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

Abbreviations: HFS: High-fat and high-sucrose, GLP-1: Glucagon-like peptide-1, RMD: Resisitant maltodextrin, FOS: Fructooligosaccharides, GIP: Glucose-dependent insulinotropic polypeptide, OGTT: Oral glucose tolerance test, PYY: Peptide YY, DPP-IV: Dipeptidyl peptidase-IV, SCFA: Short chain fatty acid

ABSTRACT

Purpose

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

Methods

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

Results

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

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.

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

INTRODUCTION

In 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 enteroendocrine L cells has multiple other functions [1, 2], including pancreatic beta-cell protection/proliferation, satiety induction, and gastric emptying suppression, as well as cardioprotective 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 the treatment of type 2 diabetes [3], but effective "GLP-1 releasers" have not yet been developed for therapeutic use. Increasing endogenous production and secretion of GLP-1 is thought to be a promising strategy for the improvement of glucose tolerance owing to its insulinotropic (incretin) effect; however, the concept has not been sufficiently proven.

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

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

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

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

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

MATERIALS AND METHODS

Animals and diets

Male Sprague–Dawley rats (5-week-old, n=32 for experiment 1, n=28 for experiment 2) were purchased from Japan SLC, Inc. (Shizuoka, Japan) and were fed an American Institute of Nutrition (AIN)-93G-based diet [26] for a one-week acclimation period. Each rat was individually housed in a separate cage and had free access to the diet and water, except for the days preceding the glucose tolerance test and killing. The experiment was performed in a temperature-controlled room maintained at 22 ± 2 °C with a 12 h light/12 h dark cycle (08.00 a.m.–20.00 p.m. light period). Rats were divided into four groups (n=7 or 8 in each group) and fed the AIN-93G diet (control), HFS diet (30% fat and 40% sucrose, wt/wt), or HFS diet in which cellulose was replaced with either RMD (Fibersol-2; Matsutani Chemical Industry Co., Hyogo, Japan) or FOS (Meioligo-P; Meiji Co., Ltd., Tokyo, Japan) (Table 1) for 8 weeks (experiment 1) or 8 days (experiment 2). The digestion resistant component in HDS+RMD diet is estimated at 4.5% by weight. In experiment 1, OGTTs 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 euthanized for tissue and blood sampling. Body weight and food intake were measured every 1–2 days. In experiment 2, the rats were fed the test diets for 8 days and euthanized on day 9 after overnight fasting (16–20 hours). Food intake was measured daily in the morning (8–9 a.m.) and 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 × *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 μmol/L; DPP4-010; Merck Millipore).

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

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

Measurement of glucagon-like peptide-1 content in intestinal tissues

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

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

Real-time polymerase chain reaction (PCR) analysis

Using a real-time PCR system [28], proglucagon mRNA expression levels were determined. Total RNA was extracted using the RNeasy Mini kit (Qiagen), according to the manufacturer's instructions. Complementary DNA was synthesized using the ReverTra Ace qPCR Master Mix with gDNA Remover (Toyobo Co., Ltd., Osaka, Japan), according to the manufacturer's instructions. Gene expression levels were determined using the Mx3000P Real-Time PCR System (Stratagene, La Jolla, CA, USA) and TaqMan Gene Expression Assay (Life Technologies, Carlsbad, CA, USA) with rat gene-specific predesigned TaqMan primers and probe sets [Rn99999916_s1 for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Rn00562293_m1 for proglucagon]. Relative expression levels were calculated for each sample after normalization to those of GAPDH as a reference gene using the standard curve method.

Measurement of cecal short-chain fatty acids

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

Statistical analyses

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

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

RESULTS

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

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

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

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

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

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

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 RMD- or 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".

