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Dietary fructo-oligosaccharides improve insulin sensitivity along with the suppression of adipocytokine secretion from mesenteric fat cells in rats

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Abstract

Short-chain fructo-oligosaccharides (FOS) are known to have beneficial effects on health. However, the effects of FOS on insulin resistance have not been fully clarified. We observed the effects of FOS feeding on insulin sensitivity and adipocytokine release from abdominal adipocytes in weaning rats. Male Sprague–Dawley rats, 3 weeks old, were divided into three groups and fed a sucrose-based American Institute of Nutrition (AIN)-93 growth diet (control), the control diet containing 5% FOS for 5 weeks (FOS-5wk) or the control diet for 2 weeks followed by the 5% FOS diet for 3 weeks (FOS-3wk). Tail blood was collected after fasting for 9 h on day 33 of feeding, and glucose and insulin levels were measured. On the last day, rats were anaesthetised and killed after the collection of aortic blood. Small- and large-intestinal mesenteric fat tissues were immediately excised, and the release of adiponectin, leptin and TNF- α was evaluated from the subsequently isolated adipocytes. The weight of the large-intestinal mesenteric fat, fasting blood insulin level and homeostatic model assessment for insulin resistance decreased in a time-dependent manner, and were much lower in the FOS-5wk group than in the control group. These values were correlated with aortic blood leptin levels. The secretion rate of leptin from the isolated mesenteric adipocytes in the small intestine, but not in the large intestine, was lower in the FOS-fed groups than in the control group. In conclusion, FOS feeding improved insulin sensitivity accompanied by the reduction in large-intestinal fat mass and leptin secretion from the mesenteric adipocytes of the small intestine.

Key words: Fructo-oligosaccharides; Insulin sensitivity; Isolated mesenteric adipocytes; Adipocytokines

Short-chain fructo-oligosaccharides (FOS) are well-known, non-digestible and fermentable sugars that induce marked changes in intestinal bacterial populations⁽¹⁾. FOS are applied to many functional foods as prebiotics and known to have beneficial influences on host health, such as promoting gut health and mineral absorption, and also ameliorating hyperlipidaemia^(2,3). It has been reported that FOS alter glucose and lipid metabolism in human subjects⁽⁴⁾ and animals^(3,5) depending on their colonic fermentation. In addition, recent research has revealed that prebiotic-induced changes of gut microbiota regulate obesity-related inflammation in mice through an improvement of gut permeability⁽⁶⁾. However, little information is available regarding the effects of FOS on insulin sensitivity.

Increasing type 2 diabetes has become a major social issue globally, and the relationship between childhood obesity and insulin resistance has been considered in recent years. It has been accepted that adipocyte dysfunction, particularly in visceral fat tissue, plays an important role in the development

of insulin resistance. Adipocytes synthesise and secrete biologically active molecules called adipocytokines^(7,8). Some of these adipocytokines secreted from hypertrophic adipocytes have been shown to directly or indirectly impair insulin sensitivity through the modulation of insulin signalling, and glucose and lipid metabolism⁽⁹⁾. Hypertrophic adipocytes produce less adiponectin, which has been reported to lead to an impairment in insulin sensitivity⁽¹⁰⁾. We previously demonstrated that the lipolytic properties of adipocytes differ between the small- and large-intestinal mesenteric fat tissues⁽¹¹⁾.

The small intestine absorbs proteins, lipids and digestible carbohydrates, whereas the large intestine absorbs bacterial components or metabolites including organic acids, which mainly consist of SCFA produced by the fermentation of non-digestible carbohydrates. These differences might be responsible for the alterations between the properties of mesenteric adipocytes from the small intestine and those from the large intestine. We examined that the change in

Abbreviations: FOS, short-chain fructo-oligosaccharides; FOS-3wk, fructo-oligosaccharide feeding for 3 weeks; FOS-5wk, fructo-oligosaccharide feeding for 5 weeks; HOMA-IR, homeostatic model assessment for insulin resistance; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1.

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the mesenteric adipocyte properties is thought to affect body metabolism, for example insulin sensitivity, and FOS may have a large impact on mesenteric adipocytes, especially those from around the large intestine.

We designed the present study to examine the effects of FOS feeding from a young age. The minimum age required to collect enough abdominal adipose tissue to enable the division of the small and large intestines of mesenteric fat cells in rats is 8 weeks. Moreover, after only 2 weeks of a high-sucrose diet from weaning, insulin sensitivity has been found to be impaired (A Shinoki, unpublished results). Therefore, we setup two groups with different terms of FOS feeding as follows: a FOS-free high-sucrose diet from weaning (3 weeks old) for 2 weeks in order to impair insulin sensitivity, followed by FOS feeding for 3 weeks; FOS feeding for 5 weeks from weaning to adjust the final age (8 weeks old). Insulin sensitivity was measured by the homeostatic model assessment for insulin resistance (HOMA-IR). HOMA-IR offers important advantages in estimating insulin sensitivity in rodent studies^(12,13), even though this index was developed for human studies.

