# Oligofructose Promotes Satiety in Rats Fed a High-Fat Diet: Involvement of Glucagon-Like Peptide-1

*Patrice D. Cani, Audrey M. Neyrinck, Nicole Maton, and Nathalie M. Delzenne*

**Abstract**

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***Objective:*** To analyze the putative interest of oligofructose (OFS) in the modulation of food intake after high-fat diet in rats and to question the relevance of the expression and secretion of intestinal peptides in that context.

***Research Methods and Procedures:*** Male Wistar rats were pretreated with standard diet or OFS-enriched (10%) standard diet for 35 days followed by 15 days of high-fat diet enriched or not with OFS (10%) treatment. Body weight, food intake, triglycerides, and plasma ghrelin levels were monitored during the treatment. On day 50, rats were food-deprived 8 hours and anesthetized for blood and intestinal tissue sampling for further proglucagon mRNA, glucagon-like peptide (GLP)-1, and GLP-2 quantification.

***Results:*** The addition of OFS in the diet protects against the promotion of energy intake, body weight gain, fat mass development, and serum triglyceride accumulation induced by a high-fat diet. OFS fermentation leads to an increase in proglucagon mRNA in the cecum and the colon and in GLP-1 and GLP-2 contents in the proximal colon, with consequences on the portal concentration of GLP-1 (increase). A lower ghrelin level is observed only when OFS is added to the standard diet of rats.

***Discussion:*** In rats exposed to high-fat diet, OFS is, thus, able to modulate endogenous production of gut peptides involved in appetite and body weight regulation. Because several approaches are currently used to treat type 2 diabetes and obesity with limited effectiveness, dietary fibers such as OFS, which promote the endogenous production of gut peptides like GLP-1, could be proposed as interesting nutrients to consider in the management of fat intake and associated metabolic disorders.

**Key words: fructans, gut peptides, ghrelin, orexigenic, anorexigenic**

## Introduction

Diet is an important factor controlling serum lipids and, consequently, the occurrence of coronary heart disease (1). Nutrients may be classified as beneficial or harmful if they decrease or increase the serum level of lipids involved in cardiovascular disease development. The role of dietary fat in energy balance has been a topic of interest for researchers and public health investigators because many epidemiological studies and animal models have characterized response to high-fat feeding in humans and rodents (2,3). High-fat diet produces a consistent and significant increase in body fat content that is dependent on both the amount of fat in the diet and the duration of feeding. Hyperphagia may be one important mechanism by which high-fat diets promote obesity because fat is less satiating than carbohydrate (4); thus, it has been suggested that fat may lead to passive diet overconsumption (5). Current recommendations for the management of obesity include an increase in dietary fibers that might help control food intake. Our previous studies have demonstrated that fructans obtained from chicory root inulin, a dietary fiber fermented largely in the caeco-colon, may be promising nutrients in the control of plurimetabolic syndrome associated with obesity. When added to the diet, they lessen steatosis in obese Zucker rats and may lower triglyceridemia in rats and in humans also (6–8). A signif-

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Unit of Pharmacokinetics, Metabolism, Nutrition, and Toxicology, Department of Pharmaceutical Sciences, Université Catholique de Louvain, B-1200 Brussels, Belgium.
Address correspondence to Nathalie M. Delzenne, Université Catholique de Louvain-Unité de Pharmacocinétique, Metabolisme, Nutrition et Toxicologie 7369, 73 Avenue Mounier, B-1200 Brussels, Belgium.
E-mail: delzenne@pmnt.ucl.ac.be

icantly reduced daily food ingestion in oligofructose (OFS)[1]-fed rats is a phenomenon contributing to the improvement of steatosis and glycemia in obese Zucker *fa/fa* rats or diabetic rats, respectively (9,10). The modulation of food intake and the decrease in epididymal fat mass by OFS has been described in normal Wistar rats, in which those effects are associated with an increase in portal serum and colonic anorexigenic glucagon-like peptide (GLP)-1 (7-36) amide and serum glucose-dependent insulinotropic polypeptide (GIP), and a decrease in serum orexigenic ghrelin (11). GLP-1 (7-36) amide is a 30-amino acid peptide secreted from the L-cells, which has been shown to decrease food intake and, consequently, body weight in rats and humans (12–16). GLP-2 is another peptide synthesized from proglucagon in L-cells, which is mostly devoted to local effect on intestine (cell proliferation) (17). Ghrelin is a circulating 28-amino acid peptide, identified primarily as the endogenous ligand of the pituitary growth hormone secretagogue-receptor (18). It emerges as one of the most powerful physiological orexigenic and adipogenic agents (19) able to orchestrate hunger and food seeking through its plasma-fluctuating concentrations (20). In the present study, we wanted to analyze the putative interest of OFS in the modulation of food intake and fat mass after high-fat diet and to question the relevance of expression and secretion of proglucagon-derived gut peptides and/or ghrelin in the effect of OFS in such a model, mimicking high-cafeteria diet in humans.

