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1 **TITLE**

2 Resistant maltodextrin or fructooligosaccharides promotes GLP-1 production in male rats fed a  
3 high-fat and high-sucrose diet, and partially reduces energy intake and adiposity

4 **AUTHORS**

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16 **RUNNING TITLE:** Increasing GLP-1 against diet-induced obesity

17

18 **Abbreviations:** HFS: High-fat and high-sucrose, GLP-1: Glucagon-like peptide-1, RMD:

19 Resistant maltodextrin, FOS: Fructooligosaccharides, GIP: Glucose-dependent insulinotropic

20 polypeptide, OGTT: Oral glucose tolerance test, PYY: Peptide YY, DPP-IV: Dipeptidyl peptidase-

21 IV, SCFA: Short chain fatty acid

22

## 23 **ABSTRACT**

### 24 Purpose

25       Increasing secretion and production of glucagon-like peptide-1 (GLP-1) by continuous  
26 ingestion of certain food components has been expected to prevent glucose intolerance and  
27 obesity. In this study, we examined whether a physiological dose (5% weight in diet) of digestion-  
28 resistant maltodextrin (RMD) has a GLP-1-promoting effect in rats fed a high-fat and high-  
29 sucrose (HFS) diet.

### 30 Methods

31       Rats were fed a control diet or the HFS (30% fat, 40% sucrose wt/wt) diet supplemented with  
32 5% RMD or fructooligosaccharides (FOS) for 8 weeks or for 8 days in separated experiments.  
33 Glucose tolerance, energy intake, plasma and tissue GLP-1 concentrations, and cecal short chain  
34 fatty acids concentrations were assessed.

### 35 Results

36       After 4 weeks feeding, HFS-fed rats had significantly higher glycemic response to oral glucose  
37 than control rats, but rats fed HFS+RMD/FOS did not (approx. 50% reduction vs HFS rats).  
38 HFS+RMD/FOS-fed rats had higher GLP-1 responses (~2-fold) to oral glucose, than control rats.  
39 After 8 weeks, visceral adipose tissue weight was significantly higher in HFS-fed rats than control  
40 rats, while HFS+RMD/FOS rats had a trend of reduced gain (~50%) of the tissue weight. GLP-1  
41 contents and luminal propionate concentrations in the large intestine increased (> 2-fold) by  
42 adding RMD/FOS to HFS. Eight days feeding of RMD/FOS-supplemented diets reduced energy  
43 intake (~10%) and enhanced cecal GLP-1 production (~2-fold), compared to HFS diet.

### 44 Conclusions

45 The physiological dose of a prebiotic fiber promptly (within 8 days) promotes GLP-1  
46 production in rats fed an obesogenic diet, which would help to prevent excess energy intake and  
47 fat accumulation.

48

49 **KEY WORDS:** Resistant maltodextrin; Fructooligosaccharides; Glucagon-like peptide-1; High-fat  
50 and high-sucrose diet; Appetite; Adiposity.

51

## 52 **INTRODUCTION**

53 In recent years, glucagon-like peptide-1 (GLP-1), an incretin hormone, has received much  
54 attention in association with glucose homeostasis. Besides the incretin effect, GLP-1 released from  
55 enteroendocrine L cells has multiple other functions [1, 2], including pancreatic beta-cell  
56 protection/proliferation, satiety induction, and gastric emptying suppression, as well as  
57 cardioprotective and neuroprotective effects. Recently, incretin "enhancers" such as GLP-1 receptor  
58 agonists and dipeptidyl peptidase-IV (DPP-IV) inhibitors have been widely and effectively used for  
59 the treatment of type 2 diabetes [3], but effective "GLP-1 releasers" have not yet been developed for  
60 therapeutic use. Increasing endogenous production and secretion of GLP-1 is thought to be a  
61 promising strategy for the improvement of glucose tolerance owing to its insulinotropic (incretin)  
62 effect; however, the concept has not been sufficiently proven.

63 GLP-1 secretion is stimulated by luminal macronutrients such as glucose, fatty acids, peptides,  
64 and amino acids [4]. Several studies demonstrated that nutrient-induced GLP-1 release is effective  
65 in attenuating glycemic responses in animals [5, 6] and humans [7, 8]. Further, stimulation of GLP-  
66 1 secretion by non-absorbable compounds would be attractive because of their relatively long-  
67 acting property throughout the intestinal lumen and less possibility for unexpected side effects after  
68 absorption into the circulation. We recently demonstrated that a water-soluble prebiotic fiber,

69 digestion-resistant maltodextrin (RMD, contains 90% soluble dietary fiber), stimulated GLP-1  
70 secretion after a single oral administration in rats [9]. Furthermore, continuous feeding of a diet  
71 containing 5% RMD increased the plasma GLP-1 concentration and cecal GLP-1 content, together  
72 with the improvement of glucose tolerance in normal rats.

73 The suppressive effects of prebiotic fibers such as RMD, oligofructose, inulin, and resistant  
74 starch on obesity, hyperglycemia, dyslipidemia, and fat accumulation have been demonstrated  
75 previously in animal and human studies [10-16]. Such effects of fermentable fibers can be mainly  
76 explained by their ability to modify gut microbiota composition, increase short-chain fatty acid  
77 production, and increase the secretion of gut hormones such as GLP-1 and peptide YY (PYY).  
78 Although effects of RMD to attenuate glucose and insulin responses [16, 17], to reduce body fat  
79 [16, 18], to increase satiety [19] and to promote GLP-1 [9] have separately been reported in  
80 different experimental models, the effect of RMD on GLP-1 secretion and production has not been  
81 clarified in the model during the development of diet-induced obesity.

82 In the present study we examined the hypothesis that the promoting effect of RMD on the  
83 secretion and production of GLP-1 could be exerted in rats fed a high-fat and high-sucrose diet as  
84 an obesogenic diet, and whether the effect would contribute to protection against glucose  
85 intolerance, fat accumulation, and excess energy intake. In contrast to the majority of animal studies  
86 employing more than 10% (w/w) dose of various prebiotic fibers such as inulin, oligofructose,  
87 resistant starch, pectin, guar-gum, etc. [10-12, 15, 20-22], we examined a 5% (w/w) dose of RMD  
88 and fructooligosaccharides (FOS) added to a diet because the 10% dose is relatively high  
89 considering the recommended dose of dietary fiber (14 g/1,000 kcal, 20-38 g/day) in humans [23,  
90 24, 25]. In experiment 1, rats were fed a control diet or high-fat and high-sucrose (HFS) diet  
91 supplemented with a physiological dose (5%) of RMD or FOS for 8 weeks. Oral glucose tolerance  
92 tests (OGTTs) were performed during the experimental period, and secretion and production of

93 GLP-1 were evaluated. In experiment 2, we monitored the energy intake daily for 8 days and then  
94 collected blood and tissue samples to examine the promoting effects of the RMD/FOS-  
95 supplemented diets on GLP-1 levels and satiety.

96

97

## 98 **MATERIALS AND METHODS**

### 99 **Animals and diets**

100 Male Sprague–Dawley rats (5-week-old, n=32 for experiment 1, n=28 for experiment 2) were  
101 purchased from Japan SLC, Inc. (Shizuoka, Japan) and were fed an American Institute of Nutrition  
102 (AIN)-93G-based diet [26] for a one-week acclimation period. Each rat was individually housed in  
103 a separate cage and had free access to the diet and water, except for the days preceding the glucose  
104 tolerance test and killing. The experiment was performed in a temperature-controlled room  
105 maintained at  $22 \pm 2$  °C with a 12 h light/12 h dark cycle (08.00 a.m.–20.00 p.m. light period). Rats  
106 were divided into four groups (n=7 or 8 in each group) and fed the AIN-93G diet (control), HFS  
107 diet (30% fat and 40% sucrose, wt/wt), or HFS diet in which cellulose was replaced with either  
108 RMD (Fibersol-2; Matsutani Chemical Industry Co., Hyogo, Japan) or FOS (Meiologo-P; Meiji Co.,  
109 Ltd., Tokyo, Japan) (Table 1) for 8 weeks (experiment 1) or 8 days (experiment 2). The digestion  
110 resistant component in HDS+RMD diet is estimated at 4.5% by weight. In experiment 1, OGTTs  
111 were conducted after 4 and 7 weeks of feeding the test diets, and the rats were fed the test diets for  
112 further one week. After 8 weeks of test diet feeding and overnight fasting (16–20 hours), rats were  
113 euthanized for tissue and blood sampling. Body weight and food intake were measured every 1–2  
114 days. In experiment 2, the rats were fed the test diets for 8 days and euthanized on day 9 after  
115 overnight fasting (16–20 hours). Food intake was measured daily in the morning (8–9 a.m.) and  
116 evening (7–8 p.m.). The study was approved by the Hokkaido University Animal Committee, and

117 the animals were handled in accordance with the Hokkaido University guidelines for the care and  
118 use of laboratory animals.

