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Viscous soluble dietary fibers alter emulsification and lipolysis of triacylglycerols in duodenal medium in vitro

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The present in vitro study was designed to test the hypothesis that soluble dietary fibers can alter the process of lipid emulsification, and possibly, subsequent triglyceride lipolysis. Three guar gums, two pectins, and gum arabic were dissolved in reconstituted duodenal medium in the range 0.3 to 2.0% (w/v). Viscosities of solutions were measured. Emulsification of a lipid mixture (triolein/phospholipids/cholesterol) was performed under mild conditions in the presence of increasing concentrations of soluble fibers. The amount of emulsified lipids was reduced and the size of emulsified droplets was increased by raising the concentration of viscous fibers only. The extent of emulsification ($r = -0.79$), the droplet size ($r = 0.88$), and the overall droplet surface area ($r = -0.59$) were strongly correlated to the medium viscosity in the range 0–20 mPa.s. Addition of solutions of viscous fibers to a preformed standard emulsion did not change the lag time and initial velocity of pancreatic lipase reaction. Conversely, when incubating emulsions prepared in the presence of fibers (with different droplet sizes) with excess enzyme for 2 hours, the high- and medium-viscosity guar gums significantly reduced the extent of triglyceride lipolysis only. Thus, reducing emulsification of dietary lipids is a mechanisms by which soluble viscous fibers can alter lipid assimilation. (J. Nutr. Biochem. 7:293–302, 1996.)

Keywords: fibers; guar gum; pectin; gum arabic; triglycerides; emulsion; pancreatic lipase

Introduction

In humans and most mammals, fat digestion begins in the stomach[1,2] and is then completed in the small intestine where absorption of lipolytic products occurs.[1] Due to the insoluble nature of triacylglycerols, the characteristic feature of gastric and pancreatic lipases is that both enzymes act at the lipid/water interface.[1–13] We recently described in healthy humans[14,15] and in the rat[16] that dietary lipids are basically emulsified in the stomach and duodenum contents, with most droplet sizes ranging from 10 to 50 μm. In those conditions of catalysis, the concentration and the properties of the emulsion interface govern the extent of enzyme binding onto the interface and the rate of lipolysis.[2,13] In fact, we recently showed in vitro that the size and composition of the emulsified droplets alter the binding[17] and the hydrolytic activity of pancreatic lipase.[17,18] This was also observed in the rat digestive tract.[16]

The effects of dietary fibers on digestion, gut physiology, and lipid metabolism have been extensively documented in laboratory animals and humans but most mechanisms involved remain to be fully elucidated.[19–21] Several studies have reported that different sources of dietary fiber can lower the activity of pancreatic lipase in vitro,[22–25] rat lipolysis in the small intestine,[26–28] and lipid absorption in vivo.[26–31] Some kinds of soluble fibers such as guar gums, pectins, or oat beta-glucans have the ability of making so-

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lutions viscous[32] and of increasing the viscosity of the meals and the digestive contents.[33–37] These kinds of fibers have also been reported to delay stomach emptying,[38] to regulate digestive organ motility[35] and to delay or reduce the absorption of nutrients.[29–31,34,36,39–42]

The mechanisms by which soluble dietary fibers might interfere with triacylglycerol hydrolysis have not yet been investigated in details. We hypothesized that soluble fibers could alter emulsification and lipolysis of dietary triacylglycerols in the digestive tract, with possible involvement of viscosity-mediated mechanisms. To test this hypothesis, we designed appropriate *in vitro* experiments. A separate experiment was dedicated to studying the effects of fibers in the acidic stomach medium.[21] In the present study, we investigated the effects of five well-known types of soluble fibers (guar gums, pectins, gum arabic) exhibiting different viscosities or electric charges in solution, in *in vitro* conditions closely mimicking those found in the duodenum content. Solution viscosity, extent of emulsification, and rate of lipolysis of triacylglycerols catalyzed by pancreatic lipase were measured. The data obtained showed that soluble fibers can reduce the extent of emulsification of dietary lipids in conditions mimicking those found in the duodenum and that viscosity is the key parameter controlling this process. This can result in a reduction of the rate of lipolysis of emulsified triacylglycerols in vitro.

Methods and materials

Dietary fibers

Five different sources of soluble dietary fibers, usual additives or thickeners in food industry, were used. Gum arabic (Sigma, St Quentin Fallavier, France), three guar gum preparations (MRS-Sanofi, Beaupte, France) with different molecular weights (HVG, high molecular weight; MVG, medium molecular weight; LVG, low molecular weight) and two apple pectins (MRS-Sanofi, Beaupte, France) with different degrees of methylation [NND (27 NAND) and BNF (Brun NF Pomme)].

Uronic acid was measured by automated metahydroxydiphenyl.[43] Individual sugars were analyzed as their alditol acetate derivatives by gas-liquid chromatography.[44] Proteins were measured by semi-automatic micro-Kjeldahl method using a conversion factor of 6.25. Methanol was determined by high pressure liquid chromatography as described.[45] Analyses were performed in duplicate.

Fibers were dissolved in buffer solutions under stirring at room temperature or 80°C in a water bath and allowed to solubilize overnight under gentle stirring. After filtration and centrifugation, the actual fiber concentration of the stock solution was measured after oven drying (100°C, 24 hours). On the whole, final concentrations ranged from 0 to 2% to fit with fiber concentrations found in the digestive tract.

The buffer solutions used for viscosity and emulsification measurements contained 50 mmol/L Tris-HCL and 150 mmol/L NaCL at pH 7.50 and were prepared with ultrapure water (Milipore MilliQ system, Saint Quentin en Yvelines, France). The pH selected during this study was 7.50 because it falls within the pH range (6.50 to 8.0) found in the intestinal contents during digestion (1) and is in the range of optimal pH (7.0 to 9.0) for pancreatic lipase activity.[13]

Viscosity measurements

Flow curves at 37.0 ± 0.1°C of solutions of arabic gum (4 to 12 g/L), guar gums (HVG: 0.1–3.1 g/L; MVG: 0.5 to 5.6 g/L; LVG: 0.7 to 24.4 g/L) and pectins (NND: 1.1–16.0 g/L; BNF: 1.1 to 16.8 g/L), in the presence or absence of bile lipids, were determined by means of a coaxial cylinder viscometer Low Shear 40 (CONTRAVES) equipped with the 412 measuring system (r_1 = 6.0 mm, r_2 = 6.5 mm, h = 18 mm) in the shear rate range 0 to 100 s-1. To take into account the fact that the shear rate in each point is proportional to the distance from the axis of the inner cylinder, small gaps between cylinders (r_1/r_2 = 0.9) were used to minimize variation in the shear rate and an average distance between cylinders have been used for calculations. Thus, no further corrections have been made in the case of non-Newtonian behavior.