## Research Methods and Procedures

### *Animals, Housing, and Diets*

Male Wistar rats (six rats per group) weighing 145 to 160 grams (Harlan, Horst, The Netherlands) were housed in a temperature- and humidity-controlled room with a 12-/12-hour light (7 AM)/dark (7 PM) cycle. After an acclimatizing period of 5 days before the experiment, we pretreated rats with standard diet or OFS-enriched standard diet for 35 days, a treatment allowing a significant increase in intestinal and portal GLP-1 in OFS-treated animals vs. controls (CTs) (11). During that period, CT rats were fed a powdered A04 standard diet (A04; UAR, Villemoisson-sur-Orge, France), whereas OFS-treated rats received a diet prepared by mixing 90 g of A04 standard diet with 10 g of Raftilose $P_{95}$ (Orafti, Tienen, Belgium). The AO4 standard diet contained the following: 19.3 g/100 g dry diet protein (consisting of equivalent mix of soy and fish proteins); 70.4 g/100 g dry diet total carbohydrates obtained from corn, wheat, barley, and bran (including 38 g/100 g dry diet starches, 3 g/100 g dry diet saccharoses, 5 g/100 g dry diet celluloses, and 8 g/100 g dry diet nondigestible carbohydrates); 3 g/100 g dry diet lipids; 6 g/100 g dry diet mineral mixture; and 1.3 g/100 g dry diet vitamins (6). The energy value for the CT diet was 3.3 kcal/g and for the OFS diet, 3.1 kcal/g. For the last 15 days of treatment (days 36 to 50), rats were fed either high-fat CT diet or the high-fat OFS diet, which was prepared by mixing 90 g of high-fat CT diet with 10 g of Raftilose $P_{95}$ (Orafti). The high-fat diet contained the following: 23 g/100 g dry diet proteins (casein), 61 g/100 g dry diet carbohydrates [including 46 g/100 g dry diet starch, 10 g/100 g dry diet sucrose, and 5 g/100 g dry diet nondigestible carbohydrates (cellulose)], 14.15 g/100 g dry diet lipids (including 10 g/100 g dry diet lard, 4 g/100 g dry diet corn oil, and 0.15 g/100 g dry diet cholesterol), 1.2 g/100 g dry diet mineral mixture, and 0.8 g/100 g dry diet vitamin mixture (9). The energy value for the high-fat CT was 4.4 kcal/g, and the high-fat OFS diet was 4.1 kcal/g.

All rat experiments were approved by the local committee, and the housing conditions were as specified by the Belgian law of November 14, 1993 on the protection of laboratory animals (agreement no. LA 1230314).

### *Chemicals*

Raftilose $P_{95}$ (Orafti) is a mixture of glucosyl-(fructosyl)$_n$-fructose and (fructosyl)$_m$-fructose but with an average degree of polymerization of 4.5 for Raftilose $P_{95}$. Other chemicals used in this study were of the purest grade available and were purchased from Sigma (St. Louis, MO) and Merck (Darmstadt, Germany).

### *Blood Samples*

At days 7, 35, and 42, rats were food deprived for 8 hours, and tail vein blood was collected in EDTA tubes (Sarstedt, Nümbrecht, Germany) for triglyceride (TG) and active ghrelin quantification. On day 50, after high-fat treatment, food was withheld during dark phase at 2 AM; 8 hours later (at 10 AM), rats were anesthetized by intraperitoneal injection of sodium pentobarbital solution (using 60 mg/kg of body weight; Nembutal Sanofi Santé Animale, Benelux Brussels). Portal vein blood samples were collected in EDTA tubes (Sarstedt) containing dipeptidyl peptidase IV (DPPIV) inhibitor (Linco Research, St. Charles, MO); after centrifugation for 10 minutes at 1500*g*, plasma was stored at −80 °C. Concentrations of GLP-1 (7-36) amide were measured using an ELISA Kit (GLP-1 active ELISA kit, Linco Research). Cava vein blood samples were collected in EDTA tubes, and plasma was stored at −80 °C. Concentrations of active ghrelin were measured using an RIA kit (Ghrelin Active RIA kit; Linco Research). Plasma hepatic TG (Elitech Diagnostics, Brussels, Belgium) concentrations were measured using coupling enzymatic reaction and spectrophotometric detection of reaction end-products. DPPIV activity was measured by quantifying the production of

