TITLE

Impact of difructose anhydride III, raffinose, and fructooligosaccharides on energy intake, gut hormones, and cecal fermentation in rats fed a high-fat and high-sucrose diet

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RUNNING TITLE:

Effect of DFA II, raffinose and FOS on diet-induced obesity

ABSTRACT

We investigated the effects of dietary supplementation of difructose anhydride III (DFA III), raffinose (Raf), and fructooligosaccharides (FOS) on diet-induced obesity development. Male rats were fed normal or high-fat and high-sucrose (HFS) diet, with or without supplementing (3%) DFA III, Raf, or FOS, for 8 or 5 weeks. Supplementing DFA III to the HFS diet decreased energy intake compared to the non-supplemented HFS diet. Accordingly, body weight gain and fat accumulation reduced in DFA III-fed rats. Cecal acetate production and plasma glucagon-like peptide-1 (GLP-1) and peptide-YY (PYY) were elevated in DFA III-fed rats, while Raf and FOS partially affected these parameters. These results demonstrate that DFA III suppressive effect on excessive energy intake driven by the palatable obesogenic diet, possibly due to combined effects of increased anorexigenic factors such as cecal acetate production and GLP-1/PYY secretion.

KEY WORDS

Difructose anhydride III, Raffinose, Fructooligosaccharides, Diet-induced obesity, GLP-1

INTRODUCTION

Overeating is one of the critical triggers of obesity that lead to metabolic diseases such as glucose intolerance, dyslipidemia, hypertension, diabetes, and artherogenesis, not only in adults but also in children [1-4]. Consuming excess amount of “normal” diet daily, or frequent ingestion of “energy-dense diets” such as high-fat and high-sugar diet can be considered as overeating. Because of their high palatability, humans and animals ingest high-fat and high-sugar diets beyond the energy requirement.

Various dietary interventions that protect from diet-induced obesity have been tested. Fermentable dietary fibers and indigestible oligosaccharides are known to prevent diet-induced obesity. Anti-obesity effects of resistant maltodextrin and inulin-type fructans including fructooligosaccharides (FOS or OFS) have been demonstrated in animal and human studies [5-11]. Fermentable fibers promote production of short chain fatty acids (SCFAs), and SCFAs stimulate secretion and production of gut hormones such as glucagon like peptide-1 (GLP-1) and peptide YY (PYY) in the distal gut. These factors affect glucose, lipid, and energy metabolisms [12]. Therefore, it is possible that other fermentable oligosaccharides also exert beneficial effects against diet-induced obesity.

Raffinose (β-D-fructofuranosyl-*O*-α-D-galactopyranosyl- (1,6)-α-D-glucopyranoside) is an indigestible trisaccharide composed of glucose, fructose, and galactose. Previous studies have demonstrated various effects of dietary raffinose on immunity [13,14], allergy [15,16], and calcium absorption [17], mainly through enhanced gut fermentation. However, it remains unclear whether dietary raffinose has any impact on obesity, glucose, or lipid metabolism.

Difructose anhydride III (di-D-fructofuranosyl 1,2′:2, 3′ anhydride, DFA III) is an

indigestible disaccharide composed of two fructose molecules. DFA III is produced from inulin [18], and it promotes intestinal mineral absorption [19-21] and shows prebiotic effects [22,23]. Although DFA III lowers cholesterol [24] in isoflavone-fed rats and suppresses energy intake in female rats with or without ovariectomy [25], its effect in diet-induced obesity in animal models has not been demonstrated.

In the present study, we first examined the effects of dietary DFA III and raffinose on glycemia and lipid accumulation in rats fed with an obesogenic high-fat and high-sucrose (HFS) diet under isocaloric feeding conditions. The experiment showed that DFA III supplementation resulted in reduced energy intake. Therefore, we further examined the effect of DFA III, as compared with FOS, on energy intake, glycemia, plasma gut hormone concentrations, and cecal fermentation in rats fed the HFS diet supplemented with or without DFA III or FOS, ad libitum.

MATERIALS AND METHODS

Animals and diets

Male Sprague–Dawley rats (5-week-old) 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 housed in a separate cage and had free access to food and water, except for the days preceding the glucose tolerance test and sacrifice. The experiment was performed in a temperature-controlled room maintained at 22 ± 2 °C with a 12 h light/12 h dark cycle (08:00–20:00 light period). The study was approved by the Hokkaido University Animal Committee, and the animals were handled in accordance with the Hokkaido University guidelines for care and use of laboratory animals.

After one week of acclimation, rats were fed individual test-diets, as shown in Table 1. The HFS diet contained 30% wt/wt fat and 40% wt/wt sucrose [11,27]. All diets contained 65 g/kg of fiber. DFA III, raffinose (Raf), and FOS were supplemented in the diet at doses of 30 g/kg diet (3%) by replacing 15 g of cellulose and 15 g of sucrose. The energy density of DFA III, FOS, and Raf was equally estimated at 2 kcal/g as the common value for non-digestible fermentable oligosaccharides, and that of cellulose was estimated at 0 kcal/g, which have been authorized by the Ministry of Health, Labor, and Welfare of Japan [28].

In experiment 1, rats were divided into four groups (n = 8–10, each group). The control group was fed the control diet ad libitum and other three groups (HFS, H-DFA, and H-Raf) were fed the HFS diet with or without DFA III/raffinose supplementation. In the first 2 days, rats were fed individual test-diets ad libitum. Then, the amount diets were adjusted to have the same energy intake among HFS, H-DFA, and H-Raf groups, based on the energy intake of the previous day. The amounts of test-diets provided to these three groups were adjusted for equal consumption, but not restricted in the lowest-consumption group, throughout the experimental period. After 2, 4, and 6 weeks of feeding the test-diet, tail vein blood was collected to measure fasting (overnight fasted) glucose and insulin concentrations in the plasma. Blood samples were immediately added with heparin (final concentration 50 IU/mL) and aprotinin (final concentration 500 KIU/mL). Plasma was separated by centrifugation at 2,300 × *g* for 10 min at 4 °C and stored at −80 °C. After 8 weeks (experiment 1) of test-diet feeding and overnight fasting, rats were anesthetized using sodium pentobarbital injection (50 mg/kg of body weight, Somnopentyl injection; Kyoritsu Seiyaku Corporation, Tokyo, Japan). Blood samples were collected from the portal vein using a syringe containing heparin (final concentration 50 IU/mL), aprotinin (final

concentration 500 KIU/mL), and DPP-IV inhibitor (final concentration 50 μmol/L; DPP4-010; Merck Millipore). Plasma was collected and stored, as described above. Adipose tissues (mesenteric, epididymal, retroperitoneal), cecal tissue, and cecal content were collected after exsanguination. The weights of the contents were calculated by subtracting tissue weight from 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 homogenates were measured using a compact pH meter (B-212, Horiba, Ltd., Kyoto, Japan).