### 119 **Glucose tolerance test**

120 OGTTs were performed on rats after 4 and 7 weeks of feeding the test diets in experiment 1. The  
121 rats were fasted overnight (16–18 hours), and basal (fasting) blood was collected from the tail vein  
122 for the measurement of glucose, insulin, and total GLP-1 levels. After the basal blood collection (0  
123 min), a glucose solution was orally administered at a dose of 2 g/kg (4 weeks) or 3 g/kg (7 weeks).  
124 Blood samples were collected from the tail vein at 0, 15, 30, 60, 90, and 120 min after the glucose  
125 load. The blood samples were collected into tubes containing heparin (final concentration 50  
126 IU/mL; Ajinomoto Company, Inc., Tokyo, Japan) and aprotinin [final concentration 500 kallikrein  
127 inhibitor units (KIU)/mL; Wako Pure Chemical Industries, Ltd., Osaka, Japan]. Plasma was  
128 separated by centrifugation at  $2,300 \times g$  for 10 min at 4 °C and frozen at –80 °C until glucose,  
129 insulin, and GLP-1 measurements were taken. Plasma glucose, insulin, and total GLP-1  
130 concentrations were measured using the Glucose CII Test Kit (Wako), rat insulin enzyme-linked  
131 immunosorbent assay (ELISA) kit (AKRIN-010T; Shibayagi Co., Ltd., Gunma, Japan; inter-assay  
132 coefficient of variability (CV): < 5%, intra-assay CV: < 5%), and multi-species GLP-1 total ELISA  
133 kit (EZGLP1T-36K; Merck Millipore, Darmstadt, Germany; intra-assay CV: < 5%, inter-assay CV:  
134 < 12%), respectively.

### 135 **Portal blood and tissue collection**

136 After 8 weeks (experiment 1) or 8 days (experiment 2) of feeding period, blood samples were  
137 collected from the portal vein and abdominal aorta of the rats under sodium pentobarbital anesthesia  
138 (50 mg/kg of body weight, somnopentyl injection; Kyoritsu Seiyaku Corporation, Tokyo, Japan)  
139 into a syringe containing heparin (final concentration 50 IU/mL), aprotinin (final concentration 500  
140 KIU/mL), and DPP-IV inhibitors (final concentration 50  $\mu\text{mol/L}$ ; DPP4-010; Merck Millipore).

141 Plasma was collected and stored as described above for the measurement of glucose, insulin, GLP-1  
142 (total and active), glucose-dependent insulintropic polypeptide (GIP), PYY, and DPP-IV. Active  
143 GLP-1 and total GIP levels were measured using the GLP-1 (active) ELISA (EGLP-35K; Merck  
144 Millipore; intra-assay CV:  $7.4 \pm 1.1\%$ , inter-assay CV:  $8 \pm 4.8\%$ ) and Rat/Mouse GIP (total) ELISA  
145 (EZRMGIP-55K; Merck Millipore; intra-assay CV: 1.0–5.9%, inter-assay CV: 1.1–5.9%) kits,  
146 respectively. Plasma PYY levels were measured using the Mouse/Rat PYY EIA kit (YK081;  
147 Yanaihara Institute, Inc., Fujinomiya, Japan; intra-assay CV: 3.1–9.8%, inter-assay CV: 4.2–  
148 14.2%). Plasma DPP-IV activity was determined based on the rate of hydrolysis of a surrogate  
149 substrate (Gly-Pro-p-nitroaniline, Gly-Pro- pNA, Sigma).

150 After the rats were killed by exsanguination, the jejunum, ileum, cecum, and colon were  
151 collected in both of experiments. Luminal contents of the jejunum, ileum, and colon were washed  
152 out with cold saline, and then a 2-cm segment and mucosae from another 5-cm segment collected  
153 from middle regions of these tissues were collected for GLP-1 content measurement and  
154 proglucagon mRNA analysis, respectively. The tissue weight of the cecum as well as the weight  
155 and pH of the cecal contents were measured, and the contents were used for short-chain fatty acid  
156 (SCFA) measurement. After washing with cold saline, the cecal tissue was divided in half; one half  
157 was collected for GLP-1 measurement, and the mucosa from the other half was scraped for  
158 proglucagon mRNA analysis. The intestinal segments were immediately frozen in liquid nitrogen  
159 and stored at  $-80\text{ }^{\circ}\text{C}$ . The mucosa samples were immediately transferred to tubes containing buffer  
160 RLT (RNeasy Mini Kit; Qiagen, Germany), frozen in liquid nitrogen, and then stored at  $-80\text{ }^{\circ}\text{C}$ .  
161 Mesenteric, retroperitoneal, and epididymal adipose tissue weights were measured, and the sum of  
162 those was presented as the visceral adipose tissue weight.

### 163 **Measurement of glucagon-like peptide-1 content in intestinal tissues**

164 Intestinal segments were homogenized in an ethanol–acid solution (ethanol/water/12 M HCl,



165 74:25:1; 5 mL/g of tissue) [27] and extracted for 24 h at 4 °C. After centrifugation (2,000 × g for 20  
166 min), the supernatants were collected and diluted (1,000-fold) with saline to measure total GLP-1  
167 levels by ELISA.

### 168 **Real-time polymerase chain reaction (PCR) analysis**

169 Using a real-time PCR system [28], proglucagon mRNA expression levels were determined.  
170 Total RNA was extracted using the RNeasy Mini kit (Qiagen), according to the manufacturer's  
171 instructions. Complementary DNA was synthesized using the ReverTra Ace qPCR Master Mix with  
172 gDNA Remover (Toyobo Co., Ltd., Osaka, Japan), according to the manufacturer's instructions.  
173 Gene expression levels were determined using the Mx3000P Real-Time PCR System (Stratagene,  
174 La Jolla, CA, USA) and TaqMan Gene Expression Assay (Life Technologies, Carlsbad, CA, USA)  
175 with rat gene-specific predesigned TaqMan primers and probe sets [Rn99999916\_s1 for  
176 glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Rn00562293\_m1 for proglucagon].  
177 Relative expression levels were calculated for each sample after normalization to those of GAPDH  
178 as a reference gene using the standard curve method.

### 179 **Measurement of cecal short-chain fatty acids**

180 The cecal contents were collected as described above. The weight of the contents was calculated  
181 by subtracting the tissue weight from the total cecal weight. The cecal contents were homogenized  
182 with deionized water (300 mg of cecal content in 1.5 ml water). The pH values of the homogenates  
183 were measured with a compact pH meter (B-212, Horiba, Ltd., Kyoto, Japan). The organic acids in  
184 the cecal homogenates were measured by an ion-exclusion chromatography method using high-  
185 performance liquid chromatography (Organic Acid Analysis System, Shimadzu Corporation,  
186 Kyoto, Japan) as previously described [29].

### 187 **Statistical analyses**

188 Data were expressed as means and standard errors (SE) of the mean. Statistical analyses were

189 performed using the JMP Pro version 10.0 software (SAS Institute, Inc., Cary, NC, USA). Statistical  
190 significance was assessed using a one-way analysis of variance (ANOVA). Significant differences  
191 ( $P < 0.05$ ) between the mean values were determined using the Tukey–Kramer or Dunnett's test, as  
192 appropriate (specified in the figure legends). For data of glycemic, insulin, and GLP-1 responses to  
193 oral glucose, two-way repeated measurement ANOVA was performed, and the results (effects of time,  
194 treatment and the interaction of time and treatment) were presented in the figure legend. Area under  
195 the curve (AUC) of plasma glucose, insulin, and GLP-1 levels during the glucose tolerance tests was  
196 calculated by the trapezoidal rule. To estimate the degree of insulin resistance during OGTT, AUC  
197 of incremental HOMA-IR (iHOMA-IR) was calculated by using incremental AUCs of glucose  
198 (mg/dL\*2 hr) and insulin (ng/mL\*2 hr) as following equation [30].  $\text{HOMA-IR} = \{\text{incremental AUC}$   
199  $\text{of glucose (mg/dL*2 hr)} \times \text{incremental AUC of insulin } (\mu\text{U/mL*2 hr})\} / 2,430$ , where 1 ng of insulin  
200 is equivalent to 26  $\mu$ units. Pearson's correlation coefficient (r) and significance of correlations were  
201 analyzed as appropriate, and shown in supplementary tables.

202

203

## 204 **RESULTS**

### 205 Experiment 1 (feeding for 8 weeks)

206 After 4 weeks, glycemic responses (Fig. 1A) in the three HFS-fed groups appeared to increase  
207 compared to that in the control group. The AUC of glucose in the HFS group was significantly  
208 higher than that in the control group, but it did not reach significant increment in the RMD- and  
209 FOS-supplemented groups. The insulin responses in the three HFS-fed groups were insignificantly  
210 higher than those in the control group. The GLP-1 responses in the HFS+RMD and HFS+FOS  
211 groups were significantly higher than those in the control group which had no increment of the  
212 GLP-1 level but rather decreased after 60 min.

213 Because no GLP-1 elevation was observed in the control group with 2 g/kg of oral glucose (Fig.  
214 1C), we increased the dose of glucose to 3 g/kg in OGTT after 7 weeks (Figs. 1E–G). The glucose  
215 response at 15 min in the HFS group was significantly larger than that in the control group, but that  
216 in the HFS+RMD and HFS+FOS groups were not significantly different from the control group.  
217 The insulin response was much higher in the HFS group than in the control group. Although the  
218 HFS+RMD and HFS+FOS groups showed increased insulin responses, the increment was smaller  
219 than that in the HFS group. The GLP-1 responses in the three HFS-fed groups tended to be higher  
220 than those in the control group, but no significant differences were detected. The AUC of  
221 incremental HOMA-IR (AUC of iHOMA-IR) was calculated to estimate the degree of insulin  
222 resistance during OGTTs (Fig. 1D, H). HFS group had significant increase in AUC of iHOMA-IR  
223 compared to control group, but RMD- or FOS-supplemented groups did not, in both of OGTTs.

