Emulsification and lipolysis of triacylglycerols are altered by viscous soluble dietary fibres in acidic gastric medium *in vitro*

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This *in vitro* study was designed to test the hypothesis that soluble dietary fibres can alter the process of intragastric lipid emulsification and possibly subsequent triacylglycerol lipolysis. Three guar gums, two pectins and gum arabic were dissolved in acidic gastric medium in the concentration range 0.3–2.0 % (w/v). Viscosities of fibre solutions were measured and apparent viscosities varied over a wide range (0.7–77 mPa·s). Emulsification of a lipid mixture (triolein/phosphatidylcholine/cholesterol) was performed under mild conditions in the presence of increasing concentrations of soluble fibres. The amount of emulsified lipid was not affected whereas the size of the emulsified droplets was increased by raising the concentration of viscous fibres only. The droplet size ($r = 0.75$, $P = 0.006$) and overall droplet surface area ($r = -0.69$, $P = 0.009$) were strongly correlated with the medium viscosity in the range 0–20 mPa·s. The addition of solutions of viscous fibres to a preformed standard emulsion did not change the initial velocity of human gastric lipase reaction. Conversely, when emulsions prepared in the presence of fibres (i.e. with different droplet sizes) were incubated with excess gastric enzyme for 2 h, the high-viscosity guar gum significantly reduced the extent of triacylglycerol lipolysis, as compared with control and low- or medium-viscosity fibres. In conclusion, the data obtained show that reducing emulsification of dietary lipids in the mildly acid medium found in the stomach is a mechanism by which soluble viscous fibres can alter lipid assimilation.

INTRODUCTION

It is now well recognized that in humans the first step of fat digestion takes place in the stomach [1–3]. The gastric lipase secreted in the gastric juice hydrolyses under acidic conditions 10–60 % of dietary triacylglycerols [4–6]. Fat digestion is then completed in the small intestine where absorption of lipolytic products occurs [2]. Because triacylglycerols are insoluble substrates, the characteristic feature of gastric and pancreatic lipases is that both enzymes act at the lipid/water interface [1,7]. We recently described in healthy humans [6,8] and in the rat [9] how dietary lipids are basically emulsified in the stomach contents with most droplets being 1–50 μm in diameter. Under these conditions of heterogeneous catalysis, the concentration and properties of the interface govern the extent of enzyme binding to the interface and lipolysis [1,3,7,10]. In this respect, we have recently shown *in vitro* that the size and composition of the emulsified droplets alter the binding [11] and activity of gastric and pancreatic lipases [11,12].

The effects of dietary fibres on digestion, gut physiology and lipid metabolism have been extensively studied but the various mechanisms involved are still under investigation [13,14]. Several studies have reported that some sources of dietary fibre can lower the activity of pancreatic lipase *in vitro* [15–18], fat lipolysis in the stomach and small intestine [19] and lipid absorption *in vivo* [20]. Moreover, viscous fibres have been reported to increase the viscosity of the digestive contents [21–23], regulate digestive organ motility [24], delay stomach emptying, delay and/or reduce the absorption of nutrients and alter postprandial response [19–21,23–27].

Since fat digestion begins in the stomach where emulsification of dietary lipids takes place [6,8], we hypothesized that soluble dietary fibres could alter emulsification and lipolysis of dietary triacylglycerols in the gastric medium. To test this hypothesis, we designed experiments in which we compared at mild acidic pH, as found in the stomach during digestion, the effects of five well-known kinds of soluble fibre (guar gums, pectins, gum arabic) that exhibit different viscosities or charges in solution.

EXPERIMENTAL

Dietary fibres

Five different sources of soluble fibre were used (Table 1): gum arabic (Sigma, St. Quentin Fallavier, France), three guar gum preparations (MRS-Sanofi, Beaupte, France) with different molecular masses [HVG, high molecular mass (1.5×10^6 Da); MVG, medium molecular mass (9×10^5 Da); LVG, low molecular mass (1×10^5 Da)] and two apple pectins (MRS-Sanofi) with different degrees of methylation [NND (42 % methylation) (27 NAND) and BNF (73.6 % methylation) (Brun NF Pomme)].