Newtonian viscosity in the low shear rate range (ηN) was determined from each solution and intrinsic viscosity [η] of the polymer was calculated by linear regression using either Huggins ($(\eta N - \eta_S)/\eta_S = [\eta]c + k_H [\eta]^2 c^2$) or Kraemer ($\text{Log}\ (\eta N/\eta_S) = [\eta]c - k_K [\eta]^2 c^2$) relations, where η_S is the viscosity of the solvent, c is the concentration of the polysaccharide, k_H is the Huggins constant, and k_K is the Kraemer constant.

Preparation of emulsions

A lipid mixture was made with 97% (wt/wt) triolein (95% pure; Sigma, Saint Quentin Fallavier, France), 2.5% egg lecithin (95% pure; Prolabo, Paris, France), and 0.5% free cholesterol (99% pure; Sigma) at a final concentration of 16% (wt/wt) in chloroform/methanol (2:1 v/v).

One mL aliquots of the lipid mixture solution were evaporated to dryness under nitrogen in 7 ml glass tubes. A fixed volume (500 µL) of a pool of porcine bile samples (obtained at the slaughterhouse just after sacrifice) was added as well as buffer solution (with or without fiber) to a final volume of 4 mL. The aqueous mixture thus contained 4% w/w lipids, 8.0 mmol/L mixed bile salts and 0 to 2% fiber, i.e., in the physiological range.[1] The tubes were stoppered, attached horizontally and shaken at 200 strokes/min for 2 hours at 37°C. These operating conditions were defined during preliminary experiments dedicated to get appropriate conditions generating lipid emulsions with droplet sizes in the range of those found in the human and rat digestive tract during fat digestion.[14–16]

Emulsification measurements

Determination of the amount of emulsified lipids. The upper limit for emulsion droplet size was set at 100 µm, given the poor stability and the negligeable interfacial area provided by lipid droplets above this value.[14,15,16] To allow accurate measurements, [carboxyl-^{14}C]triolein (98% pure, 69 mCi/mmol, CEA, Gif-sur-Yvette, France) was added in tracer amounts to the lipid mixture. Radioactivity measurements were done by scintillation counting using a Packard 1600TR equipment (Packard, Meriden, CT USA). At the end of the emulsification process, the tubes were left to stand in vertical position for a defined time: this allowed the building of a floating oily layer made of unemulsified lipids (oily material plus lipid droplets ≥100 µm) above the infranatant aqueous solution. The time needed (range: 1 to 75 min) was calculated from the Stoke's sedimentation equation for droplets ≥100 µm in the different viscosity conditions obtained, as previously reported.[14] According to the Stokes's sedimentation law, the relation between sedimentation time and particle diameter is expressed in the following equation:

$$D = (18 n_o H/(r - r_o)gt)^{1/2}$$

where D is the particle diameter (m), n_o is the viscosity coefficient of solvent (N.s/m^2), r is the density of sample (kg/m^3), r_o is the density of solvent (kg/m^3), t is the sedimentation time (sec), and H is the distance of sedimentation (m). Droplet size measurements in

the infranant confirmed the absence of droplets larger than 100 μm. Aliquots (100 μL) of the resulting infranatant containing emulsified lipid droplets were assayed for radioactivity.

Droplet size measurement. The distribution of the emulsion droplet sizes was determined by using a particle-size analyser (Capa 700, Horiba, Kyoto, Japan) as previously described.[14–18] The validity of the data were checked using calibrated microparticles in the size range of 0.2 to 100 μm (polystyrene size standard kit, Polyscience Inc., Warrington PA USA) as before.[14] Infranatants were diluted in saline buffer to obtain an optical density in the range of 0.5–1.0 O.D. at 560 nm. Measurements were done using gradient mode analysis at a constant centrifuge acceleration rate (960 rpm/min) to allow an accurate measurement of large droplets (100 μm) as well as small droplets (0.1 μm). Results are given in the form of a frequency distribution graph characterized by its median diameter (μm). From the distribution of particle size classes obtained for a given emulsion, the particle-sizer software calculated the specific interfacial area (Sw) exhibited, from $Sw = 6/dx \sum_{i=1}^{n} (F'_i/D_i)$ with d: particle density and F'_i: fraction of particles with a given diameter D_i. Sw was expressed in m^2/g of lipid particle and the total droplet surface area (m^2) was calculated from the amount (g) of emulsified lipids present in the infranatant.

Pancreatic lipase catalyzed-hydrolysis of emulsions. Purified porcine pancreatic lipase (E.C.3.1.1.3) was purchased from Boehringer Mannheim (Mannheim, Germany). Its specific activity was 1,400 units/mg. Porcine pancreatic colipase came from Boehringer Mannheim. The specific activity of this pure preparation was 1,530 units/mg of protein. In both cases, one unit was defined as 1 μ equivalent fatty acid titrated per min.

Pancreatic lipase reactions were carried out at 37°C and pH 7.50 in two different conditions allowing measurement of the enzyme initial velocity either in the presence of excess amounts of a standard emulsified substrate or with excess amount of enzyme acting on emulsions with different droplet sizes.

