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Protective Effect of Dietary Fructo-oligosaccharide in Young Rats Against Exocrine Pancreas Atrophy Induced by High Fructose and Partial Copper Deficiency

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Abstract—The objective of this investigation was to protect rats against exocrine pancreatic atrophy by adding 22% fructo-oligosaccharide (FOS), a natural fructan obtained from inulin, to the 50% copper-deficient diets containing qualitatively and quantitatively different carbohydrates. Young male Wistar rats were maintained on these diets for 10 wk, being weighed weekly then killed and autopsied. Major organs were weighed and histologically examined. Copper content in the diets was measured by flame atomic absorption spectroscopy. Incomplete (50%) copper deficiency avoided precocious mortality due to cardiovascular lesions and enabled another pathological condition to develop, consisting of the induction of exocrine pancreas atrophy. Introduction of gradually increasing percentages of fructose in diets at the level of 22, 42 and 62% induced a gradual increase in the copper-deficiency-mediated pathology in rats, expressed by an increase in exocrine pancreatic atrophy. 22% FOS introduced to the diet prevented the pathology induced by both fructose and partial copper deficiency better than starch added to diet at the level of 20 or 40%.

INTRODUCTION

Copper has long been recognized as an essential dietary component (Hart *et al.*, 1928). Cardiovascular pathology induced by dietary copper deficiency in animals has been described by numerous authors (Kelly *et al.*, 1974; Klevay, 1977 and 1981). This copper-deficiency-dependent cardiovascular pathology comprises hypercholesterolaemia, hypertriglyceridaemia, anaemia, arterial damage, necrosis and rupture as well as myocardial hypertrophy, degeneration, fibrosis, necrosis and rupture involving dramatic haemorrhage and sudden death (Fields and Lewis, 1990; Klevay, 1981). A similar copper-deficient diet can produce in rats, after 4–6 wk, considerable atrophy of the exocrine pancreas (Fell *et al.*, 1982; Lewis *et al.*, 1987; Muller, 1970; Smith *et al.*, 1982). This pancreatic atrophy is accompanied by hypertrophy and degeneration of mitochondria, degeneration of the rough endoplasmic reticulum, failure of zymogen granule synthesis and ultimately severe atrophy of the acinar cells involving reduced activity of amylase, lipase, chymotrypsinogen and tripsinogen (Fell *et al.*, 1982; Lewis *et al.*, 1987).

The aetiopathogenesis of the above-mentioned alterations linked to copper deficiency is unknown and most probably multifactorial. Anaemia (Fields *et al.*, 1991a) and hepatic iron overload (Fields *et al.*, 1991b) certainly have a major role. The involvement of the deficiency in several cuproenzymes, such as cytochrome oxidase (EC 1.9.3.1), superoxide dismutase (EC 1.15.1.1.), lysyloxidase or ceruloplasmin, should be taken in consideration (Prohaska and Heller, 1982).

Diet composition and, in particular, the type and concentration of carbohydrate has been reported to modulate the copper-deficiency-mediated pathology: high fructose and sucrose diets cause much more severe signs of copper deficiency than does glucose (Fields *et al.*, 1984 and 1986; Koh *et al.*, 1989), whereas starch protects the animals against the symptoms associated with copper deficiency. This report compares the possible modulation of pancreatic atrophy, induced by a partially copper-deficient diet, by dietary fructose, starch and fructo-oligosaccharides (FOS). This latter compound is commercialized as Raftilose, the chemical composition of which is given in Materials and Methods. It resists hydrolysis by digestive enzymes, but is highly fermented in the caecum and the colon, mainly by bifidobacteria

Abbreviations: FOS = fructo-oligosaccharides; VLDL = very-low-density lipids.

(Wang, 1993). It is prepared by enzymatic hydrolysis of inulin extracted from chicory. The fermentation products consist mainly of short-chain fatty acids which are absorbed, reach the liver by the portal vein and can diffuse through the organism, where they are expected to exert systemic effects (Rémésy *et al.*, 1992). Such compounds can thus be classified as dietary fibre (Roberfroid, 1993). It was of particular interest first to test if FOS had the same effect as fructose and, most important, if associated with fructose it would potentiate or hinder the deleterious effects induced by copper deficiency.