[1] Nonstandard abbreviations: OFS, oligofructose; GLP, glucagon-like peptide; GIP, glucose-dependent insulinotropic polypeptide; CT, control; TG, triglyceride; DPPIV, dipeptidyl peptidase IV; RT, reverse transcriptase; PCR, polymerase chain reaction.

*p*-nitroaniline (absorbance at 380 nm) from glycine-proline-*p*-nitroanilide (0.5 mM) within 10 minutes of incubation at 37 °C (pH 8.3).

### *Tissue Samples*

Segments of cecum and colon (proximal, medial, distal colon, corresponding to 2-cm segments taken just after the cecal junction, in the middle of the colon, and just before the rectum, respectively) were immediately excised, flushed with ice-cold saline, immersed in liquid nitrogen, and stored at −80 °C for further mRNA and peptide analysis. Full and empty cecum, liver, and epididymal fat pads were weighed. Liver samples were clamped immediately in liquid nitrogen. One gram of liver tissue was homogenized in 10 mL of phosphate buffer (pH 7.4). Lipids were extracted by mixing 125 μl homogenates with 1 mL of 2:1 chloroform:methanol (21). Chloroform phase was evaporated under nitrogen flux, and the dried residue was solubilized in 100 μL of isopropanol. TG content was measured as previously described for plasma samples.

### *Intestinal GLP-1 (7-36) Amide and GLP-2 Extraction*

Extraction of GLP-1 (7-36) amide from intestinal segments (cecum and colon) was carried out with ethanol/acid (100% ethanol:sterile water:12 N HCl in the following proportion: 74:25:1) solution using 5 mL/g of tissues. Samples were homogenized at 24,000 rpm and placed for 24 hours at 4 °C. Homogenates were centrifuged (20 minutes at 2000*g*), and supernatant was decanted and diluted 200-, 500-, and 500-fold in saline for cecum, ileon, and colon respectively. Concentrations of intestinal GLP-1 (7-36) amide were measured as previously described for blood samples. Concentrations of GLP-2 were measured using an RIA kit (GLP-2 RIA Kit; Phoenix Pharmaceuticals, Belmont, CA).

### *Isolation of Total RNA*

Total RNA was isolated from each intestinal segment according to the Chomczynski and Sacchi (22) method using the RNAgents Total RNA Isolation System (Promega, Leiden, The Netherlands). Approximatively 250 mg of intestinal tissue were used to extract total RNA. The quantity and the purity of RNA were determined by UV spectrophotometry at 260 and 280 nm. Total RNA (20 μg) was loaded onto agarose gel containing formaldehyde and visualized by ethidium bromide UV light staining to check ribosomal RNA 18S and 28S integrity.

### *Proglucagon Messenger and Actin mRNA: Reverse Transcriptase (RT)-Polymerase Chain Reaction (PCR)*

RT-PCR was performed with an input of 1 μg of RNA using the Kit for RT-PCR (Acces RT-PCR system; Promega). Primers used for the amplification of cDNAs of interest were for the sequences of the forward and reverse primers, respectively: 5′-GTAATGCTGGTACAAGGCA-G-3′ and 5′-TTGATGAAGTCTCTGGTGGCA-3′ for the proglucagon gene and 5′-CTGACCGAGCGTGGCTA-CAG-3′ and 5′-GGTGCTAGGAGCCAGGGCAG-3′ for the actin gene. The 23 cycles used for the detection of the proglucagon and actin transcripts correspond to the linear portion of the amplification curve (data not shown). PCR products (3 μL from each) were separated on a 1.8% agarose gel in Tris-Acetate-EDTA buffer and visualized by ethidium bromide UV light staining. Quantification of the PCR products was performed using the fluorimetric method (Rediplate 96 PicoGreen dsDNA Quantitation Kit; Molecular Probes, Eugene, OR).

