Insulin, Glucagon-like Peptide 1, Glucose-Dependent Insulinotropic Polypeptide and Insulin-Like Growth Factor I as Putative Mediators of the Hypolipidemic Effect of Oligofructose in Rats¹,²

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ABSTRACT The addition of oligofructose as a dietary fiber decreases the serum concentration and the hepatic release of VLDL-triglycerides in rats. Because glucose, insulin, insulin-like growth factor I (IGF-I) and gut peptides [i.e., glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)] are factors involved in the metabolic response to nutrients, this paper analyzes their putative role in the hypolipidemic effect of oligofructose. Male Wistar rats were fed a nonpurified diet with or without 10% oligofructose for 30 d. Glucose, insulin, IGF-I and GIP concentrations were measured in the serum of rats after eating. GIP and GLP-1 contents were also assayed in small intestine and cecal extracts, respectively. A glucose tolerance test was performed in food-deprived rats. Serum insulin level was significantly lower in oligofructose-fed rats both after eating and in the glucose tolerance test, whereas glycemia was lower only in the postprandial state. IGF-I serum level did not differ between groups. GIP concentration was significantly higher in the serum of oligofructose-fed rats. The GLP-1 cecal pool was also significantly higher. In this study, we have shown that cecal proliferation induced by oligofructose leads to an increase in GLP-1 concentration. This latter incretin could be involved in the maintenance of glycemia despite a lower insulinemia in the glucose tolerance test in oligofructose-fed rats. We discuss also the role of hormonal changes in the antilipogenic effect of oligofructose.


KEY WORDS: • oligofructose • triglycerides • insulin • incretins • rats

Because hypertriglyceridemia has been classified as a risk factor for cardiovascular disease, many attempts have been made to control serum triglycerides by modifying dietary habits. Our previous results indicated that oligofructose (OFS),³ a nondigestible fructooligosaccharide, given at a dose of 10 g/100 g in the diet of rats, significantly reduces the concentration of triglycerides in VLDL (Delzenne et al. 1993, Fiordaliso et al. 1995). At least part of the triglyceride-lowering action of OFS is due to reduction of de novo fatty acid synthesis in the liver, through inhibition of fatty acid synthase activity (Kok et al. 1996a and 1996b). The aim of this study was to investigate a possible mechanism by which a nondigestible carbohydrate such as OFS exerts such metabolic effects.

Fatty acid synthase activity and expression are regulated by glucose and insulin (Giffhorn-Katz and Katz 1986, Spence and Pitot 1982). Insulin-like growth factor I (IGF-I), referred to generically as somatomedin and structurally related to insulin, is as effective as insulin in inducing lipogenic enzymes (Hillgartner et al. 1995). In vivo, IGF-I levels are decreased by starvation and increased by refeeding in rats (Elmer and Schalch 1988) and in humans (Clemmons et al. 1981); this suggests that it may play a role in the nutritional regulation of lipogenesis.

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1(7–36)amide (GLP-1) are the major hormonal mediators regulating postprandial insulin release. Both peptides are released from endocrine cells in the intestinal mucosa after ingestion of carbohydrates and enhance postprandial insulin release from the pancreatic beta cells (for review see Morgan 1996). In addition to their insulinotropic effects, GIP and GLP-1 have direct anabolic insulin-like action on lipid metabolism, i.e., they stimulate de novo lipogenesis in both adipose tissue and liver and increase lipoprotein lipase activity (Knapper et al. 1995b, Oben et al. 1991, Zampelas et al. 1995).

We studied the influence of OFS supplementation in the diet of rats on glucose, insulin, GIP and GLP-1 concentrations...
in order to analyze their putative role in the hypolipidemic effect of such a nondigestible oligosaccharide.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats ICOPS-WY IOPS (n = 22) (Iffa Credo, Les Oncins, France) weighing initially ~120 g were individually housed with a 12-h light:dark cycle. They were given free access to a powdered nonpurified diet (UAR A04, UAR, Villemoisson-sur-Orge, France) and water before the experimental period. Rats were then randomly assigned to one of two groups. Control rats were given the nonpurified AO4 diet, whereas OFS-fed rats received the same diet containing (10 g/100g) Raftilose® P95. Rats were weighed and 24-h food intake was recorded every week. The animals were maintained and handled according to the recommendations of the Belgian State Directives.