MATERIALS AND METHODS

Young male rats of the Wistar strain, weighing approximately 50 g at the beginning of the experiment, were obtained from IFFA Credo (France). They were maintained in stainless-steel cages, five rats per cage under a standard light/dark schedule and fed *ad lib.* on a diet with the basic composition shown in Table 1. Diets varied in carbohydrate composition, which for groups 1–7 was as shown in Table 2.

Raftilose (Raffinerie Tirlemontoise, Belgium) was used as the source of FOS; it contained at least 95% fructo-oligosaccharides, with a mean degree of polymerization (n or m) of 4.8. The proportion of GF_n and F_m (G and F representing glucosyl and fructosyl moieties, respectively) was 64 and 36%, respectively.

In order to reduce the diarrhoea that may occur at the onset of administration of high doses of FOS, this carbohydrate was introduced to the diets at progressively increasing concentrations every 4 days, from 50 through 120 to 220 g/kg.

On average, the experimental diets (1–7) contained 4.10 ± 0.32 mg Cu/kg (mean $\pm$ SD), as measured by atomic absorption spectroscopy, compared with the A04 standard diet, which contained 8.9 mg Cu/kg. The subgroups 3, 4 and 6 Cu^- comprised 15 rats, subgroup 1 Cu^- contained five rats, subgroup 2 Cu^- 10 rats and subgroup 5 Cu^- had 11 rats. Each of the copper-deficient group (1–6/Cu^-) had its matched control group of rats (1–6 Cu^+), which received 5 ppm $CuSO_4$ in drinking water. Based on average water (20 ml/day) and diet (20 g/day) daily consumption, the Cu intake for rats fed a Cu^+ regimen was 120 μg/day; it was 80 μg/day/rat for the Cu^- regimen, and for rats fed the standard A04 diet it was 180 μg/day/rat.

Table 1. Composition of basic diet

Component	Concentration (g/kg)
Casein	200
Cellulose	40
Corn oil	78
DL-Methionine	2
Vitamin mixture	20
Mineral mixture (low in copper)	40
Carbohydrates	620

Table 2. Carbohydrate composition of experimental diets

Group no.	Carbohydrate content (g/kg complete diet)
1	Starch 620
2	Starch 400, fructose 220
3	Starch 200, fructose 420
4	Fructose 620
5	Starch 400, FOS 220
6	Fructose 400, FOS 220
7	Standard diet for rats AO_4*

*Supplied by U.A.R., Villemoisson-sur-Orge, France

All rats received the above-described diets for 10 wk. Each week their body weight was measured at the same day and hour. After this period, the rats were killed by ether anaesthesia, general autopsies were performed and all visible alterations were described. Major organs such as heart, liver, kidneys, spleen and brain were weighed. One specimen of a section perpendicular to the long axis of the body of the pancreas of each rat and from the major organs was fixed in 10% formalin for histological staining with standard haematoxylin and eosin and PAS techniques. The copper concentration was measured by atomic absorption spectroscopy after acid mineralization of frozen tissue using a Perkin–Elmer 1100 B spectrometer.

RESULTS

During the whole period of the experiment no deaths were noted in any group of animals, nor was any abnormal clinical sign observed in these rats, apart from slight diarrhoea during the first days of FOS administration. The mean body weight measured in rats of groups 3–6 was significantly lower ($P < 0.05$, Student's t-test) than in control rats of group 7 receiving the standard diet (Fig. 1). The copper status of the rats was assessed by measuring the copper content of liver tissue (Fig. 2). Statistical

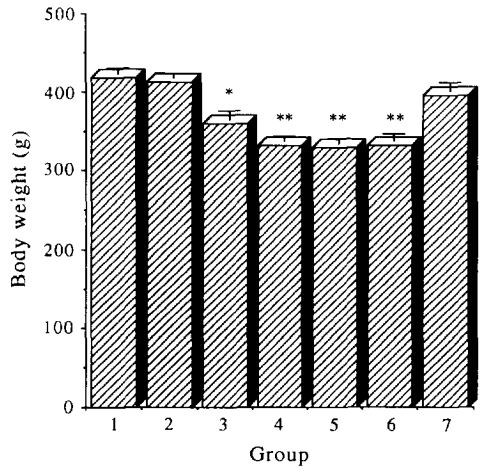

Fig. 1. Mean body weight $\pm$SEM measured at wk 10 in all groups of rats. Asterisks indicate significant difference from group 7 (controls) (*$P < 0.05$; **$P < 0.01$; Student's t-test).

