

## The hypocholesterolemic effect of guar gum depends on dietary sucrose – studies in minipigs

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**Summary:** We examined the effect of guar gum on serum lipids if fed together with either 50.3 % starch or 50.3 % sucrose in a balanced diet to pigs. For this purpose, five adult hypercholesterolemic minipigs (total serum cholesterol 9.0 mmol/l) underwent three consecutive 8-week crossover (control or guar supplementation) feeding experiments (experiment I = cornstarch plus 15 g guar, experiment II = cornstarch plus 30 g guar, experiment III = sucrose plus 30 g guar per day).

With the cornstarch-based diet neither 15 g nor 30 g guar gum had an influence on serum total cholesterol or triglyceride levels. Also, the cholesterol concentrations in the lipoprotein fractions did not change significantly during experiments I and II, yet total serum cholesterol concentration was about 20 % lower ( $p < 0.01$ ) when guar gum was added to the sucrose diet in experiment III. In the presence of sucrose the supplementation of 30 g guar led to a significant decrease ( $p < 0.05$ ) of the cholesterol concentrations in the very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL). There was also a tendency for decreased cholesterol levels in the low-density lipoproteins (LDL) after adding 30 g guar to the sucrose diet.

Thus, the study demonstrates that guar gum exerts a hypocholesterolemic effect in the presence of sucrose in the diet, but not in the case of starch consumption.

**Zusammenfassung:** In der vorliegenden Untersuchung wurde bei Miniaturschweinen der Effekt von Guar Gum auf die Serumlipide bei gleichzeitiger Gabe von entweder 50,3 % Stärke oder 50,3 % Saccharose untersucht. Dazu durchliefen 5 adulte hypercholesterolämische Miniaturschweine (Gesamtserumcholesterol 9,0 mmol/l) jeweils nach einer Cross-over-Anordnung 3 Versuchsreihen von Fütterungsexperimenten mit jeweils 8 Wochen Dauer. (Experiment I = Maisstärke plus 15 g Guar; Experiment II = Maisstärke plus 30 g Guar; Experiment III = Saccharose plus 30 g Guar pro Tag.)

In Gegenwart von Maisstärke in der Diät hatten weder 15 g noch 30 g Guar Gum einen Einfluß auf die Serum-Cholesterin- oder -Triglyceridspiegel. Die Cholesterinkonzentration in den Lipoproteinfraktionen war während der Experimente I und II

### Abbreviation index

LDL = low-density lipoprotein

HDL = high-density lipoprotein

VLDL= very low-density lipoprotein

wt = weight

ebenfalls nicht signifikant beeinflußt. War hingegen Saccharose in der Diät enthalten, so senkte Guar den Serum-Cholesterinspiegel um ca. 20 % ( $p < 0,01$ ) (Experiment III). Bei dieser Diät konnte die Zulage von 30 g Guar pro Tag ebenfalls die Cholesterinkonzentration in den VLDL- und HDL-Fraktionen signifikant ( $p < 0,05$ ) herabsetzen. In Experiment III konnte auch eine tendenzielle Reduktion des Cholesterins in der LDL-Fraktion gemessen werden.

Die Untersuchung zeigt, daß Guar Gum einen hypocholesterolemischen Effekt in Gegenwart von Saccharose in der Diät entfaltet, nicht aber bei Stärke als Kohlenhydrat in der Diät.

*Key words:* Guar gum, serum lipids, mechanism, dietary carbohydrate, sucrose

*Schlüsselwörter:* Guar Gum, Serumlipide, Mechanismus, Diätkohlenhydrat, Saccharose

## Introduction

Guar gum is a storage galactomannan from the seeds of the Indian cluster bean (*Cyamopsis tetragonoloba*). Studies in humans (3, 6, 12), as well as in experimental animals (10, 31) have demonstrated that the oral ingestion of this gel-forming dietary fiber is accompanied by a reduction of serum cholesterol levels.

However, the mode of action of guar gum in lowering cholesterol levels is not yet fully understood. There are a number of mechanisms which have been proposed to explain the hypocholesterolemic action of guar gum.

It has been proposed that gel-forming dietary fibres such as pectin and guar gum bind bile acids and reduce intestinal absorption of cholesterol (13, 21, 25). According to this proposal the cholesterol-lowering effect is largely mediated by an enhanced fecal excretion of steroids (16). However, the weak negative correlation between serum cholesterol levels and acid and neutral steroid excretion in humans (25, 35) suggests that additional mechanisms may be operative.