224 The final body weight, weight gain, and total energy intake in the HFS group were significantly  
225 higher than those in the control group after 8 weeks (Table 2), whereas the values for the RMD- and  
226 FOS-supplemented groups were slightly lower than the values for the HFS group. The visceral  
227 adipose tissue weight (sum of mesenteric, retroperitoneal, epididymal adipose tissue weights) in the  
228 HFS group was significantly higher than that in the control group (Table 2), but it did not reach  
229 significant increment in the RMD- and FOS-supplemented groups compared to the control group.  
230 When compared within the three HFS-fed groups, the visceral adipose tissue weight was  
231 significantly lower in the RMD- and FOS-supplemented groups compared to the HFS group  
232 (Dunnett's test,  $P < 0.05$ ). Figure 2 shows the change of weekly energy intake over 8 weeks.  
233 Although the HFS and HFS+FOS groups had significantly higher energy intake than control group  
234 throughout the experimental period (except week 2), energy intake at week 1, 3, and 8 in RMD-  
235 supplemented group was not significantly different from control group. When compared within the  
236 three HFS-fed groups, the HFS+RMD group had a significantly lower energy intake at weeks 1 and

237 2 compared to the HFS group (Dunnett's test,  $P < 0.05$ ). Decreased energy intake at week 5 and 8 in  
238 all the groups is due to overnight fasting for OGTT conducted after 4 and 7 weeks.

239 The portal GLP-1 (active) level was significantly higher in the RMD- and FOS-supplemented  
240 groups compared to that in the control group, but it did not significantly elevate in the HFS group  
241 (Table 3). The HFS+FOS group had the highest GLP-1 (active and total) and PYY levels, which  
242 were significantly different from that observed for the control group. Significant differences were  
243 not observed in glucose, insulin, total GIP levels, and DPP-IV activity in the portal plasma.

244 The GLP-1 contents increased in the colon (Fig. 3B) of RMD- and FOS-supplemented groups,  
245 but those in the jejunum (data not shown), ileum (data not shown), and cecum (Figs. 3A) did not  
246 differ between the treatments. The total content of GLP-1 in the cecum (Fig. 3C) significantly  
247 increased in the RMD-fed group compared to that in the HFS group, owing to an increased cecal  
248 tissue weight (Table 5). In contrast to the GLP-1 content, the proglucagon mRNA expression levels  
249 did not increase in any intestinal region in response to any diet treatment (Table 4). The HFS diet  
250 with or without RMD/FOS reduced the proglucagon mRNA expression levels in the ileum.

251 The cecal tissue and contents weights increased in the HFS+RMD and HFS+FOS groups  
252 compared to that in the control and HFS groups (Table 5). The cecal pH values (Table 5) were  
253 lower in the three HFS-fed groups than in the control group. The HFS group showed relatively low  
254 concentrations of each SCFA (Table 5). The acetic and *n*-butyric acid concentrations were higher in  
255 the HFS+RMD group than in the HFS group. The propionic acid concentration largely decreased in  
256 the HFS group than control group, but it was maintained in the RMD- and FOS-supplemented  
257 groups.

258

259 Experiment 2 (feeding for 8 days)

260 The body weight gain during the test period (9 days) was higher in the HFS-fed group than in the  
261 control group (Table 6), but the increment was significantly smaller in the HFS+FOS group than in  
262 the HFS group. The HFS+RMD group showed an intermediate increment between the HFS and  
263 HFS+FOS groups. The visceral adipose tissue weight was significantly higher in the HFS group  
264 than in the control group, but the RMD- and FOS-supplemented groups had significantly smaller  
265 visceral adipose tissue weight than the HFS group.

266 The daily energy intake (Fig. 4C) was apparently higher in the HFS group than in the control  
267 group throughout the feeding period (8 days); however, that in the HFS+RMD and HFS+FOS  
268 groups was not significantly higher than in the control group. The total energy intake (Table 6) was  
269 significantly lower in the HFS+RMD and HFS+FOS groups than in the HFS group. This reduction  
270 was attributed to less energy intake in the dark period (Fig. 4B).

271 The total plasma GLP-1 level showed an increasing trend in the RMD- and FOS-supplemented  
272 groups (Table 7), and the value in the RMD+HFS group was significantly different from that in  
273 control group when analyzed with Dunnett's test ( $P < 0.05$ ). The plasma PYY level was the highest  
274 in the HFS+FOS group. There were no significant differences in the glucose, insulin GIP levels,  
275 and DPP-IV activity among the treatments

276 The mucosal GLP-1 content in the colon tended to increase in the HFS+RMD and HFS+FOS  
277 groups (Fig. 5B) but was unchanged in the other regions by the treatments (data for jejunum and  
278 ileum not shown). Due to the increased tissue weight (Table 9), the GLP-1 content in the whole  
279 cecum (Fig. 5C) was significantly higher in the HFS+RMD and HFS+FOS groups than in the  
280 control and HFS groups. The proglucagon mRNA expression significantly decreased in the cecum  
281 of the HFS+FOS group compared to that of the control group (Table 8).

282 The cecal tissue and contents weights were significantly higher in the HFS+RMD and HFS+FOS  
283 groups than in the other two groups (Table 9). The pH values of the cecal contents were

284 significantly lower in the HFS+RMD group than in the control group (Table 9). In the HFS+RMD  
285 and HFS+FOS groups, significant increments in the propionic acid concentration were observed,  
286 while the other SCFA concentrations did not differ from those in the control group.

287 Within the three HFS-fed groups, total energy intake was significantly ( $P < 0.05$ ) correlated with  
288 visceral adipose tissue weight ( $r = 0.840$ ,  $P < 0.001$ ), total GLP-1 in the portal vein ( $r = -0.635$ ,  $P =$   
289  $0.015$ ), total GLP-1 content in the whole cecum ( $r = -0.512$ ,  $P = 0.048$ ), and propionic acid  
290 concentration in the cecum ( $r = -0.575$ ,  $P = 0.031$ ).

291

292

## 293 **Discussion**

294 In contrast to most of studies that investigated GLP-1-promoting effects of various dietary fibers  
295 added in the diet at more than 10% (wt/wt) dose [10-12, 15, 20-22], we investigated whether a  
296 relatively physiological dose (5% wt/wt) of RMD could promote GLP-1 production in rats fed a  
297 high-fat and high-sucrose diet for a long (8 weeks) and a short (8 days) period. After 8 weeks  
298 feeding of the HFS+RMD or HFS+FOS diet, the GLP-1 content in the large intestine increased  
299 compared to the HFS diet feeding. Though significant fat accumulation was induced by the HFS  
300 diet compared to the control diet, RMD- or FOS-supplemented diet did not achieve significant  
301 increment of adipose tissue weights. Reductions of energy intake and adipose tissue weight gain,  
302 and increment of GLP-1 production were observed within 8 days of feeding the RMD-  
303 supplemented diet. These parameters significantly correlated each other, and also correlated with  
304 cecal propionate production promoted by RMD or FOS ingestion. The present results suggest that  
305 the physiological dose of RMD or FOS can promote endogenous GLP-1 production by the  
306 supplementation into an obesogenic diet.

307 In Europe, per capita consumption of sugar is 124 g/day [31], which is equivalent to 496  
308 kcal/day. In U.S., per capita consumption of caloric sweeteners (refined sugar, high fructose corn  
309 syrup, glucose, dextrose, pure honey, and edible syrups) is estimated 128.9 lb (58.5 kg) /year in  
310 2015 (USDA, ERS, Sugar and Sweeteners Outlook, Table 50, <http://www.ers.usda.gov/>), which is  
311 equivalent to 160 g = 640 kcal/day. In contrast to US and Europe, Asian and African people  
312 consume less than 50 g/day (=200 kcal/day) [31]. As the energy intake in human adults is 2,000-  
313 2,500 kcal /day, 500-600 kcal from sugar contributes 20-30% energy of total intake, in average.  
314 Although the content of sucrose at 31% energy (40% wt/wt) in the HFS diet is higher than average  
315 sugar intake of Europeans and Americans (20-25% energy), the difference between our  
316 experimental diet and the sugar consumption in humans would not be extremely large. It was  
317 reported that global consumption of saturated fat was 9.4% energy in 2010 [32] with country-  
318 specific intake variation from 2.3 to 27.5% energy. The content of lard in the HFS diet at 40%  
319 energy (23% wt/wt) is higher than the human situation above, however, it has been recognized that  
320 a high consumption of saturated fats is linked to metabolic syndrome, and high fat diet containing  
321 lard is generally used in animal study as obesogenic diets [33]. Although the present study has a  
322 limitation that the HFS diet may not perfectly mimic human diet, humans having higher amount of  
323 sugar and saturated fats would easily get obese. It has not been defined the 'ideal' obesogenic diet  
324 nor established a rodent model that accurately mimic the human obesity and accompanied  
325 symptoms [33]. Based on these backgrounds and to accelerate the induction of overweight, we used  
326 the HFS diet in the present study.

327 The glycemic response significantly increased in the rats fed the HFS diet for 4 weeks but not in  
328 rats fed the HFS diet supplemented with RMD or FOS (Fig. 1A). The insulin responses were almost  
329 similar in the three HFS-fed groups (Fig. 1B), suggesting that the insulin sensitivity was not  
330 significantly impaired in the HFS+RMD and HFS+FOS groups (Fig. 1D). Interestingly, the GLP-1

331 secretory response increased in the HFS+RMD and HFS+FOS groups (Fig. 1C). Because the basal  
332 (0 min) GLP-1 levels did not differ among the treatments, the result suggests that the sensitivity of  
333 L cells in the small intestine to luminal glucose was enhanced by the chronic feeding of the RMD-  
334 or FOS-supplemented diets. Although the cecal and colonic GLP-1 contents increased in these  
335 groups, GLP-1 in the large intestine would not participate in the postprandial rapid GLP-1 response.  
336 This also supports the notion that L-cell sensitivity to glucose was enhanced by the continuous  
337 RMD or FOS intake.