Measurement of the enzyme initial velocity on a standard emulsion. The initial enzyme velocity was measured using a pH stat titrator (Metrohm, Herisau, Switzerland), as previously described.[3,13,17,18] Lipase activity was recorded until the maximum velocity was exhibited for three min. The time needed to obtain linear steady-state recording was defined as the lag time.[13] The reaction medium was a 15 mL mixture (pH 7.50) containing 5 mmol/L Tris-HCL, 150 mmol/L NaCl, 6 mmol/L $CaCl_2$, 8 mmol/L bile salts and 3.4 mmol/L phospholipids (in the form of porcine bile), 0.3% fiber or not (control), and 2.5 mL of a standard emulsion providing excess substrate. The standard emulsion (100 mL, 20% w/w) was made by sonication of the lipid mixture described above for 30 min at 4°C; its droplet median diameter was 4.52 ± 1.85 μm. The reaction medium was mechanically stirred at 200 rpm. Pancreatic lipase ($4.5\ 10^{-8}$ mol/L) and colipase ($9\ 10^{-8}$ mol/L) were added at a fixed molar ratio (1:2), as found in physiological conditions.[1] At the end of the reaction time, the liberated free fatty acids were fully titrated at pH 9.50 as in previous studies.[14,15,16,17,18,46] One lipase unit was defined as 1 μmol fatty acid liberated per min.

Hydrolysis of different emulsions by pancreatic lipase. Emulsions were prepared by using the exact procedure as used for emulsification measurements with the lipid mixture added with [carboxyl-^{14}C]triolein in the presence or not of 0.3% fibers in buffer solution (500 mmol/L Tris-HCL, 150 mmol/L NaCL; 6 mmol/L CaCL2, 8 mmol/L bile salts and 3.4 mmol/L bile phospholipids, pH 7.50). Aliquots (500 μL) of emulsions were added with pancreatic lipase (14 μg) and colipase (5.6 μg) in eppendorf tubes and incubated under moderate stirring (100 strokes/min) and 37°C for various periods of time up to 120 min. It was checked that these incubation conditions did not lead to further emulsification or de-emulsification. Aliquots (300 μL) of the reaction media were collected, generated free fatty acids were extracted according to Belfrage and Vaughan[47] and radioactivity measured. Fibers were found to slightly modify the partition coefficient of free fatty acids during this partitioning procedure. Thus, actual partition coefficients were determined in the presence of each fiber used by radioactivity measurement of free fatty acids isolated by thin-layer chromatography[14] from both phases. Actual triacylglycerol hydrolysis is expressed as percent of potentially liberable free fatty acids (45 μmoles), i.e. 2 free fatty acid moities per triglyceride molecule.

Statistics

All experiments were done in triplicate and the results are expressed as means ± SEM. The statistical significance of the differences observed was assessed by one-way analysis of variance (ANOVA) and Fisher's test ($P < 0.05$) by using Statview II microcomputer program (Abacus, Berkeley, CA). This was done for different fiber preparations at a given concentration (0.3%) or at different concentrations of a given fiber. Correlation coefficients were obtained from regression analyses using the Statview II program.

Results

Characterisation of soluble dietary fibers

As shown in *Table 1*, the fibers used showed high sugar contents and mainly differed by their monomer composition and intrinsic viscosities. The guar gums were industrial food grade and sugars (mainly galactose and mannose) accounted for 95% of dry matter. According to literature, guar gum polysaccharide is essentially a straight-chain mannan with single-membered galactose branches.[48] The three gums

Table 1 Chemical composition and viscosity of the dietary fibers used

	HVG[a]	MVG	LVG	NND	BNF	GA
Rhamnose	0.2[b]	0.2	0.0	0.9	0.9	10.2
Arabinose	0.6	0.4	0.4	0.3	1.9	25.4
Xylose	0.2	0.0	0.0	1.1	0.9	0.0
Mannose	57.8	57.4	58.5	0.0	0.3	0.0
Galactose	36.4	36.4	37.2	4.9	4.9	35.9
Glucose	0.9	0.6	0.5	15.5	16.8	0.0
Uronic acid	0.0	0.0	0.0	61.9	59.6	1.0
N × 6.25 (%)	0.5	0.6	0.9	0.8	2.8	2.6
Methylation (%)				42.0	73.6	
$[\eta]^c$	1138.0	673.7	143.9	217.4	213.2	20.4
$[\eta]^d$	1294.0	694.7	143.7	246.0	237.9	20.7
$[\eta]_{app}$[e]	21.8	10.2	1.6	1.5	1.5	0.8

[a]HVG: High viscosity guar gum; MVG: medium viscosity guar gum; LVG: low viscosity guar gum; NND and BNF: apple pectins; GA: gum arabic.
[b]The sugar composition is given as relative content (% of total) of individual sugar. $[\eta]^c$ Intrinsic viscosity calculated from Huggins relation (mPa.s). $[\eta]^d$ Intrinsic viscosity calculated from Kraemer relation (mPa.s).
[e]Apparent viscosity (mPa.s) at 0.3 % fiber concentration and pH = 7.5 (for details, see text).

have comparable chemical compositions but differed mainly by their molecular weight, i.e., 1.5 10^6 (HVG), 9 10^5 (MVG), and 1 10^5 Da (LVG) as estimated from intrinsic viscosity by using the Mark-Houwink relation according to Robinson et al.[49]

The two apple pectins (commercial grade) showed comparable overall chemical compositions with a high content in galacturonic acid, except for the degree of esterification of the uronide carboxyl group with methyl alcohol (NND, 42.0% and BNF, 73.6%). Apple pectin has been described as a polymer containing mainly homogalacturonic regions interrupted with hairy regions composed of a rhamnogalacturonic backbone carrying side-chains of arabinose, galactose and xylose.[50] The intrinsic viscosities were comparable. At the pH used (7.5), the two pectins were in their respective ionized form, negative charges being neutralized by medium counterions.

Gum arabic was mainly composed of galactose, arabinose and to a lower extent rhamnose. Compared to the other fibers, it showed a low intrinsic viscosity. According to literature, gum arabic is a highly branched polyanion with high molecular weight (0.5 to 1.4 .10^6) and is composed of six carbohydrate moities with an associated protein component.[51]

Viscosities of fiber solutions in reconstituted duodenal medium

The five soluble fibers tested were divided into three groups on the basis of their rheological properties (*Figure 1*). The apparent viscosity of a given fiber was related to its concentration and hydrodynamic volume in solution. At a given concentration, HVG and MVG yielded the highest viscosities, the LVG and the two pectins showed intermediate viscosities and gum arabic the lowest viscosity. The rheological behaviour of the pectins and gum arabic was Newtonian over the share rate range 0 to 100 s^{-1} at all tested concentrations. The solution viscosities of guar gums increased in a log linear manner up to a concentration of 0.7% for LVG, 0.3% for MVG, and 0.08% for HVG; beyond these critical concentrations, the solutions were non-Newtonian.