In experiment 2, rats were divided into six groups (n = 8, each group), and fed the respective test-diet (Table 1) ad libitum as follows: control diet, control diet supplemented with 3% DFA III (C-DFA) or 3% FOS (C-FOS), HFS diet, HFS diet supplemented with 3% DFA III (H-DFA) or 3% FOS (H-FOS). After 2 and 4 weeks of test-diet feeding, tail vein blood was collected to measure fasting (overnight fasted) glucose and insulin concentrations in the plasma. Blood samples were treated as described above. After 5 weeks of test-diet feeding and overnight fasting, rats were anesthetized and blood and tissue samples were collected as described above.

Measurement of plasma samples

Glucose and insulin concentrations were measured using a Glucose CII Test Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (AKRIN-010T; Shibayagi Co., Ltd., Gunma, Japan; inter-assay coefficient of variability (CV): < 5%, intra-assay CV: < 5%), respectively. Total GLP-1, active GLP-1, and total PYY concentrations were measured using a multi-species GLP-1 total ELISA kit (EZGLP1T-36K; Merck Millipore, Darmstadt, Germany; intra-assay CV: <

5%, inter-assay CV: < 12%), a GLP-1 (active) ELISA assay (EGLP-35K; Merck Millipore; intra-assay CV: 7.4 ± 1.1%, inter-assay CV: 8 ± 4.8%), and a MILLIPLEX MAP rat metabolic hormone magnetic bead panel metabolism multiplex assay (Merck Millipore; intra-assay CV: 3%, inter-assay CV: 11%), respectively. To estimate the degree of insulin resistance, the homeostatic model assessment for insulin resistance (HOMA-IR) was calculated using the following formula [29]: HOMA-IR = {glucose (mg/dL) x insulin (μU/mL)}/2,430, where 1 ng of insulin is equivalent to 26 μunits.

Measurement of organic acids in the cecal content

The concentration of organic acids such as succinate, acetate, propionate, and butyrate in the rat cecal contents were measured using high performance liquid chromatography (HPLC) [14]. Briefly, the cecal content homogenates were mixed with equal volumes of 0.4 N HCl. After overnight incubation at 4 °C, the supernatant was filtered through a membrane filter and loaded onto an HPLC column (RSpak KC-811; Showa Denko K.K., Tokyo, Japan), and we monitored the wavelength at 445 nm using the post-column method with bromothymol blue as a pH indicator.

Statistical analyses

Data were expressed as means and standard errors (SE) of the mean. Statistical analyses were performed using the JMP Pro version 12.0 software (SAS Institute, Inc., Cary, NC, USA). Statistical significance was assessed using a one-way (experiment 1) or two-way (experiment 2) analysis of variance (ANOVA), as appropriate. Significant differences ($P < 0.05$) between treatments were determined using the Tukey's HSD test,

as appropriate.

RESULTS

Experiment 1

Among the three HFS-fed groups, H-DFA group consumed less amount of diet from the beginning of test-diet feeding. To adjust the energy intake among the three HFS-fed groups, HFS and H-Raf groups were fed reduced amounts of the diet, while H-DFA group was fed ad libitum. As shown in Table 2, the total energy intake did not differ among the three groups. Notably, the total energy intake of HFS-fed group was not significantly higher than that of the control group which was fed the control diet ad libitum. Final body weights also did not differ significantly between the treatments. Adipose tissue weights were slightly higher in three HFS-fed groups, but not significantly different compared to that of the control group.

We monitored fasting glucose and insulin concentrations in the plasma, taken from the tail vein after 2, 4, and 6 weeks of test-diet feeding. However, no significant differences were observed in these parameters between the treatments (data not shown).

The tissue and content weights of cecum (Table 3) decreased in the HFS group compared to the control group, but these were maintained or increased by supplementation of DFA III or raffinose. Acetate concentration (μmol/g content) and amount (μmol/cecum) were highest in the H-DFA group, and the amount of acetate was lowest in the HFS group. Similar trends were observed in case of propionate and butyrate concentrations and amounts. Overall, short chain fatty acids productions were

relatively reduced in the HFS group, restored or increased in the H-DFA group, and partially restored in the H-Raf group, as compared to the control group. In contrast, succinate concentration was highest in the HFS group.

Total GLP-1 concentrations (Fig. 1) in the portal plasma after 8 weeks of test-diet feeding were measured. The GLP-1 concentration in the HFS group tended to be lower than that in the control group. In contrast, GLP-1 concentrations in H-DFA and H-Raf groups did not decrease, as compared to the control group. Moreover, concentration in the H-Raf group was significantly higher than that in the HFS group.

Although supplementation of DFA III or Raf to the HFS diet did not clearly affect adiposity and glucose tolerance under isocaloric feeding condition, DFA III showed suppressive effect on energy intake. In experiment 2, we examined effects of supplementing DFA III and FOS to the HFS diet and to the control diet under ad libitum feeding condition on energy intake, adiposity, glucose tolerance, cecal fermentation, and gut hormones involved in satiety induction.

Experiment 2

Significant effects (increments) of the HFS diet were detected using the 2-way ANOVA on body weight, weigh gain, and energy intake (Table 4). Significant effects of fiber on body weight gain and total energy intake were also detected, as rats fed DFA III-supplemented diets showed lowest values compared with rats fed cellulose- or FOS-containing diets (both control and HFS diets). Adipose tissue weights also increased by the HFS diet, but rats fed DFA III-supplemented diets showed lower epididymal fat weights than rats fed cellulose- or FOS-containing diets. Significant effect of fiber was

detected using the 2-way ANOVA.