After 30 d of treatment, at 1000 h, corresponding to the postprandial period, seven rats per group were anesthetized with pentobarbital (60 mg/kg body weight). Blood was collected from the portal vein as well as by cardiac puncture. The gastrointestinal tract was excised. Segments (± 1 cm in length) of duodenum, jejunum and ileum were taken either 2 or 10 cm after the pylorus, or 3 cm before the cecum, respectively. All segments were cleaned and immediately frozen in liquid nitrogen for storage at −20°C. The entire cecum was also excised and weighed. The cecal contents were then removed and the cecal tissue weighed, clamped and stored at −20°C.

A glucose tolerance test was also performed after 18 d of treatment. Four rats per group were deprived of food for 24 h and then force-fed with 0.3 g glucose/100g body weight. Tail blood samples were taken 0, 30, 60, 90 and 120 min after the oral glucose load for glucose and insulin measurements.

**Chemicals.** Raftilose® P95, a mixture of glucosyl-(fructosyl)-fructose (64%) and (fructosyl)n-fructose (36%) with an average degree of polymerization of 4.8, was used as the oligofructose source and was a gift from ORAFTI (Tienen, Belgium). All chemicals (Sigma Chemical, St Louis, MO) were of the purest analytical grade available.

**Analytical techniques.** Serum triglycerides and glucose were measured by using kits based on enzymatic reactions and spectrophotometric detection of end products (Elitech, Brussels, Belgium). Insulin concentrations were determined by using a commercial double antibody RIA kit (Novo Nordisk, Bagsvaerd, Denmark) with rat insulin as standard. Serum IGF-I levels were assayed in tail blood samples after 3 wk of treatment. IGF-I was determined by a commercial double antibody RIA in individual sera from which binding proteins had been depleted with the use of ODC-silica cartridges (C-18 Sep-Pak, Waters Associates, Milford, MA) as described by Maier et al. (1989).

Pure plasma-derived human IGF-I (PSI111) was used as a standard. Small intestine and cecal segments were extracted in acidified ethanol as described by Knapper et al. (1995a). Immunoreactive GIP was measured in plasma and gut extracts by double antibody RIA with human synthetic GIP as standard (Morgan et al. 1978). GLP-1 was analyzed in cecal extracts by double antibody RIA using synthetic GLP-1-(7–36)amide standards by methodology previously detailed (Elliot et al. 1993), except that no hormone-free plasma was added to the standard curve. GLP-1, glucagon, vasoactive intestinal polypeptide (VIP), secretin and pancreatic polypeptide do not cross-react in the GIP assay. The antisera used in the GLP-1 assay exhibited no cross-reactivity with GLP-2, glucagon, VIP, secretin and motilin. It is specific for the C-terminal amidated form of GLP-1, cross-reacting 100% with GLP-11–36-amide but <0.2% with GLP-17–37 and GLP-11–37. The respective interassay and intra-assay coefficients of variation were as follows: insulin, 6.2 and 5%; IGF-I, 6.8 and 6.2%; GIP, 8.4 and 4%; and GLP-1, 15 and 10%. Detection limits for insulin, IGF-I, GIP and GLP-1 were 43 pmol/L, 1.3 fmol/tube, 25 pmol/L and 5 pmol/L, respectively.

**Statistical analysis.** Data were presented as means ± SEM. Statistical analysis of the results was performed by two-way ANOVA when testing for the interaction time vs. treatment. Student’s t test was applied to compare unpaired results at one particular time point (postprandial state). Statview 512+ (BrainPower, Calabasas, CA) was used as software. The level of significance was set at P < 0.05.