The aims of the present study were to determine the effects of feeding FOS on adipocytokine release from abdominal adipocytes in relation to insulin sensitivity in weaning rats. We also evaluated adipocytokine release from freshly isolated small- and large-intestinal mesenteric fat cells from rats fed a FOS diet.

Materials and methods

Animals and diets

Male Sprague–Dawley rats (Jcl) weighing 50–54 g (3 weeks old; Japan Clea, Tokyo, Japan) were housed in individual stainless-steel cages with wire-mesh bottoms. The cages were placed in a room with a controlled temperature ($22 \pm 2^\circ\text{C}$), relative humidity (40–60%) and lighting (12 h light–12 h dark cycle, 08.00–20.00 hours) throughout the study. The rats had free access to water and a semi-purified sucrose-based diet with the American Institute of Nutrition (AIN)-93 growth formulation⁽¹⁴⁾ (control diet) or the control diet containing a 5% FOS diet for 3 or 5 weeks (Table 1). The test diets were given every day. Fructo-oligosaccharide (Meiologo-Pt; Meiji Seika Kaisha, Limited, Tokyo, Japan) is a mixture of 42% 1-kestose, 46% nystose and 9% 1F- β -fructofuranosyl nystose, and the average degrees of polymerisation was 3.55.

The present study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals.

Study design

Rats were divided into three groups of eight or nine animals each and were given the aforementioned control diet, a 5% FOS diet for 5 weeks, or the control diet for 2 weeks followed by the 5% FOS diet for 3 weeks (control, FOS-5wk and FOS-3wk, respectively). We used rats with impaired insulin sensitivity induced by feeding a high-sucrose diet for 2 weeks

Table 1. Composition of the experimental diets

Ingredients (g/kg)	Control diet	5 % FOS diet
Casein*	250	250
Maize oil	50	50
Mineral mixture†	35	35
Vitamin mixture†	10	10
Choline chloride	2.5	2.5
Crystallised cellulose‡	50	50
Sucrose	602.5	552.5
Fructo-oligosaccharide§		50

FOS, fructo-oligosaccharides.

* NZMP Acid Casein; Fonterra Limited, Auckland, New Zealand.

† The mineral and vitamin mixtures were prepared according to the

American Institute of Nutrition (AIN)-93 growth formulation.

‡ CEOLUS PH102; Asahi Chemical Industry, Tokyo, Japan.

§ Meiologo-Pt; Meiji Seika Kaisha, Limited, Tokyo, Japan.

just after weaning. Body weight and food intake were measured every day. Tail blood was collected in heparinised microtubes after fasting for 9 h on day 33. On the last day, rats were killed after the collection of abdominal aortic blood under pentobarbital anaesthesia (Nembutal: sodium pentobarbital, 50 mg/kg body weight; Abbott Laboratories, North Chicago, IL, USA). The caecum and abdominal white adipose tissues (mesenteric, retroperitoneal and epididymal) were immediately excised and weighed. Tail blood plasma and aortic blood serum were collected after centrifugation and stored at -80°C until subsequent analyses.

Blood analyses

The concentrations of glucose and TAG in tail blood plasma were measured using enzyme assay kits (Glucose CII-test from Wako Pure Chemical Industries Limited, Osaka, Japan; TG-EN from Kainos Laboratories, Tokyo, Japan), and insulin was measured using an ELISA kits (Rat Insulin ELISA kit AKRIN-010T from Shibayagi, Gunma, Japan). The concentrations of adiponectin, leptin, monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1) in the aortic plasma were measured using the ELISA kit (Mouse/Rat Adiponectin ELISA kit from Otsuka Pharmaceutical, Tokyo, Japan; Rat Leptin ELISA kit from LINCO Research, St Charles, MO, USA; Rat MCP-1 ELISA kit from Thermo Fisher Scientific, Inc., Rockford, IL, USA; ZYMUTEST Rat-PAI-1 Antigen Kit from HYPHEN Bio Med, Neuville-Sur-Oise, France). HOMA-IR was used to evaluate insulin sensitivity. It was calculated from fasting insulin and glucose concentrations using the formula of Matthews *et al.*⁽¹⁵⁾:

$$\text{HOMA-IR} = \text{fasting glucose (mmol/l)}$$

$$\times \text{fasting insulin (mIU/l)} / 22.5,$$

where 1 IU insulin = 0.038 mg.

Measurement of adipocytokine release from isolated adipocytes

Excised mesenteric fat tissue was immediately separated into the small- and large-intestinal regions along the portal vein. The isolated adipocytes were prepared from the rat mesenteric

