Biochemical Basis of Oligofructose-Induced Hypolipidemia in Animal Models[1]

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ABSTRACT   Oligofructose (OFS), a mixture of nondigestible/fermentable fructooligosaccharides, decreases serum triacylglycerol (TAG) when it is included in the standard, fiber-free or high fat diet of rats. This paper summarizes in vivo and in vitro data to establish a biochemical mechanism underlying the hypolipidemic effect of OFS. When OFS is added to the standard (carbohydrate-rich) diet of rats at the dose of 10 g/100 g, a TAG-lowering action occurs as a consequence of a reduction of de novo liver fatty acid synthesis. The depression in the activity of all lipogenic enzymes and fatty acid synthase mRNA suggests that OFS modifies the gene expression of lipogenic enzymes. Through its modulation of de novo lipogenesis, OFS can protect against liver lipid accumulation induced by providing 10% fructose-enriched water for 48 h. OFS also significantly decreases serum insulin and glucose, which are both known to participate in the nutritional regulation of lipogenesis. It also increases the intestinal production of incretins, namely, glucose-dependent insulinotropic peptide and glucagon-like peptide 1. This latter phenomenon results mainly from promotion of intestinal tissue proliferation by oligofructose fermentation end-products. Collectively, a link likely exists between the modulation of hormone and incretin production by OFS, and its antilipogenic effect.   J. Nutr. 129: 1467S–1470S, 1999.

KEY WORDS: • *fructan* • *triglycerides* • *lipogenesis* • *rat* • *liver* • *oligofructose*

The hypotriglyceridemic effect of nondigestible but fermentable carbohydrates, including resistant starch or oligofructose, has been described in both humans (Glore et al. 1994) and animals (de Deckere et al. 1995, Overton et al. 1994, Roberfroid and Delzenne 1998, Tokunaga et al. 1986). The mechanism of their lowering effect on serum lipids remains incompletely elucidated. Oligofructose (OFS)[3] is a natural food ingredient that can also be prepared by enzymic hydrolysis of chicory inulin. This commercially available OFS is called Raftilose. Because of the presence of β-linkages, OFS is undigestible in the upper gastrointestinal tract. Consequently, it enters the cecum/large bowel intact, where it is largely fermented and causes proliferation of selected anaerobic bacteria, mostly bifidobacteria (Wang and Gibson 1993).

Feeding rats a diet supplemented with 10 g/100 g OFS significantly lowers triacylglycerol (TAG) and phospholipid (PL) serum concentrations (Delzenne et al. 1993). This article will review probable biochemical mechanism(s) accounting for the hypolipidemic effect of OFS.

MATERIALS AND METHODS

Chemicals.   Raftilose P_{95}, a mixture of glucosyl-$(fructosyl)_n$-fructose (64%) and $(fructosyl)_m$-fructose (36%) with an average degree of polymerization of 4.8, was used as the source of OFS. It was a gift from Orafti (Tirlemont, Belgium).

Animals.   Male Wistar rats ICOPS-WY IOPS from Iffa Credo (Les Oncins, France), weighing initially ~120 g, were housed individually on a 12-h dark/12-h light alternating schedule. Rats were assigned to one of two experimental groups. Treated rats received diets containing 10 g/100 g Raftilose P_{95}. The basal diet was obtained from a commercial source (UAR A04, UAR, Villemoisson-sur-Orge, France). The composition of all diets is described in the legend of **Table 1**. The measurement of hepatic enzyme activities and metabolites, and of fatty acid synthase mRNA was performed on tissue samples taken at 0900 h, corresponding to the postprandial period. The entire cecum was excised, weighed and stored at −20°C.

Measurements.   Serum and liver triacylglycerols, nonesterified fatty acids, phospholipids, cholesterol and glucose were measured using enzymatic kits (Sopar-Biochem, Brussels, Belgium). The same kits were used for liver lipids analysis, after chloroform-methanol extraction according to Folch et al. (1957). Insulin concentrations were determined using a RIA kit from Novo Nordisk (Bagsvaerd, Denmark). Immunoreactive glucose-dependent insulinotropic polypeptide (GIP) was measured in serum by double antibody RIA with human synthetic GIP as standard (Morgan et al. 1978). Glucagon-like peptide 1 (GLP-1) was analyzed in cecal extracts by double antibody RIA as previously described (Elliott et al. 1993, Knapper et

[3] Abbreviations used: ACC, acetyl-coenzyme A carboxylase; ATPLC, ATP citrate lyase; BSA, bovine serum albumin; CPT I, carnitine palmitoyl transferase; FAS, fatty acid synthase; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; GPAT, glycerol-3-phosphate acyltransferase; G6PDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme; OFS, oligofructose; PAP, phosphatidate phosphohydrolase; PL, phospholipid; TAG, triacylglycerol; TG, triglyceride.

