be sufficient to activate the
371 function of GLP-1 to enhance insulin secretion in a glucose dependent manner. According to this
372 assumption, the combination of AGIQ and soybean fiber also improved glucose tolerance and
373 insulin sensitivity in rats fed a high-fat high-sucrose diet (Fig. 5 and 6). Relatively, but
374 insignificantly, lower energy consumption and visceral fat accumulation cannot be used for
375 explanation because both HS and HSQ groups have shown similar results for these parameters

376 (Fig. 1). Soybean fiber supplementation has been shown to mitigate plasma glucose and
377 triglyceride levels without body weight changes in both male random-bred albino control and
378 streptozotocin-induced diabetic rats.⁴⁶ Reportedly, quercetin acts on 5' AMP-activated protein
379 kinase (AMPK) stimulation and glucose transporter type 4 (GLUT4) translocation, promoting
380 glucose uptake in skeletal muscle and liver.⁴⁷ These data partly explain the preventive effects of
381 combined soybean fiber and AGIQ against metabolic impairments induced by excessive energy
382 intake. Possibly, an extension on the feeding period may show clearer effects, therefore, a longer
383 chronic feeding of this combination diet is needed in the future.

384 Throughout the experiment, we found a significant correlation between basal GLP-1 levels
385 and total quercetin derivatives in plasma (Fig. 8). The basal GLP-1 level was possibly promoted
386 by increased methylquercetin metabolites because a high concentration of these metabolites was
387 detected in the plasma, cecum, urine, and feces of AGIQ fed rats (Fig. 3 and 4). It is well known
388 that methylquercetin metabolites are resistant to degradation by intestinal bacteria,^{33,48} and long-
389 term quercetin consumption can extend the methylation of quercetin.⁴⁹ The stimulatory effect of
390 AGIQ on GLP-1 secretion has been demonstrated following an acute infusion into the rat small
391 intestine, as well as following acute exposure in a murine GLP-1-producing cell line (GLUTag
392 cells).⁵⁰ Luminal amount (Fig. 4) and concentrations (0.62 ± 0.17 mM in HQ, 5.57 ± 0.67 mM in
393 HSQ) of quercetin in the cecum were much higher in HSQ group than HQ group, but significant
394 correlations between plasma GLP-1 concentrations were not observed ($r = 0.36$, $P = 0.2080$ for
395 amount of luminal quercetin; $r = 0.37$, $P = 0.1869$ for concentration of luminal quercetin). We did
396 not measure luminal quercetin in the small intestine, but luminal quercetin in the ileum might be
397 involved in the increment of basal GLP-1 secretion. Another possible explanation is the
398 involvement of increased mass of the cecal tissue for the increment of GLP-1 secretion. There

399 were no differences in tissue GLP-1 concentrations among treatments (Fig. 7a), however, the
400 amount of GLP-1 in the whole cecum was higher than in HSQ group than in HQ group (Fig. 7b).
401 This suggests that the number of GLP-1 producing cells was simultaneously increased together
402 with the increased mass of cecal tissue (Fig. 1d). The correlation analysis (Fig. 8c, d) revealed the
403 significant and positive correlation between plasma GLP-1 concentrations and total amount of
404 cecal tissue GLP-1 ($r = 0.72$, $P = 0.0037$) in data from Q-fed groups (HQ vs HSQ), but not in the
405 data from non-Q-fed groups (H vs HQ). Therefore, not only tissue expansion by soybean fiber but
406 the presence of quercetin (in the intestinal lumen and/or plasma) could be responsible for
407 increasing basal GLP-1 secretion. Further studies are needed to clarify the molecular mechanisms
408 by which quercetin and its metabolites promote GLP-1 secretion.

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

415

416 **Abbreviations**

417	AGIQ	α -glycosyl-isoquercitrin
418	C	Control
419	GLP-1	Glucagon-like peptide-1
420	H	High-fat high-sucrose
421	HS	High-fat high-sucrose + Soybean fiber

422	HSQ	High-fat high-sucrose + Soybean fiber + α -glycosyl-isoquercitrin
423	HQ	High-fat high-sucrose + α -glycosyl-isoquercitrin
424	MTT	Meal tolerance test
425	SCFA(s)	Short-chain fatty acid(s)