**RESULTS**

**Diet intake and growth.** The average daily food intake did not differ (P > 0.05) between OFS-fed and control rats (16.4 ± 0.7 and 16.4 ± 0.8 g/d, respectively). Body weight at the end of the feeding period was 284 ± 8 and 272 ± 12 g, in OFS and control rats, respectively (P > 0.05).

**Serum metabolites and hormones.** In the postprandial state, chronic OFS feeding significantly decreased serum tri-glycerides (Table 1). Glucose and insulin levels were significantly reduced in the cardiac blood collected from OFS-fed rats, whereas circulating GIP was significantly greater in OFS-fed than in control rats (Table 1). In contrast, serum IGF-I-circulating level was not modified after 3 wk of 10% OFS feeding (Table 1). Glucose concentration measured in portal blood was also significantly (P < 0.05) lower in OFS-fed rats than in controls (7.23 ± 0.35 vs. 8.95 ± 0.64 mmol/L).

**Gut hormones and cecal development.** In both groups of rats, GIP concentration was significantly greater in the upper part of the intestinal tract (duodenum and jejunum) than in the distal part (ileum) (Table 2). Chronic OFS feeding did not significantly modify intestinal GIP level.

**Diet supplementation resulted in a significant enlargement of the cecum (Table 2). GLP-1 concentration (pmol/g) was not modified by OFS treatment (Table 2). However, because of cecal hypertrophy, immunoreactive GLP-1 content (pmol) was significantly greater in OFS than in control rats (Table 2).

**Glucose tolerance test.** In the glucose tolerance test, before the glucose load, glycemia did not differ in the two groups (Fig. 1A), whereas insulin concentration was significantly lower in the serum of OFS-treated rats (Fig. 1B). Even though OFS treatment (19 d) did not modify the glycemic response to an oral load of glucose (Fig. 1A), it significantly (P < 0.05) reduced the insulin response (Fig. 1B). Total area under the curve for insulin was significantly (P < 0.05) lower in OFS-fed than in control rats (34 ± 8 vs. 58 ± 14 fmol/l h · L, respectively), whereas total areas under the glucose curves did not differ.

<table>
<thead>
<tr>
<th>Table 1: Effect of dietary oligofructose on triglyceride, glucose and hormone concentrations in serum of rats in the postprandial state.</th>
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<tbody>
<tr>
<td><strong>Diet</strong></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
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<tr>
<td>Glucose, mmol/L</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
</tr>
<tr>
<td>GIP, pmol/L</td>
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<tr>
<td>IGF-I, nmol/L</td>
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</table>

1 Values are means ± SEM, n = 7. Asterisks indicate values significantly different than control rats: *P < 0.05, **P < 0.01.
2 GIP, glucose-dependent insulinotropic polypeptide; IGF-I, insulin-like growth factor I.

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6 AO4 diet contained per kilogram: 193 g protein, 704 g carbohydrates (including 380 g starch and 80 g nondigestible carbohydrates), 30 g lipids, 60 g mineral mixture and 18 g vitamins, providing an energy value of 14.7 kJ/g. The mineral mixture contained (calculated in mg/kg AO4 diet): P, 5900; Ca, 8300; Na, 1900; K, 6700; Mg, 2000; Mn, 90; Fe, 240; Cu, 30; Zn, 85; Co, 1.5; i. 0.3. The vitamin mixture contained (calculated in mg/kg AO4 diet): thiamin, 7; riboflavin, 6.5; niacin, 81.5; pyridoxine, 2.6; cyanocobalamin, 0.02; all-rac-a-tocopherol, 30; menadione, 2.5; folic acid, 0.5; biotin, 0.04; choline, 1600; all-trans-retinol, 2.25; cholecalciferyl, 0.0375 (UAR, Villemoisson-sur-Orge, France).
TABLE 2