### *Statistical Analysis*

Results are expressed as means ± SE. Statistical differences between groups were evaluated by independent sample Student's *t* test using SPPS version 9.0.0 for Windows (SPSS, Chicago, IL). The level of significance was set at $p < 0.05$.

## Results

### *Food Energy Consumption and Growth*

Energy intake during the experimental period was significantly different between groups (Figure 1). Total energy consumption was significantly lower in high-fat OFS group than in the high-fat CT group. Body weight was significantly different between groups at the end of the 35-day pretreatment and at the end of the high-fat treatment. Weight gain throughout the 15-day high-fat treatment was significantly lower in animals receiving a diet enriched in OFS than in animals receiving the high-fat diet only (Figure 2).

### *Organ Weight*

The epididymal adipose tissue weight was ~2-fold smaller in high-fat OFS rats than in high-fat CT rats (high-fat CT, 2.6 ± 0.1 g/100 g body weight; high-fat OFS, 1.1 ± 0.1 g/100 g body weight; $p < 0.05$). Macroscopic analysis of the organs revealed cecum enlargement in high-fat OFS vs. high-fat CT rats (cecal content weight, high-fat CT, 4.1 ± 0.3 g; high-fat OFS, 12.2 ± 1.2 g; $p < 0.05$; cecal tissue weight, high-fat CT, 1.1 ± 0.1 g; high-fat OFS, 2.1 ± 0.1 g; $p < 0.05$). The liver weight was similar between groups (high-fat CT, 3.6 ± 0.1 g/100 g body weight; high-fat OFS, 3.4 ± 0.1 g/100 g body weight).

### *Intestinal Proglucagon mRNA, GLP-1, and GLP-2 Concentrations*

Proximal colon and cecum proglucagon mRNA levels were significantly higher in samples taken from high-fat

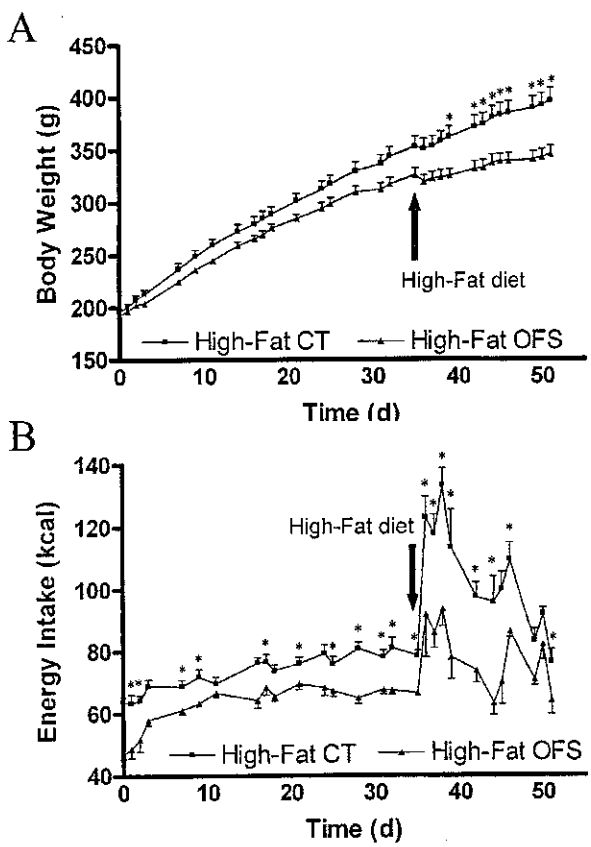


*Figure 1:* (A) Body weight (grams) and (B) dietary intake (kilocalories per day) of rats pretreated for 35 days with a CT diet or a diet supplemented with OFS, followed by 15 days of high-fat CT or high-fat OFS treatment. Time (d) indicates day after beginning of treatment. Values are means ± SE, $n = 6$. * Significantly different from CT rats, $p < 0.05$.