426 **References**

- 427 1. Wach, A.; Pyrzyńska, K.; Biesaga, M. Quercetin content in some food and herbal samples.
428 Food Chem. 2007, 100, 699–704.
- 429 2. Srivastava, S.; Somasagara, R. R.; Hegde, M.; Nishana, M.; Tadi, S. K.; Srivastava, M.;
430 Choudhary, B.; Raghavan, S. C. Quercetin, a natural flavonoid interacts with dna, arrests
431 cell cycle and causes tumor regression by activating mitochondrial pathway of
432 apoptosis. Sci Rep. 2016, 6, 24049.
- 433 3. Patel, R. V.; Mistry, B. M.; Shinde, S. K.; Syed, R.; Singh, V.; Shin, H. S. Therapeutic
434 potential of quercetin as a cardiovascular agent. Eur J Med Chem. 2018, 155, 889–904.
- 435 4. Seo, M. J.; Lee, Y. J.; Hwang, J. H.; Kim, K. J.; Lee, B. Y. The inhibitory effects of
436 quercetin on obesity and obesity-induced inflammation by regulation of MAPK
437 signaling. J Nutr Biochem. 2015, 26, 1308–1316.
- 438 5. Zhao, Y.; Chen, B.; Shen, J.; Wan, L.; Zhu, Y.; Yi, T.; Xiao, Z. The beneficial effects of
439 quercetin, curcumin, and resveratrol in obesity. Oxid Med Cell Longev. 2017, 1459497.
- 440 6. Shi, G.; Li, Y.; Cao, Q.; Wu, H.; Tang, X.; Gao, X.; Yu J.; Chen Z. Y.; Yong Y. *In vitro*
441 and *in vivo* evidence that quercetin protects against diabetes and its complications: A
442 systematic review of the literature. Biomed Pharmacother. 2019, 109, 1085-1099.
- 443 7. Gao, L.; Liu, G.; Wang, X.; Liu, F.; Xu, Y.; Ma, J. Preparation of a chemically stable
444 quercetin formulation using nanosuspension technology. Int J Pharm. 2011, 404(1-2), 231-
445 237.
- 446 8. Mullen, W.; Rouanet, J. M.; Auger, C.; Teissèdre, P. L.; Caldwell, S. T.; Hartley, R. C.;
447 Lean, M.; Edwards, C.; Crozier, A. Bioavailability of [2-14C] quercetin-4'-glucoside in
448 rats. J Agric Food Chem. 2008, 56, 12127–12137.

- 449 9. Almeida, A. F.; Borge, G. I.; Piskula, M.; Tudose, A.; Tudoreanu, L.; Valentová, K.;
450 Williamson, G.; Santos, C. N. Bioavailability of Quercetin in humans with a focus on
451 interindividual variation. *Compr Rev Food Sci Food Saf.* 2018, 17(3), 714-731.
- 452 10. Peng, X.; Zhang, Z.; Zhang, N.; Liu, L.; Li, S.; Wei, H. *In vitro* catabolism of quercetin by
453 human fecal bacteria and the antioxidant capacity of its catabolites. *Food Nutr Res.* 2014,
454 58(1), 23406.
- 455 11. Trakooncharoenvit, A.; Tanaka, S.; Mizuta, E.; Hira, T.; Hara, H. Water-soluble dietary
456 fibers enhance bioavailability of quercetin and a fiber derived from soybean is most
457 effective after long-term feeding in rats. *Eur J Nutr.* 2019, 59, 1389-1398.
- 458 12. Murakami, H.; Yasui-Furukori, N.; Otaka, H.; Nakayama, H.; Murabayashi, M.; Mizushiri,
459 S.; Matsumura, K.; Tanabe, J.; Matsuhashi, Y.; Yanagimachi, M.; Yanagimachi, M.;
460 Sugawara, N.; Shimoda, K.; Daimon, M. Coping styles associated with glucose control in
461 individuals with type 2 diabetes mellitus. *J Diabetes Investig.* 2020, 11, 1215-1221.
- 462 13. Cavaghan, M. K.; Ehrmann, D. A.; Polonsky, K. S. Interactions between insulin resistance
463 and insulin secretion in the development of glucose intolerance. *J Clin Invest.* 2000, 106,
464 329–333.
- 465 14. Drucker, D. J.; Sherman, S. I.; Gorelick, F. S.; Bergenstal, R. M.; Sherwin, R. S.; Buse J.
466 B. Incretin-based therapies for the treatment of type 2 diabetes: evaluation of the risks and
467 benefits. *Diabetes Care.* 2010, 33, 428–433.
- 468 15. Holst, J. J.; The physiology of glucagon-like peptide 1. *Physiol Rev.* 2007, 87, 1409–1439.
- 469 16. Drucker, D. J. Mechanisms of action and therapeutic application of glucagon-like peptide-
470 1. *Cell Metab.* 2018, 27, 740–756.

- 471 17. Pinyo, J.; Hira, T.; Hara, H. Enhanced postprandial glucagon-like peptide-1 secretion
472 during obesity development has a protective role against glucose intolerance induction in
473 rats. *Br J Nutr.* 2019, 122, 411–422.
- 474 18. Tolhurst, G.; Reimann, F.; Gribble, F. M. Nutritional regulation of glucagon-like peptide-
475 1 secretion. *J Physiol.* 2009, 587, 27–32.
- 476 19. Tsuda, T. Possible abilities of dietary factors to prevent and treat diabetes via the
477 stimulation of glucagon-like peptide-1 secretion. *Mol Nutr Food Res.* 2015, 59, 1264–
478 1273.
- 479 20. Phuwamongkolwiwat, P.; Suzuki, T.; Hira, T.; Hara, H. Fructooligosaccharide augments
480 benefits of quercetin-3-O- β -glucoside on insulin sensitivity and plasma total cholesterol
481 with promotion of flavonoid absorption in sucrose-fed rats. *Eur J Nutr.* 2013, 53, 457–468.
- 482 21. Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. AIN-93 purified diets for laboratory rodents:
483 final report of the American Institute of Nutrition ad hoc writing committee on the
484 reformulation of the AIN-76A rodent diet. *J Nutr.* 1993, 123, 1939–1951.
- 485 22. Nakajima, S.; Hira, T.; Hara, H. Postprandial glucagon-like peptide-1 secretion is increased
486 during the progression of glucose intolerance and obesity in high-fat/high-sucrose diet-fed
487 rats. *Br J Nutr.* 2015, 113, 1477–1488.
- 488 23. Williamson, G.; Manach, C. Bioavailability and bioefficacy of polyphenols in humans. II.
489 Review of 93 intervention studies. *Am J Clin Nutr.* 2005, 81.
- 490 24. Stewart, L. K.; Soileau, J. L.; Ribnicky, D.; Wang, Z. Q.; Raskin, I.; Poulev, A.; Majewski,
491 M.; Cefalu, W. T.; Gettys, T. W. Quercetin transiently increases energy expenditure but
492 persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-
493 fat diet. *Metabolism.* 2008, 57, S39-46.