Figure 2 shows daily changes in the difference in cumulative energy intake of test groups compared with that in the control group fed the control diet containing only cellulose as dietary fiber. The result clearly demonstrates that rats fed with the HFS diet had larger energy intake from beginning of the test period, and the cumulative difference continuously increased throughout the experimental period. H-FOS group showed incremental changes, similar to the HFS group, while H-DFA group showed only slight increment until end of the experiment. The C-DFA group had lower energy intake than the control group throughout the experiment.

We monitored fasting glucose and insulin levels to assess glucose tolerance after 2 and 4 weeks of test-diet feeding (Fig. 3). Significant effects were not detected using the 2-way ANOVA, but HFS diet showed a tendency of increased glucose level after 2 weeks ($P = 0.053$). Plasma GLP-1 levels were significantly affected by replacing the fiber ($P = 0.034$ and 0.001, after 2 and 4 weeks, respectively). After 4 weeks, rats fed DFA-supplemented diets had highest GLP-1 levels among all treatments. When compared within the three HFS diet-fed groups, plasma GLP-1 concentrations in the H-DFA rats were significantly higher than those in the HFS-fed rats (Tukey's test, $P< 0.05$).

After 5 weeks of test-diet feeding, we collected portal plasma under anesthesia. Total GLP-1 and active GLP-1 concentrations in the portal plasma (Fig. 4) were significantly affected by fiber (2-way ANOVA). Active and total GLP-1 concentrations in C-DFA and C-FOS groups were significantly higher than those in the control group, and GLP-1 concentrations in the H-DFA and H-FOS groups had higher tendency than those in the HFS group. The concentration of PYY, another L-cell-derived hormone, in the portal

plasma of the C-DFA and C-FOS groups were significantly higher than that in the control group. Although the HFS diet significantly elevated PYY levels ($P < 0.0001$, 2-way ANOVA), supplementing DFA III further increased plasma PYY concentration as it is significantly higher than that in HFS group.

Cecal content weight (Table 5) increased significantly in the C-DFA group, as compared to the control group, while the H-DFA group showed insignificant increments, compared to the HFS group, suggesting suppressive effect of the HFS diet on the DFA III-enhanced cecal fermentation. This is supported by lower concentrations of acetate and propionate in the H-DFA group compared to the C-DFA group. However, when compared within the three HFS-fed groups, acetate and propionate concentrations in the H-DFA group, but not in the H-FOS group, were significantly higher than those in the HFS group ($P < 0.05$, Tukey's test). Succinate concentration was more than 3-fold higher in the HFS group compared to that in the control group, while H-DFA and H-FOS had modestly increased concentrations compared to the control group.

DISCUSSION

The present study aimed to investigate the effects of indigestible oligosaccharides on development of diet-induced obesity. FOS are known to exert anti-obesity or anti-hyperglycemic effects, but effects of DFA III and Raf on diet-induced obesity remained unclear. In the first experiment comparing the effects of DFA III and Raf under isocaloric feeding conditions, DFA III seemed to prevent excess energy intake. The anorexigenic effect of DFA III was reproduced in the second experiment under ad libitum feeding

conditions. FOS or Raf did not reduce the energy intake at identical doses (3% wt in the diet). Rats fed DFA III showed highest acetate content in the cecum and increased plasma GLP-1 and PYY concentrations compared to rats fed the diet containing only cellulose as fiber. These results suggest anti-obesity effect of dietary DFA III as one of fermentable oligosaccharides with relatively higher potency than FOS and raffinose.

In experiment 1, except for the control group, rats were fed the test-diets (HFS, H-DFA, H-Raf) under isocaloric conditions to examine the effects of DFA III and raffinose on adiposity and glucose metabolism, independent of energy intake. The results show no apparent effects of DFA III and raffinose on parameters related to adiposity and glucose metabolism. However, energy intake was lower in the H-DFA group than in the HFS and H-Raf groups, when rats were fed the diet ad libitum at the beginning of experimental period. The HFS group did not show significant increment in body weight, adipose tissue weights, and glycemia, compared to the control group because of adjustment of energy intake among the three HFS-fed groups. These results suggest that DFA III has preventive effect against diet-induced obesity and glucose intolerance through reduced consumption of the high-energy diet.

Experiment 2 was conducted to examine the effects of DFA III and FOS on energy intake and on the development of diet-induced obesity under ad libitum feeding conditions. Supplementation of DFA III reduced the energy intake when it was added either in the control diet or the HFS diet, while FOS had no effects on the energy intake. Accordingly, body weight gain and epididymal fat weight were also reduced by feeding DFA III-supplemented diets (C-DFA and H-DFA). As a result, the H-DFA group showed almost similar values of body weight, adipose tissue weight, and fasting glucose

concentrations as those of the control group. FOS and inulin-type fructans have been reported to increase SCFA production, increase GLP-1 production, and decrease energy intake in rodent models, on adding these oligosaccharides in the diet at around 5–10% (wt/wt) [5-9,30-32]. The dose of FOS at 3% in the present study seems insufficient to exert an anorexic effect in the diet-induced obesity model. In contrast, DFA III at 3% in the diet is effective in preventing excess energy intake. Although DFA III reduced energy intake when it was added to the control diet, the growth of rats was not impaired in the present study. The relatively higher efficacy of DFA III than FOS and raffinose on appetite suppression against the palatable obesogenic diet indicates the translational potential of DFA III in preventing excess energy intake in humans at a low dose and with low risk of unfavorable effects such as hyperosmotic diarrhea. In a human study, repeated ingestion of DFA III at 5 g/day for 12 days did not show any harmful side effects [33].

Cecal SCFA (acetate, propionate, butyrate) contents (μmol/cecum) decreased after continuous feeding with HFS diet in both of the experiments. SCFA contents were restored overall by supplementing with DFA III, raffinose, and FOS, although these oligosaccharides had different impacts on individual SCFA levels. Acetate contents were increased by DFA III in both experiments, while raffinose and FOS had relatively smaller impact on the acetate production. These results indicate that oligosaccharides are utilized to produce SCFAs in the cecum, even if they are supplemented in the HFS diet that suppresses gut fermentation.