fat tissues by the method of Rodbell with minor modifications as described previously^(16,17). Briefly, mesenteric fat tissues were cut into small pieces with scissors before digestion for 1 h in isolation medium (Krebs–Ringer bicarbonate buffer, pH 7.4; 118 mM-NaCl, 4.7 mM-KCl, 2.7 mM-CaCl₂, 1.2 mM-KH₂PO₄ and 24.9 mM-NaHCO₃) containing 4% (w/v) bovine serum albumin (fatty acid free; Wako Pure Chemical Industries Limited), 0.1% (w/v) collagenase (type I; Sigma, St Louis, MO, USA), 0.01% (w/v) trypsin inhibitor (type II-S; Sigma) and 2.5 mM-glucose in a shaking water-bath (70 rpm, 37°C). After incubation, the medium including isolated fat cells was filtered through a 1000 µm nylon mesh. The mature adipocytes floated and formed a layer on the surface of the filtered medium (packed fat cells), and the stromal vascular fraction (capillary, progenitor cells, fibroblastic cells and macrophages) was precipitated. The matured fat cells were washed twice with collagenase-free Krebs–Ringer phosphate buffer containing 3% (w/v) bovine serum albumin. The isolated mesenteric fat cells (50 µl packed volume) were incubated at 37°C in 950 µl of medium (Krebs–Ringer phosphate buffer, pH 7.4; 122 mM-NaCl, 4.9 mM-KCl, 1.2 mM-MgSO₄ and 16.7 mM-phosphate buffer) containing 3% (w/v) bovine serum albumin and 5 mM-glucose. After incubation for 1 h, the reaction was stopped on ice. The floating fat cells were removed, and the medium was used for adipocytokine measurement.

The rate of adipocytokine release from the isolated small- or large-intestinal mesenteric fat cells is expressed as unit volume of fat cells used^(17,18). The adipocytokine secretion rate from the whole mesenteric fat tissue (small- and large-intestinal regions) was calculated from the secretion rates from the small- and large-intestinal mesenteric fat cells by multiplying the whole mesenteric fat tissue weight.

TNF-α released into the incubation medium by the fat cells was quantified with an ELISA kit (Rat TNF-α Ultra Sensitive ELISA kit from Invitrogen Corporation, Camarillo, CA, USA). Levels of adiponectin and leptin released by the fat cells were measured as described earlier for blood serum.

Measurement of fat cell size

Isolated adipocytes from mesenteric fat tissues were stained with oil red O. Fat cell size was evaluated by the area of each isolated cell, and the mean size of the cell was calculated for more than 100 cells per preparation from each rat. Cell areas were measured using Image J version 1.41 software (National Institutes of Health, Bethesda, MD, USA).

Statistical analyses

All values are expressed as means with their standard errors of the mean for seven to nine rats. Statistical analyses were performed by one-way ANOVA, and the differences among treatment groups were determined using Tukey–Kramer's test if the difference was found to be significant using one-way ANOVA. A difference with $P < 0.05$ was considered to be significant. Pearson's correlation coefficients were also calculated.

Results

Body weight and fat tissue weight

There were no differences in initial or final body weight or food intake among the three groups (Table 2), and energy intake was also not different among the three groups (data not shown). However, the relative wet weight of the caecum was higher in the FOS-3wk and FOS-5wk groups than that in the control group ($P < 0.001$). Relative wet weights of the small-intestinal mesenteric fat ($P = 0.024$), retroperitoneal fat ($P = 0.02$) and epididymal fat ($P = 0.036$) were lower in the FOS-5wk group than those in the control group. The relative weight of the large-intestinal mesenteric fat tissue in both the FOS-3wk and FOS-5wk groups was lower than that in the control group ($P = 0.005$). There were no differences in the relative weights of the perirenal fat tissue among the three groups.

Table 2. Initial and final body weight, food intake, caecum, and small- and large-intestinal mesenteric, perirenal, retroperitoneal and epididymal fat relative weight in rats fed the experimental diets for 5 weeks

(Mean values with their standard errors, n 7–9)

	Control		FOS-3wk		FOS-5wk		P^*
	Mean	SEM	Mean	SEM	Mean	SEM	
Initial body weight (g)	51.9	1.0	52.4	0.9	52.5	0.8	0.910
Final body weight (g)	341.2	8.5	339.9	5.3	334.6	5.1	0.734
Food intake (g/d)	18.8	0.6	18.5	0.4	18.0	0.3	0.412
Tissue weight (wet g/100 g body weight)							
Caecum	0.99 ^b	0.05	2.31 ^a	0.16	2.37 ^a	0.13	<0.001
Mesenteric fat							
Small intestine	0.58 ^a	0.04	0.55 ^{a,b}	0.03	0.46 ^b	0.02	0.024
Large intestine	0.55 ^a	0.05	0.39 ^b	0.02	0.42 ^b	0.02	0.005
Perirenal fat	0.25	0.02	0.26	0.01	0.24	0.03	0.803
Retroperitoneal fat	1.49 ^a	0.06	1.30 ^{a,b}	0.08	1.17 ^b	0.08	0.020
Epididymal fat	1.34 ^a	0.10	1.12 ^{a,b}	0.07	0.97 ^b	0.11	0.036

FOS-3wk, fructo-oligosaccharide feeding for 3 weeks; FOS-5wk, fructo-oligosaccharide feeding for 5 weeks.

^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$; Tukey–Kramer's test).

* ANOVA.