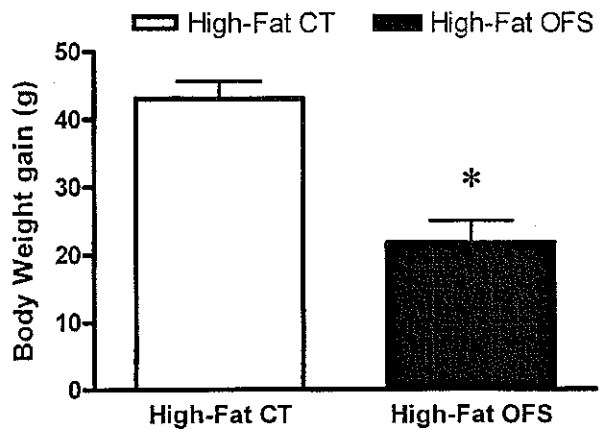


*Figure 2:* Body weight gain (grams) during high-fat treatment of rats fed high-fat CT diet or a high-fat diet supplemented with OFS. Values are means ± SE, $n = 6$ per group. (*) Significantly different from CT rats, $p < 0.05$.

OFS rats as compared with high-fat CT rats ($p < 0.05$) (Table 1). Proglucagon mRNA levels did not differ between groups in distal colon. Proximal and medial colon GLP-1 (7-36) amide concentration was almost doubled in high-fat OFS rats as compared with CTs (Figure 3). Due to the important cecal tissue enlargement in high-fat OFS rats and despite a similar peptide concentration per gram of tissue, GLP-1 (7-36) amide content expressed in picomoles per total cecum was 2-fold higher in high-fat OFS than in high-fat CT rats ($p < 0.05$) (Figure 3). Because proglucagon mRNA and GLP-1 were increased mostly in the proximal colon of high-fat OFS rats, we measured another product of intestinal proglucagon processing, namely GLP-2, in this segment. GLP-2 concentration was 2-fold higher in high-fat OFS than in high-fat CT rats (high-fat CT, 292.52 ± 25.94 pg/g tissue; high-fat OFS, 455.98 ± 25.14 pg/g tissue; $p < 0.05$).

### *Plasma GLP-1 (7-36) Amide and Ghrelin Concentrations, and DPPIV Activity*

Portal vein GLP-1 (7-36) amide concentration was doubled in high-fat OFS rats, as compared with high-fat CT rats (Table 2). DPPIV activity was 30% lower in high-fat OFS rats, as compared with high-fat rats (Table 2).

Active ghrelin concentration was measured 8 hours after the last meal, 35 days after CT or OFS pretreatment and 7 and 15 days after high-fat treatment (corresponding to days 42 and 50, respectively). At day 35, active ghrelin concentration was 30% lower in OFS than in CT rats ($p = 0.1$) (Figure 4); active ghrelin concentration measured in high-fat CT rats at days 42 and 50 decreased to reach the same levels in both groups as the one measured at day 35 in high-fat OFS rats (Figure 4).

### *Plasma and Hepatic TG Concentrations*

The OFS supplementation significantly decreased plasma TG during both standard diet OFS pretreatment and high-fat OFS treatment. At days 7 and 35 of pretreatment, TGs were 2-fold lower in the OFS group than in the CT group (Figure 5). Seven days after the beginning of high-fat treatment (at day 42), TGs significantly increased by ~35% in the high-fat CT group as compared with the value measured during the pretreatment period, whereas TGs increased by 14% in the high-fat OFS group as compared with the same rats during OFS pretreatment. Fifteen days after the beginning of high-fat treatment (at day 50), serum TG went back to same levels as those measured before high-fat treatment. Liver TG levels were ~30% lower in high-fat OFS than in high-fat CT rats (high-fat CT, 370.81 ± 72.83 nmol/mg protein; high-fat OFS, 251.6 ± 34.9 nmol/mg protein; $p = 0.1$).

**Table 1.** Effect of high-fat CT and high-fat OFS diets on intestinal proglucagon mRNA concentration (RFU)†

| | Cecum (RFU) | Proximal colon (RFU) | Medial colon (RFU) | Distal colon (RFU) |
|---|---|---|---|---|
| High-fat CT | 0.81 ± 0.05 | 0.90 ± 0.11 | 1.38 ± 0.14 | 1.17 ± 0.12 |
| High-fat OFS | 1.30 ± 0.14* | 1.28 ± 0.29* | 1.51 ± 0.16 | 0.93 ± 0.08 |

RFU, relative fluorescence unit; CT, control; OFS, oligofructose.
Values are means ± SEM, $n = 6$ per group; statistical analysis has been performed through independent sample Student's $t$ test separately for each organ.
* Significantly different from high-fat CT, $p < 0.05$.
† RFU, relative fluorescence units, proglucagon mRNA/$\beta$ actin mRNA.