Uronic acid was automatically measured by using meta-hydroxydiphenyl reagent [28]. Individual sugars were analysed as their alditol acetate derivatives by GLC [29]. Proteins were measured by a semi-automatic micro-Kjeldahl method using a conversion factor of 6.25. Methanol was determined by HPLC as described previously [30].

Fibres were dissolved in buffers with stirring at room temperature or 80 °C in a water bath and allowed to solubilize overnight at room temperature with gentle stirring. After filtration and centrifugation, the actual fibre concentration of the stock solution was measured after oven drying (100 °C, 24 h). Final concentrations ranged from 0 to 2 % to fit with fibre concentrations found in the digestive tract.

The buffers used for viscosity and emulsification measurements contained 50 mM sodium acetate and 150 mM NaCl at pH 5.4 and were prepared with ultrapure water. This pH was because it falls within the range (4.0–6.0) found in the stomach during

Abbreviations used: HVG, high-viscosity guar gum; MVG, medium-viscosity guar gum; LVG, low-viscosity guar gum.

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Table 1 Chemical composition and intrinsic and apparent viscosity of the dietary fibres used

HVG, high-viscosity guar gum; MVG, medium-viscosity guar gum; LVG, low-viscosity guar gum; NND and BNF, apple pectins; GA, gum arabic. The sugar composition is given as relative content (% of total) of the individual sugar. Apparent viscosity ($[\eta]_{app.}$) was calculated at 0.3% fibre concentration and pH 5.4. For details, see the text.

	HVG	MVG	LVG	NND	BNF	GA
Rhamnose	0.2	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
$N \times 6.25$ (%)	0.5	0.6	0.9	0.8	2.8	2.6
Methylation (%)	–	–	–	42.0	73.6	–
$[\eta]$ (ml/g)	1318.3*	981.6*	401.7*	313.3*	302.8*	20.6*
	1402.4†	1041.2†	407.8†	313.9†	320.6†	22.4†
$[\eta]_{app.}$ (mPa·s)	45.7	9.8	1.3	1.6	2.2	0.8

* Intrinsic viscosity was calculated from Huggins relation.
† Intrinsic viscosity was calculated from the Kraemer relation.

digestion [1,2] and is the optimal pH for human gastric lipase activity [1,31–33].

Viscosity measurements

Flow curves of solutions of gum arabic (8.96–129.85 g/l) and guar gum (HVG, 0.2–5.17 g/l; MVG, 0.18–8.97 g/l; LVG, 0.89–27.15 g/l) were determined by means of a Low Shear 40 viscometer (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 0–100 s^{-1}. Apparent viscosities of the pectin solutions (NND, 0.43–8.6 g/l; BNF, 0.43–7.8 g/l) were determined using a Viscomatic viscometer (Contraves). All measurements were performed at 37.0 ± 0.1 °C. Apparent viscosity was defined as the ratio of stress applied to the shear rate generated.

Whatever the concentration in the range studied, gum arabic and pectin solutions exhibited Newtonian behaviour over the shear rate range 0–100 s^{-1}, i.e. apparent viscosity was independent of shear rate. The guar gum solutions exhibited classical shear-thinning behaviour in relation to coil overlap and entanglement. At low concentrations, at which coils were free to move through the solvent with little mutual interference, apparent viscosity was independent of shear rate (Newtonian behaviour). As the concentration of guar gum solutions increased, a state was reached at which coils were forced to interpenetrate: at low shear rates, where no net change in entanglement density occurred during flow, apparent viscosity remained constant at the limiting zero-shear value. In contrast, with increasing shear rate, the rate of re-entanglement became lower than the rate of forced disentanglement by flow and the apparent viscosity decreased and became highly dependent on shear rate. Classically, critical coils overlap concentration increases with decreasing molecular mass of guar gums: it was found between 0.16 and 0.2% for HVG, 0.25 and 0.3% for MVG and about 0.68% for LVG. For HVG at a concentration of 0.3%, the critical shear rate for the onset of shear-thinning behaviour was about 10 s^{-1}. Since we could not precisely estimate shear rates equivalent to the emulsification process used in this study, Newtonian viscosity or limiting zero-shear value in the case of shear-thinning behaviour was selected to characterize the viscometric behaviour of guar gum solutions although this could lead to some overestimation of the apparent viscosity of solutions with zero-shear viscosity higher than 4–5 mPa·s.