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

After 7 weeks (Figs. 1E-H), the glycemic response at 15 min was the highest in the HFS group, while the RMD- and FOS-supplemented groups showed slightly smaller responses than the HFS group, which were accompanied by smaller insulin responses. This still suggests that the 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 thier 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 (μmol/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

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

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

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

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Author's contributions to the manuscript

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

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Tables

Table 1. Test diet composition

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

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

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

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);

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 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^{b}	607 ±	11^{a}	579 ±	17^{a}	596 ±	14^{a}	0.001
Body weight gain (g)	306 ±	10^{b}	401 ±	13^{a}	372 ±	16^{a}	390 ±	13^{a}	<0.001
Visceral adipose (g/100 g BW)	8.18 ±	0.49^{b}	11.92 ±	0.61^{a}	10.07 ±	0.54^{ab}	10.10 ±	0.42^{ab}	0.001
Total Energy intake (kcal)	5440 ±	154^{b}	6280 ±	148^{a}	6142 ±	179^{a}	6275 ±	176^{a}	0.004

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

Table 3. Glucose, insulin, GLP-1, GIP, PYY levels and DPP-IV activity in portal 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^{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

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

Table 4. Proglucagon mRNA expression in intestinal tissues of rats fed test diets for 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^{a}	0.51 ±	0.10^{ab}	0.47 ±	0.09^{b}	0.55 ±	0.13^{ab}	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

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

Table 5. Cecal tissue, content weights, pH and short chain fatty acid concentrations 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^{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

The values are the means ± SE (n = 7–8). The values that do not share the same letter 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 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^{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

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

Table 7. Glucose, insulin, GLP-1, GIP, PYY levels and DPP-IV activity in portal 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

The values are the means ± SE (n = 7–8). The values that do not share the same letter 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^{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

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).

701

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^{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

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).

Figure legends

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

OGTTs were performed in rats fasted overnight, after 4 weeks (A–C; oral glucose at 2 g/kg) and 7 weeks (D–F; oral glucose at 3 g/kg) of test diet feeding. Glucose solution was orally administered at 0 min after the basal blood collection, and then blood samples were collected from the tail vein over 120 min. Glucose (A, E), insulin (B, F), and total GLP-1 (C, G) levels were measured in the plasma. The area under the curve (AUC) was calculated using the trapezoidal rule. AUC of incremental HOMA-IR (D, H) was calculated by using incremental AUC of glucose and insulin. The values are the means ± SE (n = 7–8). Two-way repeated ANOVA P values for time were all < 0.05; for treatment were all < 0.05; for time x treatment were A: 0.224, B: 0.754, C: 0.116, D: 0.792, E: 0.124, F: 0.949. The plots that do not share the same letter differ significantly between the treatments at the same time point (P < 0.05, Tukey–Kramer test). The bars that do not share the same letter differ significantly between the treatments (P < 0.05, Tukey–Kramer test). n.s., no significant difference 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 (HFS+RMD), or HFS diet supplemented with 5% FOS (HFS+FOS) for 8 weeks, ad libitum. The values are the means ± 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 < 0.05, Tukey–Kramer test).

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

Intestinal tissues (cecum and colon) were separately collected after overnight fasting from rats fed respective test diets (control, HFS, HFS+RMD, or HFS+FOS) for 8 weeks. After acid–ethanol extraction, GLP-1 concentrations were measured, and the values were corrected according to the tissue weight (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 values are the means ± SE (n = 7–8). The bars that do not share the same letter differ significantly between the treatments ($P < 0.05$, Tukey–Kramer test). n.s., no significant 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 ± 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.

Intestinal tissues (cecum and colon) were separately collected after overnight fasting from rats fed respective test diets (control, HFS, HFS+RMD, or HFS+FOS) for 8 days. After acid–ethanol extraction, GLP-1 concentrations were measured, and the values were corrected 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 values are the means ± SE (n = 6–7). The bars that do not share the same letter differ significantly between the treatments ($P < 0.05$, Tukey–Kramer test). n.s., No significant difference among the treatments.