## Discussion

Several papers support the possibility that dietary fibers, namely fructan-type prebiotics, can exert beneficial effects on lipid metabolism and risk factors for cardiovascular disease (7). Curiously, few data are available concerning the potential modulation of high-fat diet-induced hyperphagia by dietary fibers (23,24). We have recently shown that the addition of inulin-type fructans such as OFS in the standard diet of male Wistar rats was able, despite the fact that those fibers have no gel-forming or viscous properties, to decrease food intake with consequences on epididymal fat mass. Such effects are linked to an increased synthesis of proglucagon-derived peptide, namely GLP-1, in the colon (11). In the present study, we have tested the putative effect of OFS added to a high-fat diet in rats. The effects of high-fat diet on food intake and body weight gain are well documented in several reviews (2,3). We confirm here that high-fat CT diet induces severe hyperphagia, which is maintained for at least 14 days; this is associated with a significantly higher body weight gain during this period. Interestingly, the presence of OFS in the high-fat diet allows the avoidance of the large increase in food intake and body weight. In a recent paper, we have shown that GLP-1, a key intestinal peptide involved in the regulation of food intake, could play a role in the effect of OFS when added in a standard diet because its intestinal content was increased by 2-fold in OFS fed rats, as compared with CTs (11,25). Therefore, we have analyzed in more detail the relevance of the intestinal modifications occurring through OFS fermentation when it is added in a high-fat diet. In several studies, we have shown that inulin-type fructans added at the concentration of 100 g/kg standard diet often produce a severalfold enlargement of cecal tissue weight (9,11,26). This is also the case in high-fat OFS rats, which exhibit cecal tissue enlargement and cecal con-

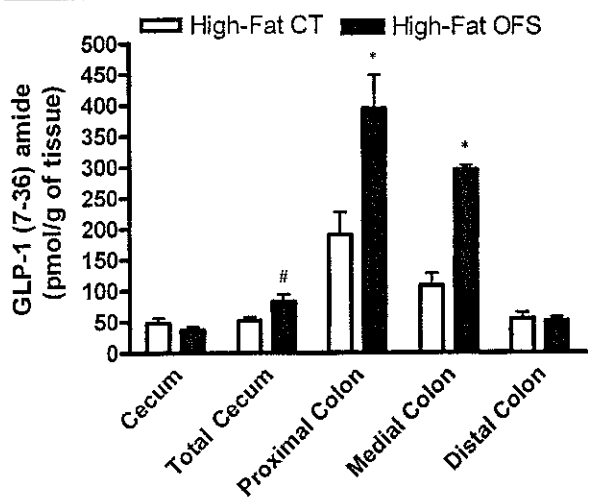


*Figure 3:* Intestinal GLP-1 (7-36) amide concentration of rats pretreated 35 days with a CT diet or a diet supplemented with OFS, followed by 15 days of high-fat CT or high-fat OFS treatment. Values are means ± SE, $n = 6$ per group. * Significantly different from CT rats, $p < 0.05$. # $p = 0.1$ vs. high-fat CT. Cecum, cecal tissue; total cecum, cecum tissue and content.

**Table 2.** Effect of high-fat CT and high-fat OFS diets on portal GLP-1 (7-36) amide content and DPPIV activity

| | GLP-1 (pM) | DPPIV (nmol/mL per minute) |
|---|---|---|
| High-fat CT | 21.3 ± 3.9 | 18.6 ± 1.3 |
| High-fat OFS | 42.4 ± 3.8* | 14.3 ± 1.3* |

CT, control; OFS, oligofructose; GLP-1, glucagon-like peptide-1 (7–36) amide; DPPIV, dipeptidyl peptidase IV.
* Values are means ± SEM, $n = 6$ per group; statistical analysis has been performed through independent sample Student's $t$ test. Significantly different from high-fat CT, $p < 0.05$.