SCFAs are known to promote secretion and production of GLP-1 in the large intestine [34,35]. Elevated plasma GLP-1 levels on supplementation of DFA III, raffinose, and FOS were demonstrated in the present study. Although changes in SCFA production and

plasma GLP-1 levels are not simply parallel among all the diet-treatments, increased SCFAs probably enhanced GLP-1 release in the large intestine. GLP-1 has an anorexic effect, along with an incretin effect [36]. Moreover, acetate also shows an anorexic effect [37,38]. Acetate production was largely increased by DFA III in the present study. A previous study showed similar effects of DFA III, FOS and raffinose on cecal SCFA production in rats [39], while several following studies demonstrated higher impacts of DFA III in rats on cecal SCFA production than FOS, especially on acetate content [40-42]. Although experimental conditions vary among studies, the latter studies support the finding that DFA III has potent effect on gut fermentation compared to FOS. Thus, the suppressive effect of DFA III on energy intake could be attributed to combined effects of increased acetate and GLP-1 levels. Plasma GLP-1 concentrations increased rapidly (within 2 weeks) by DFA III and FOS, and the effect sustained by end of the experimental period, suggesting that cecal fermentation was already modified after 2 weeks of feeding these oligosaccharides. Another anorexic gut hormone, PYY [43], was significantly elevated by DFA III, but not by FOS, when added to the HFS diet. These results suggest that additive or synergistic effects of increased plasma GLP-1, PYY, and acetate are involved in suppressive effects of DFA III on excess energy intake. There may be other mechanisms also involved. Although DFA III failed to stimulate GLP-1 secretion from a murine GLP-1-producing enteroendocrine cell line (GLUTag) in our preliminary study (data not shown), postprandial GLP-1/PYY secretions might be transiently potentiated by the supplementation of DFA III to the diet, through direct/indirect action of DFA III on enteroendocrine L cells in vivo. Further studies would be need in the future to assess the hypothesis.

Interestingly, increased production of succinate by the HFS diet was attenuated by supplementation of DFA-III, raffinose, and FOS. Luminal succinate has been reported to have unfavorable effects such as inhibition of colonic epithelial proliferation, inflammatory and hypoxic effects [44-46]. Therefore, these oligosaccharides may protect the host from any harmful effects by reducing succinate production. Clarifying the underlying mechanism will be an attractive issue for future studies.

In summary, rats were fed the HFS diet with or without supplementation of DFA III, raffinose, and FOS for 5 or 8 weeks. Rats fed DFA III-containing diet showed lower energy intake compared to rats fed with the HFS diet, resulting in reduced fat accumulation in DFA III-fed rats. Cecal SCFA productions were increased by these oligosaccharides, but acetate production was largely increased by DFA III. Plasma GLP-1 concentrations were elevated in DFA III and FOS-fed groups after 2 weeks of the diet intervention. In addition, plasma PYY concentration was increased in the DFA III fed group. These results demonstrate that DFA III has suppressive effect on energy intake against palatable obesogenic diet, possibly through combined effects of increased anorexigenic factors, including cecal acetate, plasma GLP-1, and PYY concentrations.

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TABLES

Table 1. Test diet composition.

Test diet	Control	Control +DFA III (C-DFA)	Control +FOS (C-FOS)	HFS	HFS +DFA III (H-DFA)	HFS +FOS (H-FOS)	HFS +Raffinose (H-Raf)
Ingredient	g/kg of diet						
Cornstarch	397.486	397.486	397.486	-	–	–	–
Casein[1)]	200	200	200	200	200	200	200
Dextrinized cornstarch[2)]	132	132	132	–	–	–	–
Sucrose	85	70	70	384.486	369.486	369.486	369.486
Soybean oil	70	70	70	70	70	70	70
Lard oil	–	–	–	230	230	230	230
Fiber							
Cellulose[3)]	65	50	50	65	50	50	50
DFA III[4)]	–	30	–	–	30	–	–
FOS[5)]	–	–	30	–	–	30	–
Raffinose[6)]	–	–	–	–	–	–	35.3
Mineral mixture[7)]	35	35	35	35	35	35	35
Vitamin mixture[7)]	10	10	10	10	10	10	10
L-Cystine	3	3	3	3	3	3	3
Choline	2.5	2.5	2.5	2.5	2.5	2.5	2.5
tert-Butyl-hydroquinone	0.014	0.014	0.014	0.014	0.014	0.014	0.014
Energy density (kcal/g)	3.90	3.90	3.90	5.05	5.05	5.05	5.03

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) Difructose anhydrate III (Nippon Beet Sugar Manufacturing Co., Ltd., Tokyo, Japan);

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

6) Raffinose (Nippon Beet Sugar Manufacturing Co., Ltd., Tokyo, Japan); contains 15% of water as hydrate.

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

Table 2. Body weight, energy intake, and visceral adipose tissue weights of rats fed with test-diets for 8 weeks (Experiment 1).

	Control	HFS	H-DFA	H-Raf	ANOVA *P* value
Body Weight (g)	444 ± 9	459 ± 5	469 ± 6	460 ± 6	0.096
Total Energy Intake (kcal)	4481 ± 61	4655 ± 28	4656 ± 86	4632 ± 65	0.126
Mesenteric fat (g)	10.06 ± 0.74	11.34 ± 0.61	11.22 ± 0.67	9.57 ± 0.78	0.249
Mesenteric fat (g/100 g BW)	2.25 ± 0.14	2.47 ± 0.13	2.39 ± 0.13	2.07 ± 0.15	0.230
Epididymal fat (g)	10.92 ± 1.05	13.92 ± 0.70	13.87 ± 0.85	11.67 ± 0.74	0.037
Epididymal fat (g/100 g BW)	2.44 ± 0.20	3.03 ± 0.15	2.95 ± 0.16	2.53 ± 0.13	0.039
Retroperiotoneal fat (g)	15.52 ± 1.05	17.52 ± 0.98	17.72 ± 1.58	15.06 ± 1.13	0.306
Retroperiotoneal fat (g/100 g BW)	3.47 ± 0.18	3.81 ± 0.20	3.76 ± 0.29	3.27 ± 0.23	0.335
Visceral fat [#] (g)	36.5 ± 2.7	42.8 ± 2.0	42.8 ± 2.6	36.3 ± 2.2	0.096
Visceral fat (g/100 g BW)	8.16 ± 0.48	9.31 ± 0.40	9.10 ± 0.45	7.87 ± 0.41	0.088

[#] Visceral fat weight is the sum of mesenteric, retroperitoneal, epididymal fat weights

Values shown are the means ± SE (n = 8–10). Significant differences among the treatments were not detected by post-hoc analysis (P < 0.05, Tukey–Kramer test).