Table 3. Plasma concentrations of TAG, glucose, insulin and homeostatic model assessment for insulin resistance (HOMA-IR) in the tail blood after 9 h fasting, and adiponectin, leptin, monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1) in the aortic blood of rats fed the experimental diets for 5 weeks

(Mean values with their standard errors, n 7–9)

	Control		FOS-3wk		FOS-5wk		P^*
	Mean	SEM	Mean	SEM	Mean	SEM	
TAG (mmol/l)	1.87	0.32	1.24	0.14	1.40	0.15	0.151
Glucose (mmol/l)	7.39	0.22	7.12	0.15	7.06	0.10	0.326
Insulin (nmol/l)	0.15 ^a	0.02	0.11 ^{a,b}	0.02	0.08 ^b	0.02	0.058
HOMA-IR	7.65 ^a	1.01	5.46 ^{a,b}	1.17	3.82 ^b	0.86	0.047
Adiponectin (mg/l)	2.50 ^a	0.28	1.76 ^b	0.17	1.77 ^b	0.08	0.021
Leptin (μ g/l)	7.69 ^a	0.91	5.59 ^{a,b}	0.84	3.84 ^b	0.28	0.005
MCP-1 (μ g/l)	2.88	0.24	2.72	0.16	2.79	0.13	0.833
PAI-1 (μ g/l)	0.73	0.05	0.76	0.07	0.86	0.09	0.408

FOS-3wk, fructo-oligosaccharide feeding for 3 weeks; FOS-5wk, fructo-oligosaccharide feeding for 5 weeks.

^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$; Tukey–Kramer's test).

* ANOVA.

Plasma analyses

There were no differences in the plasma concentrations of TAG and glucose after a 9 h fast among the three groups, whereas plasma insulin concentrations decreased by FOS feeding, and the concentration in the FOS-5wk group was much lower than that in the control group (Table 3). Changes in HOMA-IR, an index of insulin sensitivity, were also lower in the FOS-fed groups than in the control group ($P = 0.047$). The value in the FOS-5wk group was half that in the control group. In the aortic blood plasma, adiponectin concentration was significantly lower in both the FOS-fed groups ($P = 0.021$), and leptin concentration was lower in the FOS-5wk group compared with the control group ($P = 0.005$), whereas there were no differences in MCP-1 and PAI-1 among the three groups.

Secretion rates of adipocytokines from isolated mesenteric fat cells

Leptin release from the small-intestinal mesenteric fat cells was lower in FOS-3wk group than in the control group ($P = 0.04$; Fig. 1), whereas there were no differences in the secretion rate from the large-intestinal fat cells among the three groups. There were no differences in TNF- α release from the large-intestinal mesenteric fat cells among the three groups; however, the secretion rate from the small intestine was lower, though not significantly, in the FOS-fed groups. Feeding with the FOS diet had no effect on the secretion rate of adiponectin from either type of intestinal fat cell.

A comparable mean size of isolated mesenteric fat cells was measured in rats fed the test diets for 5 weeks. Mean size in the small-intestinal region was 3.53 (SEM 0.24) $\times 10^3 \mu\text{m}^2$ in the control group (n 7); 3.66 (SEM 0.19) $\times 10^3 \mu\text{m}^2$ in the FOS-3wk group (n 8); 3.49 (SEM 0.24) $\times 10^3 \mu\text{m}^2$ in the FOS-5wk group (n 9). Mean size in the large-intestinal region was 3.52 (SEM 0.13) $\times 10^3 \mu\text{m}^2$ in the control group (n 8); 3.62 (SEM 0.25) $\times 10^3 \mu\text{m}^2$ in the FOS-3wk group (n 8); 3.22 (SEM 0.18) $\times 10^3 \mu\text{m}^2$ in the FOS-5wk group (n 9). Mean size

of adipocytes in the small- and large-intestinal mesenteric fat cells showed no differences among the three groups.

Secretion rates of adipocytokines from the whole mesenteric fat tissue

The secretion rates of leptin ($P = 0.007$) and TNF- α ($P = 0.047$) from the whole mesenteric fat tissue were lower in both the FOS-3wk and FOS-5wk groups than in the control group (Fig. 2). There were no differences in the adiponectin secretion rates from the whole mesenteric fat tissue among the three groups.

Discussion

In the present study, we evaluated the effects of feeding FOS on mesenteric fat tissue, divided into small- and large-intestinal portions. The results show that FOS feeding led to a clear reduction in abdominal fat weight, which was associated with increases in insulin sensitivity as evaluated by HOMA-IR. We also examined adipocytokine release from the isolated mesenteric fat cells in rats fed the FOS diet. We found that leptin release was significantly reduced by FOS feeding, and TNF- α release showed a similar tendency to leptin release.

HOMA-IR was significantly decreased in the FOS-5wk group, indicating the promotion of insulin sensitivity. The HOMA-IR values in rats fed the FOS diet for 3 weeks were intermediate between those in the FOS-5wk and control groups, though the differences were not significant. These results suggest that it takes a considerable period for FOS feeding to affect insulin sensitivity. Recently, obese dogs were found to show improved insulin resistance after FOS feeding with modulation of the gene transcription of fatty acid or glucose metabolism⁽¹⁹⁾, which is in agreement with the present results. We showed caecal hypertrophy by FOS feeding, which indicates that caecal fermentation associated with SCFA production may be increased. It has been reported that FOS feeding largely increased acetic acid production in