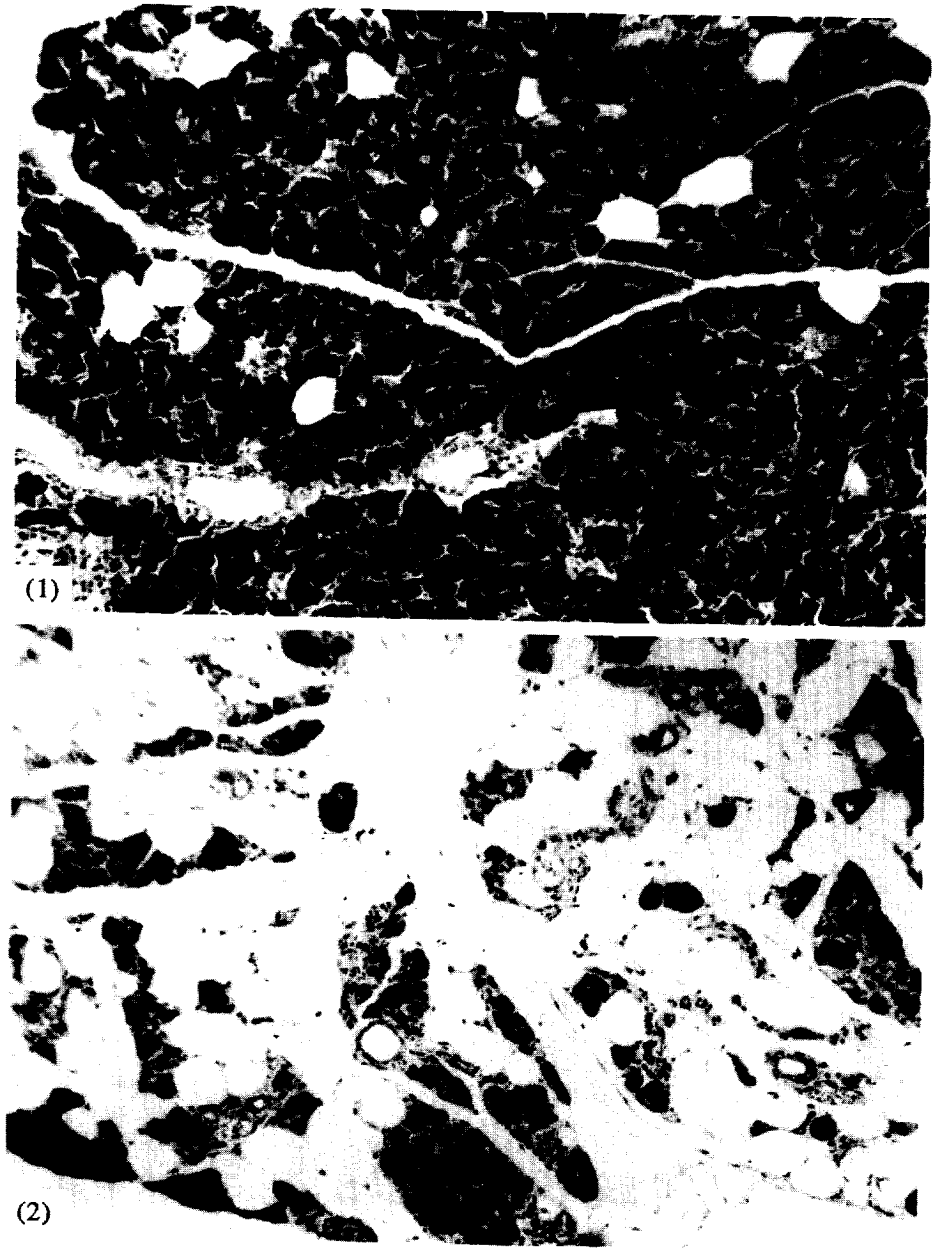

Plate 1. Grade+ pancreatic atrophy in copper-deficient rat fed 62% fructose (group 4). Less than 10% of pancreatic exocrine parenchyma is replaced by adipose tissue (empty holes). Haematoxylin and eosin. × 100.