Table 3. Cecal tissue, content weights, pH, and short chain fatty acid concentrations of rats fed test-diets for 8 weeks (Experiment 1).

	Control	HFS	H-DFA	H-Raf	ANOVA *P* value
Cecal tissue (g)	1.26 ± 0.06 [b]	0.97 ± 0.05 [c]	1.66 ± 0.07 [a]	1.47 ± 0.10 [ab]	<.0001
Cecal content (g)	2.55 ± 0.19 [b]	1.54 ± 0.17 [c]	3.79 ± 0.43 [a]	2.88 ± 0.36 [ab]	<.0001
Cecal pH	6.8 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	0.138
Acetate (μmol/g content)	20.7 ± 1.7 [b]	19.0 ± 1.6 [b]	32.8 ± 3.3 [a]	23.9 ± 1.9 [ab]	0.001
Acetate (μmol/cecum)	52.1 ± 5.3 [bc]	28.6 ± 3.3 [c]	118.8 ± 16.3 [a]	70.1 ± 10.4 [b]	<.0001
Propionate (μmol/g content)	4.61 ± 0.35	3.57 ± 0.24	4.70 ± 0.44	4.42 ± 0.29	0.087
Propionate (μmol/cecum)	11.26 ± 0.44 [bc]	5.66 ± 0.88 [c]	18.51 ± 3.18 [a]	13.20 ± 2.52 [ab]	0.000
Butyrate (μmol/g content)	2.03 ± 0.19 [ab]	1.75 ± 0.30 [b]	3.19 ± 0.50 [a]	2.20 ± 0.27 [ab]	0.025
Butyrate (μmol/cecum)	5.08 ± 0.56 [bc]	2.68 ± 0.50 [c]	11.87 ± 1.76 [a]	6.07 ± 0.80 [b]	<.0001
Succinate (μmol/g content)	20.7 ± 3.1 [b]	37.1 ± 1.8 [a]	21.5 ± 1.5 [b]	22.2 ± 2.7 [b]	<.0001
Succinate (μmol/cecum)	50.5 ± 7.3	58.0 ± 8.5	84.9 ± 14.9	64.0 ± 10.1	0.127

Values shown are the means ± SE (n = 8–10). Values that do not share the same letter differ significantly between the treatments (P < 0.05, Tukey–Kramer test).

Table 4. Body weight, energy intake, and adipose tissue weights of rats fed the control or the HFS diet supplemented with DFA III or FOS for 5 weeks (Experiment 2).

		Fiber in the diet			ANOVA	2-way ANOVA		
		Cellulose	DFA III	FOS	*P* value	HFS	Fiber	HFS x Fiber
Body Weight (g)	Control	370 ± 12 ^{ab}	355 ± 7 ^{b}	377 ± 8 ^{ab}	0.003	0.000	0.050	0.967
	HFS	395 ± 11 ^{a}	384 ± 6 ^{ab}	404 ± 4 ^{a}				
Body Weight Gain (g)	Control	175 ± 9 ^{bc}	160 ± 5 ^{c}	182 ± 7 ^{abc}	0.000	<.0001	0.015	0.971
	HFS	200 ± 11 ^{ab}	189 ± 6 ^{abc}	209 ± 3 ^{a}				
Total Energy Intake (kcal)	Control	2769 ± 66 ^{ab}	2584 ± 38 ^{b}	2750 ± 74 ^{ab}	0.000	<.0001	0.012	0.833
	HFS	2942 ± 92 ^{a}	2815 ± 38 ^{ab}	2992 ± 42 ^{a}				
Mesenteric fat (g)	Control	6.01 ± 0.62 ^{ab}	5.56 ± 0.48 ^{b}	6.03 ± 0.46 ^{ab}	0.006	0.001	0.139	0.625
	HFS	7.50 ± 0.66 ^{ab}	6.58 ± 0.29 ^{ab}	7.98 ± 0.30 ^{a}				
Mesenteric fat (g/100 g BW)	Control	1.62 ± 0.15	1.56 ± 0.11	1.59 ± 0.10	0.047	0.004	0.357	0.566
	HFS	1.89 ± 0.12	1.71 ± 0.07	1.98 ± 0.08 ^{NS}				
Epididymal fat (g)	Control	8.16 ± 0.65 ^{ab}	6.58 ± 0.66 ^{b}	8.26 ± 0.70 ^{ab}	0.001	0.000	0.031	0.994
	HFS	10.35 ± 0.93 ^{a}	8.87 ± 0.25 ^{ab}	10.42 ± 0.68 ^{a}				
Epididymal fat (g/100 g BW)	Control	2.20 ± 0.16 ^{ab}	1.85 ± 0.17 ^{b}	2.18 ± 0.17 ^{ab}	0.012	0.002	0.073	0.975
	HFS	2.61 ± 0.18 ^{a}	2.31 ± 0.05 ^{ab}	2.58 ± 0.17 ^{a}				
Retroperiotoneal fat (g)	Control	9.21 ± 0.90 ^{bc}	8.38 ± 0.63 ^{c}	9.42 ± 0.71 ^{abc}	0.002	<.0001	0.362	0.917
	HFS	11.76 ± 1.37 ^{ab}	11.42 ± 0.34 ^{abc}	12.59 ± 0.46 ^{a}				
Retroperiotoneal fat (g/100 g BW)	Control	2.47 ± 0.18 ^{ab}	2.35 ± 0.15 ^{b}	2.49 ± 0.18 ^{ab}	0.006	0.000	0.698	0.876
	HFS	2.95 ± 0.26 ^{ab}	2.98 ± 0.07 ^{ab}	3.11 ± 0.10 ^{a}				
Visceral fat $^{\#}$ (g)	Control	23.4 ± 2.0 ^{bc}	20.5 ± 1.7 ^{c}	23.7 ± 1.8 ^{bc}	0.001	<.0001	0.102	0.9483
	HFS	29.6 ± 2.9 ^{ab}	26.9 ± 0.7 ^{abc}	31.0 ± 1.0 ^{a}				
Visceral fat (g/100 g BW)	Control	6.29 ± 0.44 ^{ab}	5.76 ± 0.41 ^{b}	6.26 ± 0.41 ^{ab}	0.005	2E-04	0.259	0.9429
	HFS	7.44 ± 0.53 ^{a}	7.00 ± 0.14 ^{ab}	7.68 ± 0.26 ^{a}				

