

Title	Resistant maltodextrin or fructooligosaccharides promotes GLP-1 production in male rats fed a high-fat and high- sucrose diet, and partially reduces energy intake and adiposity
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Citation	European Journal of Nutrition, 57(3), 965-979 https://doi.org/10.1007/s00394-017-1381-7
Issue Date	2017
Doc URL	http://hdl.handle.net/2115/85305
Rights	The final publication is available at link.springer.com
Туре	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Eur J Nutr 57_965.pdf



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
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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	Resisitant 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	
40		

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

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

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

51

52 INTRODUCTION

53In recent years, glucagon-like peptide-1 (GLP-1), an incretin hormone, has received much attention in association with glucose homeostasis. Besides the incretin effect, GLP-1 released from 5455enteroendocrine L cells has multiple other functions [1, 2], including pancreatic beta-cell 56protection/proliferation, satiety induction, and gastric emptying suppression, as well as 57cardioprotective and neuroprotective effects. Recently, incretin "enhancers" such as GLP-1 receptor agonists and dipeptidyl peptidase-IV (DPP-IV) inhibitors have been widely and effectively used for 5859the 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) 62effect; however, the concept has not been sufficiently proven. 63 GLP-1 secretion is stimulated by luminal macronutrients such as glucose, fatty acids, peptides, 64and 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.

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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 105maintained at 22 ± 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 107diet (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 (Meioligo-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 further one week. After 8 weeks of test diet feeding and overnight fasting (16-20 hours), rats were 112113euthanized for tissue and blood sampling. Body weight and food intake were measured every 1-2 114days. In experiment 2, the rats were fed the test diets for 8 days and euthanized on day 9 after 115overnight 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

the animals were handled in accordance with the Hokkaido University guidelines for the care anduse of laboratory animals.

119 Glucose tolerance test

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

135 **Portal blood and tissue collection**

After 8 weeks (experiment 1) or 8 days (experiment 2) of feeding period, blood samples were collected from the portal vein and abdominal aorta of the rats under sodium pentobarbital anesthesia (50 mg/kg of body weight, somnopentyl injection; Kyoritsu Seiyaku Corporation, Tokyo, Japan) into a syringe containing heparin (final concentration 50 IU/mL), aprotinin (final concentration 500

140 KIU/mL), and DPP-IV inhibitors (final concentration 50 µmol/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 insulinotropic 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 °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 °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.
169	Massurement of glussgan like nontide 1 content in intestinal tiggues

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 $(2,000 \times g \text{ for } 20 \text{ min})$, 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., Carv, 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 192appropriate (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, 194treatment and the interaction of time and treatment) were presented in the figure legend. Area under 195the 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]. HOMA-IR = {incremental AUC 199 of glucose (mg/dL*2 hr) x incremental AUC of insulin (μ U/mL*2 hr)}/2,430, where 1 ng of insulin 200is equivalent to 26 µunits. Peason's correlation coefficient (r) and significance of correlations were 201analyzed as appropriate, and shown in supplementary tables.

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204 **RESULTS**

205 Experiment 1 (feeding for 8 weeks)

After 4 weeks, glycemic responses (Fig. 1A) in the three HFS-fed groups appeared to increase compared to that in the control group. The AUC of glucose in the HFS group was significantly higher than that in the control group, but it did not reach significant increment in the RMD- and FOS-supplemented groups. The insulin responses in the three HFS-fed groups were insignificantly higher than those in the control group. The GLP-1 responses in the HFS+RMD and HFS+FOS groups were significantly higher than those in the control group which had no increment of the 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 <i>n</i> -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.

259 Experiment 2 (feeding for 8 days)

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

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

The total plasma GLP-1 level showed an increasing trend in the RMD- and FOS-supplemented groups (Table 7), and the value in the RMD+HFS group was significantly different from that in control group when analyzed with Dunnett's test (P < 0.05). The plasma PYY level was the highest

in the HFS+FOS group. There were no significant differences in the glucose, insulin GIP levels,

and DPP-IV activity among the treatments

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

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

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).