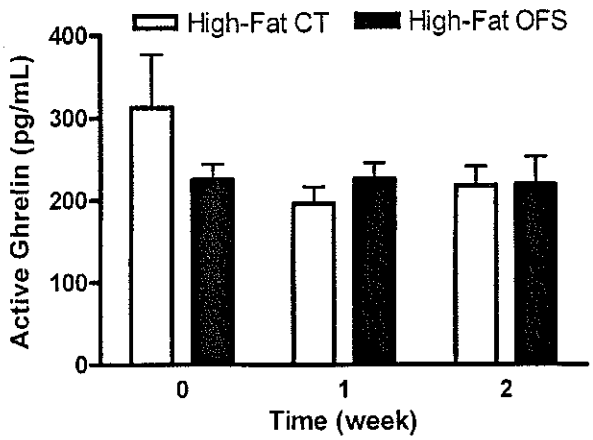


*Figure 4:* Plasma active ghrelin (nanograms per liter) of rats pretreated for 35 days with a CT diet or a diet supplemented with OFS (T0), followed by 15 days of high-fat CT or high-fat OFS treatment. T1 and T2 correspond to 7 and 15 days after high-fat diet treatment, respectively. Values are means ± SE, $n = 6$ per group.

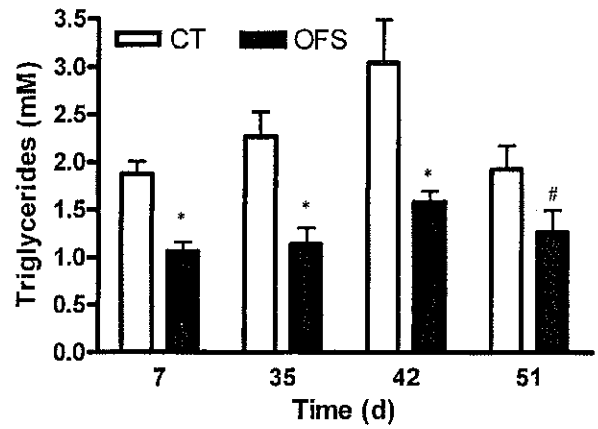


*Figure 5:* Plasma TG concentrations (millimoles per liter) of rats pretreated 35 days with a CT diet or a diet supplemented with OFS, followed by 15 days of high-fat CT or high-fat OFS treatment. Time (d) indicates day after beginning of treatment, with 42 and 51 corresponding to 7 and 15 days after high-fat diet treatment, respectively. Values are means ± SE, $n = 6$ per group. * Significantly different from CT rats, $p < 0.05$. # $p = 0.08$ vs. high-fat CT.

tent accumulation even 8 hours after the last meal. Is the cecum the main organ responsible for proglucagon/GLP-1 production after fructans feeding? By analyzing both proglucagon mRNA and GLP-1 (7-36) amide tissue concentration, we may assess that GLP-1 content, both in high-fat CT and high-fat OFS rats, is produced mainly in the proximal and medial colon and not in the cecum. The presence of OFS in the high-fat diet, as compared with high-fat diet alone, increases mRNA proglucagon in the proximal colon and in the cecum. We did not investigate whether the increase in proglucagon mRNA concentration is due to a higher gene expression or to an increase in mRNA stability, but in islet cell lines, butyrate activates proglucagon expression (27); this product of fructan fermentation could constitute an interesting candidate because its intestinal concentration is almost doubled after fructan feeding in rats (28).

The increase in GLP-1 concentration was observed only in the proximal and medial colon. Even if GLP-1 concentration per gram of cecal tissue remained unchanged after fructan feeding, cecum enlargement, observed in rats having received high-fat OFS as compared with high-fat CT, allows the GLP-1 pool to be increased 2-fold in the whole organ. Therefore, both cecal and colonic pools of GLP-1 (7-36) amide are promoted by an OFS-containing diet, with consequences on GLP-1 serum concentration. Indeed, we show that the addition of OFS to the high-fat diet doubles GLP-1 (7-36) amide concentration in the portal vein, as compared with high-fat diet alone. Moreover, OFS reduces DPPIV activity by ~30%. This enzyme is involved in the cleavage and inactivation of GLP-1 (7-36) amide. Thus, a lower DPPIV due to OFS may contribute, together with the higher intestinal production, to the higher GLP-1 (7-36) amide concentration and biological activity in the portal vein. The increase in this venous compartment is physiologically relevant because glucose sensors and the terminal vagal nerve responsive to GLP-1 are located mainly in the portal vein (29).