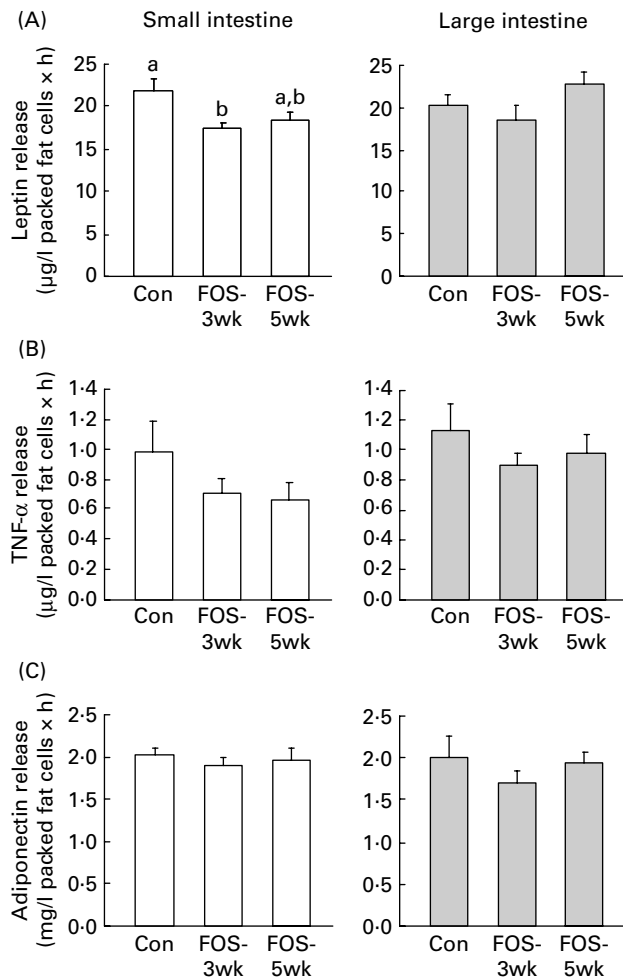


Fig. 1. Secretion rates of (A) leptin, (B) TNF- α and (C) adiponectin in isolated small- and large-intestinal mesenteric fat cells from rats fed the test diets. Values are means, with their standard errors represented by vertical bars (n 7–9). P values (one-way ANOVA) were 0.040 in the small intestine and 0.197 in the large intestine (leptin); 0.303 in the small intestine and 0.548 in the large intestine (TNF- α); and 0.770 in the small intestine and 0.524 in the large intestine (adiponectin). ^{a,b} Mean values with unlike letters were significantly different ($P < 0.05$). Con, control; FOS-3wk, fructo-oligosaccharide feeding for 3 weeks; FOS-5wk, fructo-oligosaccharide feeding for 5 weeks.

the colon⁽²⁰⁾. Acetic acid treatment suppresses body fat accumulation by down-regulating lipogenesis and lowering energy intake⁽²¹⁾. It has also been reported that butyrate supplementation at 5% (w/w) in a high-fat diet prevents the development of dietary obesity and insulin resistance⁽²²⁾. Moreover, the fermentation of dietary fibre including FOS suppresses endotoxaemia and improves metabolic disturbances⁽²³⁾. Hence, SCFA may contribute to the reduction of the mass of mesenteric fat tissue in the large intestine shown in the present study. However, the present results revealed a reduction in fat mass also in small-intestinal mesenteric fat tissue. Factors other than SCFA production by FOS might lead to the loss of body fat.

Adiponectin is as an adipocyte-specific protein^(24–26), and high adiponectin levels are known to enhance insulin

sensitivity in general. In contrast, recent studies have shown controversial results, i.e. high adiponectin levels were associated with an increased risk of CVD and/or mortality^(27,28), and the authors have speculated that elevations in adiponectin represent acute compensatory mechanisms to counteract metabolic and vascular stress. The present study shows that plasma adiponectin level was decreased in FOS-fed rats though insulin sensitivity was increased, which seems to