- 494 25. Hara, H.; Saito, Y.; Idei, A.; Kiriya, S. Evaluation of short-chain fatty acid production
495 in the cecum of fiber fed rats. *Food Hydrocoll.* 1994, 485–490.
- 496 26. Hira, T.; Suto, R.; Kishimoto, Y.; Kanahori, S.; Hara, H. Resistant maltodextrin or
497 fructooligosaccharides promotes GLP-1 production in male rats fed a high-fat and high-
498 sucrose diet, and partially reduces energy intake and adiposity. *Eur J Nutr.* 2017, 57, 965–
499 979.
- 500 27. Manach, A.; Morand, C.; Demigné, C.; Texier, O.; Régéat, F.; Rémésy, C. Bioavailability
501 of rutin and quercetin in rats. *FEBS Letters.* 1997, 409, 12–16.
- 502 28. Stefova, M.; Kulevanova, S.; Stafilov, T. Assay of flavonols and quantification of quercetin
503 in medicinal plants by HPLC with UV-diode array detection. *J Liq Chromatogr Relat*
504 *Technol.* 2001, 24, 2283–2292.
- 505 29. Pinyo, J.; Hira, T.; Hara, H. Continuous feeding of a combined high-fat and high-sucrose
506 diet, rather than an individual high-fat or high-sucrose diet, rapidly enhances the glucagon-
507 like peptide-1 secretory response to meal ingestion in diet-induced obese rats. *Nutrition.*
508 2019, 62, 122–130.
- 509 30. Pinyo, J.; Hara, H.; Hira, T. Diet-induced obesity enhances postprandial glucagon-like
510 peptide-1 secretion in Wistar rats, but not in diabetic goto-kakizaki rats. *Br J Nutr.* 2020,
511 1-13.
- 512 31. Cacho, J.; Sevillano, J.; Castro, J. D.; Herrera, E.; Ramos, M. P. Validation of simple
513 indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats.
514 *Am J Physiol Endocrinol Metab.* 2008, 295, 1269-1276.
- 515 32. Holota, Y.; Dovbynychuk, T.; Kaji, I.; Vareniuk, I.; Dzyubenko, N.; Chervinska, T.;
516 Zakordonets, L.; Stetska, V.; Ostapchenko, L.; Serhiychuk, T.; Tolstanova, G. The long-

- 517 term consequences of antibiotic therapy: Role of colonic short-chain fatty acids (SCFA)
518 system and intestinal barrier integrity. *PloS One*. 2019, 14, e0220642.
- 519 33. Matsukawa, N.; Matsumoto, M.; Shinoki, A.; Hagio, M.; Inoue, R.; Hara, H. Nondigestible
520 saccharides suppress the bacterial degradation of quercetin aglycone in the large intestine
521 and enhance the bioavailability of quercetin glucoside in rats. *J Agric Food Chem*. 2009,
522 57, 9462–9468.
- 523 34. Hollman, P. C. Absorption, bioavailability, and metabolism of flavonoids. *Pharm Biol*.
524 2004, 42, 74–83.
- 525 35. Matsumoto, K.; Watanabe, Y.; Yokoyama, S. I. Okara, soybean residue, prevents obesity
526 in a diet-induced murine obesity model. *Biosci Biotechnol Biochem*. 2007, 71, 720–727.
- 527 36. Choi, H.; Kim, C. S.; Yu, R. Quercetin upregulates uncoupling protein 1 in white/brown
528 adipose tissues through sympathetic stimulation. *J Obes Metab Syndr*. 2018, 27, 102–109.
- 529 37. Taciak, M.; Barszcz, M.; Tuśnio, A.; Pastuszewska, B. Interactive effects of indigestible
530 carbohydrates, protein type, and protein level on biomarkers of large intestine health in
531 rats. *PloS One*. 2015, 10, e0142176.
- 532 38. Farmer A. D. Caecal pH is a biomarker of excessive colonic fermentation. *World J*
533 *Gastroenterol*. 2014, 20, 5000-5007.
- 534 39. Lesser, S.; Cermak, R.; Wolfram, S. Bioavailability of quercetin in pigs is influenced by
535 the dietary fat content. *J Nutr*. 2004, 134, 1508–1511.
- 536 40. Chen, I. L.; Tsai, Y. J.; Huang, C. M.; Tsai, T. H. Lymphatic absorption of quercetin and
537 rutin in rat and their pharmacokinetics in systemic plasma. *J Agric Food Chem*. 2010, 58,
538 546–551.