Newtonian viscosity in the low shear-rate range (ηN) was determined for each solution, and the intrinsic viscosity $[\eta]$ of the polymer was calculated by the usual double-linear regression method using Huggins and Kraemer relations.

Preparation of emulsions

A lipid mixture was prepared with 97% (w/w) triolein (95% pure; Sigma), 2.5% egg phosphatidylcholine (95% pure; Prolabo, Paris, France) and 0.5% free cholesterol (99% pure; Sigma) at a final concentration of 16% (w/w) in chloroform/methanol (2:1, v/v). Aliquots of the lipid mixture solution (1 ml each) were evaporated to dryness under nitrogen in 7 ml glass tubes. The buffer (with or without fibre) was added to a final volume of 4 ml. The aqueous mixture thus contained 4% (w/w) lipids and 0–2% fibre, i.e. in the physiological range [5,6,23,24]. The tubes were stoppered, attached horizontally and shaken at 200 strokes/min for 2 h at 37 °C. These operating conditions resulted from preliminary experiments designed to discover conditions that would generate lipid emulsions with droplet sizes in the range found in human and rat stomach during fat digestion [6,8,9].

Emulsification measurements

Determination of the amount of emulsified lipids

The upper limit for emulsion droplet size was set at 100 μm, given the instability and negligible interfacial area of lipid droplets above this value [6]. To allow accurate measurements, [*carboxyl*-^{14}C]triolein (98% pure; 69 mCi/mmol; CEA, Gif-sur-Yvette, France) was added in trace amounts to the lipid mixture. Radioactivity was measured by liquid scintillation counting using a Packard 1600TR instrument (Packard, Meriden, CT, U.S.A.) from 100 μl aliquots of two fractions collected at the end of the emulsification process. One fraction was the floating oily layer composed of unemulsified lipids (oily material plus lipid droplets $\geqslant 100$ μm) which collect above the aqueous solution surface when tubes are left to stand for a given time (0.5–10 min at 1 g) calculated from the Stoke's sedimentation equation for droplets $\geqslant 100$ μm under the present conditions, as previously reported [6]. According to Stoke's sedimentation law, the relation between sedimentation time and particle diameter is expressed in the following equation:

$$D = [18\eta_0 H/(\rho - \rho_0) g t]^{1/2}$$

where D is the particle diameter (m), η_0 is the viscosity coefficient of the solvent ($N \cdot s/m^2$), ρ is the density of the sample (kg/m^3), ρ_0 is the density of the solvent (kg/m^3), g is acceleration due to gravity ($m \cdot s^{-2}$), t is the sedimentation time (s) and H is the distance of sedimentation (m). The second fraction was the resulting infranatant containing emulsified lipid droplets.

Measurement of droplet size

The distribution of the emulsion droplet sizes in the infranatant solution was determined by using a particle-size analyser (Capa 700; Horiba, Kyoto, Japan) as previously described [6,8,9,10,11]. The validity of the method has previously been evaluated and calibrations made using microparticles in the size range 0.2–100 μm (polystyrene size standard kit; Polyscience Inc., Warrington, PA, U.S.A.). Measurements were carried out using gradient-mode analysis at a constant centrifuge acceleration rate (960 rev./min) to allow an accurate measurement of large drop-

lets (100 μm) as well as small droplets (0.1 μm). The particle-sizer software calculated the droplet-size distribution which is expressed as a fraction of the total droplet volume. Results are given as a frequency-distribution graph characterized by its median diameter (μm) and a specific interfacial area (m^2/g). The droplet surface area (m^2) was calculated from the amount (g) of emulsified lipids in the infranatant solution.