TABLE 1

Effects of 10% oligofructose (OFS) on postprandial triglyceridemia in rats

Diet	Duration of the experiment (wk)	% of serum TG compared with controls
Standard diet[1]	4	61
Fiber-free diet[2]	5	27
High fat diet[3]	3	43

[1] Standard diet composition (per kg): carbohydrates 704 g, including starch, 380 g; proteins, 193 g; lipids, 30 g; vitamin and mineral mix, to 1000 g (AO4, UAR, Villemoisson-sur-Orge, France). The mineral mix contained (calculated in mg/kg): 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 mix contained (calculated in mg/kg, except as noted): thiamin, 7; riboflavin, 6.5; niacin, 81.5; pyridoxin, 2.6; cyanocobalamin, 0,02; all-*rac*-α-tocopherol, 30; menadione, 2.5; folic acid, 0,5; biotin, 0,04; choline, 1600; retinol, 7500 IU; cholecalciferol, 1500 IU. Adapted from Kok et al. 1996a and 1996b.

[2] Fiber-free diet composition (per kg): starch 320 g; saccharose, 320 g; casein, 200 g; corn oil 80 g; vitamin and mineral mix, to 1000 g (INRA, Jouy-en-Josas, France). Adapted from Delzenne et al. 1993.

[3] High fat composition (per kg): starch, 460 g; saccharose, 100 g; nondigestible carbohydrate, 50 g; protein 230 g; lard, 100 g; corn oil, 40 g; cholesterol, 1.5 g; vitamin and mineral mix to 1000 g (INRA, Jouy-en-Josas, France). Adapted from Kok et al. 1998.

al. 1995). The esterification pathway was assessed by measuring glycerol-3-phosphate hepatic content (Hohorst 1965) and glycerol-3-phosphate acyltransferase (GPAT) as well as Mg^{2+}-dependent phosphatidate phosphohydrolase (PAP) activities (Bates et al. 1977, Cheng and Saggerson 1978). The oxidation of fatty acyl-SCoA was assessed by measuring carnitine palmitoyl transferase (CPT I) (Schäfer et al. 1993). Fatty acid synthesis was assessed by measuring acetyl-coenzyme A carboxylase (ACC) (Maeda et al. 1976), ATP citrate lyase (ATPCL) (Takeda et al. 1969), fatty acid synthase (FAS) (Linn 1981), malic enzyme (ME) (Hsu and Lardy 1969) and glucose-6-phosphate deshydrogenase (G6PDH) (Löhr and Waller 1974) activities. Protein was assayed by the method of Lowry et al. (1951), using bovine serum albumin (BSA) as standard. All enzymes assays were linear with respect to protein concentration. Total liver RNA was isolated with an acid guanidinium-thiocyanate-phenol-chloroform mixture (Chomczynski and Sacchi 1987) after liver glycogen removal (Sambrook et al. 1989). Northern blot analysis was performed as previously described (Brichard et al. 1994). To normalize the amount of total RNA loaded on each lane, specific mRNA levels were expressed relative to those of 18S rRNA assessed by methylene blue staining of the blot.

Statistical analysis. Statistical analysis of the results was performed by two-way ANOVA testing for the interaction time vs. treatment and using Statview 512+ (Abacus Concepts, Berkeley, CA) as software. Student's *t* test was used to compare results at one particular time point.

RESULTS AND DISCUSSION

Our results were obtained by analyzing the systemic effects of dietary OFS (Raftilose) on lipid metabolism in male Wistar rats. Table 1 summarizes the results obtained by comparing the influence of OFS given at the final concentration of 10 g/100 g in several diets, i.e., a standard diet (AO4), a fiber-free diet (semisynthetic diet without any digestible compound, except OFS), or a high fat diet known to induce hypertriglyceridemia and hypercholesterolemia. In all of these protocols, the constant response was a decrease in TAG concentration. This triglyceride (TG)-lowering effect was significant ($P < 0.05$) after 1 wk of feeding for the standard diet and lasted for up to 16 wk ($P < 0.05$); it was significant as well in the postprandial

as in the postabsorbtive period (Fiordaliso et al. 1995). Analysis of serum lipoproteins, after ultracentrifugation of the serum collected from food-deprived rats, showed that OFS feeding affected only the VLDL fraction, whereas the composition of both LDL and HDL remained unchanged (Fiordaliso et al. 1995).