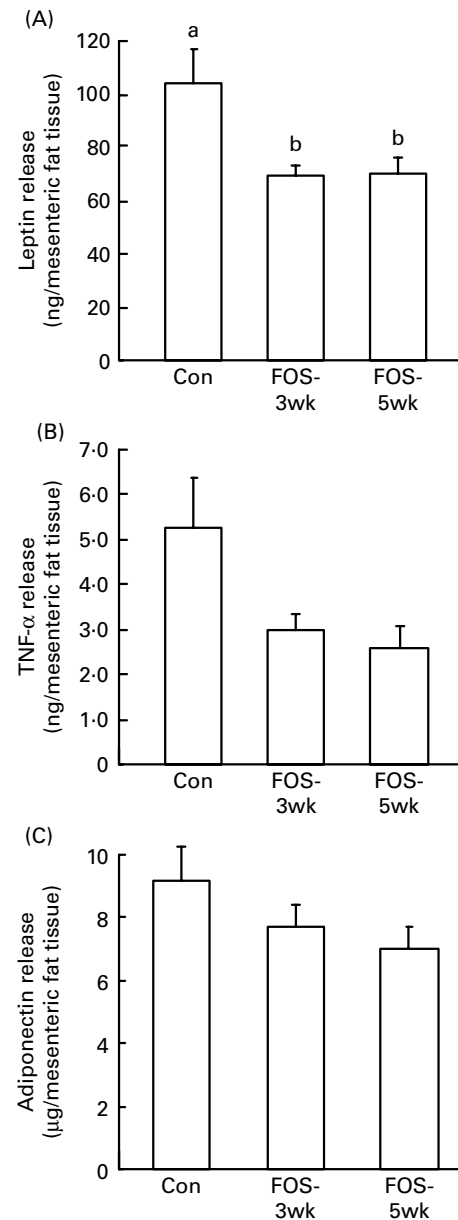


Fig. 2. Secretion rates from the whole mesenteric fat tissue of (A) leptin, (B) TNF- α and (C) adiponectin of rats fed the test diets, which were calculated from the secretion rates from the small- and large-intestinal mesenteric fat cells by multiplying the whole mesenteric fat tissue weight based on the weight per unit volume of the fat cells used. Values are means, with their standard errors represented by vertical bars (n 7–8). P values (one-way ANOVA) were 0.007 for leptin, 0.047 for TNF- α and 0.182 for adiponectin. ^{a,b} Mean values with unlike letters were significantly different ($P < 0.05$). Con, Control; FOS-3wk, fructo-oligosaccharide feeding for 3 weeks; FOS-5wk, fructo-oligosaccharide feeding for 5 weeks.

agree with the previous observations. It has been reported that liver is the main site of adiponectin clearance⁽²⁹⁾. This clearance rate is relatively stable with long-term fasting⁽³⁰⁾. However, both genetically (ob/ob mice) and diet-induced obesity resulted in slower clearance rates of adiponectin⁽²⁹⁾. Increased clearance rather than diminished secretion possibly results in lower plasma adiponectin levels as observed in the present study. The present results showed that adiponectin did not contribute, but other adipocytokines may contribute, to the improvement of insulin sensitivity in the FOS feeding groups.

The concentration of plasma leptin was clearly decreased by the feeding of FOS for 5 weeks, and a concomitant decrease in abdominal fat tissue weight was observed. The secretion rate of leptin from the whole mesenteric fat tissue was also markedly decreased by FOS feeding. Plasma leptin concentrations were positively correlated with the leptin secretion rate from mesenteric fat (R 0.492, $P=0.017$, n 7–8), which suggests that leptin release from the mesenteric fat contributes to changes in plasma leptin levels. Leptin increases in obesity, type 2 diabetes mellitus, hypertension and the metabolic syndrome. Numerous publications in human studies have suggested leptin as a biomarker for obesity, insulin resistance and the metabolic syndrome⁽³¹⁾. HOMA-IR was positively correlated with both plasma leptin levels (R 0.568, $P=0.005$, n 7–8) and the leptin release rate from mesenteric fat tissue (R 0.427, $P=0.048$, n 7–8). These results strongly suggest that suppression of leptin release from mesenteric fat tissue is involved in the FOS-mediated promotion of insulin sensitivity. Leptin is involved in the suppression of appetite^(32–34) and is also known to regulate energy balance, thereby modulating energy expenditure. Until recently, no direct action of leptin outside the brain was anticipated; however, there is some indication that leptin has additional effects in peripheral tissues^(35–37), as leptin receptors have been found in tissues other than the brain. There is some evidence demonstrating that leptin inhibits insulin gene transcription^(38,39) and insulin secretion^(40–42). By activating ATP-dependent K channels or via interactions with the cyclic AMP protein kinase A signalling pathway⁽⁴³⁾, perhaps by activating phosphodiesterase B₃⁽⁴⁴⁾. In white adipose tissue, leptin reduces insulin-dependent glucose uptake^(45–47) and impairs insulin-stimulated glucose transport⁽⁴⁸⁾ and lipogenesis⁽⁴⁹⁾. These observations are shown in the physiological range of insulin concentrations (0.1–0.5 nmol/l) and leptin concentrations (0.5–3 nmol/l), which strongly suggests that leptin reduces insulin sensitivity under physiological conditions^(50,51). The present results regarding the decreased leptin secretion rate from the whole mesenteric fat tissue and enhanced insulin sensitivity in FOS-fed rats agree with the results from these previous studies, and indicate that the reduction in leptin secretion may contribute to increase insulin sensitivity associated with FOS feeding. The secretion rates of TNF- α from the small- and large-intestinal mesenteric fat cells and from the whole mesenteric fat tissue were reduced by FOS-fed rats without significant differences. However, changes in TNF- α release were very similar to those observed for leptin release. The reduction in TNF- α release

may also be associated with an increase in insulin sensitivity. In contrast, there were no changes in plasma concentrations of MCP-1 and PAI-1 by FOS feeding. These results indicate that MCP-1 and PAI-1 are not involved in the improvement of insulin sensitivity by FOS feeding, and also reveal that suppression of leptin and TNF- α release are specific changes with feeding of this oligosaccharide.