Gastric lipase-catalysed hydrolysis of emulsions

Human gastric juice was obtained from gastric aspirates collected for diagnostic purposes in adult patients under pentagastrin stimulation in hospital facilities (gifts from Dr. J. Peyrot and Dr. J. Salducci, Gastroenterology Department, North University Hospital, Marseille, France). Samples with comparatively high activity were neutralized, pooled and stored at -70 °C. The gastric lipase activity and the specific activity of the pooled samples were 45 units/ml and 25 units/mg of protein respectively with tributyrin as substrate [31,33].

To measure the initial velocity of human gastric lipase on a standard emulsion using a pH-stat titrator [11,31–33], the reaction medium was a 15 ml mixture containing 5 mM sodium acetate, 150 mM NaCl, 6 mM $CaCl_2$, 2 mM sodium taurodeoxycholate (Sigma), 1.5 μM fatty acid-free BSA (Sigma) at pH 5.4, 0.3 % or no (w/v) fibre 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 rev./min and 37 °C, and, for each assay, 1 ml of gastric juice was used. At the end of the reaction time (1 min), the liberated fatty acids were fully titrated at pH 9.4 as previously described [11,31–33]. One lipase unit is defined as 1 μmol of fatty acid liberated/min.

For hydrolysing different emulsions by gastric lipase, emulsions were prepared by using the exact procedure used for the emulsification measurements in the presence of various fibres at a 0.3 % (w/v) concentration in buffer (500 mM sodium acetate, 150 mM NaCl, 6 mM $CaCl_2$, 1.75 mM BSA, pH 5.4) with the lipid mixture added with [*carboxyl*-^{14}C]triolein (880 d.p.m./μmol). Aliquots (250 μl) of emulsions were added to gastric lipase (22.5 units; 500 μl of gastric juice) in Eppendorf tubes and incubated with moderate stirring (100 strokes/min) and at 37 °C for 30 min, since triacylglycerol lipolysis *in vivo* has been reported to be maximum after 15–60 min [4–6,8]. It was verified that these incubation conditions did not lead to further emulsification or coalescence. Aliquots (300 μl) of the reaction media were collected, non-esterified fatty acids generated were extracted as described by Belfrage and Vaughan [34] and radioactivity was measured; calculations were performed using the actual non-esterified fatty acid partition coefficients determined with the samples used.

Statistics

All experiments were performed in triplicate and the results are expressed as means $\pm$ S.E.M. The statistical significance of the differences observed was assessed by one-way analysis of variance and Fisher's test ($P < 0.05$) using the Statview II micro-computer program (Abacus, Berkeley, CA, U.S.A.).

RESULTS

Viscosities of fibre solutions at acidic pH

Variations in Newtonian viscosity and zero-shear values of the six fibre solutions in the concentration range 0–20 % are shown in Figure 1. Values corresponding to solutions at 0.3 % concentration are given in Table 1 together with calculated intrinsic viscosities of the six polymers at pH 5.4. Gum arabic was markedly smaller than the other polymers resulting in very low apparent viscosities of solutions, and Newtonian behaviour was exhibited even for high concentrations. The two pectin samples were very similar in size under the present solvent conditions at infinite dilution and showed similar apparent viscosities. The three kinds of guar gum exhibited different intrinsic viscosities in close relationship to their molecular mass: the larger the guar gum molecule, the higher the apparent viscosity of the solution. At 0.3 % concentration, the apparent viscosity of the uncharged LVG was comparable with those of the two charged pectins whereas MVG and even more so HVG exhibited much higher apparent viscosities in solution.