Plate 2. Grade + + pancreatic atrophy in rat fed similar diet to that in Plate 1. About 50% of pancreatic exocrine parenchyma is replaced by adipose tissue (empty holes). Haematoxylin and eosin. × 100.

Plate 3. Grade + + + pancreatic atrophy in rat fed similar diet to that in Plate 1. More than 90% of pancreatic exocrine parenchyma is replaced by adipose tissue (empty space). The preserved darkly stained acini are marked with a single arrow. Islet of Langerhans indicated by double arrow. Haematoxylin and eosin. × 100.

Plate 4. Normal pancreatic architecture in a control rat fed standard laboratory diet (group 7). The polarity of acinar cells with dark (basophilic) basal pole containing nuclei and clear (eosinophilic) pole are distinctly limited and their cytoplasm is homogeneous. Islet of Langerhans (X); excretory ducts (arrow). Haematoxylin and eosin. × 400.

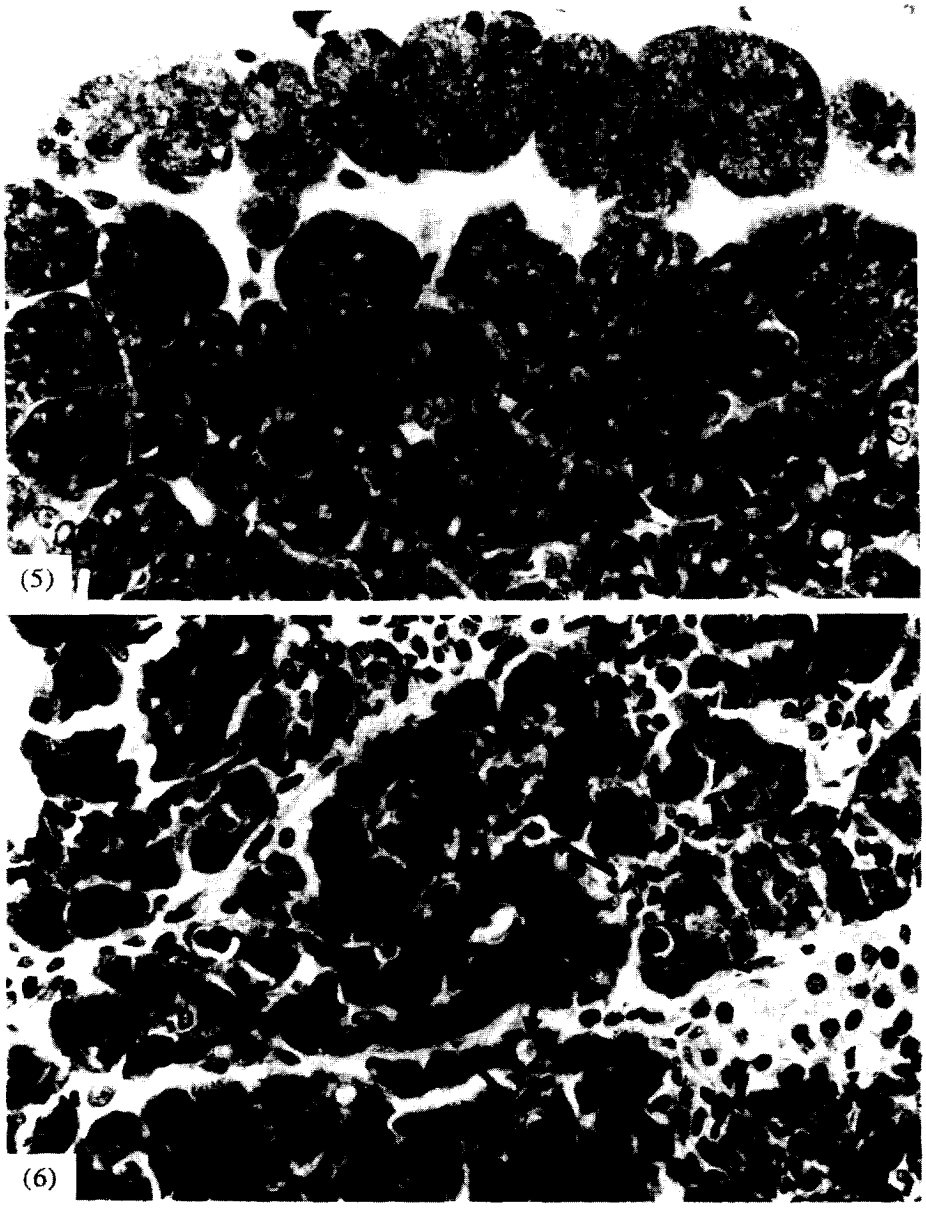

Plate 5. Early signs preceding pancreatic atrophy in a copper-deficient rat fed 40% starch and 22% fructose (group 2). The polarity of acinar cells has disappeared, their cytoplasm has a vacuolar pattern and is considerably less stained than in Plate 4. Acinar architecture is severely disrupted. Haematoxylin and eosin. ×400.

Plate 6. Early signs preceding pancreatic atrophy in another rat receiving the same diet as that in Plate 5. Numerous apoptotic bodies (arrows) indicate increased death rate of individual acinar cells. The acinar architecture is markedly disrupted. Haematoxylin and eosin. ×400.

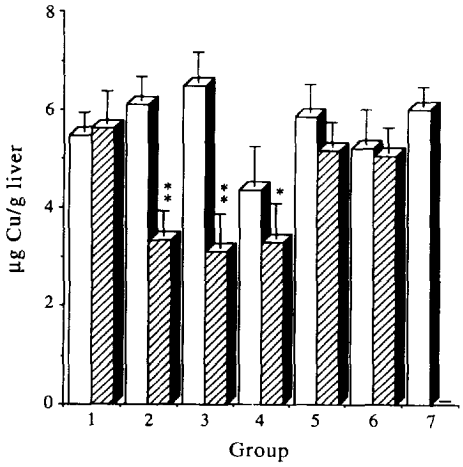