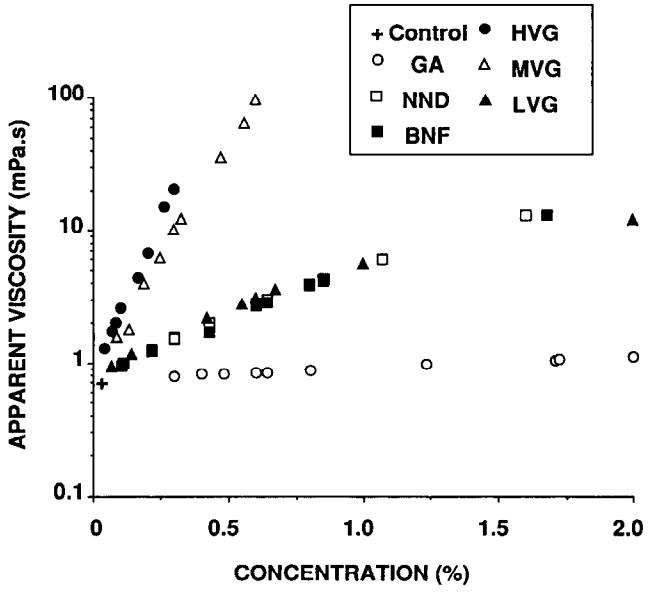

Figure 1 Effect of concentration of soluble fibers on the viscometric behaviour of reconstituted duodenum medium at 37°C. Apparent viscosity at low shear rates for each final composition of the medium was deduced from the flow curve measured with the Low Shear 40 in the 0–100 s^{-1} shear rate range. G.A.: Gum Arabic; HVG: High Viscosity Guar gum; MVG: Medium Viscosity Guar gum; LVG: Low Viscosity Guar gum. NND: 42.0% methylated pectin; BNF: 73.6% methylated pectin.

Table 2 Effects of fibers on the extent of lipid emulsification and the dropet size of emulsions

Medium	Fiber concentration (%)	Emulsified triglycerides (%)	Median droplet diameter (μm)
Gum arabic	2.0	85.84 ± 3.36^{b}	4.92 ± 0.60^{y}
	0.6	90.42 ± 1.38^{c}	9.02 ± 0.78^{y,z}
	0.3	57.71 ± 1.80^{d}	14.96 ± 1.63^{x,z}
	0	68.46 ± 0.41^{a}	18.52 ± 2.45^{x}
HV guar	0.3	24.74 ± 0.43^{b}	31.48 ± 1,28^{y,z}
	0.2	61.94 ± 2.64^{c}	35.40 ± 6.67^{y}
	0.1	39.35 ± 1.04^{d}	17.81 ± 1.20^{x,z}
	0	68.46 ± 0.41^{a}	18.52 ± 2.45^{x}
MV guar	0.6	26.89 ± 0.77^{b}	26.26 ± 2.87^{y}
	0.3	32.31 ± 1.24^{c}	35.70 ± 1.80^{y}
	0.1	38.17 ± 0.67^{d}	17.20 ± 2.40^{x}
	0	68.46 ± 0.41^{a}	18.52 ± 2.45^{x}
LV guar	2.0	26.97 ± 0.72^{b}	29.07 ± 3.71^{y}
	0.6	48.72 ± 0.80^{a,b}	16.56 ± 1.91^{x}
	0.3	72.02 ± 1.30^{a}	16.66 ± 2.79^{x}
	0	68.46 ± 0.41^{a}	18.52 ± 2.45^{x}
Pectin NND	0.8	38.21 ± 1.55^{b}	21.75 ± 5.06^{x}
	0.6	35.27 ± 1.04^{b}	16.87 ± 2.20^{x}
	0.3	66.07 ± 1.93^{a}	13.80 ± 1.73^{x}
	0	68.46 ± 0.41^{a}	18.52 ± 2.45^{x}
Pectin BNF	0.8	36.99 ± 1.41^{b}	24.34 ± 2.09^{y}
	0.6	52.50 ± 2.51^{c}	12.65 ± 1.43^{x,z}
	0.3	64.28 ± 2.37^{a}	10.84 ± 0.78^{z}
	0	68.46 ± 0.41^{a}	18.52 ± 2.45^{x,y}

Emulsified material was defined as emulsified droplets with diameter below 100 μm and is expressed as % of total lipid present and median diameter was calculated from droplet size distribution by the particule sizer software (for details, see text). HVGuar: high viscosity guar gum; MVGuar: medium viscosity guar gum; LVGuar: low viscosity guar gum; NND Pectin: 42% methylation; BNF Pectin: 73.6% methylation. Values are mean ± SEM of three determinations. For a given fiber, different superscript letters indicate significant differences (one-way ANOVA; $P < 0.05$), in either emulsified triglycerides (^{a,b,c}) or droplet diameter (^{x,y,z}).

Effects of fibers on the extent of emulsification

To assess the effects of soluble fibers on fat emulsification we measured the amount of emulsified lipids and the size of the emulsified lipid droplets produced and finally, calculated the surface area generated.

Amount of emulsified lipids. As shown in *Table 2,* an important extent of emulsification (68.5% of lipid present) was reached in the selected control conditions. At low concentrations (0.1 to 0.3%), LVGuar, NND, and BNF pectins and gum arabic did not change the extent of emulsification. LVGuar significantly decreased the amount of emulsified

fat at higher concentrations (0.6% and 2.0%) whereas gum arabic had an opposite effect. MVGuar and HVGuar markedly reduced emulsification even at a low concentration (0.1%) and further lowered these figures at higher concentrations (0.6 and 0.3%, respectively). The two pectins lowered the amount of lipids emulsified to a comparable extent at 0.6% and more markedly, at 0.8% concentration. The highest reduction in the extent of emulsification was 2.7 fold with HVGuar at 0.3%. At a comparable concentration (0.3%), the effect of fibers on the amount of emulsified lipids ranged in the following order: HVGuar > MVGuar > LVGuar = NNDpectin = BNFpectin = gum arabic.