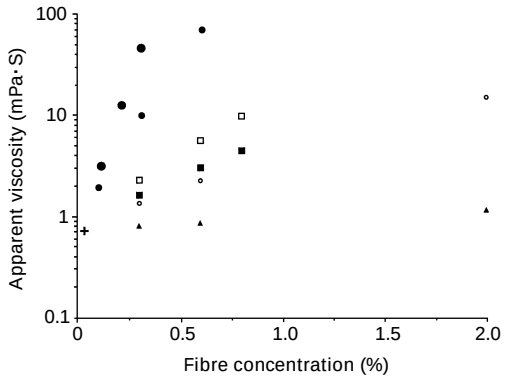

Figure 1 Effect of soluble-fibre concentration on the viscometric behaviour of reconstituted gastric medium at pH 5.4 and 37 °C

Apparent viscosity at low shear rates for each final composition of the medium was deduced from the flow curves measured with the Low Shear 40 in the 0–100 s^{-1} shear-rate range. +, Control; ▲, gum arabic; ■, NND pectin; □, BNF pectin; ●, HVG; ●, MVG; ○, LVG.

Effects of fibres on lipid emulsification

To determine the effects of soluble fibres on the extent of fat emulsification, we measured the amount of emulsified lipid and the size of the emulsified lipid droplets produced and calculated the surface area generated.

Lipid emulsification occurred to a moderate extent (24.6 %) under the conditions selected in the absence of fibre (control). Adding fibres did not markedly change this. For instance, 0.3 % solutions of the fibres caused the following extents of emulsification: gum arabic, 23.8 %; HVG, 29.7 %; MVG, 25.6 %; LVG, 24.2 %; NND pectin, 27.8 %; BNF pectin, 26.1 %.

The data obtained from droplet-size measurements of emulsified lipids are given in Table 2. In control buffer, the median diameter exhibited by the emulsion was 7.19 μm. Gum arabic (0.3–2.0 %), LVG and MVG at low concentrations did not significantly alter the droplet size. In contrast, LVG at 2.0 %, MVG at 0.6 % and HVG at 0.3 % significantly increased the median size of the droplets (45.6, 41.7 and 25.4 μm respectively). Also, BNF pectin and NND pectin significantly increased the droplet median diameter to a comparable extent at concentrations of 0.6 % and 0.8 % respectively. A comparison of the effects of the fibres tested at a given concentration (0.3 %; Table 2) provides the following information: the highest median diameters were elicited by HVG (25.41 ± 4.09 μm) and to a lesser degree MVG (14.36 ± 4.78 μm) and NND pectin (15.42 ± 4.34 μm) whereas the other fibres [LVG (8.71 ± 0.29 μm),

Table 2 Effects of fibres on the droplet size of emulsions

Median diameters were calculated from droplet-size distribution by the particle-sizer software (for details, see the text). Values are means ± S.E.M. of three determinations. Different superscript letters (a, b, c) indicate significant differences (analysis of variance; $P < 0.05$).

	Fibre concentration (%)	Median diameter[d] (μm)
Control	0.0	7.19 ± 0.5[a]
Gum arabic	2.0	9.30 ± 1.55[a]
	0.6	7.80 ± 1.30[a]
	0.3	7.12 ± 1.22[a]
HVG	0.3	25.41 ± 4.09[b]
	0.2	21.59 ± 5.80[ab]
	0.1	14.29 ± 2.67[ab]
MVG	0.6	41.66 ± 6.62[c]
	0.3	14.36 ± 4.78[a]
	0.1	12.82 ± 7.31[a]
LVG	2.0	45.59 ± 5.67[b]
	0.6	11.57 ± 0.29[a]
	0.3	8.71 ± 0.29[a]
NND pectin	0.8	36.22 ± 3.75[b]
	0.6	14.70 ± 2.04[a]
	0.3	15.42 ± 4.34[a]
BNF pectin	0.8	41.83 ± 7.25[b]
	0.6	27.78 ± 2.01[b]
	0.3	14.42 ± 2.34[a]

Table 3 Effects of different guar gums (0.3%, w/v) on droplet-size distribution (0.1–100 μm) of emulsions in reconstituted gastric medium

Droplet-size distribution was calculated by the particle-sizer software (for details, see the text). Values are means ± S.E.M. of three determinations.