Because a positive relationship exists between VLDL-TG hepatic output and liver lipogenesis, OFS was hypothesized to reduce de novo liver fatty acid synthesis (lipogenesis). This hypothesis was first investigated by measuring incorporation of ^{14}C-acetate (a lipogenic precursor) into TG in hepatocytes isolated from control and OFS-fed rats. OFS feeding significantly reduced TG synthesis and secretion from acetate in isolated hepatocytes (**Fig. 1**) (Kok et al. 1996b).

The activities of the enzymes driving the esterification pathway (GPAT, which catalyzes the first reaction of the pathway, and PAP, which converts phosphatidate to diacylglycerol, the common intermediate for PL and TAG biosynthesis) were not different in OFS-fed rats compared with controls (**Table 2**). Fatty acid esterification is also known to depend on the availability of glycerol-3-phosphate and fatty acyl-CoA. Table 2 provides evidence that hepatic glycerol-3-phosphate concentrations were significantly higher in OFS-fed rats than in controls. This relative increase in glycerol-3-phosphate liver content might be due to a decrease in its utilization for fatty acid esterification. Indeed, the daily administration of OFS slighly but significantly reduced the hepatocyte capacity to esterify ^{14}C-palmitate into TAG (Fiordaliso et al. 1995).

Fatty acid availability is considered to be the rate-limiting factor for TAG synthesis under physiologic conditions (Stals et al. 1994). The hypothesis of an increased fatty acid catabolism to explain a lower availability is unlikely. The activity of CPT I, the rate-limiting enzyme in the hepatic mitochondrial β-oxidation (MacGarry et al. 1978), was not increased in the liver of OFS-fed rats (Table 2). Moreover, Kok et al. (1993) have previously shown that mitochondria-enriched fractions prepared from the liver of OFS-fed rats had the same capacity to oxidize various fatty acids as those prepared from untreated rats.

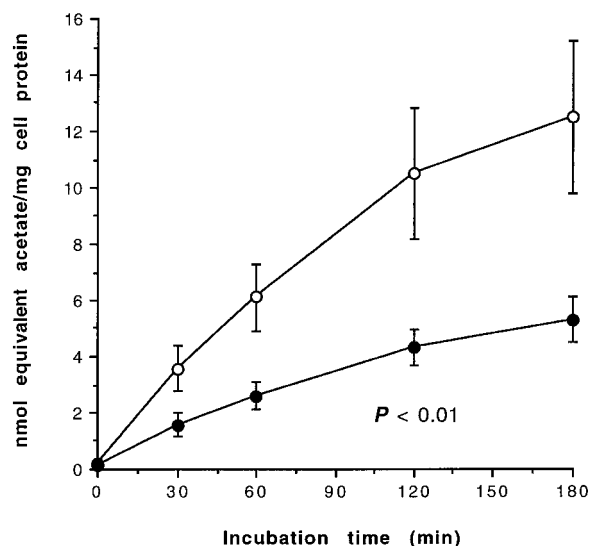Fatty acid synthesis is controlled by several key enzymes

FIGURE 1 Effect of oligofructose (OFS) feeding on triacylglycerol synthesis from ^{14}C-acetate. Hepatocytes from control (open circles) or OFS-fed rats (closed circles) were incubated for 180 min in the presence of 2 mmol/L [1-^{14}C] acetate. Adapted from Kok et al. 1996b.

that respond coordinately to physiologic stimuli. The activities of all lipogenic enzymes were found to be lower in the livers of OFS-fed rats compared with controls (Table 2), suggesting that OFS feeding could decrease lipogenic flux and thus liver TAG-VLDL secretion capacity (Arbeeny et al. 1992, Gibbons 1990).

The coordinate inhibition of all of the lipogenic enzymes as well as the fact that the activity of FAS is regulated only by the protein content, essentially at the transcriptional level (Hill-gartner et al. 1995), suggests that OFS might decrease the lipogenic flux in the liver by reducing the expression of the genes coding for lipogenic enzymes. FAS mRNA was reduced by 40% in OFS-fed rats compared with controls, as was found for FAS activity (Table 2).

Collectively, the TAG-lowering effect of OFS in rats appears to be due to its antilipogenic action in the liver, i.e., by reducing the activity and possibly the expression of all lipogenic enzymes.

Other possible mechanisms. Whatever metabolic change is responsible for the hypolipidemic effect, one must still explain how a nondigestible carbohydrate can regulate systemic lipid metabolism if, as shown in previous studies (Fiordaliso et al. 1995, Kok et al. 1998a and 1998b), it has no significant increasing effect on the fecal excretion of lipids.