Effect of dietary oligofructose (OFS) on cecal development and gut peptide concentrations in rats\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Diet</th>
<th>GIP\textsuperscript{3}</th>
<th>Control</th>
<th>OFS</th>
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</thead>
<tbody>
<tr>
<td>Intestine, pmol/g duodenum</td>
<td>177 ± 24\textsuperscript{a}</td>
<td>192 ± 17\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>Intestine, pmol/g jejunum</td>
<td>168 ± 13\textsuperscript{b}</td>
<td>199 ± 13\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>Intestine, pmol/g ileum</td>
<td>12 ± 2\textsuperscript{b}</td>
<td>16 ± 2\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>Cecum, g</td>
<td>0.8 ± 0.03</td>
<td>1.3 ± 0.06\textsuperscript{**}</td>
<td></td>
</tr>
</tbody>
</table>
| GLP-1
| Cecum, pmol/g              | 148 ± 8                   | 166 ± 23                  |
| Cecum, pmol                | 120 ± 7                   | 218 ± 28\textsuperscript{**} |

\textsuperscript{1} Values are means ± SEM, n = 7.

\textsuperscript{2} Means in a column with different superscript letters are significantly different (P < 0.05); asterisks indicate values significantly different than control rats; ** P < 0.01.

\textsuperscript{3} GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1(7–36)amide.

not differ \([1089 ± 41 \text{ vs. } 1100 ± 51 \text{ mmol}/(120 \text{ min} \cdot \text{L})\), respectively].

**DISCUSSION**

As previously shown, chronic OFS feeding significantly reduced postprandial triglyceridemia (Delzenne et al. 1993, Fiordaliso et al. 1995, Kok et al. 1996b). To identify the mechanism by which such a nondigestible compound exerts hypolipidemic effects, the aim of this study was to investigate the influence of chronic OFS feeding on the concentration of several hormones regulating hepatic lipid synthesis.

**Influence of oligofructose feeding on hormonal status.** Several studies have reported that GIP and GLP-1 synthesis and secretion by the gut are under the control of nutrients. Glucose is considered an important modulator of incretin secretion; moreover, compounds delaying glucose absorption such as guar gum or acarbose also reduce the production of GIP (Nunes and MalmloÈf 1992, Renard et al. 1991). In our studies, we report that the enrichment of rat diet with a nondigestible but fermentable carbohydrate, i.e., OFS, also modifies both GLP-1 and GIP levels; surprisingly, despite a lower postprandial glycemia, OFS feeding increases GIP serum concentration and cecal production of GLP-1. Several mechanisms may be proposed to explain this effect.

Feeding fermentable carbohydrates such as inulin or OFS leads to considerable enlargement of the cecum and to an increased blood flow in cecal tissue, both effects being proportional to their percentage in the diet (Reimer and McBurney 1996, Younes et al. 1995). This relative cecal hypertrophy was observed after OFS intake in this study and might be related to the trophic effects of short-chain fatty acids (SCFA) produced by OFS fermentation in the cecocolon (Roberfroid 1993).

GLP-1 is produced in intestinal L cells from the proglucagon molecule by a cell-specific proteolytic event (Holst 1994). Moreover, SCFA, either directly infused or produced by microbial fermentation of fiber, increase intestinal proglucagon expression as well as GLP-1 secretion in rats (Reimer and McBurney 1996, Reimer et al. 1997). The higher GLP-1 pool could be due to changes in SCFA production, consequent to OFS fermentation. In addition, in rats, GIP from the duodenal mucosa also stimulates GLP-1 release, suggesting an enteroen-
We also noted that the glycemic response was identical in OFS-fed and control rats after an oral load of glucose despite a lower insulin response in the former group. This suggests that OFS intake might improve glucose disposal in rats, a phenomenon that can be ascribed either to a higher insulin sensitivity or to an enhanced insulin-independent glucose disposal. Concerning the last hypothesis, good candidates could be incretins. Indeed, GLP-1 has been shown to have antidiabetogenic effects; it improves glucose tolerance by increasing insulin-independent glucose disposal (D’Alessio et al. 1994, Gutniak et al. 1992). Consequently, insulin requirements to maintain euglycemia are decreased. A similar mechanism has been proposed by Reimer and Mc Burney (1996) to explain improvements of glycemic control observed with chronic consumption of 30% dietary fiber in Sprague-Dawley rats.