- 539 41. Makino, R.; Takano, K.; Kita, K.; Nishimukai, M. Influence of long-term feeding of high-
540 fat diet on quercetin and fat absorption from the small intestine in lymph duct-cannulated
541 rats. *Biosci Biotechnol Biochem.* 2018, 82, 2007–2011.
- 542 42. Murota, K.; Cermak, R.; Terao, J.; Wolfram, S. Influence of fatty acid patterns on the
543 intestinal absorption pathway of quercetin in thoracic lymph duct-cannulated rats. *Br J*
544 *Nutr.* 2012, 109, 2147–2153.
- 545 43. Zhang, Z.; Peng, X.; Li, S.; Zhang, N.; Wang, Y.; Wei, H. Isolation and identification of
546 quercetin degrading bacteria from human fecal microbes. *PLoS ONE.* 2014, 9(3).
- 547 44. Singh, R. K.; Chang, H.; Yan, D.; Lee, K. M.; Ucmak, D.; Wong, K.; Abrouk, M.;
548 Farahnik, B.; Nakamura, M.; Zhu, T.; Bhutani, T.; Liao, W. Influence of diet on the gut
549 microbiome and implications for human health. *J Transl Med.* 2017, 15(1).
- 550 45. Gibson, G. R.; Roberfroid, M. B. Dietary modulation of the human colonic microbiota:
551 Introducing the concept of prebiotics. *J Nutr.* 1995, 125(6), 1401-1412.
- 552 46. Madar, Z. Effect of brown rice and soybean dietary fiber on the control of glucose and lipid
553 metabolism in diabetic rats. *Am J Clin Nutr.* 1983, 38, 388–393.
- 554 47. Haddad, P.; Eid, H.; Nachar, A.; Thong, F.; Sweeney, G. The molecular basis of the
555 antidiabetic action of quercetin in cultured skeletal muscle cells and hepatocytes.
556 *Pharmacogn Mag.* 2015, 11, 74-81.
- 557 48. Spencer, J. P.; Kuhnle, G. G.; Williams, R. J.; Rice-Evans, C. Intracellular metabolism and
558 bioactivity of quercetin and its *in vivo* metabolites. *Biochem J.* 2003, 372, 173-181.
- 559 49. Manach, B.; Texier, O.; Morand, C.; Crespy, V.; Régéat, F.; Demigné, C.; Rémésy, C.
560 Comparison of the bioavailability of quercetin and catechin in rats. *Free Radic Biol Med.*
561 1999, 27, 1259–1266.

562 50. Phuwamongkolwivat, P.; Hira, T.; Hara, H. A nondigestible saccharide,
563 fructooligosaccharide, increases the promotive effect of a flavonoid,
564 α -glucosyl-isoquercitrin, on glucagon-like peptide 1 (GLP-1) secretion in rat intestine and
565 enteroendocrine cells. *Mol Nutr Food Res*. 2014, 58, 1581–1584.

566

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570

571 **Author Contributions**

572 A. T., T. H. and H. H. designed the research; A. T. conducted the research and analysed the data;
573 T. H. provided essential reagents; A. T. and T. H. wrote the paper; T. H. had primary responsibility
574 for the final content. All authors read and approved the final manuscript.

575

576 **Conflicts of interest**

577 The authors declare that they have no conflicts of interest.

Figure captions

Figure 1. 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 test). N.S. indicates the absence of a significant difference among treatments.

Figure 2. 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 test).

Figure 3. 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 ($n = 7$). 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).

Figure 4. 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 ($n = 7$). 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).

Figure 5. 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 test) among all treatments (panels b, d, f, and g). N.S. indicates the absence of a significant difference among treatments.

Figure 6. 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 test) among all treatments (panels b, d, f, and g). N.S. indicates the absence of a significant difference among treatments.

Figure 7. 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.

Figure 8. 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).

Table 1 Composition of test diets

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

AIN, American Institute of Nutrition; C, control diet; H, high-fat high-sucrose diet; HS, high-fat high-sucrose diet + soybean fiber; HSQ, high-fat high-sucrose diet + soybean fiber + α -glycosyl-isoquercitrin; HQ, high-fat high-sucrose diet + α -glycosyl-isoquercitrin.

^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)

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

Values represent mean \pm 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 ($\mu\text{mol/day}$) = total aglycone derivatives in urine + total aglycone derivatives in feces

^b Degraded aglycone ($\mu\text{mol/day}$) = Ingested aglycone - Excreted aglycone

^c Excreted aglycone (%) = (Excreted aglycone / Ingested aglycone) \times 100

^d Degraded aglycone (%) = [100 - (Excreted aglycone / Ingested aglycone)] \times 100