338 We recently demonstrated that 8 weeks feeding of the HFS diet enhanced postprandial GLP-1  
339 secretion based on a meal tolerance test [34]. This effect was observed after 7 weeks of HFS  
340 feeding but not after 4 weeks. In the present study, the GLP-1 response to oral glucose was already  
341 enhanced by feeding the HFS diet for 4 weeks. It appears that the sensitivity of L cells to glucose is  
342 readily adaptive compared to the sensitivity to a “meal”.

343 It is still controversial whether GLP-1 secretion is impaired under obesity, glucose intolerance,  
344 and diabetic conditions [35-37]. We speculate that the enhanced GLP-1 response to a meal [34], as  
345 well as to oral glucose as observed in the present study and in a previous human study [38], has a  
346 protective role against the development of postprandial hyperglycemia induced by continuous  
347 feeding of high-energy diets. The enhanced GLP-1 secretion caused by repeated feeding of the HFS  
348 and RMD/FOS-supplemented diets might be induced through distinct mechanisms. Further studies  
349 are needed to understand the physiological relevance and underlying mechanisms of the RMD- and  
350 FOS-enhanced L-cell sensitivity.

351 After 7 weeks (Figs. 1E-H), the glycemic response at 15 min was the highest in the HFS group,  
352 while the RMD- and FOS-supplemented groups showed slightly smaller responses than the HFS  
353 group, which were accompanied by smaller insulin responses. This still suggests that the  
354 postprandial insulin sensitivity was not significantly impaired in the RMD- and FOS-supplemented



355 groups after 7 weeks (Fig. 1H). Although the GLP-1 response to oral glucose in RMD- and FOS-  
356 supplemented groups did not differ from the HFS group, increased fasting GLP-1 levels (Table 3)  
357 might contribute to the attenuation of insulin resistance since improving insulin sensitivity is one of  
358 the multiple effects of GLP-1 receptor activation [2, 39, 40].

359 The body and adipose tissue weights were expectedly increased by feeding the HFS diet  
360 compared to feeding the control diet, even after 8 days of feeding (Table 2 and 6). However, the  
361 RMD or FOS supplementation did not result in significant increments in the adipose tissue weight.  
362 The suppressive effect of RMD on the HFS diet-induced excessive energy intake in the early period  
363 (Fig. 2) was reproduced in experiment 2 by monitoring daytime (light period) and nighttime (dark  
364 period) food intake everyday (Fig. 4). This was clearly observed during nighttime, the usual eating  
365 time for rodents. The reduced fat accumulation (Table 2 and 6) could be attributed to these effects.  
366 In addition, there are several possible mechanisms for reducing fat accumulation by prebiotic fibers.  
367 RMD is reported to reduce lipid absorption [41], and FOS or oligofructose modulates lipid  
368 metabolism [42, 43].

369 Oligofructose [44-49] and RMD [19] have been reported for their satiety effects on subjective  
370 appetite accompanied by increased GLP-1 and/or PYY levels in animals or healthy humans,  
371 suggesting a possibility of these prebiotics to contribute to control energy intake and body weight  
372 for a long-term. Although elevated portal GLP-1 levels in RMD- and FOS-supplemented groups  
373 were not significantly different from that in the HFS group in experiment 2, portal GLP-1 level (but  
374 not PYY level) and cecal GLP-1 content were inversely correlated with total energy intake. Thus,  
375 the increased plasma GLP-1 level after 8 days of feeding the RMD- and FOS-supplemented diet is  
376 likely responsible for the reduced energy intake in the present study.

377 One of the major effects of prebiotic fibers is modulation of gut fermentation. A number of  
378 studies have demonstrated effects of the fibers on gut microbiota in animals and humans. SCFAs

379 are potent stimuli for GLP-1 and PYY secretion [50]. The plasma (active or total) GLP-1 levels  
380 tended to be increased by the HFS diet compared to the control diet in experiment 1, but the  
381 increment became statistically significant in the RMD- and FOS-supplemented groups (Table 3).  
382 The increased production of SCFAs such as acetate, propionate, and *n*-butyrate could be responsible  
383 for the significant elevation of production and secretion of GLP-1. Recent reports [51, 52]  
384 demonstrated that GLP-1/PYY secretion was potently stimulated by luminal propionate in vivo  
385 (rats and mice, and humans), and by exposure to propionate in human and mice colonic culture  
386 models in vitro, suggesting roles for propionate in inducing GLP-1/PYY secretion and reducing  
387 appetite. Therefore, the increased levels of propionate induced by RMD or FOS in both experiments  
388 are likely involved in reduced energy intake through increased GLP-1 and PYY secretion. Other  
389 SCFA functions [53], besides increasing these gut hormones, could also contribute to reducing  
390 energy intake and fat mass.

391 In comparison among HFS-fed 3 groups, RMD and FOS supplementations increased propionate  
392 production in the cecum (both per g content and per total content) in both of experiments. The HFS  
393 diet reduced propionate production after 8-weeks feeding, but the effect did not appear after 8-days  
394 feeding. These results indicate that the HFS diet has suppressive effect on propionate production  
395 after a relatively long feeding period (~8 weeks), but prebiotics such as RMD and FOS exert  
396 promoting effect on propionate production immediately (~8 days) after the intervention.

397 By correlation analysis (Supplemental Table 1 and 2) among HFS-fed 3 groups, significant  
398 correlations were observed between following combinations, in experiment 2 (8 day-feeding):  
399 [cecal propionate ( $\mu\text{mol/g}$  content) and cecal GLP-1 ( $\text{pmol/tissue}$ );  $r = 0.6166$ ,  $P = 0.0188$ ], [cecal  
400 GLP-1 ( $\text{pmol/tissue}$ ) and portal total GLP-1 ( $\text{pM}$ );  $r = 0.5845$ ,  $P = 0.0282$ ], [portal total GLP-1  
401 ( $\text{pM}$ ) and total energy intake ( $\text{kcal}$ );  $r = -0.6353$ ,  $P = 0.0146$ ]. However, portal PYY did not  
402 significantly correlated with various parameters tested. These results suggests that propionate

403 production increased by RMD/FOS promoted GLP-1 production in the large intestine, which  
404 contributed to elevated fasting plasma GLP-1 level and resulted in energy intake.

405 In experiment 1 (8 weeks feeding), while a significant correlation was observed between cecal  
406 propionate (pmol/tissue) and cecal GLP-1 (pmol/g tissue) ( $r = 0.6415$ ,  $P = 0.0013$ ), cecal GLP-1 did  
407 not significantly correlated with plasma GLP-1. Furthermore, plasma GLP-1 and PYY did not  
408 inversely correlate with energy intake. Possibly, above pathway raised from experiment 2 might  
409 have been impaired by a long time feeding with HFS. Although we did not examined in the present  
410 study, postprandial GLP-1 secretions might be enhanced in RMD/FOS-treated groups due to  
411 increased GLP-1 pool in the large intestine.

412 GLP-1 is produced in enteroendocrine L cells scattered throughout the intestinal epithelium, with  
413 a larger population in the ileum and large intestine than in the proximal small intestine [54]. This  
414 has been confirmed by measuring the GLP-1 content in separated intestinal segments from the  
415 control rats in the present study (Figs. 3 and 5). The GLP-1 concentration (pmol/g of tissue)  
416 significantly changed only in the colon after 8 weeks of feeding the RMD- or FOS-containing diet,  
417 but the increment was not significant in the short-term experiment (8 days). This indicates that  
418 colonic tissue is more sensitive to the continuous feeding of prebiotic fibers than the other intestinal  
419 regions, including the cecum. It is quite interesting how such a difference could appear between the  
420 cecum and colon. Possibly, the compositions of luminal SCFAs and microbiota differ between these  
421 regions. Present results reveled that the changes in the mRNA expression levels at the time  
422 (overnight fasted) do not directly explain the differences in the GLP-1 content between the  
423 treatments since the mRNA expression levels was not proportional to GLP-1 contents in each  
424 region in both experiments. There might be other factors affecting GLP-1 contents in the tissue, for  
425 example, DPP-IV activity that degrades GLP-1, and prohormone convertase 1/3 activity, which is

426 involved in posttranslational processing of the proglucagon peptide to produce the GLP-1 peptide  
427 [55, 56].

428 We evaluated the effects of RMD and FOS added at 5% (wt/wt) to the HFS diet in the present  
429 and previous studies [9]. The dose of 5% (wt/wt) in an animal study is considered equivalent to 20–  
430 30 g/day in the case of humans [23, 24], and the recommended dose of dietary fiber is 20–38 g/day  
431 (14 g/1000 kcal) in human adults [25]. Therefore, the 5% supplementation in an experimental diet  
432 in the present study is thought to be more suitable than the 10% dose that has been used in the  
433 majority of animal studies on prebiotic fibers, and the present results could have translational  
434 potential for human applications.