We hypothesised that FOS feeding might have larger effects on the secretion rate of adipocytokines from the large-intestinal mesenteric fat cells compared with the small-intestinal region. However, the secretion rate in the small, but not the large, intestinal region was reduced by FOS feeding. Though the mechanism remains unclear, these findings suggest that FOS directly or indirectly acts on the small-intestinal mesenteric fat cells after absorption in intact forms or by fermentation products. A report has shown that raffinose, a non-digestible oligosaccharide in the same category as FOS, was absorbed intact in the intestine⁽⁵²⁾. FOS are also known to have direct or indirect immunomodulating effects on the intestine⁽⁵³⁾, which is a possible mechanism to change adipocytokine release by FOS in the small intestine. Even though the absorbed proportion of the orally administered FOS may be quite small, it still seems possible that the FOS absorbed from the small intestine suppressed leptin secretion in the mesenteric adipocytes of the small-intestinal region.

In conclusion, the ingestion of FOS decreased abdominal fat tissue weight and improved insulin sensitivity may be associated with changes in the properties of the small-intestinal mesenteric adipocytes and a reduction in the small- and large-intestinal mesenteric fat mass. The present findings may have implications for the prevention of insulin resistance in human subjects.

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References

1. Sakai K, Aramaki K, Takasaki M, *et al.* (2001) Effect of dietary short-chain fructooligosaccharides on the cecal microflora in gastrectomized rats. *Biosci Biotechnol Biochem* **65**, 264–269.
2. Tahiri M, Tressol J, Arnaud J, *et al.* (2003) Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. *Am J Clin Nutr* **77**, 449–457.
3. Kauffhold J, Hammon H & Blum J (2000) Fructo-oligosaccharide supplementation: effects on metabolic, endocrine and hematological traits in veal calves. *J Vet Med A Physiol Pathol Clin Med* **47**, 17–29.
4. Luo J, Rizkalla S, Alamowitch C, *et al.* (1996) Chronic consumption of short-chain fructooligosaccharides by healthy

- subjects decreased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism. *Am J Clin Nutr* **63**, 939–945.
5. Diez M, Hornick J, Baldwin P, *et al.* (1997) Influence of a blend of fructo-oligosaccharides and sugar beet fiber on nutrient digestibility and plasma metabolite concentrations in healthy beagles. *Am J Vet Res* **58**, 1238–1242.
 6. Cani PD, Possemiers S, Van de Wiele T, *et al.* (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091–1103.
 7. Funahashi T, Nakamura T, Shimomura I, *et al.* (1999) Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity. *Intern Med* **38**, 202–206.
 8. Matsuzawa Y (2006) The metabolic syndrome and adipocytokines. *FEBS Lett* **580**, 2917–2921.
 9. Kershaw E & Flier J (2004) Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* **89**, 2548–2556.
 10. Kadowaki T, Yamauchi T, Kubota N, *et al.* (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* **116**, 1784–1792.
 11. Shinoki A & Hara H (2010) Adrenaline-induced lipolysis in isolated rat mesenteric adipocytes is higher in the large intestine than in the small intestine. *Biosci Biotechnol Biochem* **74**, 670–672.
 12. Cacho J, Sevillano J, de Castro J, *et al.* (2008) Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague–Dawley rats. *Am J Physiol Endocrinol Metab* **295**, E1269–E1276.
 13. Muniyappa R, Chen H, Muzumdar R, *et al.* (2009) Comparison between surrogate indexes of insulin sensitivity/resistance and hyperinsulinemic euglycemic clamp estimates in rats. *Am J Physiol Endocrinol Metab* **297**, E1023–E1029.
 14. Reeves PG, Nielsen FH & Fahey GC Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* **123**, 1939–1951.
 15. Matthews D, Hosker J, Rudenski A, *et al.* (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412–419.
 16. Rodbell M (1964) Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J Biol Chem* **239**, 375–380.
 17. Shinoki A & Hara H (2010) Calcium deficiency in the early stages after weaning is associated with the enhancement of a low level of adrenaline-stimulated lipolysis and reduction of adiponectin release in isolated rat mesenteric adipocytes. *Metabolism* **59**, 951–958.
 18. Morimoto C, Kameda K, Tsujita T, *et al.* (2001) Relationships between lipolysis induced by various lipolytic agents and hormone-sensitive lipase in rat fat cells. *J Lipid Res* **42**, 120–127.
 19. Respondek F, Swanson K, Belsito K, *et al.* (2008) Short-chain fructooligosaccharides influence insulin sensitivity and gene expression of fat tissue in obese dogs. *J Nutr* **138**, 1712–1718.
 20. Lara-Villoslada F, de Haro O, Camuesco D, *et al.* (2006) Short-chain fructooligosaccharides, in spite of being fermented in the upper part of the large intestine, have anti-inflammatory activity in the TNBS model of colitis. *Eur J Nutr* **45**, 418–425.
 21. Yamashita H, Fujisawa K, Ito E, *et al.* (2007) Improvement of obesity and glucose tolerance by acetate in Type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem* **71**, 1236–1243.
 22. Gao Z, Yin J, Zhang J, *et al.* (2009) Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* **58**, 1509–1517.
 23. Cani PD, Neyrinck AM, Fava F, *et al.* (2007) Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* **50**, 2374–2383.
 24. Scherer P, Williams S, Fogliano M, *et al.* (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* **270**, 26746–26749.
 25. Hu E, Liang P & Spiegelman B (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* **271**, 10697–10703.
 26. Maeda K, Okubo K, Shimomura I, *et al.* (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apm1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* **221**, 286–289.
 27. Hung WC, Wang CP, Lu LF, *et al.* (2010) Circulating adiponectin level is associated with major adverse cardiovascular events in type 2 diabetic patients with coronary artery disease. *Endocr J* **57**, 793–802.
 28. Maiolino G, Cesari M, Sticchi D, *et al.* (2008) Plasma adiponectin for prediction of cardiovascular events and mortality in high-risk patients. *J Clin Endocrinol Metab* **93**, 3333–3340.
 29. Halberg N, Schraw T, Wang Z, *et al.* (2009) Systemic fate of the adipocyte-derived factor adiponectin. *Diabetes* **58**, 1961–1970.
 30. Combs T, Wagner J, Berger J, *et al.* (2002) Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. *Endocrinology* **143**, 998–1007.
 31. Moran O & Phillip M (2003) Leptin: obesity, diabetes and other peripheral effects – a review. *Pediatr Diabetes* **4**, 101–109.
 32. Cioffi J, Shafer A, Zupancic T, *et al.* (1996) Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nat Med* **2**, 585–589.
 33. Müller G, Ertl J, Gerl M, *et al.* (1997) Leptin impairs metabolic actions of insulin in isolated rat adipocytes. *J Biol Chem* **272**, 10585–10593.
 34. Wang J, Chinookoswong N, Scully S, *et al.* (1999) Differential effects of leptin in regulation of tissue glucose utilization *in vivo*. *Endocrinology* **140**, 2117–2124.
 35. Rouru J, Cusin I, Zakrzewska K, *et al.* (1999) Effects of intravenously infused leptin on insulin sensitivity and on the expression of uncoupling proteins in brown adipose tissue. *Endocrinology* **140**, 3688–3692.
 36. Ceddia R, William WJ, Lima F, *et al.* (1998) Leptin inhibits insulin-stimulated incorporation of glucose into lipids and stimulates glucose decarboxylation in isolated rat adipocytes. *J Endocrinol* **158**, R7–R9.
 37. Ceddia R, William WJ, Lima F, *et al.* (2000) Leptin stimulates uncoupling protein-2 mRNA expression and Krebs cycle activity and inhibits lipid synthesis in isolated rat white adipocytes. *Eur J Biochem* **267**, 5952–5958.
 38. Seufert J, Kieffer T & Habener J (1999) Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient ob/ob mice. *Proc Natl Acad Sci U S A* **96**, 674–679.