Another explanation is the effect of guar gum on carbohydrate absorption and metabolism, because it has been shown to reduce postprandial hyperglycemia (9) and to diminish postprandial insulin secretion (17). This could be due to delayed gastric emptying (5, 23, 30), to altered transport barriers along the small intestine (15, 20), or a reduction in the secretion of the insulin stimulating gastrointestinal hormone GIP (gastric inhibitory polypeptide) (26) after guar gum ingestion. In this respect the lower postprandial insulin levels may decrease lipoprotein secretion, because insulin stimulates the secretion of VLDL lipoproteins (36).

A third mechanism involving an inhibition of hepatic cholesterol synthesis by short-chain fatty acids generated by a cecal bacterial fermentation (2) has been invalidated by earlier experiments from this laboratory (1).

In the aim to further our understanding of the hypocholesterolemic action of guar gum, we initiated studies to define the dietary conditions which make the hypolipidemic effect appear. During these studies, we observed the specific kind of dietary carbohydrate essential for lowering serum cholesterol levels by guar gum.

## Material and Methods

All chemicals used were of reagent grade and obtained from Merck, Darmstadt, FRG. The guar gum (Glucotard) was granulated and was a generous gift from Boehringer, Mannheim, FRG.

In all experiments the same five adult (4–5 years old) male Göttingen miniature pigs were used. They were individually housed in a room maintained at  $20 \pm 2$  grade C and 60%–70% humidity with 13 h (0600–1900 h) of light. The pigs were given 380 g per day dry matter of a semisynthetic diet (see below) to maintain constant body weight. This food was given in two equal amounts at 0600 and 1600 hours and was consumed completely in all experiments. Water was provided *ad libitum*.

### Diets

The diet (Table 1) simulated the gross composition of a western-style diet for humans, providing 18, 52, and 30% of energy from protein, carbohydrate, and fat, respectively. Crystalline cholesterol (1% wt/wt) and cholic acid (0.5% wt/wt) were mixed with the diet to increase the serum cholesterol levels, which in pigs normally range around 2 mmol/l (7, 33). The animals were therefore comparable to hypercholesterolemic patients needing hypolipidemic therapy.

### Experimental procedures

The whole investigation consisted of three sets of experiments (Experiment I, II, and III). All experiments were carried out as follows:

Table 1. Composition of diets.

Ingredient	Experiments I and II Amount g/100 g diet	Experiment III Amount g/100 g diet
Cornstarch <sup>1</sup>	50.3	—
Sucrose <sup>2</sup>	—	50.3
Casein <sup>3</sup>	20.0	20.0
Margarine <sup>4</sup>	7.3	7.3
Lard <sup>5</sup>	7.3	7.3
Cellulose <sup>6</sup>	5.6	5.6
Mineral/vitamin <sup>7</sup> mixture	8.0	8.0
Cholesterol <sup>8</sup>	1.0	1.0
Cholic acid <sup>9</sup>	0.5	0.5

<sup>1</sup>Cornstarch was obtained from Maizena Industrieprodukte, Krefeld, FRG (Sirona).

<sup>2</sup>Sucrose was obtained from Merck, Darmstadt, FRG (No. 7651). <sup>3</sup>Casein was an acid-precipitated preparation from Bayerische Milchversorgungsbetriebe, Nuremberg, FRG (Biogen-Serolact P 65, 15% N on moisture-free basis). <sup>4</sup>Margarine („Sana“ from Seibel, Kiel, FRG) had a P/S ratio of 0.60. <sup>5</sup>Lard („Comberg Lard“ from Comberg, Kiel, FRG) had a P/S ratio of 0.20. <sup>6</sup>Cellulose used was a wood cellulose powder (No. 123) from Schleicher & Schüll, Dassel, FRG. <sup>7</sup>Composition (grams/kilogram diet): CaCO<sub>3</sub>, 18.0; CaHPO<sub>4</sub> · 2 H<sub>2</sub>O, 22.0; Ca (H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> · H<sub>2</sub>O, 5.1; K<sub>2</sub>CO<sub>3</sub>, 7.5; MgO, 6.8; NaCl, 7.6; (milligrams/kilogram diet): FeSO<sub>4</sub> · 7 H<sub>2</sub>O, 610; ZnO, 313; MnO<sub>2</sub>, 198; CuSO<sub>4</sub> · 5 H<sub>2</sub>O, 197; KI, 6; CoSO<sub>4</sub> · 7 H<sub>2</sub>O, 5; Na<sub>2</sub>Se, 1; retinyl acetate, 53, 000 IU; cholecalciferol, 6200 IU; allrac- $\alpha$ -tocopheryl acetate, 314; ascorbic acid, 931; thiamin HCl, 9.6; pyridoxine HCl, 16; riboflavin, 23; cyanocobalamin, 0.17; menadione, 23; Ca-pantothenate, 66; nicotinic acid, 77; choline chloride, 8600; biotin, 0.9; and folic acid, 5. <sup>8</sup>Cholesterol (No. 3670) and <sup>9</sup>cholic acid (No. 222) were obtained from Merck, Darmstadt, FRG.