Several other gut peptides that may be linked to GLP-1 production (30) act to markedly alter food intake in humans and rodents. GLP-2 is a 33-amino-acid peptide hormone derived by posttranslational processing of proglucagon in entero-endocrine cells in both the small and large bowel and in specific regions of the brainstem. Circulating levels of GLP-2 rise rapidly after ingestion of nutrients, and the intact peptide is rapidly degraded to an inactive metabolite, GLP-2 (3-33), using the enzyme DPPIV (31). In addition to its potent trophic effects on the intestinal mucosa, GLP-2 inhibits gastric emptying and gastric acid secretion (32–34). Interestingly, in the present study, we found a 2-fold increase of GLP-2 concentrations in the proximal colon of OFS-fed rats.

Serum GIP concentration is also increased through OFS feeding. We had previously demonstrated that plasma GIP level was markedly increased when OFS was added to a standard diet for 4 weeks (67 ± 5 and 52 ± 4 pM in OFS and CT rats, respectively); this increase was accompanied by a higher GIP content in the ileum of OFS-fed rats (35). These significant modulations of GIP peptide could be, in this study, partly involved in the OFS effects. Despite the fact that GIP does not modulate gastric emptying per se (36), GIP is a hormone involved in the stimulation of GLP-1

production/secretion and, thus, indirectly participates in a reduced gastric emptying (37). Taken together, these modulations of gut peptides, namely GLP-1, GLP-2, and GIP, could be involved in the complex response to OFS.

Ghrelin could constitute a potential relay of the effect of OFS on satiety because GLP-1 and ghrelin concentrations are inversely correlated after glucose ingestion, and GLP-1 reduces ghrelin secretion (38,39). We have shown here that serum ghrelin concentration, even if it always remains lower in OFS-fed than in CT rats at the end of pretreatment period, was equivalent in both groups during high-fat treatment. It is interesting to note that a high-fat diet compared to a standard diet leads per se to a decrease in serum ghrelin concentration. Furthermore, the reduction in plasma active ghrelin level with the high-fat CT diet agrees with a decreased circulating level observed in obese humans (40). The effect of high-fat diet may be consecutive to an inhibition of ghrelin secretion by luminal fat (or fatty acid) itself or it may be the result of the intervention of gastrointestinal or peripheral peptides including neurotensin, cholecystokinin, peptide YY, leptin and resistin, or GLP-1 itself (41). The hypothesis of a decreased ghrelin linked to GLP-1 is consistent, as basal portal GLP-1 is approximatively 2-fold higher in high-fat CT as compared with CT diets.

Therefore, we believe that the increase in GLP-1 (7-36) amide is one key event explaining the effect of OFS, when given in a high-fat diet, on energy intake, body weight gain, and epidydimal fat mass development, whereas the implication of ghrelin remains doubtful in this protocol. A high-fat diet also induces hypertriglyceridemia, as previously described (9,42,43). The strong increase in serum TG observed 1 week after high-fat CT treatment as compared with the CT pretreatment period could be partly explained by a significant increase in food intake, whereas at the end of the treatment, reduction of triglyceridemia correlates with a lower food ingestion. When OFS is added to the standard diet, the decrease in triglyceridemia is attributable to a lower hepatic lipogenesis (44,45). Because the high-fat diet abolishes hepatic lipogenic activity, the protective effect of OFS against high-fat diet-induced hypertriglyceridemia is probably due to its effect on dietary intake and not to direct metabolic effect on liver lipogenic enzymes.

In conclusion, the addition of OFS in the diet protects against high-fat diet modulation of energy intake, body weight gain, and fat mass development through a mechanism that includes OFS fermentation in the cecum and in the proximal colon. This leads to an increase in proglucagon/GLP-1 synthesis, with consequences on the portal concentration of GLP-1; interestingly, a lower DPPIV activity might also contribute to the OFS effect on portal GLP-1 in this model. Thus, in rats exposed to high-fat diet, OFS is able to modulate endogenous production of gut peptides involved in appetite and body weight regulation. Because several approaches are currently used to treat type 2 diabetes and obesity with limited effectiveness, dietary fibers such as fructans, which promote the endogenous production of satietogenic peptides, could be proposed as interesting nutrients to consider in the dietary advice to control obesity and associated syndromes. Human studies have also shown the interest of fructans in controlling hypertriglyceridemia (7). Their effects in obese or overweight people need to be studied to assess their interest in human nutrition.

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