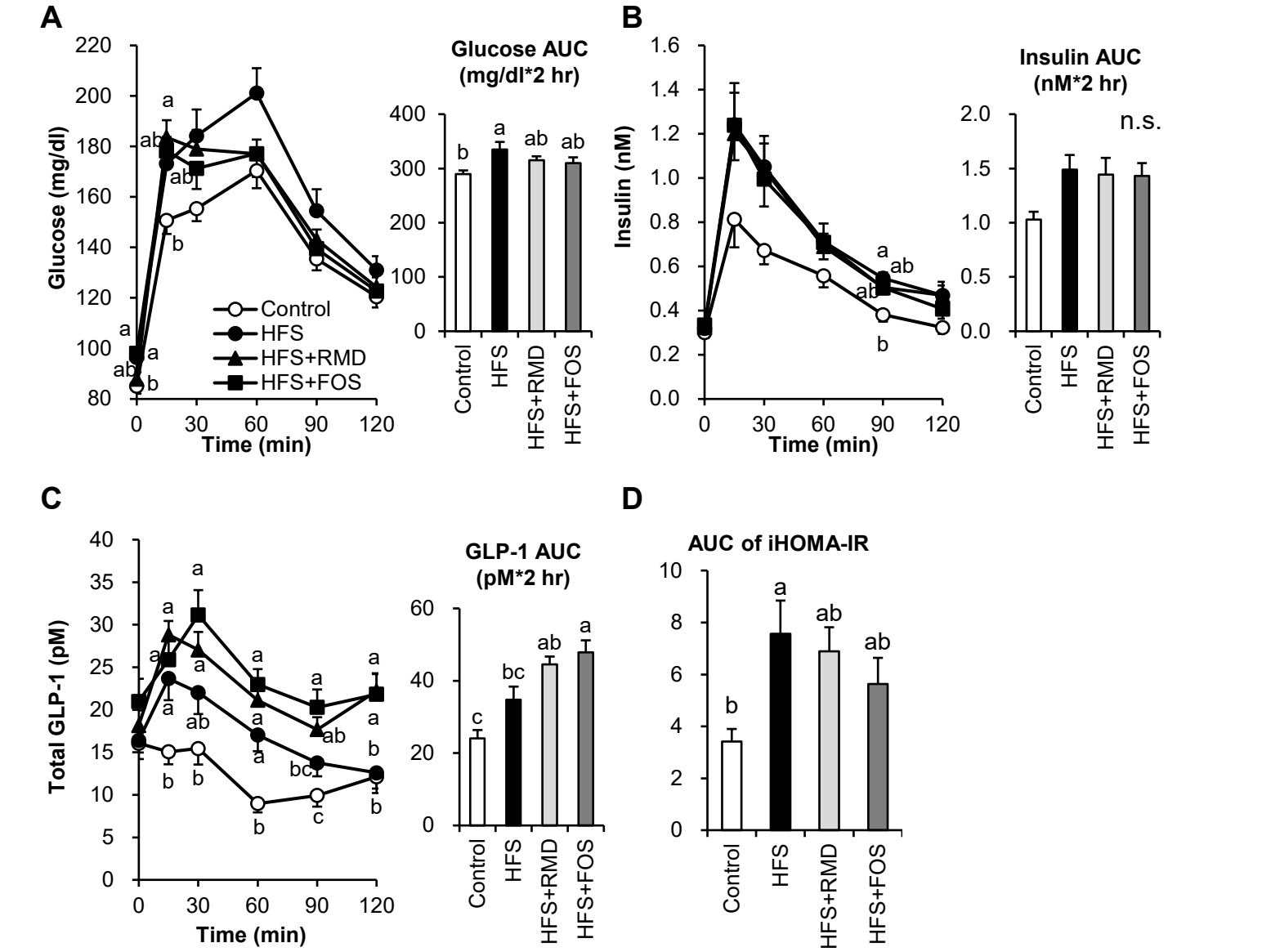

OGTT, 4 wk
A
Glucose (mg/dl)
Time (min)
Control
HFS
HFS+RMD
HFS+FOS
Glucose AUC
(mg/dl*2 hr)
B
Insulin (nM)
Insulin AUC
(nM*2 hr)
n.s.
C
Total GLP-1 (pM)
GLP-1 AUC
(pM*2 hr)
D
AUC of iHOMA-IR