The experiment was preceded by a 4-week adaptation period. During this adaptation period all animals consumed the diets listed in Table 1. Following adaptation the nutritional experiment consisted of two 4-week periods (periods A and B). In period A, three pigs continued on that diet, whereas the other two had an additional guar gum supplementation. In period B the treatments were changed (crossover). The guar gum was mixed immediately before the feeding with the diet and was completely consumed.

Blood samples were taken by puncture of the vena jugularis on days 21, 26, and 28 of periods A and B before the morning meal. Body weights were recorded twice a month. The average body weight was  $34.2 \pm 2.9$  kg (mean  $\pm$  SEM) at the beginning of the investigation and did not change significantly during any experiment.

*Experiment I:* This experiment was carried out with cornstarch as the carbohydrate component. During the guar period each animal received 15 g guar gum per day.

*Experiment II:* This experiment was carried out in the same manner and with the same diet as experiment I. The only difference was an increased guar gum dose of 30 g per animal and day.

*Experiment III:* During experiment III, 50.3 % (wt/wt) sucrose was fed instead of cornstarch (Table 1). The guar gum dose of 30 g per animal and day was as in experiment II.

#### *Analytical procedures*

Blood samples were analyzed for total serum and lipoprotein cholesterol and total triglyceride levels. Cholesterol levels were determined according to Röschlau et al. (32). Separation of VLDL was achieved by flotation at 1.006 g/ml in an Airfuge (Beckman Instruments, Munich, FRG) according to Bronzert and Brewer (8). High-density lipoprotein (HDL) cholesterol was determined in the supernatant obtained after adding 1.3 g/l of dextrane sulfate plus 0.13 mol/l of  $MgCl_2$  to whole plasma (24). Low-density lipoprotein (LDL) was calculated by subtraction. Total recovery of cholesterol in the three lipoprotein fractions was greater than 92 % during all three experiments. The determination of triglyceride levels was done according to Wahlefeld (38). Statistical evaluation was done by using an ANOVA, followed by Tukey's test (14).

## **Results**

The high total cholesterol concentrations during all control periods (see Tables 2 and 4) of about 9 mmol/l (i.e., 347 mg/dl) are similar to the typical values observed in hypercholesterolemic patients for whom hypolipidemic treatment is desirable.

Neither 15 g nor 30 g guar gum per day significantly changed serum cholesterol levels, nor did it affect serum triglyceride levels when supplied with a cornstarch based diet (Table 2). Also, the cholesterol concentrations in the lipoprotein fractions were not changed significantly by one of the guar doses with the cornstarch diet (Table 3).

Figure 1 shows total serum cholesterol concentrations obtained at each day of blood sampling. Statistical evaluation showed that the cholesterol levels did not change significantly between days 21 and 28 within an experimental period, indicating that a metabolic steady state was attained. Figure 1 confirms that guar gums was not hypocholesterolemic in the case of starch feeding. Only in the presence of sucrose did the supplementation of 30 g guar gum per day lead to a significant 20 % decrease of cholesterol levels ( $p < 0.01$ ).

Table 2. Total serum cholesterol and triglyceride concentration of minipigs ( $n = 5$ ) after supplementation of a diet rich in cornstarch with 15 g/day (experiment I) or 30 g/day (experiment II) of guar gum (Means  $\pm$  SEM). Control and guar-supplemented values not sharing the same superscript are significantly different at the  $p < 0.05$  level.