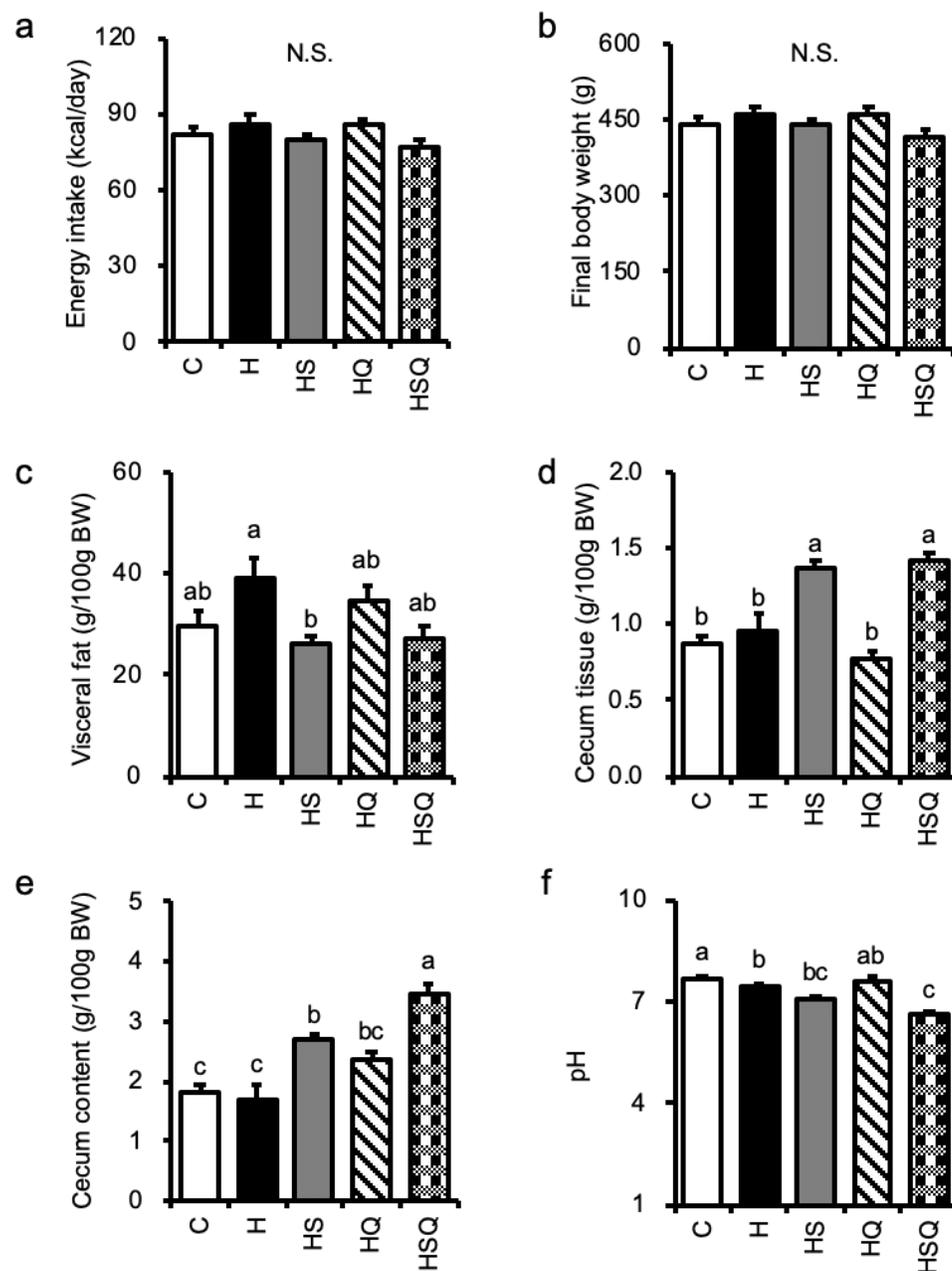
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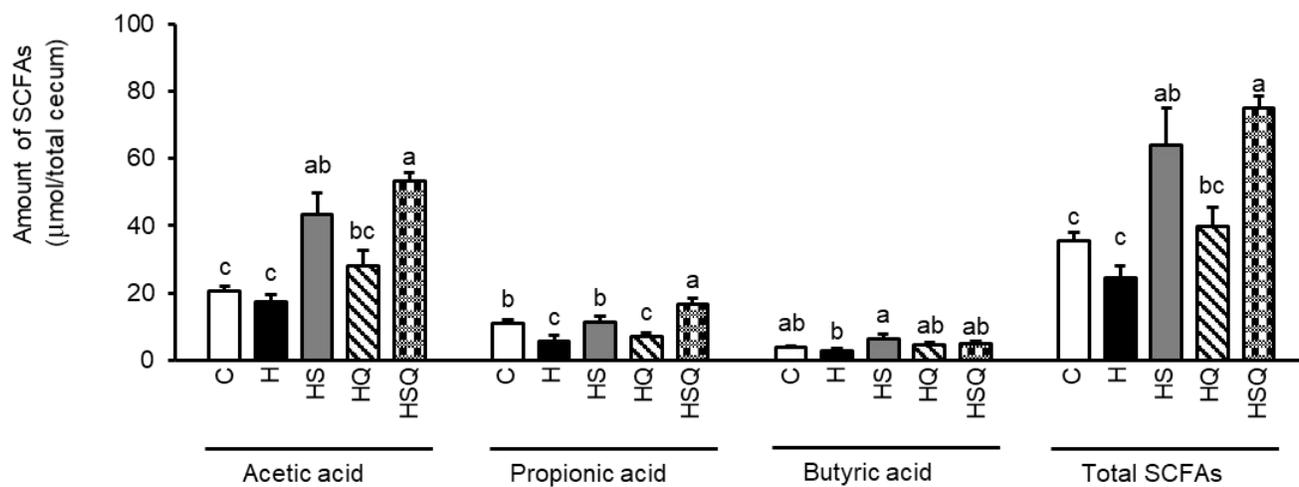