Size of the emulsified lipid droplets. Values obtained from droplet size measurements of emulsified lipids are given in *Table 2*. In the control buffer solution, the median diameter exhibited by the emulsion was 18.5 μm. LVGuar did not change the median particle size at 0.1 and 0.3% but markedly increased this figure at 2.0% concentration (29.1 μm). MVGuar and HVGuar significantly increased the median diameter of the droplets from 0.3% (35.7 μm) and 0.2% (35.4 μm), respectively. On the contrary, gum arabic significantly reduced the droplet size at 0.6% (9.0 μm) and above. Also, NNDpectin and BNFpectin increased the droplet median diameter to a comparable extent at 0.8% concentration (21.8 and 24.3 μm, respectively). The comparative effect of fibers at a given low concentration (0.3%) is illustrated in *Figure* 2A. The data clearly show that only highly viscous solutions of fibers, namely MVGuar and HVGuar, have the capability of increasing the median diameter of the emulsified droplets in duodenum conditions. The effects of guar gums at 0.3% were evaluated in a more detailed way. As shown in *Figure 3*, solutions of MVGuar and HVGuar significantly lowered the proportion of small-sized droplets (1 to 20 μm) and generated large-sized droplets (50 to 90 μm), as compared with the control and LVGuar solution.

Table 3 Effects of gum arabic and guar gums (0.3%) on pancreatic lipase hydrolytic activity

	Standard emulsion ± fiber solution		Triglyceride hydrolyzed (% FFA releasable)
	Initial velocity (μmoles FFA/min)	Lag time (min)	Emulsion prepared with/ without fiber
Control	10.1 ± 0.5[a]	4.9 ± 0.1[a]	55.7 ± 4.9[a]
Gum arabic	9.5 ± 0.5[a]	5.0 ± 0.5[a]	56.2 ± 1.1[a]
LV guar	9.5 ± 0.5[a]	4.2 ± 0.2[a]	58.1 ± 3.8[a]
MV guar	9.7 ± 0.2[a]	5.0 ± 0.1[a]	43.9 ± 3.2[b]
HV guar	8.5 ± 0.5[a]	4.5 ± 0.5[a]	44.3 ± 3.3[b]

HVGuar: high viscosity guar gum; MVGuar: medium viscosity guar gum; LVGuar: low viscosity guar gum. For the initial velocity measurements, a standard emulsion was used as the substrate provided in excess and fiber solutions added extemporaneously. For time-courses of triglyceride hydrolysis (120 min), the emulsions were made in the presence of fibers as described for emulsification measurements (for details, see "Methods and Materials"). Values are mean ± SEM of three determinations. Different superscript letters in the same column indicate significant differences (ANOVA; $P < 0.05$).

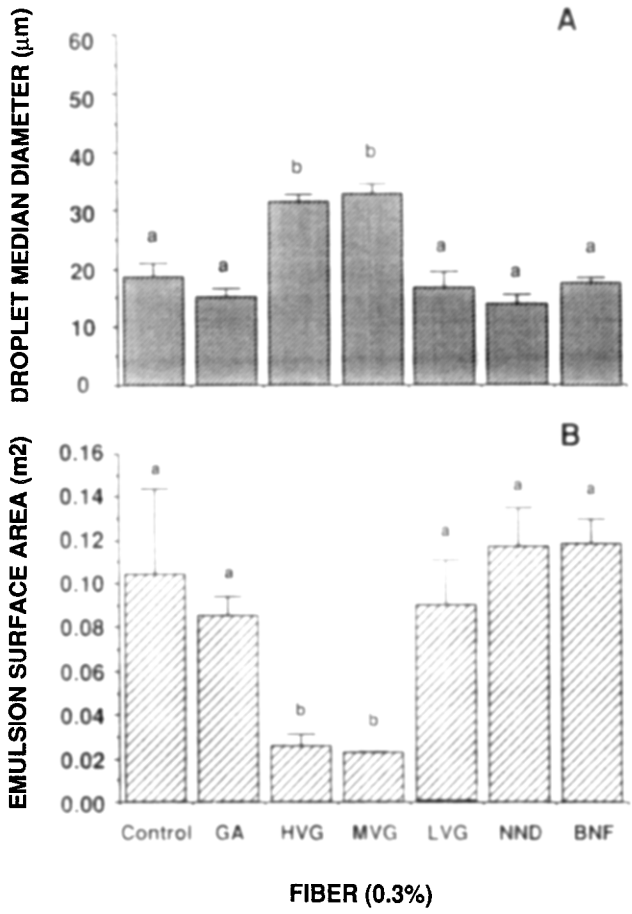

Figure 2 Effects of soluble fibers (0.3% w/v) on the median diameter (A) and surface area (B) of emulsified droplets generated in reconstituted duodenum medium. Calculations were made as described in details in the text. G.A.: Gum Arabic; HVG: High Viscosity Guar gum; MVG: Medium Viscosity Guar gum; LVG: Low Viscosity Guar gum. NND: 42.0% methylated pectin; BNF: 73.6% methylated pectin. Values are mean ± SEM of three determinations. Different superscript letters indicate significant differences (ANOVA; $P < 0.05$).

Emulsion surface area. The overall effect of soluble fibers on the emulsification process was evaluated by calculating the total surface area provided by the emulsified droplets generated at a given low fiber concentration (0.3%), as shown in *Figure* 2B. The value given by the control (about $0.1 m^2$) was not changed in the presence of NND and BNF pectins, LVGuar and gum arabic. Conversely, HVGuar and MVGuar markedly lowered (by 4.0 and 4.5 fold) the emulsion surface area obtained in the absence of fibers.

Because the charged pectins did not exhibit any particular effects as compared to guar gum at comparable solution viscosity, further experiments were done only with guar gums and gum arabic.