		Total cholesterol	Total triglyceride
		mmol/l	
Experiment I	Control	8.90 <sup>a</sup> $\pm$ 1.44	0.336 <sup>c</sup> $\pm$ 0.083
	15 g guar	8.46 <sup>a</sup> $\pm$ 1.26	0.349 <sup>c</sup> $\pm$ 0.073
Experiment II	Control	9.18 <sup>b</sup> $\pm$ 1.48	0.357 <sup>d</sup> $\pm$ 0.062
	30 g guar	9.40 <sup>b</sup> $\pm$ 1.67	0.396 <sup>d</sup> $\pm$ 0.099

Table 3. Cholesterol concentrations in serum lipoprotein fractions of minipigs ( $n = 5$ ) after supplementing a diet based on cornstarch with 15 g/day (experiment I) or 30 g/day (experiment II) of guar gum (Means  $\pm$  SEM). Control and guar values not sharing the same superscript are significantly different at the  $p < 0.05$  level.

		VLDL	HDL	LDL
		mmol/l		
Experiment I	Control	1.16 <sup>a</sup> $\pm$ 0.50	2.48 <sup>c</sup> $\pm$ 0.28	4.68 <sup>e</sup> $\pm$ 1.07
	15 g guar	1.37 <sup>a</sup> $\pm$ 0.45	2.70 <sup>c</sup> $\pm$ 0.36	3.75 <sup>e</sup> $\pm$ 0.70
Experiment II	Control	1.86 <sup>b</sup> $\pm$ 0.62	2.41 <sup>d</sup> $\pm$ 0.26	4.47 <sup>f</sup> $\pm$ 0.62
	30 g guar	1.46 <sup>b</sup> $\pm$ 0.60	2.04 <sup>d</sup> $\pm$ 0.19	5.51 <sup>f</sup> $\pm$ 1.02

Table 4. Cholesterol concentrations in lipoprotein fractions and total serum as well as total triglyceride concentrations in serum of minipigs ( $n = 5$ ) after supplementation of a diet rich in sucrose (experiment III) with 30 g/day of guar gum (Means  $\pm$  SEM). Means in the same horizontal line not sharing a common superscript letter are significantly different ( $p < 0.05$ ).

Fraction	Control	30 g guar
mmol/l		
VLDL cholesterol	1.64 <sup>a</sup> $\pm$ 0.60	0.81 <sup>b</sup> $\pm$ 0.44
HDL cholesterol	2.28 <sup>a</sup> $\pm$ 0.13	1.78 <sup>b</sup> $\pm$ 0.17
LDL cholesterol	4.49 <sup>a</sup> $\pm$ 0.84	4.01 <sup>a</sup> $\pm$ 0.93
Total serum cholesterol	9.04 <sup>a</sup> $\pm$ 1.52	7.18 <sup>b</sup> $\pm$ 1.44
Total serum triglyceride	0.302 <sup>a</sup> $\pm$ 0.056	0.318 <sup>a</sup> $\pm$ 0.062

Serum total triglycerides were not changed by 30 g of guar (Table 4). This table shows that the addition of 30 g guar per day to the sucrose diet lowered the VLDL and HDL cholesterol levels significantly ( $p < 0.05$ ). With the LDL cholesterol concentration there was also a tendency of decrease, but it failed to reach the 5 % level of significance.