Fig. 2. Copper concentration in liver tissues expressed as µg/g (mean ± SD). Asterisks indicate significant differences between groups given copper-containing (□) or copper-deficient (▨) diets (*$P < 0.05$; **$P < 0.01$; Student's t-test).

analysis of the results (Student's t-test) showed that, in groups of rats fed the fructose-supplemented diet, the liver copper content was significantly lower (groups 2–4) in Cu^- than Cu^+ groups, except for group 6, which received fructose and FOS. Moreover, in groups receiving starch and/or FOS, without fructose, the concentration of copper in liver tissue did not differ significantly between the Cu^+ or Cu^- groups.

Weights of the liver, heart, kidneys, spleen and brain after 10 wk of dietary treatment did not present any striking difference between the various dietary groups of rats.

At autopsy the pancreas had a normal volume and macroscopic appearance. No macroscopically detectable alteration could be found in any other organ examined, as was also the case on microscopic examination of the haematoxylin- and eosin-stained slides of all tissues except the pancreas. In that organ, different grades of atrophy were observed microscopically in some experimental groups. In order to present the histological alterations in pancreas in a semi-quantitative way the microscopic results for this organ were classified into five different grades of atrophy as follows: grade 0 = no atrophy, normal histological pattern; ± = no atrophy but early signs preceding atrophy; + = minor atrophy (disappearance of 1–10% of pancreatic tissue surface in histological slides); + + = intermediate atrophy (around 50%); + + + = very intense atrophy (more than 90%).