Effects of soluble fibers on emulsion hydrolysis catalyzed by pancreatic lipase

Fibers and initial velocity of pancreatic lipase on a standard lipid emulsion. To evaluate the possible effects of fibers present in solution on the catalytic reaction, a stan-

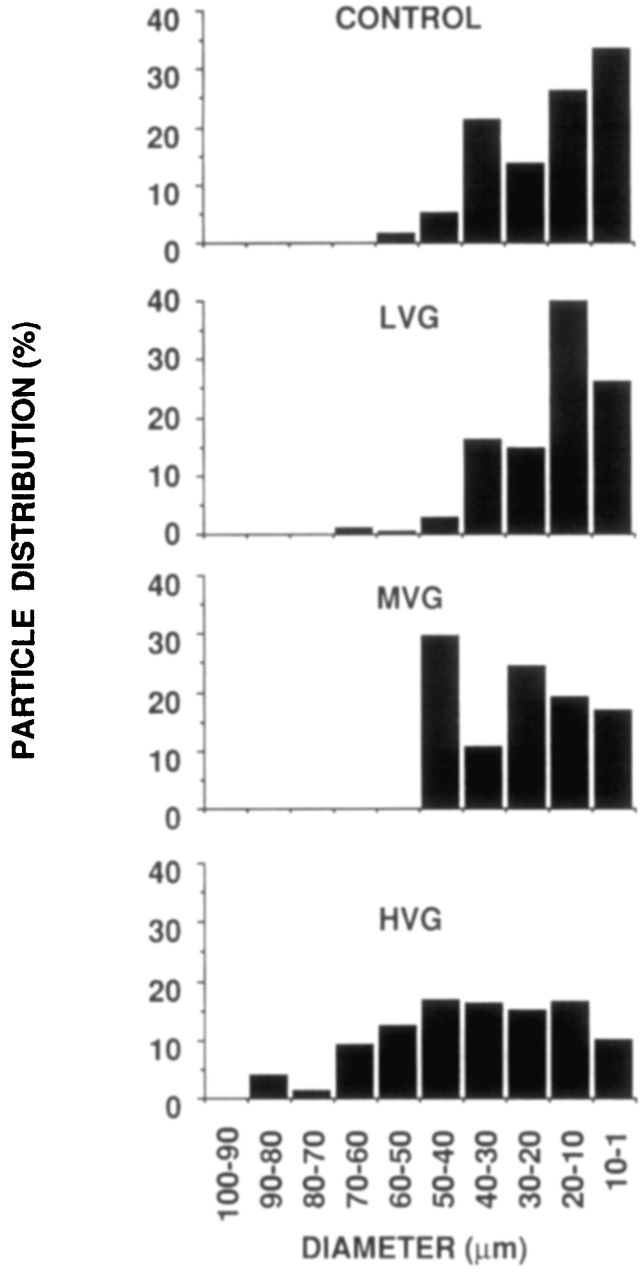

Figure 3 Effects of different guar gums (0.3% w/v) on the droplet size distribution (1 to 100 μm) of emulsions in the reconstituted duodenum medium. Control: duodenum medium; HVG: High Viscosity Guar gum; MVG: Medium Viscosity Guar gum; LVG: Low Viscosity Guar gum. Values are mean ± SEM of three determinations.

dard emulsion with a fixed median diameter (4.52 ± 1.85 μm) providing excess amounts of triacylglycerol substrate was used. As shown in *Table 3,* neither gum arabic nor any kind of guar gum at 0.3% concentration in medium solution altered the initial velocity of pancreatic lipase on a standard pre-formed emulsion. The lag time, i.e., the time needed to reach steady state maximum enzyme velocity, was unaltered by the presence of fibers in the solutions (*Table 3*).

Hydrolysis of emulsions with different droplet sizes. In this experiment, we aimed to study the possible effect of fibers on lipolysis as mediated by alterations in emulsion properties. Thus, emulsions prepared in the presence of 0.3% fibers by using the shaking procedure used for emulsification as above exhibited comparable (control, gum arabic and LVGuar) or higher (MVGuar and HVGuar) droplet sizes (*Table 2*). After 30 and 60 min incubation with pancreatic colipase-lipase, the rate of triglyceride hydrolysis was lower in the presence of HVGuar and MVGuar as compared with the control, LVGuar, and gum arabic. After 120 min incubation, as shown in *Table 3,* 55.7% of potentially available free fatty acids were released under lipase action in control conditions. Very comparable data were obtained in the presence of 0.3% gum arabic or LVGuar whereas a significantly lower extent of lipolysis was measured in the presence of 0.3% MVGuar or HVGuar.

Discussion

In the present study, we aimed to evaluate for the first time the possible effects of soluble fibers on dietary fat emulsification and lipolysis in vitro and to investigate some physico-chemical mechanisms involved. For this purpose, it was necessary to define in vitro experimental conditions mimicking as closely as possible those prevailing in the duodenum content where most triglycerides are hydrolyzed and from which lipid molecules are absorbed.[1] The reconstituted media contained all bile lipids provided in the form of porcine bile given their reported importance for emulsification, lipase binding and hydrolysis.[1,2,5,7,8,13] Mild emulsification conditions were developed to emulsify most triglycerides present (68.5% in controls) with droplet sizes basically ranging from 1 to 100 μm with median diameters from 10 to 40 μm, as recently described by our laboratory in the stomach and duodenum in humans[14,15] and rats.[16] Finally, the kinds of fiber sources tested have previously been widely used in metabolic studies as well in animals as in humans and we selected concentration ranges (0 to 1%) likely present in the digestive tract after ingestion of fiber-rich diets (5 to 8% of food dry matter). Measurements of viscosity of digesta in rats and pigs in the presence of soluble fibers support this assumption.[33–36]

The data obtained clearly indicate that a slight increase in the medium viscosity is not sufficient to alter the process of triglyceride emulsification. This was obtained herein either with high concentrations (up to 2%) of fibers with negligeable apparent viscosity (gum arabic), moderate concentration (0.3%) of fibers with moderate apparent viscosities (NND and BNF pectins, LVGuar) or very low concentrations (0.1%) of fibers displaying high apparent viscosities (MVGuar and HVGuar). The well-known facilitating effect of gum arabic on lipid emulsification[3,51,52] was observed in the present conditions only above a concentration of 0.6%. Conversely, it appeared that reaching a sufficiently elevated medium viscosity with higher concentrations of moderately or highly viscous fibers dramatically reduced the overall process of emulsification of dietary fat. Data obtained with individual fiber sources indicate that an increased viscosity markedly reduces the amount of triglyceride emulsified, increases the droplet size of emulsions and decreases the generated emulsion interface area.