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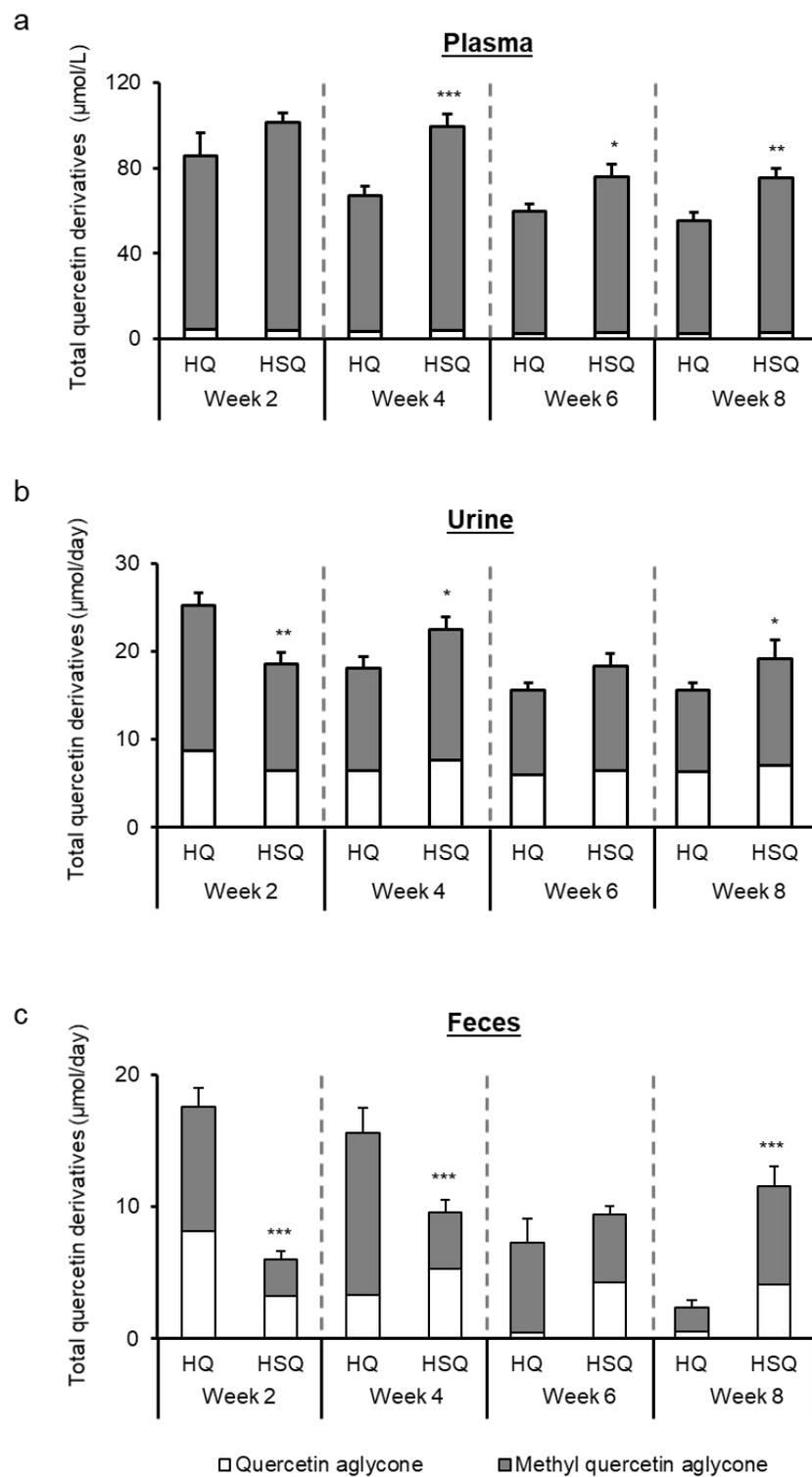
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