Fig. 1A-D

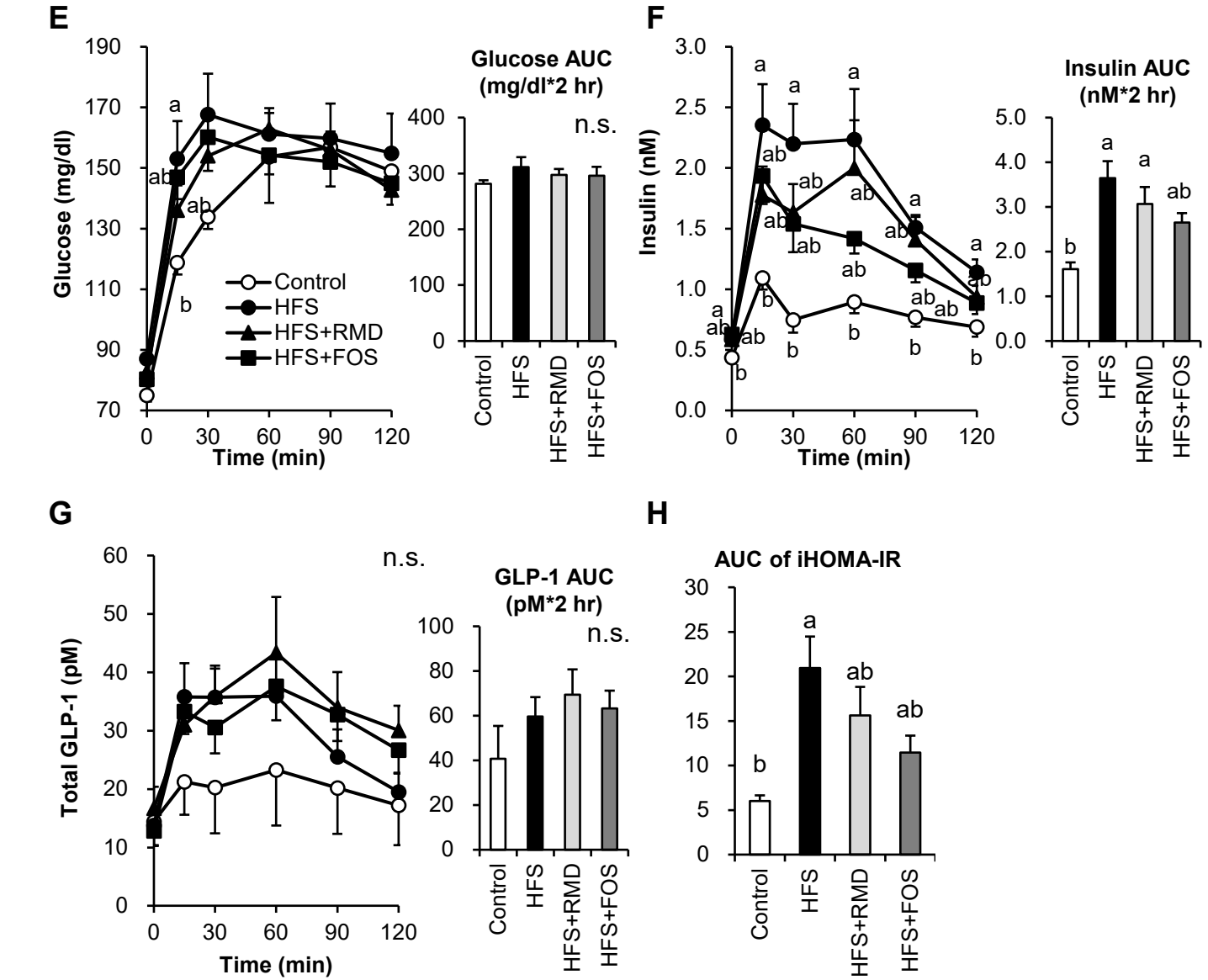

OGTT, 7 wk
E
Glucose (mg/dl)
Time (min)
Control
HFS
HFS+RMD
HFS+FOS
Glucose AUC
(mg/dl*2 hr)
n.s.
F
Insulin (nM)
Time (min)
Insulin AUC
(nM*2 hr)
G
Total GLP-1 (pM)
Time (min)
n.s.
GLP-1 AUC
(pM*2 hr)
n.s.
H
AUC of iHOMA-IR

Fig. 1E-H

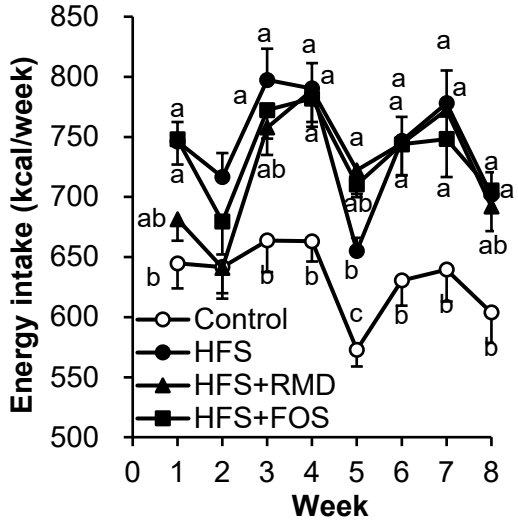

Energy intake (kcal/week)
850
800
750
700
650
600
550
500
0 1 2 3 4 5 6 7 8
Week
Control
HFS
HFS+RMD
HFS+FOS