435 In summary, rats were fed a high-fat and high-sucrose diet with or without 5% RMD/FOS for 8  
436 weeks. Feeding the HFS diet resulted in increased glycemic response to oral glucose, but  
437 supplementation of RMD or FOS did not achieve significant increments in glycemia and an index  
438 of insulin resistance, with increased GLP-1 secretion. Feeding RMD or FOS-supplemented diet  
439 increased GLP-1 content in the large intestine. Energy intake and adipose tissue weight gain were  
440 reduced by feeding the RMD or FOS-supplemented diet for 8 days, and these parameters inversely  
441 correlated with cecal propionate, plasma and tissue GLP-1 levels. These results demonstrate that a  
442 physiological dose of prebiotic fiber rapidly promotes GLP-1 production in rats fed an obesogenic  
443 high-fat and high-sucrose diet, which would effectively help to prevent excess energy intake and fat  
444 accumulation.

445

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447

448 **Conflict of Interest:** Y. Kishimoto and S. Kanahori are employees of Matsutani Chemical Industry.  
449 T. Hira, R. Suto, and H. Hara, no conflicts of interest.

450

451 **Author's contributions to the manuscript**

452 T. H., R. S., Y. K. and H. H. designed research; T. H. and R. S. conducted research and analyzed  
453 data; T. H., R. S., Y. K., S. K. and H. H. wrote the paper. T. H. had primary responsibility for final  
454 content. All authors read and approved the final manuscript.

455

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645

646 **Tables**647 **Table 1. Test diet composition**

Ingredient	Control	HFS	HFS+RMD	HFS+FOS
	g/kg of diet			
Cornstarch	397.486	–	–	–
Casein <sup>1)</sup>	200	200	200	200
Dextrinized cornstarch <sup>2)</sup>	132	–	–	–
Sucrose	100	399.486	399.486	399.486
Soybean oil	70	70	70	70
Lard oil	–	230	230	230
Fiber				
Cellulose <sup>3)</sup>	50	50	–	–
RMD <sup>4)</sup>	–	–	50	–
FOS <sup>5)</sup>	–	–	–	50
Mineral mixture <sup>6)</sup>	35	35	35	35
Vitamin mixture <sup>6)</sup>	10	10	10	10
L-Cystine	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5
<i>tert</i> -Butylhydroquinone	0.014	0.014	0.014	0.014
Energy density (kcal/g)	3.96	5.11	5.16	5.21

648 1) Acid Casein (Fonterra, Ltd., Auckland, New Zealand);

649 2) TK-16 (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan);

650 3) Avicel PH102 (Asahi Kasei Chemicals Corporation, Tokyo, Japan);

651 4) Resistant maltodextrin (Fibersol 2, Matsutani Chemical Industry, Hyogo, Japan);

652 5) Fructooligosaccharides (Meiologo-P, Meiji Co., Ltd., Tokyo, Japan);

653 6) Mineral and vitamin mixtures were prepared according to the AIN-93G formulation.

654

655 **Table 2. Body weight, visceral adipose tissue weight, and energy intake of rats fed**  
 656 **test diets for 8 weeks.**

	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Initial body Weight (g)	209 ±	3	205 ±	4	207 ±	3	206 ±	3	0.882
Final body Weight (g)	515 ±	12 <sup>b</sup>	607 ±	11 <sup>a</sup>	579 ±	17 <sup>a</sup>	596 ±	14 <sup>a</sup>	0.001
Body weight gain (g)	306 ±	10 <sup>b</sup>	401 ±	13 <sup>a</sup>	372 ±	16 <sup>a</sup>	390 ±	13 <sup>a</sup>	<0.001
Visceral adipose (g/100 g BW)	8.18 ±	0.49 <sup>b</sup>	11.92 ±	0.61 <sup>a</sup>	10.07 ±	0.54 <sup>ab</sup>	10.10 ±	0.42 <sup>ab</sup>	0.001
Total Energy intake (kcal)	5440 ±	154 <sup>b</sup>	6280 ±	148 <sup>a</sup>	6142 ±	179 <sup>a</sup>	6275 ±	176 <sup>a</sup>	0.004

657 The values are the means ± SE ( $n = 7-8$ ). The values that do not share the same letter  
 658 differ significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test).

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662 **Table 3. Glucose, insulin, GLP-1, GIP, PYY levels and DPP-IV activity in portal**  
 663 **plasma of rats fed test diets for 8 weeks.**

	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Glucose (mg/dl)	102.6 ±	2.3 <sup>NS</sup>	120.8 ±	6.3	105.6 ±	4.6	118.4 ±	4.3	0.023
Insulin (nM)	13.8 ±	1.6 <sup>NS</sup>	12.8 ±	2.9	10.3 ±	1.5	9.4 ±	2.7	0.493
Total GLP-1 (pM)	45.1 ±	3.8 <sup>b</sup>	60.4 ±	3.2 <sup>ab</sup>	59.4 ±	7.2 <sup>ab</sup>	67.8 ±	5.6 <sup>a</sup>	0.050
Active GLP-1 (pM)	11.2 ±	1.3 <sup>b</sup>	32.2 ±	3.4 <sup>ab</sup>	38.7 ±	7.3 <sup>a</sup>	49.9 ±	10.4 <sup>a</sup>	0.007
Total GIP (pM)	12.1 ±	1.6 <sup>NS</sup>	13.4 ±	0.7	12.7 ±	0.7	9.5 ±	1.3	0.133
PYY (ng/mL)	1.15 ±	0.12 <sup>b</sup>	1.37 ±	0.07 <sup>ab</sup>	1.66 ±	0.13 <sup>ab</sup>	1.86 ±	0.22 <sup>a</sup>	0.010
DPP-IV (mU/mL)	15.8 ±	1.9 <sup>NS</sup>	18.1 ±	1.1	17.2 ±	2.2	18.6 ±	1.9	0.732

664 The values are the means ± SE ( $n = 7-8$ ). The values that do not share the same letter  
 665 differ significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test).

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668 **Table 4. Proglucagon mRNA expression in intestinal tissues of rats fed test diets for**  
 669 **8 weeks.**

Proglucagon/GAPDH (relative to Control)	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Jejunum	1.00 ± 0.23		1.16 ± 0.20		1.02 ± 0.09		1.16 ± 0.17		0.526
Ileum	1.00 ± 0.18 <sup>a</sup>		0.51 ± 0.10 <sup>ab</sup>		0.47 ± 0.09 <sup>b</sup>		0.55 ± 0.13 <sup>ab</sup>		0.022
Cecum	1.00 ± 0.27		1.55 ± 0.27		1.46 ± 0.19		0.97 ± 0.15		0.165
Colon	1.00 ± 0.25		0.85 ± 0.15		0.91 ± 0.11		1.12 ± 0.31		0.097

670 Total RNA was used for real-time PCR analysis. Proglucagon mRNA expression levels  
 671 were normalized to that of GAPDH, and the data are expressed as relative changes  
 672 compared to the control group. The values are the means ± SE ( $n = 7-8$ ). The values are  
 673 the means ± SE ( $n = 7-8$ ). The values that do not share the same letter differ significantly  
 674 between the treatments ( $P < 0.05$ , Tukey–Kramer test).

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678 **Table 5. Cecal tissue, content weights, pH and short chain fatty acid concentrations**  
 679 **of rats fed test diets for 8 weeks.**

	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Cecal tissue (g/100 g BW)	0.24 ± 0.02 <sup>b</sup>		0.18 ± 0.01 <sup>b</sup>		0.35 ± 0.02 <sup>a</sup>		0.36 ± 0.04 <sup>a</sup>		<0.001
Cecal content (g/100 g BW)	0.34 ± 0.04 <sup>b</sup>		0.27 ± 0.02 <sup>b</sup>		0.64 ± 0.09 <sup>a</sup>		0.87 ± 0.08 <sup>a</sup>		<0.001
Cecal pH	6.87 ± 0.12 <sup>a</sup>		6.36 ± 0.08 <sup>b</sup>		6.36 ± 0.12 <sup>b</sup>		6.04 ± 0.06 <sup>b</sup>		<0.001
Acetic acid (μmol/g content)	18.9 ± 1.5 <sup>ab</sup>		16.2 ± 1.2 <sup>b</sup>		23.8 ± 1.8 <sup>a</sup>		17.3 ± 1.9 <sup>b</sup>		0.012
Propionic acid (μmol/g content)	5.32 ± 0.55 <sup>a</sup>		1.82 ± 0.35 <sup>b</sup>		4.99 ± 0.33 <sup>a</sup>		4.14 ± 0.45 <sup>a</sup>		<0.001
n-Butyric acid (μmol/g content)	1.17 ± 0.11 <sup>ab</sup>		0.54 ± 0.28 <sup>b</sup>		1.88 ± 0.33 <sup>a</sup>		1.06 ± 0.25 <sup>ab</sup>		0.011
iso-Butyric acid (μmol/g content)	2.06 ± 0.45 <sup>a</sup>		0.89 ± 0.18 <sup>ab</sup>		1.22 ± 0.35 <sup>ab</sup>		0.67 ± 0.14 <sup>b</sup>		0.025

680 The values are the means ± SE ( $n = 7-8$ ). The values that do not share the same letter  
 681 differ significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test).