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293 Discussion

294In contrast to most of studies that investigated GLP-1-promoting effects of various dietary fibers 295added in the diet at more than 10% (wt/wt) dose [10-12, 15, 20-22], we investigated whether a 296relatively physiological dose (5% wt/wt) of RMD could promote GLP-1 production in rats fed a 297high-fat and high-sucrose diet for a long (8 weeks) and a short (8 days) period. After 8 weeks 298feeding 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 301increment 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 305the 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.

The glycemic response significantly increased in the rats fed the HFS diet for 4 weeks but not in rats fed the HFS diet supplemented with RMD or FOS (Fig. 1A). The insulin responses were almost 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

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

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

343 It is still controversial whether GLP-1 secretion is impaired under obesity, glucose intolerance, 344and diabetic conditions [35-37]. We speculate that the enhanced GLP-1 response to a meal [34], as 345well 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 347feeding 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

groups after 7 weeks (Fig. 1H). Although the GLP-1 response to oral glucose in RMD- and FOSsupplemented groups did not differ from the HFS group, increased fasting GLP-1 levels (Table 3)
might contribute to the attenuation of insulin resistance since improving insulin sensitivity is one of
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. 362The 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 364period) food intake everyday (Fig. 4). This was clearly observed during nighttime, the usual eating 365time 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 thier satiety effects on subjective 370 appetite accompanied by increased GLP-1 and/or PYY levels in animals or healthy humans, 371suggesting a possibility of these prebiotics to contribute to control energy intake and body weight 372for 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 374not PYY level) and cecal GLP-1 content were inversely correlated with total energy intake. Thus, 375the 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 <i>n</i> -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 (μ mol/g content) and cecal GLP-1 (pmol/tissue); r = 0.6166, P = 0.0188], [cecal
- 400 GLP-1 (pmol/tissue) and portal total GLP-1 (pM); r = 0.5845, P = 0.0282], [portal total GLP-1
- 401 (pM) and total energy intake (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 peptide427 [55, 56].

428We evaluated the effects of RMD and FOS added at 5% (wt/wt) to the HFS diet in the present 429and 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 432in 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. 435In summary, rats were fed a high-fat and high-sucrose diet with or without 5% RMD/FOS for 8 436weeks. 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 439increased GLP-1 content in the large intestine. Energy intake and adipose tissue weight gain were 440reduced by feeding the RMD or FOS-supplemented diet for 8 days, and these parameters inversely 441correlated with cecal propionate, plasma and tissue GLP-1 levels. These results demonstrate that a 442physiological dose of prebiotic fiber rapidly promotes GLP-1 production in rats fed an obesogenic 443high-fat and high-sucrose diet, which would effectively help to prevent excess energy intake and fat 444accumulation. 445

446 Financial support: The research was supported by JSPS KAKENHI Grant Number 26252016.447

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.

451Author's contributions to the manuscript 452T. H., R. S., Y. K. and H. H. designed research; T. H. and R. S. conducted research and analyzed 453data; T. H., R. S., Y. K., S. K. and H. H. wrote the paper. T. H. had primary responsibility for final 454content. All authors read and approved the final manuscript. 455456References 4571. Cho YM, Fujita Y, Kieffer TJ (2014) Glucagon-like peptide-1: glucose homeostasis and 458beyond. Annu Rev Physiol 76:535-559. doi:10.1146/annurev-physiol-021113-170315 4592. Kim YO, Schuppan D (2012) When GLP-1 hits the liver: a novel approach for insulin 460resistance and NASH. Am J Physiol Gastrointest Liver Physiol 302 (8):G759-761. 461doi:10.1152/ajpgi.00078.2012 4623. Nauck M (2016) Incretin therapies: highlighting common features and differences in the 463modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 464inhibitors. Diabetes Obes Metab 18 (3):203-216. doi:10.1111/dom.12591 4654. Diakogiannaki E, Gribble FM, Reimann F (2012) Nutrient detection by incretin hormone 466secreting cells. Physiol Behav 106 (3):387-393. doi:10.1016/j.physbeh.2011.12.001 467Higuchi N, Hira T, Yamada N, Hara H (2013) Oral Administration of Corn Zein Hydrolysate 5. 468 Stimulates GLP-1 and GIP Secretion and Improves Glucose Tolerance in Male Normal Rats 469 and Goto-Kakizaki Rats. Endocrinology 154 (9):3089-3098. doi:10.1210/en.2012-2275 4706. Ishikawa Y, Hira T, Inoue D, Harada Y, Hashimoto H, Fujii M, Kadowaki M, Hara H (2015) 471Rice protein hydrolysates stimulate GLP-1 secretion, reduce GLP-1 degradation, and lower the 472glycemic response in rats. Food Funct 6 (8):2525-2534. doi:10.1039/c4fo01054j 4737. Greenfield JR, Farooqi IS, Keogh JM, Henning E, Habib AM, Blackwood A, Reimann F,