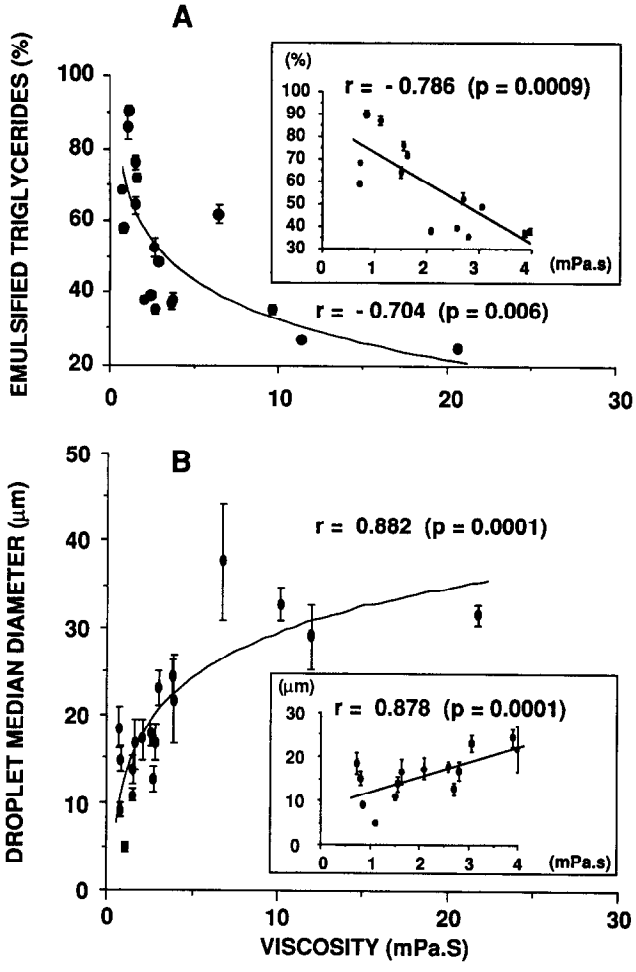

Figure 4 Regression analyses between (A) the amount of triglyceride emulsified (% of lipid present) or (B) the droplet median diameter (µm) and the apparent viscosities in the range 0 to 25 mPa.s or 0 to 4 mPa.s (insert) elicited by the control and fiber solutions used. Mean values of triplicate determinations obtained in different conditions were used.

The key role of medium viscosity on dietary fat emulsification was more clearly assessed by plotting some measured emulsification parameters against solution apparent viscosities exhibited by the different fibers and concentrations tested (as provided in *Figure 1*). As shown in *Figure 4*A, the amount of triglyceride emulsified, defined as droplets exhibiting a diameter less than 100 µm, was negatively and linearly correlated with solution viscosities in the range 0 to 4 mPa.s (r = –0.786) and in a curvilinear fashion in the overall range 0–22 mPa.s (r = –0.704). An average 63% reduction in the amount of emulsified triglycerides was obtained in a linear fashion with a medium viscosity of about 4 mPa.s. An opposite relationship was obtained when plotting the median diameters of the emulsified droplets against solution viscosity, as shown in *Figure 4*B, a strong positive linear correlation was found between droplet diameter and viscosity in the range 0–4 mPa.s (r = 0.878) and a curvilinear relation was observed on the entire range of viscosities tested (0–22 mPa.s; r = 0.882). An average 65.5% increase in droplet size was obtained in a linear fashion within the range 0–4 mPa.s. The overall effect of increasing medium viscosity on emulsion surface area which includes both the amount of lipid emulsified and the droplet size of emulsified material is shown in *Figure 5*A. A negative correlation was observed between the two parameters in the range 0 to 4 mPa.s (r = –0.591) as well in the overall range 0 to 22 mPa.s of viscosities (r = –0.598).

Thus, in line with basic knowledge on the mechanisms involved in emulsion building,[53] the data obtained herein point out that soluble dietary fibers that sufficiently increase the apparent viscosity of (reconstituted) duodenal medium significantly reduce the extent of dietary fat emulsification. In this respect, the effect on medium viscosity appears to be a key parameter whereas the electric charges beared by some fiber molecules do not play a specific role, at least with the kinds of fibers used, i.e., pectins and gum arabic.

One can question what the mechanisms behind the effects of viscous fibers on dietary fat emulsification are. With the exception of, gum arabic, which is well-known to display emulsifier properties due to its important protein moities,[51,52] the other fibers tested, i.e., guar gums and pectins, are essentially branched polysaccharide chains that only