682 **Table 6. Body weight, visceral adipose tissue weight, and energy intake of rats fed**  
 683 **test diets for 8 days.**

	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Initial body weight (g)	169 ±	2	172 ±	3	171 ±	2	169 ±	2	0.723
Final body weight (g)	218 ±	3 <sup>b</sup>	237 ±	5 <sup>a</sup>	234 ±	3 <sup>a</sup>	225 ±	4 <sup>ab</sup>	0.008
Body weight gain (g)	49.0 ±	1.1 <sup>c</sup>	65.0 ±	3.0 <sup>a</sup>	63.1 ±	2.4 <sup>ab</sup>	56.0 ±	1.9 <sup>bc</sup>	<0.001
Visceral fat (g/100 g BW)	2.89 ±	0.16 <sup>b</sup>	4.25 ±	0.23 <sup>a</sup>	3.50 ±	0.12 <sup>b</sup>	3.46 ±	0.22 <sup>b</sup>	<0.001
Light period energy intake (kcal)	49.1 ±	5.9	61.4 ±	11.3	49.3 ±	8.6	60.3 ±	8.9	0.637
Dark period energy Intake (kcal)	520 ±	13 <sup>b</sup>	646 ±	27 <sup>a</sup>	588 ±	11 <sup>ab</sup>	549 ±	21 <sup>b</sup>	0.001
Total energy intake (kcal)	569 ±	15 <sup>b</sup>	708 ±	22 <sup>a</sup>	637 ±	11 <sup>b</sup>	609 ±	19 <sup>b</sup>	<0.001

684 The values are the means ± SE ( $n = 7-8$ ). The values that do not share the same letter  
 685 differ significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test).

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689 **Table 7. Glucose, insulin, GLP-1, GIP, PYY levels and DPP-IV activity in portal**  
 690 **plasma of rats fed test diets for 8 days.**

	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Glucose (mg/dl)	105.3 ±	4.8	118.1 ±	5.8	126.1 ±	5.6	122.9 ±	7.1	0.090
Insulin (nM)	0.7 ±	0.1	1.1 ±	0.1	1.0 ±	0.1	0.9 ±	0.1	0.152
Total GLP-1 (pM)	24.9 ±	3.7	29.3 ±	3.6	38.8 ±	4.7	38.0 ±	4.2	0.062
Active GLP-1 (pM)	7.2 ±	1.3	13.4 ±	2.0	15.9 ±	4.4	14.4 ±	1.9	0.141
Total GIP (pM)	1.8 ±	0.5	3.7 ±	1.1	2.6 ±	1.1	2.6 ±	0.7	0.567
PYY (ng/mL)	0.93 ±	0.05 <sup>b</sup>	0.97 ±	0.07 <sup>ab</sup>	1.09 ±	0.04 <sup>ab</sup>	1.18 ±	0.08 <sup>a</sup>	0.038
DPP-IV (mU/mL)	23.4 ±	0.9	24.7 ±	1.3	23.5 ±	1.2	24.4 ±	1.0	0.781

691 The values are the means ± SE ( $n = 7-8$ ). The values that do not share the same letter  
 692 differ significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test).

693 **Table 8. Proglucagon mRNA expression in intestinal tissues of rats fed test diets for**  
 694 **8 days.**

695

Proglucagon/GAPDH (relative to Control)	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Jejunum	1.00 ± 0.22		0.73 ± 0.13		1.08 ± 0.17		1.04 ± 0.17		0.526
Ileum	1.00 ± 0.17		0.49 ± 0.03		0.58 ± 0.10		0.62 ± 0.18		0.078
Cecum	1.00 ± 0.07 <sup>a</sup>		0.81 ± 0.06 <sup>ab</sup>		0.88 ± 0.13 <sup>ab</sup>		0.64 ± 0.06 <sup>b</sup>		0.044
Colon	1.00 ± 0.15		0.53 ± 0.08		1.17 ± 0.18		1.17 ± 0.27		0.097

696 Total RNA was used for real-time PCR analysis. Proglucagon mRNA expression levels  
 697 were normalized to that of GAPDH, and the data are expressed as relative changes  
 698 compared to the control group. The values are the means ± SE ( $n = 6-7$ ). The values that  
 699 do not share the same letter differ significantly between the treatments ( $P < 0.05$ , Tukey–  
 700 Kramer test).

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702

703 **Table 9. Cecal tissue, content weights, pH and short chain fatty acid concentrations**  
 704 **of rats fed test diets for 8 days.**

	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Cecal tissue (g/100 g BW)	0.34 ± 0.03 <sup>b</sup>		0.29 ± 0.01 <sup>b</sup>		0.57 ± 0.02 <sup>a</sup>		0.56 ± 0.03 <sup>a</sup>		<0.001
Cecal content (g/100 g BW)	1.07 ± 0.10 <sup>b</sup>		1.00 ± 0.09 <sup>b</sup>		2.40 ± 0.16 <sup>a</sup>		2.23 ± 0.15 <sup>a</sup>		<0.001
Cecal pH	6.60 ± 0.21 <sup>a</sup>		6.27 ± 0.19 <sup>ab</sup>		5.86 ± 0.12 <sup>b</sup>		5.93 ± 0.19 <sup>ab</sup>		0.030
Acetic acid (μmol/g content)	16.5 ± 2.9		19.2 ± 2.6		16.3 ± 1.6		20.9 ± 2.4		0.494
Propionic acid (μmol/g content)	3.36 ± 0.51 <sup>b</sup>		2.81 ± 0.24 <sup>b</sup>		6.89 ± 0.62 <sup>a</sup>		6.27 ± 0.74 <sup>a</sup>		<0.001
n-Butyric acid (μmol/g content)	1.40 ± 0.31		3.37 ± 1.17		4.28 ± 1.19		4.31 ± 1.29		0.223
iso-Butyric acid (μmol/g content)	1.58 ± 0.56		2.75 ± 0.59		0.79 ± 0.18		0.58 ± 0.38		0.092

705 The values are the means ± SE ( $n = 6-7$ ). The values that do not share the same letter  
 706 differ significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test).

707 **Figure legends**

708 **Fig. 1. Plasma glucose, insulin, and GLP-1 levels measured by OGTTs after 4 and 7**  
709 **weeks.**

710 OGTTs were performed in rats fasted overnight, after 4 weeks (A–C; oral glucose at 2  
711 g/kg) and 7 weeks (D–F; oral glucose at 3 g/kg) of test diet feeding. Glucose solution was  
712 orally administered at 0 min after the basal blood collection, and then blood samples were  
713 collected from the tail vein over 120 min. Glucose (A, E), insulin (B, F), and total GLP-1 (C,  
714 G) levels were measured in the plasma. The area under the curve (AUC) was calculated  
715 using the trapezoidal rule. AUC of incremental HOMA-IR (D, H) was calculated by using  
716 incremental AUC of glucose and insulin. The values are the means  $\pm$  SE ( $n = 7-8$ ). Two-  
717 way repeated ANOVA P values for time were all  $< 0.05$ ; for treatment were all  $< 0.05$ ; for  
718 time x treatment were A: 0.224, B: 0.754, C: 0.116, D: 0.792, E: 0.124, F: 0.949. The plots  
719 that do not share the same letter differ significantly between the treatments at the same  
720 time point ( $P < 0.05$ , Tukey–Kramer test). The bars that do not share the same letter differ  
721 significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test). n.s., no significant  
722 difference among the treatments.

723  
724 **Fig. 2. Weekly energy intake of rats fed test diets for 8 weeks.**

725 Rats were fed control diet, HFS diet, HFS diet supplemented with 5% RMD  
726 (HFS+RMD), or HFS diet supplemented with 5% FOS (HFS+FOS) for 8 weeks, ad libitum.  
727 The values are the means  $\pm$  SE ( $n = 7-8$ ). Two-way repeated ANOVA P values for time  
728 was  $< 0.001$ ; for treatment was  $< 0.001$ ; for time x treatment was 0.085. The plots that do  
729 not share the same letter differ significantly between the treatments at the same week ( $P <$   
730  $0.05$ , Tukey–Kramer test).

731 **Fig. 3. GLP-1 content in intestinal tissues of rats fed test diets for 8 weeks.**

732 Intestinal tissues (cecum and colon) were separately collected after overnight fasting  
733 from rats fed respective test diets (control, HFS, HFS+RMD, or HFS+FOS) for 8 weeks.  
734 After acid–ethanol extraction, GLP-1 concentrations were measured, and the values were  
735 corrected according to the tissue weight (pmol/g of tissue, A and B). GLP-1 contents in the  
736 whole cecum (C) and colon (D) were calculated based on the whole tissue weights. The  
737 values are the means  $\pm$  SE ( $n = 7-8$ ). The bars that do not share the same letter differ  
738 significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test). n.s., no significant  
739 difference among the treatments.

740

741 **Fig. 4. Energy intake by rats fed test diets for 8 days.**

742 Rats were fed control, HFS, HFS+RMD, or HFS+FOS diet for 8 days, ad libitum. Food  
743 intake was measured at 08:00 a.m. and 20:00 p.m. every day. Energy intake was  
744 calculated for the light period (A), for the dark period (B), and for the entire day (C). The  
745 values are the means  $\pm$  SE ( $n = 6-7$ ). Two-way repeated ANOVA  $P$  values for time were  
746 all  $< 0.001$ ; for treatment were A: 0.19, B and C:  $< 0.001$ ; for time  $\times$  treatment were A:  
747 0.592, B: 0.600, C: 0.934. The plots that do not share the same letter differ significantly  
748 between the treatments on the same day ( $P < 0.05$ , Tukey–Kramer test).