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646 **Tables**

g/kg (of diet 	- 200 - 399,486
_ 200 _ 999.486 70	- 200 - 399.486	- 200 - 399,486
200 - 99.486 70	200 - 399.486	200 - 399.486
– 99.486 70	_ 399.486	- 399.486
99.486 70	399.486	399,486
70		
	70	70
230	230	230
50	_	_
_	50	_
-	-	50
35	35	35
10	10	10
3	3	3
2.5	2.5	2.5
0.014	0.014	0.014
	5 46	5 .04
	- 35 10 3 2.5 0.014	 35 35 10 10 3 3 2.5 2.5 0.014 0.014 5.11 5.16

647 **Table 1. Test diet composition**

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);

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

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

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

Table 2. Body weight, visceral adipose tissue weight, and energy intake of rats fed

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

656 test diets for 8 weeks.

657 The values are the means \pm 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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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.

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

664 The values are the means \pm 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

Proglucagon/GAPDH	Contr	Control		HFS		HFS+RMD		HFS+FOS	
(relative to Control)	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	0.52
lleum	1.00 ±	0.18 ^a	0.51 ±	0.10 ^{ab}	0.47 ±	0.09 ^b	0.55 ±	0.13 ^{ab}	0.02
Cecum	1.00 ±	0.27	1.55 ±	0.27	1.46 ±	0.19	0.97 ±	0.15	0.16
Colon	1.00 +	0.25	0.85 +	0.15	0.91 ±	0.11	1.12 ±	0.31	0.09

672 compared to the control group. The values are the means \pm SE (n = 7-8). The values are 673 the means \pm 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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8 weeks.

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

680 The values are the means \pm 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).

Table 6. Body weight, visceral adipose tissue weight, and energy intake of rats fed

	Control		HFS	HFS		HFS+RMD		HFS+FOS	
	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 ^b	237 ±	5 ^a	234 ±	3 ^a	225 ±	4 ^{ab}	0.008
Body weight gain (g)	49.0 ±	1.1 ^c	65.0 ±	3.0 ^a	63.1 ±	2.4 ^{ab}	56.0 ±	1.9 ^{bc}	<0.001
Visceral fat (g/100 g BW)	2.89 ±	0.16 ^b	4.25 ±	0.23 ^a	3.50 ±	0.12 ^b	3.46 ±	0.22 ^b	<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 ^b	646 ±	27 ^a	588 ±	11 ^{ab}	549 ±	21 ^b	0.001
Total energy intake (kcal)	569 ±	15 ^b	708 ±	22 ^a	637 ±	11 ^b	609 ±	19 ^b	<0.001

683 test diets for 8 days.

684 The values are the means \pm 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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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 ^b	0.97 ± 0.07 ^{ab}	1.09 ± 0.04 ^{ab}	1.18 ± 0.08 ^a	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 \pm 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).