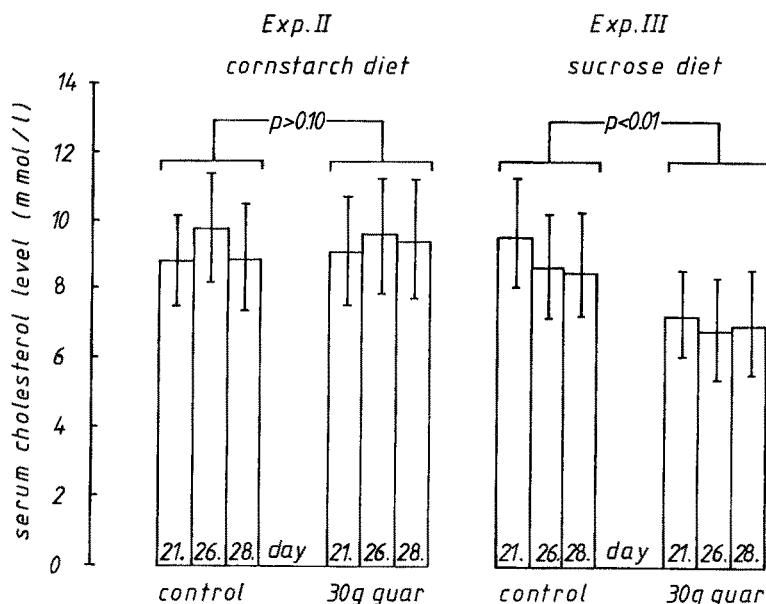


Fig. 1. Response of total serum cholesterol levels to guar gum during a cornstarch or sucrose diet. Guar gum supplementation amounted to 30 g/day. Means  $\pm$  SEM are given ( $n = 5$ ).

## Discussion

We report herein that guar gum exerts a hypocholesterolemic effect in the Göttingen miniature pig if the dietary carbohydrate consists of sucrose, but not in the case of starch consumption.

We think that this finding is of significance for the mechanism of guar action and for the dietary guidance of hyperlipidemic patients.

As mentioned above, two major biochemical mechanisms have been considered for the hypocholesterolemic action of guar:

- 1) a drainage of steroids by a rise of fecal excretion (13, 16, 21, 25) or
- 2) an interference of guar gum with nutrient absorption, especially of carbohydrates (5, 9, 15, 20, 23, 30). It is our proposal that the data presented herein strongly suggest the second mechanism to be operative. The following evidence may be cited in favor of this proposal.

There is ample biochemical evidence linking high glucose and/or fructose concentration and insulin with a high rate of hepatic secretion of VLDL cholesterol into the plasma compartment. For example, glucose and insulin have been repeatedly shown to enhance VLDL secretion by the isolated perfused rat liver (36).

Moreover, the same authors have shown that there was a parallel rise of hepatic cholesterol biosynthesis. Insulin has also been shown to raise the activity of  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA reductase (E.C. 1.1.1.34) in cultured hepatocytes, this being the rate-controlling enzyme of cholesterol biosynthesis (34). All these biochemical changes can metabolically con-

tribute to hypercholesterolemia and any agent which is able to reduce the postprandial rise of glucose, fructose or insulin can be supposed to prevent hypercholesterolemia.

However, starch and sucrose intake cause a different postprandial plasma glucose, fructose, and insulin responses. Whereas sucrose consumption is followed by a steep rise of glucose, fructose, and insulin, this is much less so in the case of starch (11).

Because sucrose has been reported to raise serum cholesterol above levels found in starch feeding (27, 29) and guar gum is known to delay carbohydrate absorption and to reduce postprandial hyperinsulinemia (17), we propose that guar gum exerts its hypocholesterolemic effect mainly via interference with carbohydrate absorption and subsequent modification of plasma insulin and hepatic VLDL cholesterol secretion. This mechanism is obviously more operative than is a gastrointestinal mechanism that affects fecal steroid excretion via bile acid and cholesterol binding by the dietary fiber.

It is up to future research to prove a delay of glucose absorption and reduced serum insulin concentrations following guar under our experimental conditions a pursuit which was, unfortunately, not feasible in the experiments reported here now, because of biochemical reasons.

Moreover, these considerations help to clarify when and when not the intake of guar gum may be of therapeutical benefit. Guar gum can be expected to be of particular value where a hyperlipidemia is based on states of hyperglycemia and hyperinsulinemia. This is particularly the case in maturity onset diabetes and obesity. In other cases of hyperlipidemia, guar gum seems unlikely to be very effective.

Guar gum did not lower serum triglycerides under our experimental conditions. This is in agreement with the majority of clinical studies that showed no significant hypotriglyceridemic effect of guar gum (18, 19, 39). From the proposed action mechanism a lowering of triglyceride concentrations had to be anticipated. However, as the plasma triglyceride concentration results from the rate of secretion of VLDL triglyceride and from the rate of removal by lipoprotein lipase it might be that a compensatory change of the latter enzyme annihilates any hypotriglyceridemic effect. The importance of lipoprotein lipase activity for carbohydrate-induced hypertriglyceridemia has been observed and discussed by several authors (4, 22, 28, 37).

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