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755 **Fig. 5. GLP-1 content in intestinal tissues of rats fed test diets for 8 days.**

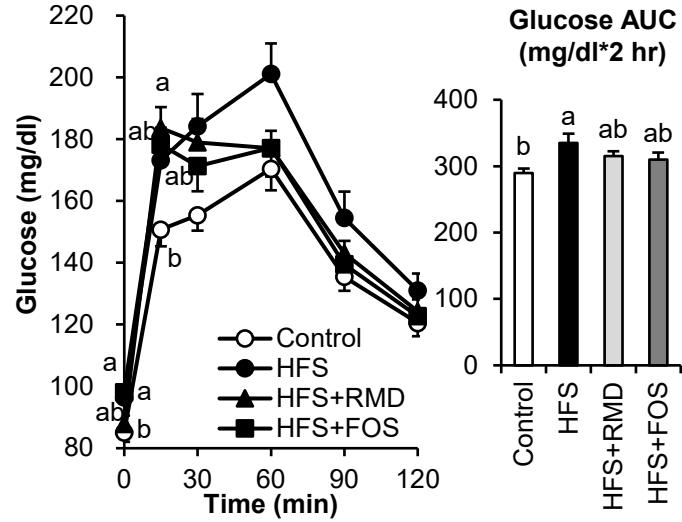
756 Intestinal tissues (cecum and colon) were separately collected after overnight fasting  
757 from rats fed respective test diets (control, HFS, HFS+RMD, or HFS+FOS) for 8 days.  
758 After acid–ethanol extraction, GLP-1 concentrations were measured, and the values were  
759 corrected according to tissue weights (pmol/g of tissue, A and B). GLP-1 contents in the  
760 whole cecum (C) and colon (D) were calculated based on the whole tissue weights. The  
761 values are the means  $\pm$  SE ( $n = 6-7$ ). The bars that do not share the same letter differ  
762 significantly between the treatments ( $P < 0.05$ , Tukey–Kramer test). n.s., No significant  
763 difference among the treatments.

764

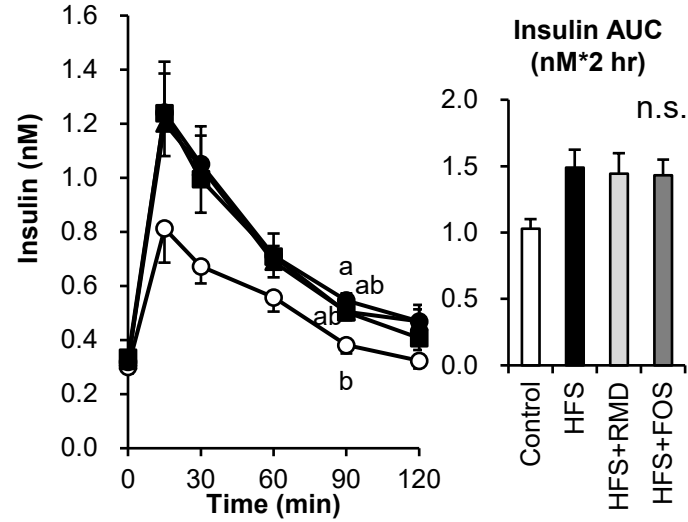
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# OGTT, 4 wk

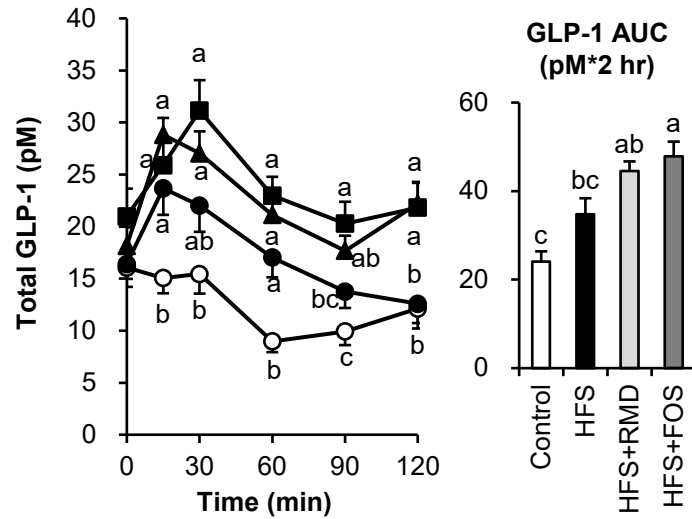
**A**



**B**



**C**



**D**

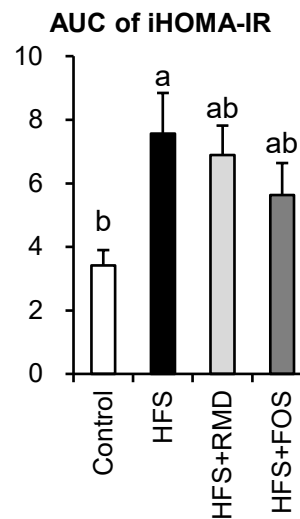
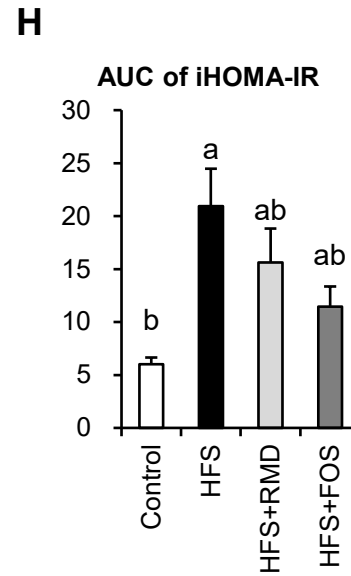
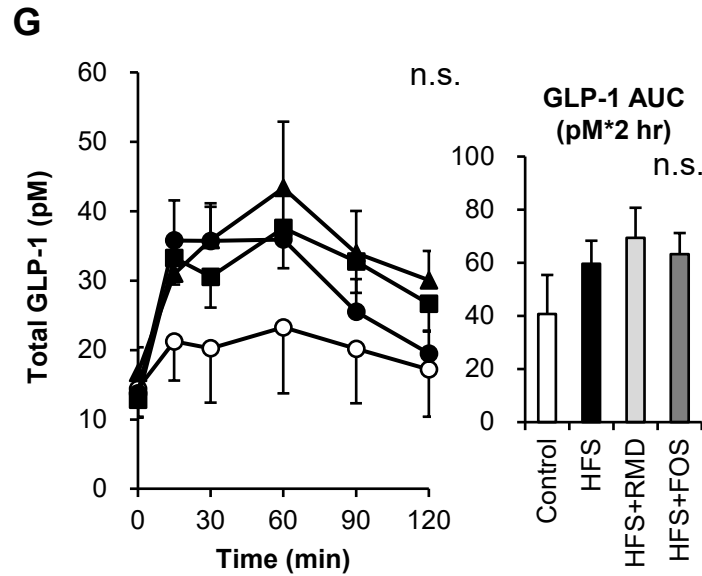
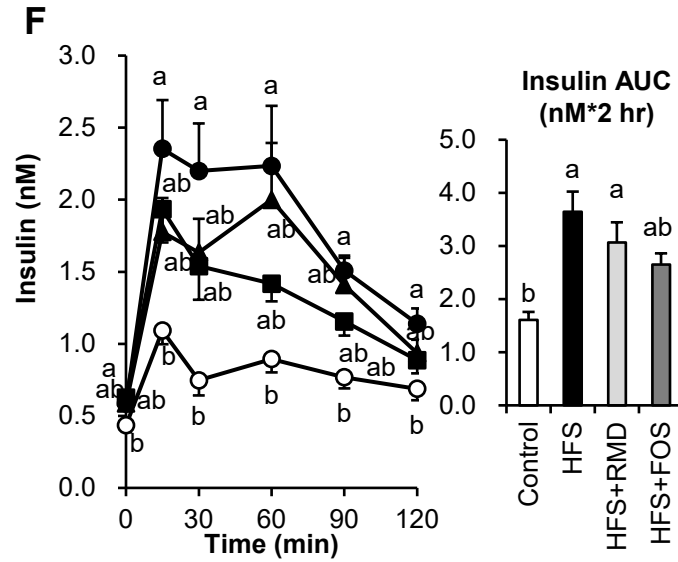
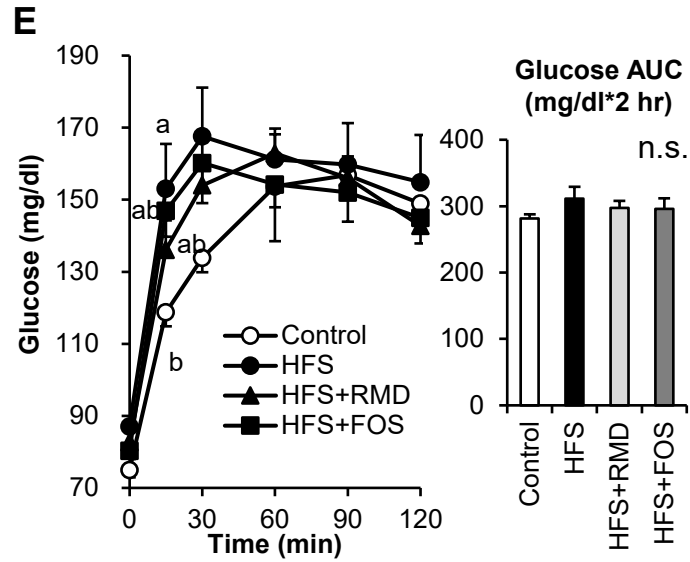