$^{\#}$ Visceral fat weight is the sum of mesenteric, retroperitoneal, epididymal fat weights

Values shown are the means ± SE (n = 8). 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 the control or the HFS diet supplemented with DFA III or FOS for 5 weeks (Experiment 2).

		Fiber in the diet			ANOVA	2-way ANOVA		
		Cellulose	DFA III	FOS	*P* value	HFS	Fiber	HFS x Fiber
Cecal tissue (g)	Control	1.01 ± 0.03 ^{c}	1.49 ± 0.06 ^{ab}	1.49 ± 0.06 ^{ab}	<0.001	<0.002	<0.003	<0.004
	HFS	1.71 ± 0.09 ^{a}	1.32 ± 0.04 ^{bc}	1.78 ± 0.15 ^{a}				
Cecal content (g)	Control	2.27 ± 0.17 ^{b}	4.22 ± 0.64 ^{a}	2.03 ± 0.19 ^{b}	0.000	0.052	<.0001	0.076
	HFS	2.03 ± 0.18 ^{b}	2.79 ± 0.31 ^{b}	2.06 ± 0.22 ^{b}				
Cecal pH	Control	7.71 ± 0.07 ^{a}	7.48 ± 0.06 ^{ab}	7.29 ± 0.14 ^{ab}	0.000	<.0001	0.003	0.065
	HFS	7.07 ± 0.14 ^{bc}	7.35 ± 0.11 ^{ab}	6.84 ± 0.11 ^{c}				
Acetate (µmol/g content)	Control	22.6 ± 1.4	26.6 ± 2.2	25.3 ± 1.8	0.194	0.126	0.075	0.961
	HFS	19.9 ± 1.5	24.1 ± 1.9	23.6 ± 2.0				
Acetate (µmol/cecum)	Control	52.1 ± 6.4 ^{b}	112.2 ± 20.3 ^{a}	51.2 ± 5.6 ^{b}	0.000	0.013	<.0001	0.085
	HFS	39.8 ± 3.8 ^{b}	65.6 ± 7.2 ^{b}	46.9 ± 4.0 ^{b}				
Propionate (µmol/g content)	Control	8.57 ± 0.63 ^{ab}	10.10 ± 0.62 ^{a}	9.92 ± 0.68 ^{a}	0.001	0.000	0.024	0.556
	HFS	5.69 ± 0.47 ^{b}	7.22 ± 0.56 ^{ab}	8.43 ± 1.19 ^{ab}				
Propionate (µmol/cecum)	Control	19.5 ± 2.0 ^{b}	42.5 ± 6.6 ^{a}	20.6 ± 2.9 ^{b}	0.000	0.000	0.000	0.018
	HFS	11.7 ± 1.5 ^{b}	19.7 ± 2.1 ^{b}	17.4 ± 2.6 ^{b}				
Butyrate (µmol/g content)	Control	3.71 ± 0.26 ^{a}	2.79 ± 0.22 ^{ab}	3.27 ± 0.41 ^{ab}	0.022	0.002	0.292	0.507
	HFS	2.43 ± 0.36 ^{ab}	2.30 ± 0.44 ^{b}	2.38 ± 0.22 ^{ab}				
Butyrate (µmol/cecum)	Control	8.39 ± 0.85 ^{ab}	11.49 ± 1.63 ^{a}	6.53 ± 0.90 ^{b}	0.000	0.000	0.000	0.000
	HFS	5.01 ± 0.87 ^{b}	6.05 ± 0.95 ^{b}	4.62 ± 0.28 ^{b}				
Succinate (µmol/g content)	Control	8.0 ± 1.2 ^{b}	3.3 ± 1.0 ^{b}	7.5 ± 1.6 ^{b}	0.000	<.0001	<.0001	0.0026
	HFS	30.1 ± 2.7 ^{a}	13.0 ± 3.4 ^{b}	12.8 ± 3.0 ^{b}				
Succinate (µmol/cecum)	Control	18.1 ± 3.2 ^{b}	11.1 ± 3.1 ^{b}	16.0 ± 3.7 ^{b}	0.000	<.0001	0.008	0.0336
	HFS	61.6 ± 8.4 ^{a}	33.8 ± 9.7 ^{b}	25.0 ± 7.0 ^{b}				

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

FIGURE LEGENDS

Fig. 1. GLP-1 concentration in the portal plasma after 8 weeks of test-diet feeding (Experiment 1).

Blood samples were collected from the portal vein of rats fasted overnight after 8 weeks of test-diet feeding, and the concentrations of total GLP-1 in the plasma were measured. Values are the means ± SE (n = 8–10). Bars that do not share the same letter differ significantly between treatments ($P < 0.05$, Tukey–Kramer test).

Fig. 2. Differences in cumulative energy intake compared to energy intake of the control group (Experiment 2).

Cumulative energy intake was calculated from the amount of consumed diet in each group, and the differences between cumulative energy intakes of each group and the control group are presented as means ± SE (n = 8). Two-way ANOVA p values for treatment, day, and treatment x day were <.0001, 0.947, and 0.023, respectively.

Fig. 3. Plasma glucose, insulin, GLP-1 concentrations, and HOMA-IR under fasting condition 2 and 4 weeks after test-diet feeding (Experiment 2).