By analogy with fermentable dietary fibers such as pectin (Roberfroid 1993), and knowing their effect on the physiology of the gastrointestinal tract, it could be possible that dietary OFS modifies the kinetics of absorbtion of dietary carbohydrates, leading to modifications of both serum glucose and hormones (insulin, glucagon). Moreover, dietary modulation of lipogenic activity is often linked to modifications of glucose and/or insulin serum levels. Indeed, acarbose, an intestinal glucosidase inhibitor that delays starch digestion, reduces glucose absorption and postprandial glycemia and insulinemia, and also decreases FAS activity in the liver (Maury et al.

TABLE 2

Effect of oligofructose (OFS) feeding on hepatic fatty acid metabolism[1]

Liver content, nmol/mg protein	Control rats (n)		OFS-fed rats (n)	
Triacylglycerols	51.2	± 3.9 (10)[1]	37.9	± 2.5 (10)*
Phospholipids	126.6	± 3.7 (10)	111.4	± 3.3 (10)*
Total cholesterol	25.3	± 0.01 (10)	23.2	± 0.8 (10)
Glycerol-3-phosphate, μmol/g liver	0.24	± 0.01 (10)	0.38	± 0.04 (10)**
Enzyme activities, mU/mg protein				
GPAT[2]	3.35	± 0.07 (5)	2.98	± 0.06 (4)*
PAP	5.5	± 0.4 (10)	4.9	± 0.53 (10)
CPT I	24.3	± 1.8 (10)	22.4	± 1.4 (10)
FAS	63.7	± 4.8 (8)	37.3	± 3.4 (8)**
ACC	0.53	± 0.05 (6)	0.32	± 0.05 (7)*
ATPCL	22.3	± 1.5 (8)	12.3	± 2.2 (8)**
ME	31.4	± 2.2 (8)	15.5	± 2.4 (8)**
G6PDH	36.0	± 4.3 (4)	19.3	± 2.9 (4)*
FAS mRNA/18S rRNA	0.655	± 0.09 (7)	0.378	± 0.08 (7)*

[1] Values are means ± SEM of *n* rats. *, ** Indicate significant differences (Student's *t* test) between control and OFS-fed rats; $P < 0.05$ and $P < 0.01$, respectively.
[2] ACC, acetyl CoA carboxylase; ATPCL, ATP citrate lyase; CPT I, carnitine palmitoyl transferase I; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; GPAT, glycerol-3-phosphate acyl transferase; ME, malic enzyme; PAP, phosphatidate phosphohydrolase.

TABLE 3

Effects of 10% oligofructose (OFS) on postprandial glucose, insulin and glucose-dependent insulinotropic polypeptide (GIP) serum concentrations in rats[1]

Diet	Glucose[2] mmol/L (n)	Insulin[2] pmol/L (n)	GIP pmol/L (n)
Control	10.13 ± 0.38 (10)	946 ± 85 (10)	52 ± 4 (7)
OFS-fed	8.44 ± 0.35 (10)**	638 ± 85 (10)*	67 ± 5 (7)*

[1] Values are means ± SEM of *n* rats. *, ** Indicate significant difference (Student's *t* test); $P < 0.05$ and 0.01, respectively.
[2] Adapted from Kok et al. 1996b.

1993). Similarly, resistant starch decreases serum TG concentration in rats (de Deckere et al. 1995), reduces FAS activity by 50 and 20% in adipose tissue and liver, respectively, and concommitantly lowers postprandial insulin response by 30% without affecting glucose response (Takase et al. 1994).

Recently, OFS ingestion was reported to reduce postprandial glycemia and insulinemia by 17 and 26%, respectively (**Table 3**) (Kok et al. 1996b). This could explain the lower FAS activity and mRNA. The transcription level of FAS is recognized to be primarily activated by glucose and insulin (Hillgartner et al. 1995). Studies are now in progress to analyze the putative involvement of glucose and insulin homeostasis in the hypolipidemic effect of oligofructose.

OFS also modulates the concentration of intestinal peptides, namely, GIP and GLP-1. Both peptides are known to regulate postprandial insulin release and also to have direct insulin-like actions on lipid metabolism (Morgan 1996). Serum GIP concentrations are enhanced in OFS-fed rats (Table 3). OFS increases GLP-1 twofold in the cecum (218 ± 28 vs. 120 ± 7 pmol/cecum in OFS-fed and control rats, respectively) ($P < 0.01$). This latter increase is a consequence of cecal hypertrophy and might be related to the trophic effects of short-chain fatty acids produced by OFS fermentation in the cecocolon (Roberfroid 1993).

CONCLUSION

In conclusion, the main systemic effect of OFS feeding in the rat is a decrease in serum TG. If this observation with dietary OFS or similar compounds is confirmed in humans, it would have profound importance because hypertriglyceridemia is a known risk factor for coronary heart disease (Davignon and Cohn 1996).

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