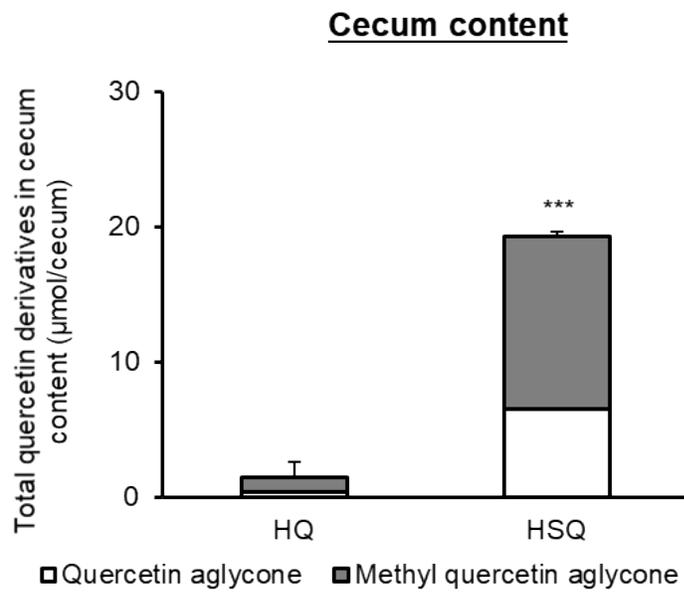
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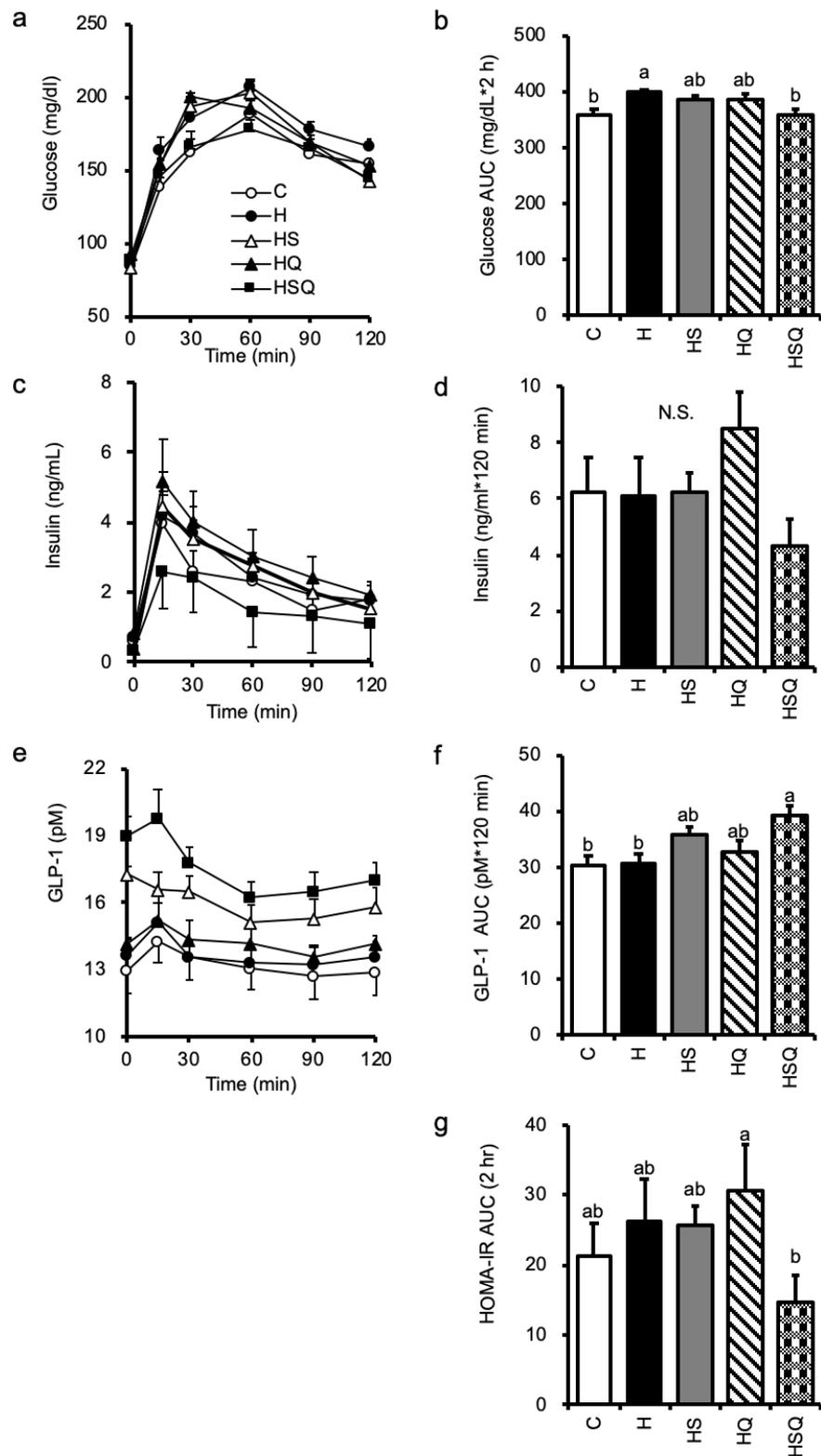
Figure 5**Week 4**