Table 8. Proglucagon mRNA expression in intestinal tissues of rats fed test diets for

694 **8 days.**

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Proglucagon/GAPDH	Control		HFS		HFS+RMD		HFS+FOS		ANOVA
(relative to Control)	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
lleum	1.00 ±	0.17	0.49 ±	0.03	0.58 ±	0.10	0.62 ±	0.18	0.078
Cecum	1.00 ±	0.07 ^a	0.81 ±	0.06 ^{ab}	0.88 ±	0.13 ^{ab}	0.64 ±	0.06 ^b	0.044
Colon	1.00 ±	0.15	0.53 ±	0.08	1.17 ±	0.18	1.17 ±	0.27	0.097

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

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703 Table 9. Cecal tissue, content weights, pH and short chain fatty acid concentrations

of rats fed test diets for 8 days.

	Control		HFS	HFS		HFS+RMD		HFS+FOS	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P value
Cecal tissue (g/100 g BW)	0.34 ±	0.03 ^b	0.29 ±	0.01 ^b	0.57 ±	0.02 ^a	0.56 ±	0.03 ^a	<0.001
Cecal content (g/100 g BW)	1.07 ±	0.10 ^b	1.00 ±	0.09 ^b	2.40 ±	0.16 ^a	2.23 ±	0.15 ^a	<0.001
Cecal pH	6.60 ±	0.21 ^a	6.27 ±	0.19 ^{ab}	5.86 ±	0.12 ^b	5.93 ±	0.19 ^{ab}	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 ^b	2.81 ±	0.24 ^b	6.89 ±	0.62 ^a	6.27 ±	0.74 ^a	<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

The values are the means \pm SE (*n* = 6–7). The values that do not share the same letter

differ significantly between the treatments (P < 0.05, Tukey–Kramer test).

- 707 Figure legends
- Fig. 1. Plasma glucose, insulin, and GLP-1 levels measured by OGTTs after 4 and 7
 weeks.

710OGTTs were performed in rats fasted overnight, after 4 weeks (A-C; oral glucose at 2 711g/kg) and 7 weeks (D-F; oral glucose at 3 g/kg) of test diet feeding. Glucose solution was 712orally administered at 0 min after the basal blood collection, and then blood samples were 713collected from the tail vein over 120 min. Glucose (A, E), insulin (B, F), and total GLP-1 (C, 714G) levels were measured in the plasma. The area under the curve (AUC) was calculated 715using the trapezoidal rule. AUC of incremental HOMA-IR (D, H) was calculated by using 716incremental AUC of glucose and insulin. The values are the means \pm SE (n = 7-8). Two-717way repeated ANOVA P values for time were all < 0.05; for treatment were all < 0.05; for 718time x treatment were A: 0.224, B: 0.754, C: 0.116, D: 0.792, E: 0.124, F: 0.949. The plots 719that 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 721significantly between the treatments (P < 0.05, Tukey–Kramer test). n.s., no significant 722difference among the treatments.

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

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.

The values are the means \pm SE (*n* = 7–8). Two-way repeated ANOVA P values for time

was < 0.001; for treatment was < 0.001; for time x treatment was 0.085. The plots that do

not share the same letter differ significantly between the treatments at the same week (P <

730 0.05, Tukey–Kramer test).

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 (<i>n</i> = 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.

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

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

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

756Intestinal tissues (cecum and colon) were separately collected after overnight fasting 757from rats fed respective test diets (control, HFS, HFS+RMD, or HFS+FOS) for 8 days. 758After acid-ethanol extraction, GLP-1 concentrations were measured, and the values were 759corrected according to tissue weights (pmol/g of tissue, A and B). GLP-1 contents in the whole cecum (C) and colon (D) were calculated based on the whole tissue weights. The 760761values are the means \pm SE (*n* = 6–7). The bars that do not share the same letter differ 762significantly between the treatments (P < 0.05, Tukey–Kramer test). n.s., No significant 763difference among the treatments.

764



Fig. 1A-D







а

HFS

ab

HFS+RMD

ab

HFS+FOS