Therefore, from our data, we speculate that cecal proliferation induced by OFS leads to an increase in GLP-1 concentration. This latter incretin could be involved in the maintenance of glycemia despite a lower insulinemia in the glucose tolerance test in OFS-fed rats.

Nunes and Malmlöf (1992) have shown that a guar-gum diet lowers IGFI plasma concentration in pigs. We showed that OFS supplementation at a dose of 10% in the diet of rats does not affect IGFI serum concentration. This latter result suggests that such treatment does not modify the balance between IGFI production by the liver and its clearance through IGF binding proteins (Thissen et al. 1994). Although food intake and nutritional status are important regulators of IGFI, the main hormonal regulator of circulating IGF-I is growth hormone (Thissen et al. 1994). Food intake was not impaired by OFS supplementation; this may be the reason why OFS feeding did not affect IGFI serum concentration. In addition, rat growth was not impaired by OFS supplementation.

**Involvement of insulin, insulin-like-growth factor-I, glucose-dependent insulinotrophic polypeptide and glucagon-like peptide-1 in the hyptolipidemic effect of oligofructose.** The key event in the hypotriglyceridemia resulting from OFS feeding is the inhibition of hepatic lipogenesis, through modulation of fatty acid synthase activity (Kok et al. 1996a and 1996b). The expression of the fatty acid synthase gene (which constitutes the sole mechanism of regulation of this enzyme activity described to date) in the liver is dependent on glucose availability. This phenomenon is potentiated by insulin (Giffhorn-Katz and Katz 1986, Spence and Pitt 1982). We may thus postulate that the lower glucose and insulin levels contribute to the reduction in hepatic fatty acid and triglyceride synthesis and are part of the mechanism of the hypotriglyceridemic effect of OFS.

A similar hypothesis was proposed by Maury et al. (1993) to explain the antilipogenic effect of acarbose, an intestinal α-glucosidase inhibitor. By a similar mechanism, resistant starch, by reducing the digestion rate of starch, lowers postprandial insulin response and decreases by 50 and 20% fatty acid synthase activity in adipose tissue and liver, respectively (Takase et al. 1995).

Serum GIP circulating level was significantly greater in OFS-fed than in control rats. This could be important because GIP is also directly involved in fatty acid metabolism. Both endogenous and exogenous GIP have been shown to lower the plasma triglyceride response to a fat load in rats (Ebert et al. 1991). GIP directly stimulates lipoprotein lipase activity in rat adipose tissue (Knapper et al. 1995b). Lipoprotein lipase is the capillary-bound enzyme responsible for the clearance of dietary (chylomicron) and endogenous (VLDL) triglycerides (Braun and Severson 1992). Moreover, in rat liver explants, GIP decreases incorporation of 14C-labeled glucose into total lipids, thus suggesting that in vivo, GIP decreased hepatic lipogenesis. However, the contribution of GIP in the regulation of lipogenesis remains to be clarified, because GIP paradoxically stimulates fatty acid synthesis in explants of rat adipose tissue (Oben et al. 1991). Moreover, GIP potentiates the stimulatory action of insulin on hepatic lipogenesis (Zampelas et al. 1995).

In vitro, GLP-1 binds to receptors and exerts insulinomimetic effects in liver, muscle and fat (for review see Valverde et al. 1996). Physiological doses of GLP-1 increase lipogenesis in explants of rat adipose tissue (Oben et al. 1991), whereas it enhances glucose oxidation as well as glycogen synthesis in isolated hepatocytes (Valverde et al. 1996). The putative influence of GLP-1, if any, on hepatic lipogenesis and triglyceride secretion remains to be explored.

In conclusion, this study suggests that supplementation of diet with OFS improves glucose disposal and decreases postprandial insulin level in rat. It increases GIP secretion, as well as GLP-1 concentration. The exact contribution of these hormones in the antilipogenic effect of nondigestible carbohydrate remains to be clarified.

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**LITERATURE CITED**


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