Fig. 1A-D

**OGTT, 7 wk**



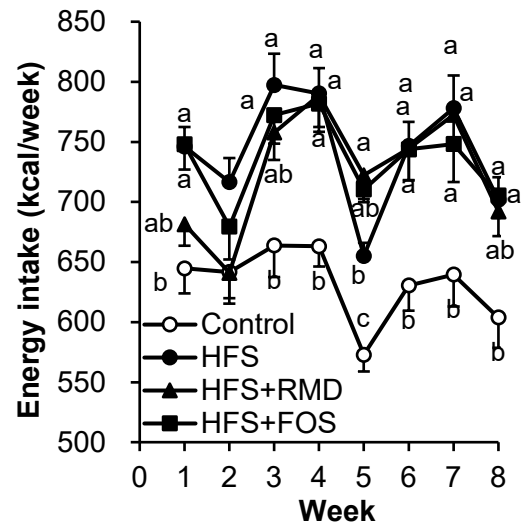
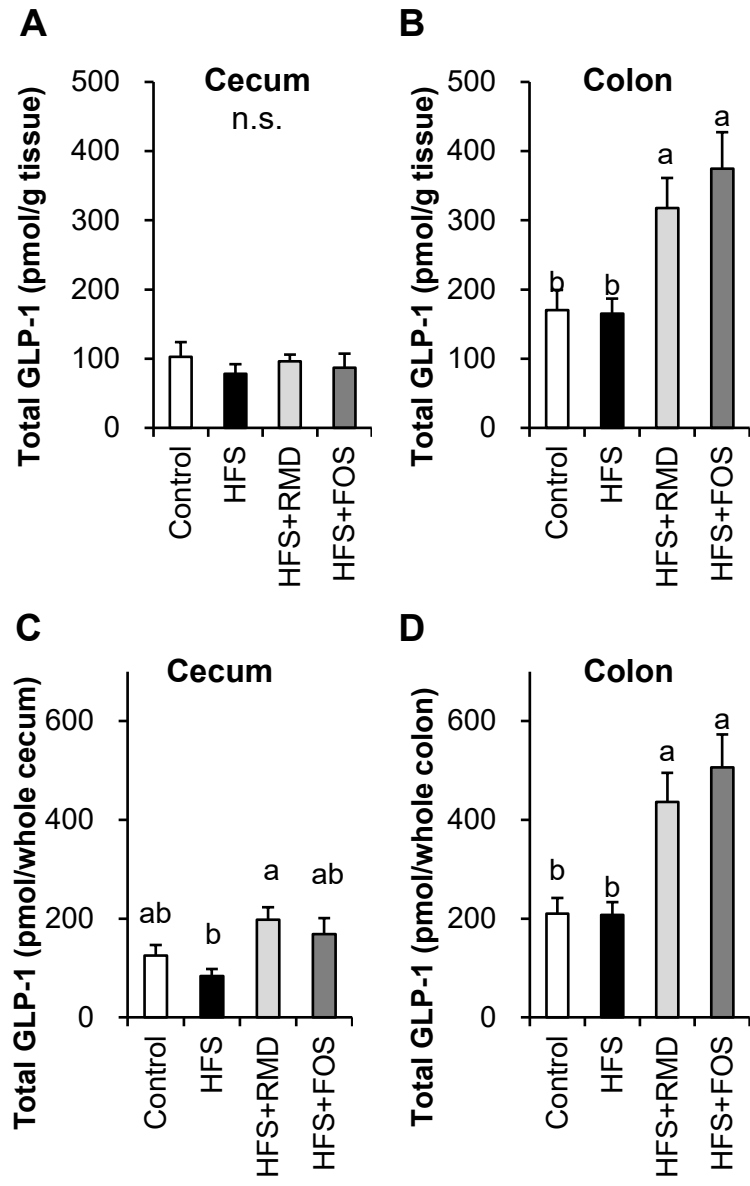


Fig. 2





**Fig. 3**

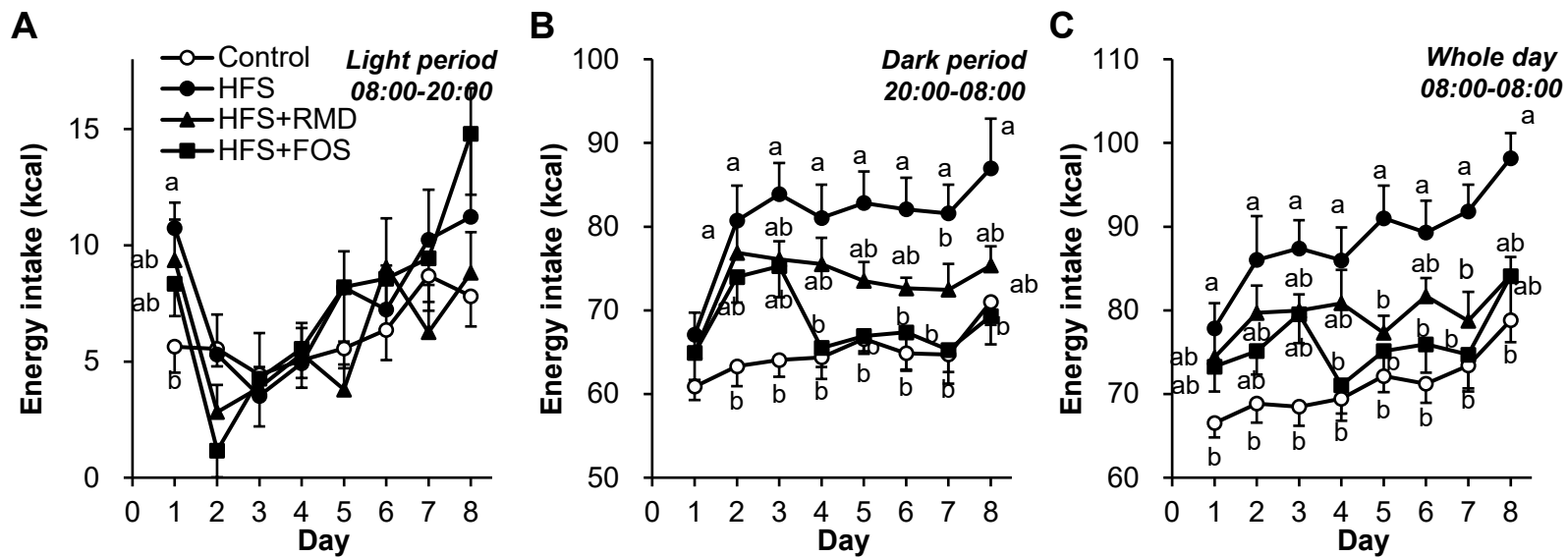


Fig. 4

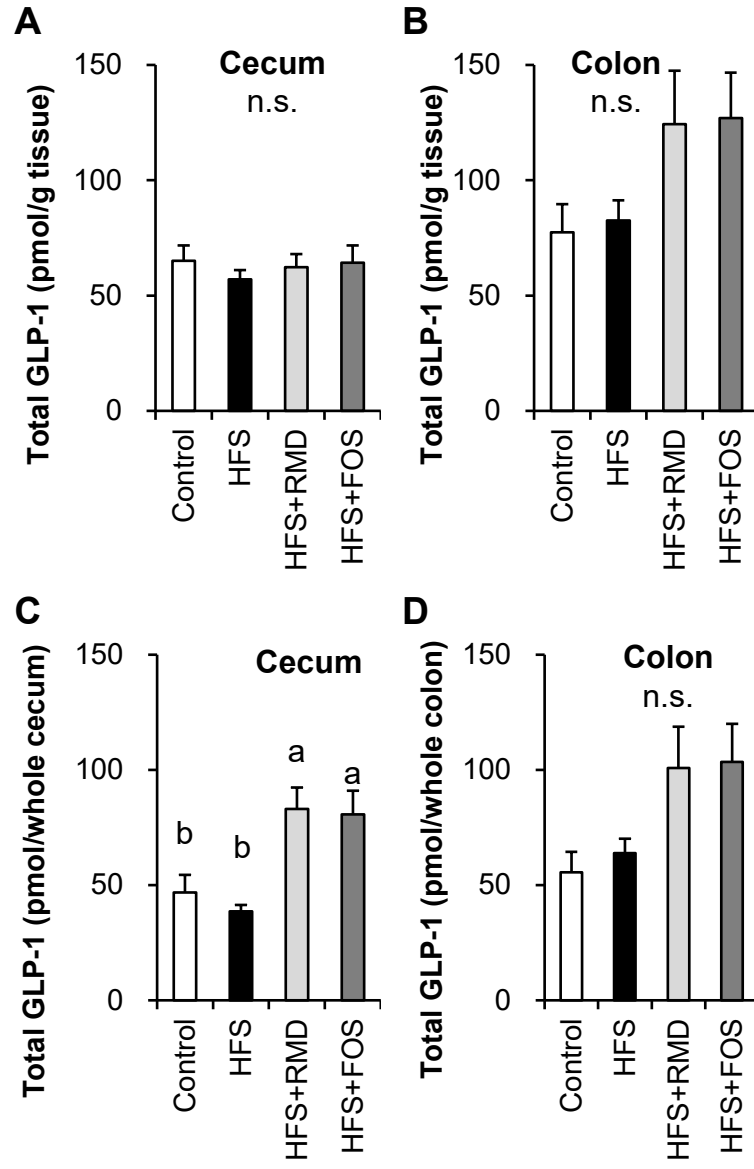


Fig. 5