Fig. 2

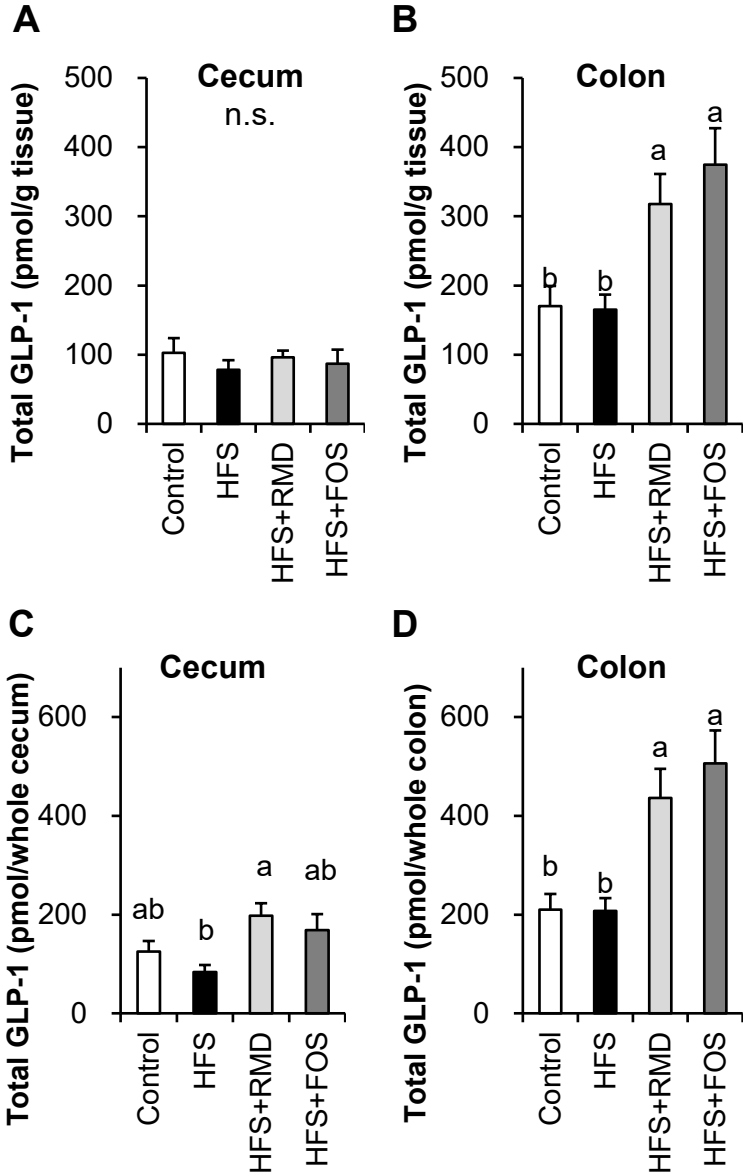

A
Cecum
n.s.
Total GLP-1 (pmol/g tissue)
500
400
300
200
100
0
Control
HFS
HFS+RMD
HFS+FOS
B
Colon
Total GLP-1 (pmol/g tissue)
b
b
a
a
C
Cecum
Total GLP-1 (pmol/whole cecum)
600
400
200
0
ab
b
a
ab
D
Colon