Statistical analysis concerning comparison of grades of pancreatic atrophy in different groups was based on the chi-square test. The results of this semi-quantitative evaluation of the histopathological lesions of pancreas in different groups of rats fed Cu^- regimens are presented in Table 3. The same

Table 3. Semiquantitative evaluation of different grades of pancreatic atrophy in rats fed Cu^- diet containing quantitatively and qualitatively different carbohydrates

Atrophy grade	Group* 1	2	3	4	5	6
0	5/5†	7/10	4/15	2/15	10/11	10/15
±	0/5	3/10	0/15	0/15	1/11	5/15
+	0/5	0/10	5/15	3/15	0/11	0/15
+ +	0/5	0/10	5/15	3/15	0/11	0/15
+ + +	0/5	0/10	1/15	7/15	0/11	0/15

*See Materials and Methods for description of diets of groups 1–7.
†Values are no. of rats with pancreatic atrophy per no. of rats in each group.

semi-quantitative evaluation of results has been done in corresponding groups of rats fed a Cu^+ regimen. In none of these animals was atrophy (or even early signs preceding pancreatic atrophy) found (data not shown).

The most prevalent (13/15) and most intense pancreatic atrophy was observed in Cu^- group 4 (62% fructose). Only 2/15 rats had a histologically normal pancreas. Three of 15 rats had atrophy of less than 10% of the microscopical surface of the pancreatic parenchyma (grade+). Exocrine acini were replaced by adipocytes, giving the impression of empty holes in the histological slides (Plate 1). The endocrine pancreas (islets of Langerhans) and the excretory ducts were not altered in these or in any other pancreatic specimens examined in this study. In the same Cu^- group 4 there were 3/15 rats with exocrine pancreatic atrophy grade + +, which involved about 50% of the pancreatic surface (Plate 2). At a higher magnification it was seen that in this, as in some other cases, the pancreatic acini were replaced both by adipocytes (visible as empty holes) and by solid aggregates of fibrocytes and fibroblasts (fibrosis). The persisting pancreatic acini exhibited different degrees of disorganization. In most rats of the Cu^- group 4 (7/15) the highest grade (+ + +) of pancreatic atrophy was recognized, which involved more than 90% of the tissue surface (Plate 3). Persisting pancreatic acini were visible as rarely dispersed, isolated foci with a dark staining surrounded by a thin layer of fibroblasts in a very extensive background of adipose tissue. As can be seen from Table 3, there was a gradual intensification of

Table 4. Statistical comparison of groups* in Table 1 based on chi-square test: probability of null hypothesis

Group	1	2	3	4	5	6
1						
2	—					
3	**	**				
4	**	**	—			
5	—	—	**	**		
6	—	—	**	**	—	

*As in Table 1.
Asterisks indicate significant differences (*$P \leq 0.05$; **$P \leq 0.01$; Student's t-test); — = not significant ($P > 0.05$).

pancreatic atrophy corresponding to the gradual increase of dietary content of fructose in Cu groups 1–4. The above-described atrophy was homogeneously distributed over the whole section. Its degree differed only between experimental animals. Isolated and more intensively stained foci of persisting exocrine pancreatic tissue demonstrated alterations that may be considered as preceding atrophy (decreased volume of densely stained cells and frequent apoptoses). They are similar to the pattern shown in Plate 6.

Table 4 indicates that the difference between groups 1 and 2 (0 and 22% fructose, respectively) is not significant whereas the differences between groups 1 and 3 (0 and 42% fructose, respectively) as well as between groups 1 and 4 (0 and 62% fructose, respectively) are highly statistically significant. There was no pancreatic atrophy in rats of Cu⁻ groups 6 and 5 which received, respectively, a diet containing fructose 40%/FOS 22% and starch 40%/FOS 22%. The results obtained in these two groups (6 and 5) show a highly statistically significant difference when compared with groups 3 and 4 receiving fructose 42%/starch 20% and fructose 62%, respectively (see Table 4).

It is important to emphasize that the addition of 22% FOS had a better preventive effect on the pancreatic atrophy in fructose-dependent copper deficiency groups than did the addition of 20% or even 40% of starch in the diet.