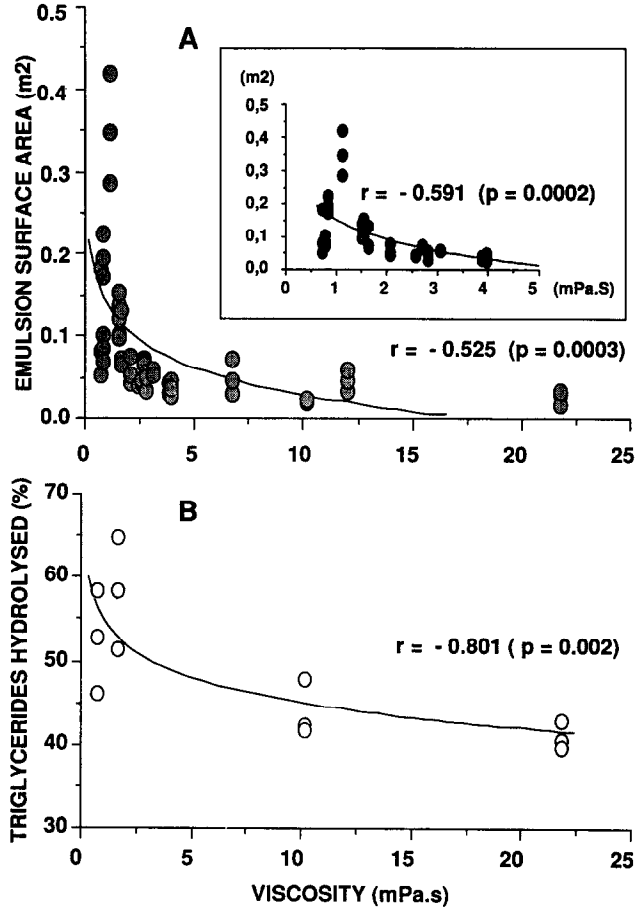

Figure 5 Regression analyses between (A) the emulsion surface area (m^2) or (B) the extent of triglyceride hydrolysis (%) after 2 hours incubation with excess pancreatic colipase-lipase and the apparent viscosities in the range 0 to 25 mPa.s or 0 to 4 mPa.s (insert) elicited by the control and fiber solutions used. Mean values of triplicate determinations obtained in different conditions were used.

marginally lower the interfacial tension[53–55] and thus, cannot be referred to as emulsifying agents.[53,54] With the exception of a stabilizing effect on pre-formed emulsified droplets,[53,54] the viscous fibers cannot display significant emulsifying properties. Conversely, medium viscosity has been considered for a long time by food scientists as an important factor in the emulsification process of various hydrophobic compounds.[53–57] Taking into account this basic knowledge and the data obtained herein, an overall concept can be proposed as follows: at a given composition and concentration of the dispersed lipid phase and under a constant and low to moderate input of mechanical energy as likely found within the digestive tract,[1] medium viscosity is a limiting factor for emulsification of dietary fat by reducing the energy efficiency to deforming and then disrupting the lipid mass to large and finally small droplets. Because great care was taken in the relative amounts of all dietary and biliary lipids and low fiber concentrations have been used herein in vitro, this mechanism is likely to take place in the duodenum. Experiments are in progress in our laboratory to provide such in vivo data.

In a second step, some experiments have been carried out to evaluate the possible effects of medium viscosity as altered by soluble fibers, on the hydrolytic activity of pancreatic lipase in reconstituted duodenal medium. Although several descriptive studies have already reported the possible interference of soluble fibers on pancreatic lipase-catalyzed triglyceride lipolysis,[22–27] the mechanisms involved have not been investigated in details, as recently underlined.[21]

In the first set of experiments, we showed that guar gums and gum arabic solubilized in the medium do not modify the lipase initial velocity or the lag time needed to reach maximum initial velocity, when the triglyceride substrate is provided in the form of a given pre-formed fine and stable emulsion (4.5 μm) as generally used during lipase kinetic studies.[2,13] These data indicate that the addition of viscous soluble fibers on a preformed emulsion does not alter the efficiency of the binding step of pancreatic lipase at the emulsion surface[3,5–13,17] and does not affect the catalytic reaction per se. As previously observed with high concentrations of gum arabic,[8] it could be suggested that the building of a surface fiber monolayer surrounding the lipid droplet[53–55] and/or the increased solution viscosity might reduce the interaction occurring between the enzyme binding-site and the substrate [58]; within the physiologic concentration range of fiber used, the enzyme initial velocity is not affected.

In a second set of experiments, emulsions were prepared in the presence or not of 0.3% solutions of guar gums and gum arabic and were provided as substrate (with different droplet sizes) to excess pancreatic lipase for 2 hours incubation to mimick intra-duodenal digestion. Whereas the initial rate of lipolysis was unchanged (up to 15 min), the overall extent of lipolysis significantly decreased for longer periods of time up to 2 hours, with the emulsions prepared in the presence of the MVGuar and HVGuar. It is noteworthy that only two fibers (HVG and MVG), which significantly increase the emulsion droplet diameter, reduce the extent of triglyceride lipolysis. Thus, this points out that the viscous gums which alter droplet size and possibly the droplet interface, can reduce the rate of triglyceride lipolysis catalyzed by pancreatic colipase-lipase, as illustrated in *Figure 5*B. This agrees well with our recent in vitro findings[17,18] that the lower the droplet diameter, the higher the lipase maximum velocity in the presence of excess substrate. This was interpreted as a result of changes in some interfacial properties linked to surface curvature. A second possibility could be that the increase in surface area generated under the lipolytic process as shown in vitro[59] could be empeded in the presence of a high-viscosity medium. A third possible mechanism could involve the building of a gum monolayer[53–55] around the lipid droplets which could be more tightly bound or rigid as the medium viscosity increases. Consequently, the release of the lipolytic products from the droplet interface to the aqueous phase might be limited to some extent, thus leading to surface accumulation; as lipolysis proceeds, this may in turn lower the rate of reaction. Finally, one could hypothesize that fibers may have bound to some extent biliary lipids present in solution as already shown,[24,60,61] thus leading to an impaired dispersion and solubilisation of lipolytic products. This possibility cannot be ruled out, but the amplitude of this phenomenon is not expected to be sufficient in the case of low physiological concentrations of fibers such as below 1%.[24,61]

The present in vitro data show that viscous fibers reduce the emulsification of dietary lipids and subsequently, lower the extent of triglyceride lipolysis in duodenal conditions. Two additional pieces of information, already obtained with these types of guar gums, lead to point out that this mechanism is likely to occur within the digestive tract. First, when looking at the viscosity of pig digesta,[35] it was observed that the HVGuar, and the MVGuar to a lower extent, increase the medium viscosity following meal intake. Secondly, it was observed in the pig[62] following ingestion of different meals containing or not 6% LVG, MVG or HVG, that the postprandial rise in plasma triglyceride (area under the 0 to 7 h curve) is significantly reduced only in the presence of the MVGuar and the HVGuar.

In conclusion, viscous soluble fibers such as guar gums, in addition to documented effects on gastric emptying, rate of intestinal uptake of nutrients and sterol binding, can interfere with the normal process of emulsification of dietary fat and subsequent lipolysis. This, in turn, could alter the uptake of lipid nutrients by the intestine and the postprandial response[21,63] which are implicated in the mechanism of action of fibers on the overall lipid metabolism.

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