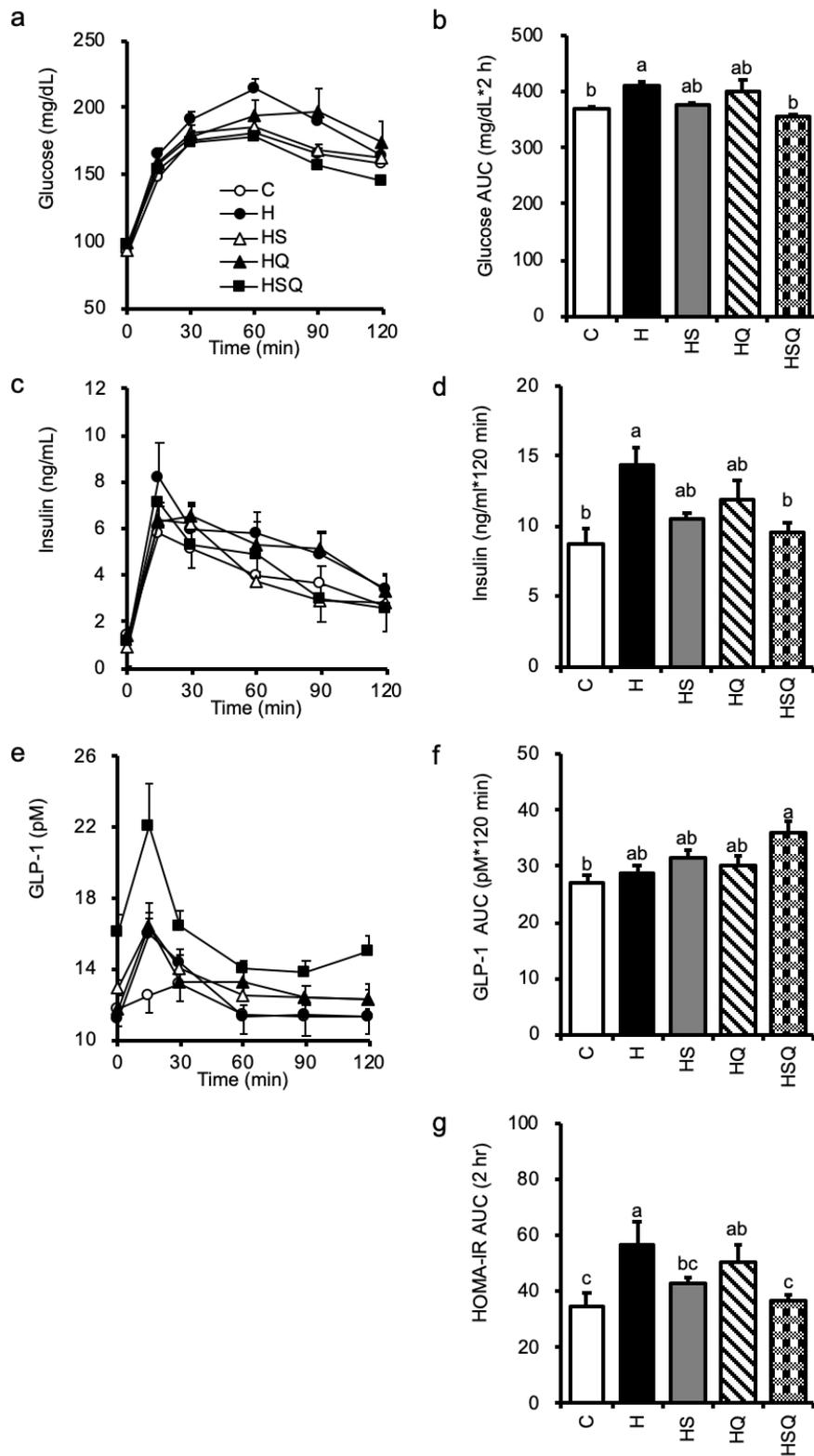
Figure 6**Week 8**

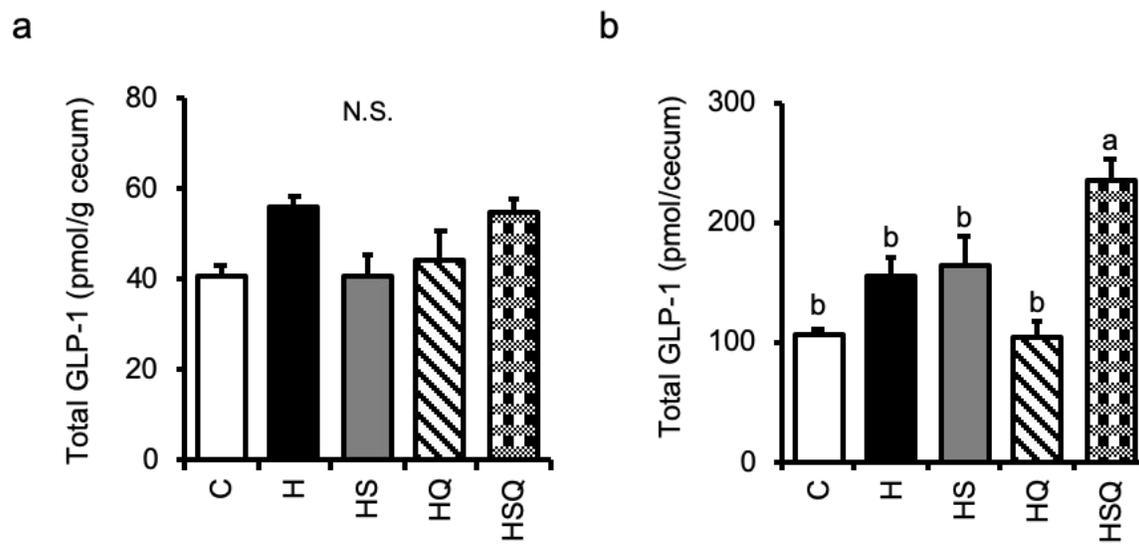
Figure 7

Figure 8