Total GLP-1 (pmol/whole colon)
b
b
a
a

Fig. 3

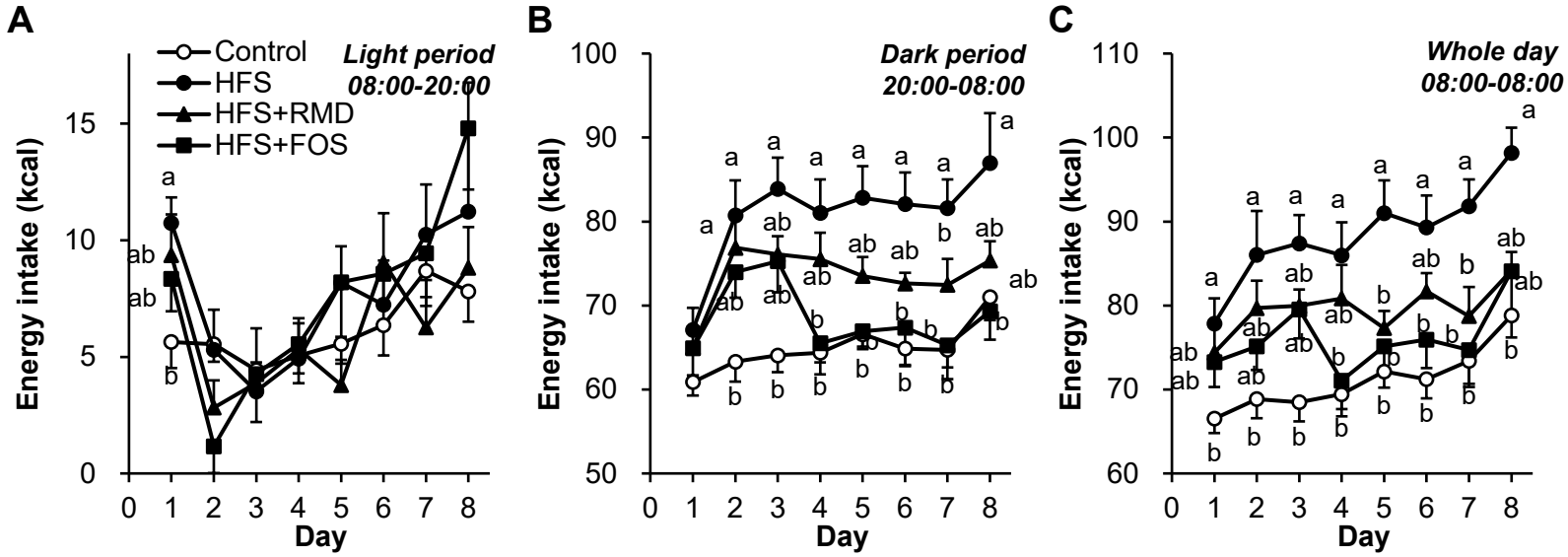

A
Control
HFS
HFS+RMD
HFS+FOS
Light period
08:00-20:00
Energy intake (kcal)
Day
B
Dark period
20:00-08:00
Energy intake (kcal)
Day
C
Whole day
08:00-08:00
Energy intake (kcal)
Day