39. Melloul D, Marshak S & Cerasi E (2002) Regulation of insulin gene transcription. *Diabetologia* **45**, 309–326.
40. Kieffer T & Habener J (2000) The adipoinsular axis: effects of leptin on pancreatic beta-cells. *Am J Physiol Endocrinol Metab* **278**, E1–E14.
41. Margetic S, Gazzola C, Pegg G, *et al.* (2002) Leptin: a review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord* **26**, 1407–1433.
42. Havel P (2004) Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes* **53**, Suppl. 1, S143–S151.
43. Ahrén B & Havel P (1999) Leptin inhibits insulin secretion induced by cellular cAMP in a pancreatic B cell line (INS-1 cells). *Am J Physiol* **277**, R959–R966.
44. Zhao A, Bornfeldt K & Beavo J (1998) Leptin inhibits insulin secretion by activation of phosphodiesterase 3B. *J Clin Invest* **102**, 869–873.
45. Frederich R, Hamann A, Anderson S, *et al.* (1995) Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* **1**, 1311–1314.
46. Zhou Y, Wang Z, Higa M, *et al.* (1999) Reversing adipocyte differentiation: implications for treatment of obesity. *Proc Natl Acad Sci U S A* **96**, 2391–2395.
47. Frederich R, Löllmann B, Hamann A, *et al.* (1995) Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. *J Clin Invest* **96**, 1658–1663.
48. Barzilai N, Wang J, Massilon D, *et al.* (1997) Leptin selectively decreases visceral adiposity and enhances insulin action. *J Clin Invest* **100**, 3105–3110.
49. Funahashi T, Shimomura I, Hiraoka H, *et al.* (1995) Enhanced expression of rat obese (ob) gene in adipose tissues of ventromedial hypothalamus (VMH)-lesioned rats. *Biochem Biophys Res Commun* **211**, 469–475.
50. Considine R, Sinha M, Heiman M, *et al.* (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* **334**, 292–295.
51. Dagogo-Jack S, Fanelli C, Paramore D, *et al.* (1996) Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* **45**, 695–698.
52. Watanabe H, Sonoyama K, Watanabe J, *et al.* (2004) Reduction of allergic airway eosinophilia by dietary raffinose in Brown Norway rats. *Br J Nutr* **92**, 247–255.
53. Fukasawa T, Murashima K, Matsumoto I, *et al.* (2007) Identification of marker genes for intestinal immunomodulating effect of a fructooligosaccharide by DNA microarray analysis. *J Agric Food Chem* **55**, 3174–3179.