Blood samples were collected from the tail vein of rats fasted overnight after 2 and 4 weeks of test-diet feeding. Glucose (A, E), insulin (B, F), and total GLP-1 (D, H) levels were measured in the plasma. HOMA-IR (C, G) was calculated from glucose and insulin concentrations. Values are the means ± SE (n = 8). *P* values of 2-way ANOVA are presented below each panel. Bars that do not share the same letter differ significantly between treatments ($P < 0.05$, Tukey–Kramer test).

Fig. 4. GLP-1 and PYY concentrations in the portal plasma of rats fed the control or the HFS diet supplemented with DFA III or FOS for 5 weeks (Experiment 2).

Blood samples were collected from the portal vein of rats fasted overnight after 5 weeks of test-diet feeding, and concentrations of total GLP-1, active GLP-1, and PYY were measured in the plasma. Values are the means ± SE (n = 8). *P* values of 2-way ANOVA were presented below each panel. Bars that do not share the same letter differ significantly between treatments ($P < 0.05$, Tukey–Kramer test).

Fig. 1.

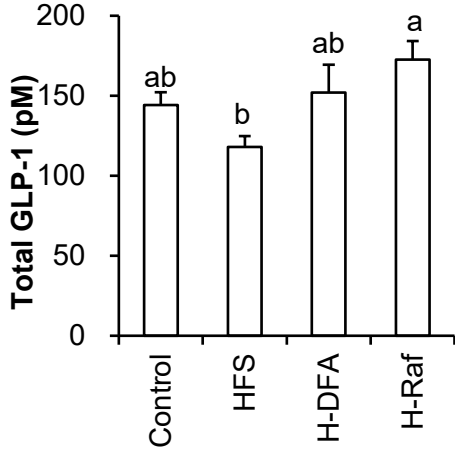


Fig. 2.

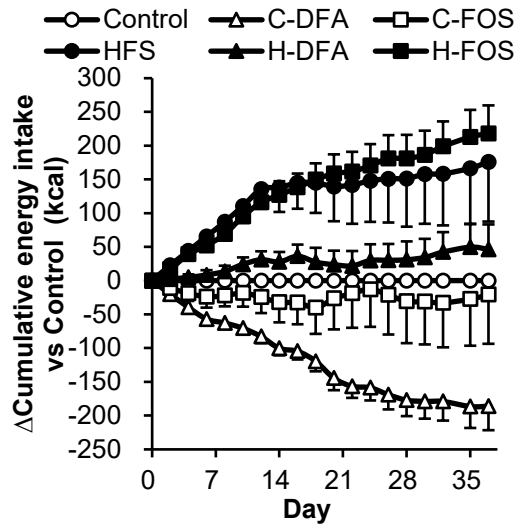
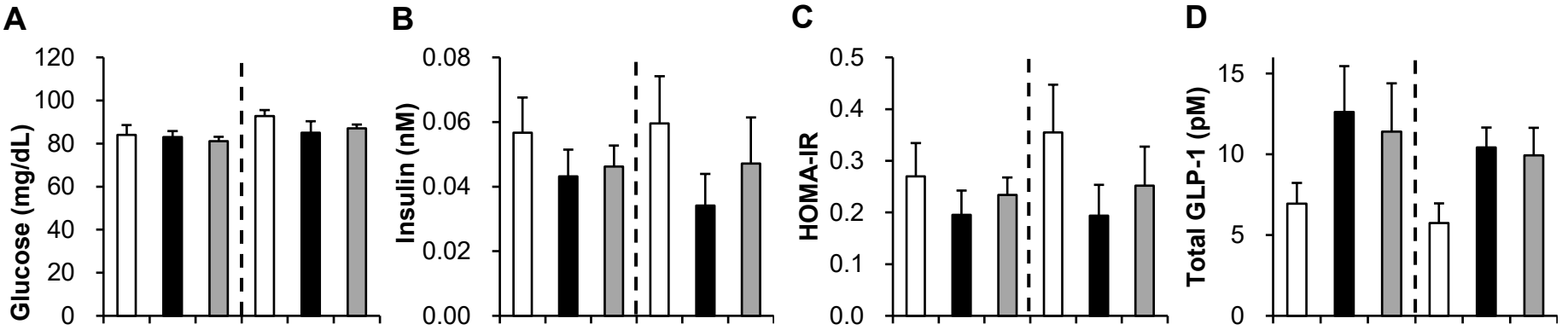

Control
C-DFA
C-FOS
HFS
H-DFA
H-FOS
ΔCumulative energy intake vs Control (kcal)
300
250
200
150
100
50
0
-50
-100
-150
-200
-250
0
7
14
21
28
35
Day

Fig. 3.

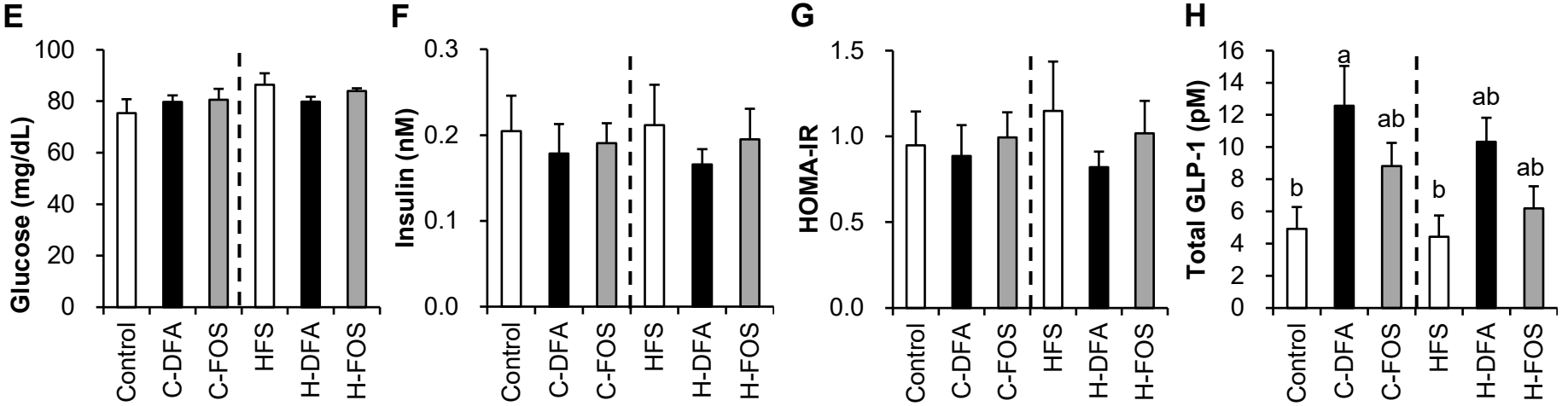
After 2 weeks



2-way ANOVA *P* values

HFS	0.053	HFS	0.849	HFS	0.640	HFS	0.342
Fiber	0.368	Fiber	0.213	Fiber	0.279	Fiber	0.034
HFS x Fiber	0.620	HFS x Fiber	0.848	HFS x Fiber	0.676	HFS x Fiber	0.968