Fig. 4

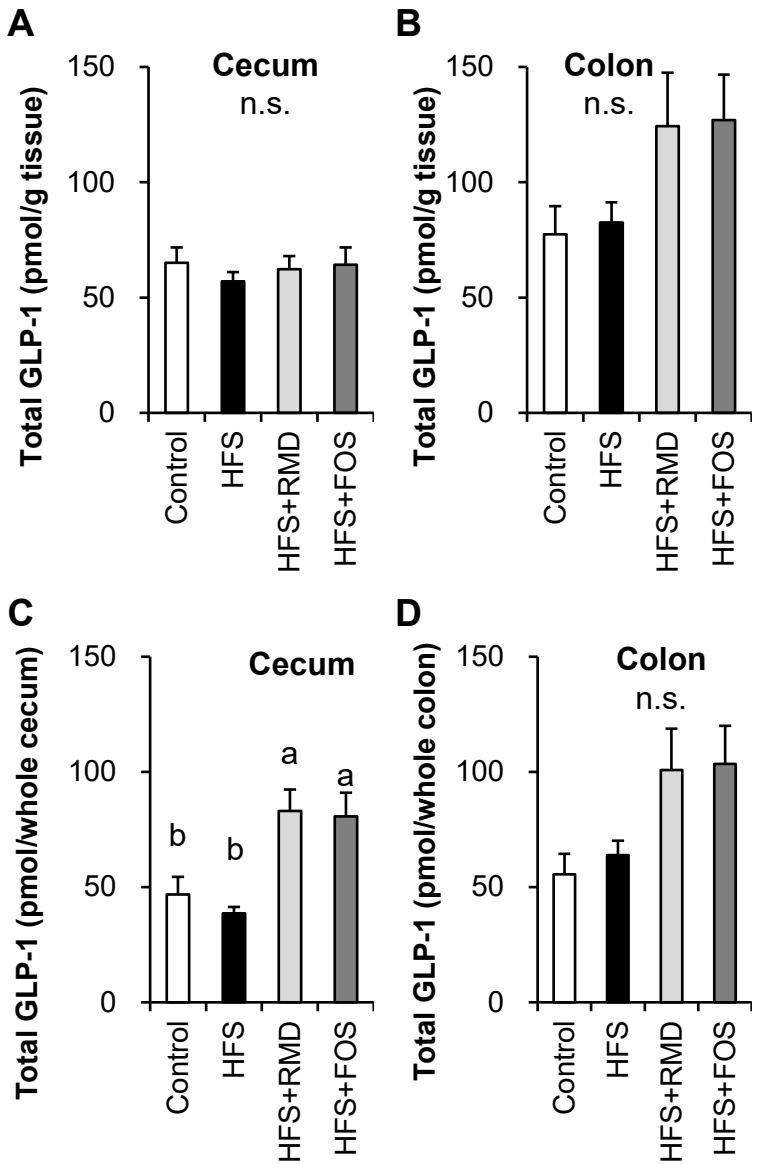

A
Cecum
n.s.
Total GLP-1 (pmol/g tissue)
150
100
50
0
Control
HFS
HFS+RMD
HFS+FOS
B
Colon
n.s.
Total GLP-1 (pmol/g tissue)
150
100
50
0
Control
HFS
HFS+RMD
HFS+FOS
C
Cecum
Total GLP-1 (pmol/whole cecum)
150
100
50
0
b
b
a
a
Control
HFS
HFS+RMD
HFS+FOS
D
Colon
n.s.
Total GLP-1 (pmol/whole colon)
150
100
50
0
Control
HFS
HFS+RMD
HFS+FOS

Fig. 5