In groups 2, 5 and 6 there was no atrophy, but only early morphological signs preceding pancreatic atrophy. These signs comprised various degenerative events in the acinar cells, such as disappearance of polarity, thinned basal pole with decreased or absent basophilia, decrease of eosinophilic zymogen granules in the apical pole, and blurred limits between the basal and apical poles of acinar cells. These alterations are particularly obvious when they are compared with the normal pancreatic architecture in a control rat maintained on the standard diet (group 7) (Plate 4) The entire cytoplasm in the acinar cells in groups 2, 5 and 6 had a vacuolar or foamy pattern (Plate 5). In the altered exocrine pancreatic tissue, apoptotic bodies were frequent and the glandular architecture was disorganized, with dissociation, scattering and disappearance of acinar cells (Plate 6).

DISCUSSION

Conclusions relating to this study are limited to three main points:

1. Partial (50%) copper deficiency did not cause precocious mortality due to cardiovascular lesions, as reported for more extensive copper deficiency (Fields, 1990; Klevay, 1981), but it did induce another pathological condition—exocrine pancreatic atrophy.
2. Introduction of gradually increasing percentages of fructose in diets at the level of 22, 42 and 62% (conditions not previously described in the literature related to copper deficiency) induced a gradual increase in copper-deficiency-mediated pathology in rats, as shown by a gradual increase of exocrine pancreatic atrophy. This atrophy involved the exocrine pancreas exclusively and was preceded by degenerative lesions and apoptosis. No signs of necrosis but the presence of apoptotic bodies confirmed the process of atrophy in the exocrine pancreas.
3. 22% FOS added to the diet prevented the pathology induced by both fructose and partial copper deficiency, better than did starch added to the diet at 20 or even 40%. The beneficial effect of FOS on copper-deficiency-induced pancreatic atrophy could result either from decreased bioavailability of copper or from a metabolic effect of FOS. Differences in the rate of development or severity of copper deficiency among rats fed different carbohydrates (starch, fructose, glucose, maltose) are apparently not caused by effects of the compounds on absorption or endogenous excretion of copper (Johnson, 1988). We have recently shown that 10% FOS added the diet of rats did not modify dietary intake and faecal excretion of copper (N. Delzenne, unpublished data). The mechanism(s) of dietary fructose–copper interaction in rats are not known, but could result from biochemical effects mediated by the metabolism of glucose, fructose or sorbitol (Lewis *et al.*, 1990). Therefore, we postulate that FOS exerts its beneficial effect through metabolic regulation. FOS has already been shown to have systemic effects on lipid homoeostasis by modifying liver metabolism (Kok *et al.*, 1993). The putative mediators of such systemic effects of this non-digestible compound could be the products of fermentation, namely acetate, propionate and L-lactate, which have been known to regulate cellular metabolism in the liver and adipose tissue (Wright *et al.*, 1990).

Obviously, the above-described results, mainly the utilization of FOS to prevent pathological effects of fructose in a copper-deficient regimen, open up very interesting perspectives. Several unsolved questions, however, call for further investigation of this topic, as follows:

1. Detailed study of the intermediate steps and long-term observation of copper deficiency (partial and complete) associated with the supply of qualitatively and quantitatively different types of carbohydrate in rats of different ages, sex and strains, using biochemical, histological and histochemical parameters. The involvement of the redox system, mainly of such oxidative enzymes as cytochrome oxidase, which contains copper, seems to be an interesting subject for investigation.

2. Study of the possible reversal of pancreatic atrophy by means of a re-established copper-supplemented diet and the possible role of various carbohydrates, especially FOS, therein.
3. With respect to lipid metabolism, elevation of serum cholesterol and sometimes of triglycerides has been reported in rats fed a fructose-enriched diet (Ovecka *et al.*, 1987). This treatment is associated with an increase in triglyceride very-low-density lipids (VLDL), probably resulting from increased hepatic formation of VLDL (Cunnane *et al.*, 1990; Verschoor *et al.*, 1985). As FOS is able to decrease triglyceride, cholesterol and phospholipid content in VLDL (Fiordaliso *et al.*, 1993), we can postulate that FOS could have a preventive role in complete copper deficiency associated with the development of cardiovascular pathology.

Such studies may elucidate the mechanisms involved in the pathology of copper deficiency and the role of different carbohydrates therein, thus opening new preventive and, perhaps, therapeutic perspectives.

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