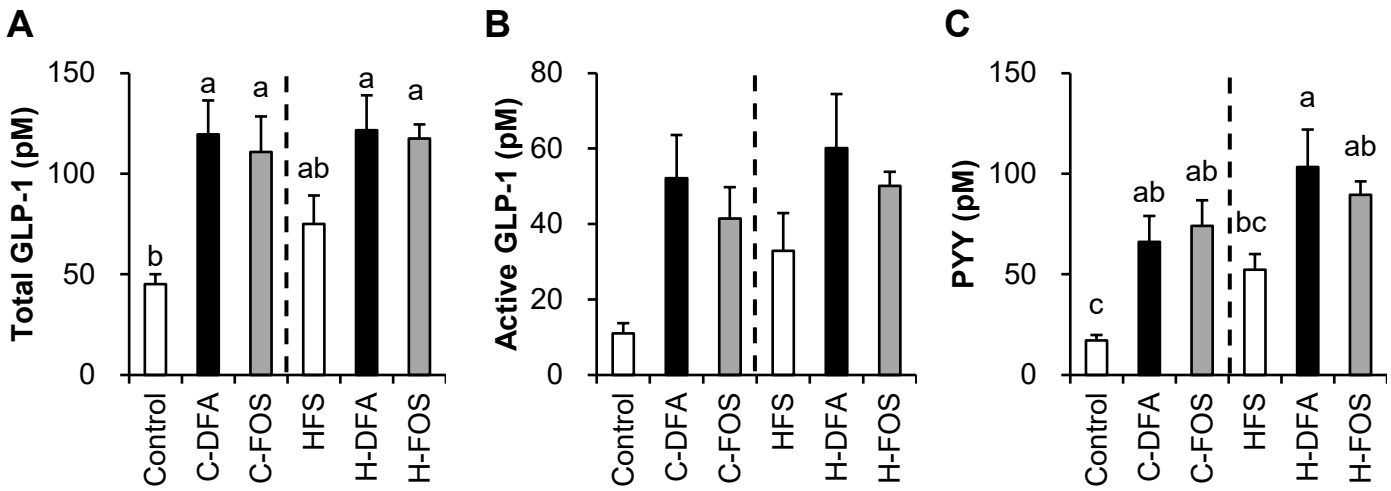
After 4 weeks



2-way ANOVA *P* values

HFS	0.107	HFS	0.984	HFS	0.729	HFS	0.190
Fiber	0.775	Fiber	0.581	Fiber	0.552	Fiber	0.001
HFS x Fiber	0.306	HFS x Fiber	0.953	HFS x Fiber	0.773	HFS x Fiber	0.792

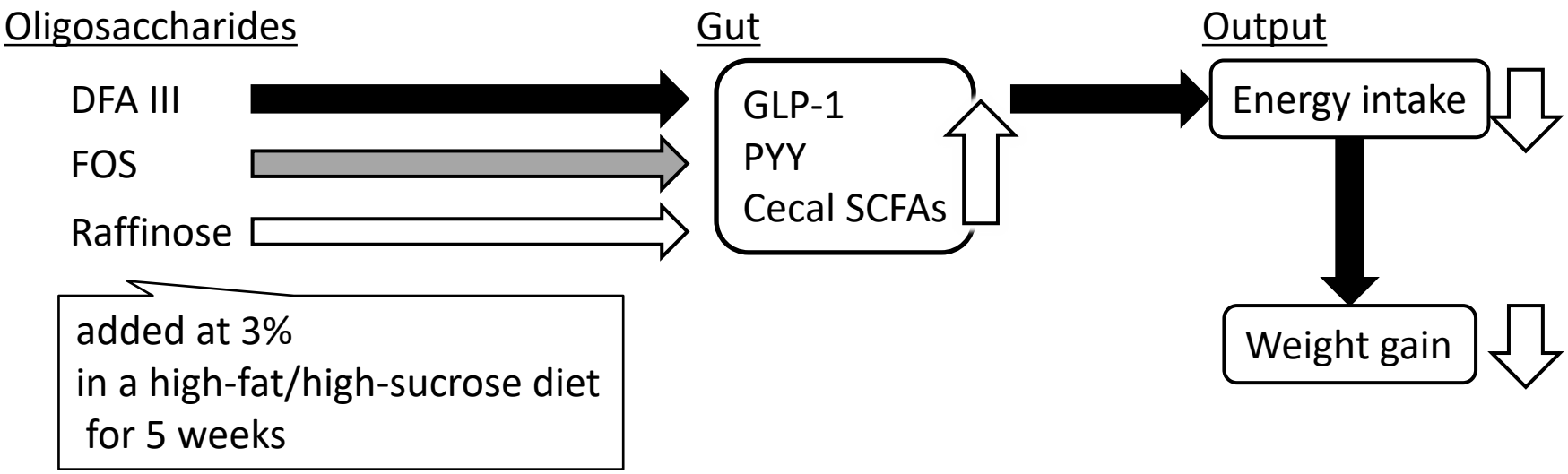
Fig. 4.



2-way ANOVA *P* values

HFS	0.265	HFS	0.151	HFS	<.0001
Fiber	0.000	Fiber	0.014	Fiber	0.003
HFS x Fiber	0.576	HFS x Fiber	0.796	HFS x Fiber	0.065

Graphic abstract



Supplementation of 3% DFA III attenuated energy intake of rats fed a obesogenic a high-fat/high-sucrose diet