Droplet size (μm)	Control	Guar gums		
		LVG	MVG	HVG
100–90	0	0	0	3.4 ± 2.8
90–80	0	0	0	5.0 ± 3.4
80–70	0	0	1.0 ± 1.0	4.0 ± 2.5
70–60	0	0	3.9 ± 2.2	5.1 ± 2.9
60–50	0.0 ± 1.6	0	3.3 ± 3.3	10.8 ± 4.9
50–40	1.7 ± 1.7	0	9.1 ± 5.3	7.6 ± 2.2
40–30	0	0.2 ± 0.2	3.8 ± 2.2	9.2 ± 3.1
30–20	0	8.4 ± 6.8	6.3 ± 2.5	13.9 ± 2.6
20–10	27.4 ± 4.1	28.9 ± 5.5	24.8 ± 6.0	15.6 ± 4.3
10–1	60.1 ± 3.6	66.4 ± 7.0	41.8 ± 8.4	24.4 ± 3.8
1–0.1	10.8 ± 2.3	4.8 ± 3.1	6.1 ± 1.9	2.3 ± 1.5

BNF pectin (14.42 ± 2.34 μm) and gum arabic (7.12 ± 1.22 μm)] gave values the same as the control (7.19 ± 0.5 μm). As shown in Table 3, a more detailed comparison of the distribution of the droplet-size classes clearly showed that, compared with the control and the 0.3 % solution of LVG, the 0.3 % solution of MVG and to a greater extent HVG markedly shifted the droplet spectrum towards large droplets. For instance, the proportion of droplets with a diameter above 50 μm was zero (control and LVG), 1.3 % (gum arabic), 7.4 % (NND pectin), 8.1 % (MVG), 8.6 % (BNF pectin) and 25.1 % (HVG) at 0.3 % fibre concentration. These data suggested that viscosity could be the key determinant of the increase in droplet size elicited by some fibres. This was clearly evidenced, as shown in Figure 2(A), by the finding of a strong positive correlation ($r = 0.75$; $P = 0.006$)

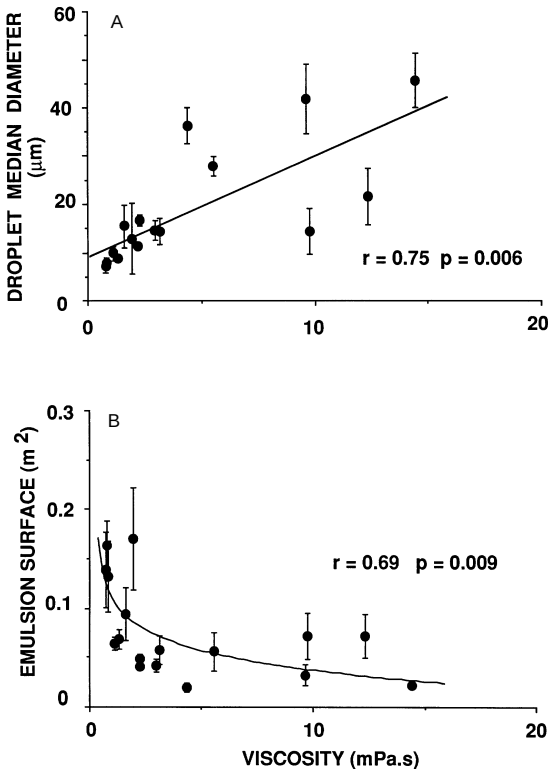

Figure 2 Regression analysis between (A) the droplet median diameter (μm) and (B) the emulsion surface area (m^2) and the apparent viscosity in the selected range 0–20 mPa·s elicited by the control and fibre solutions

Mean ± S.E.M. values of triplicate determinations obtained under different conditions were plotted.

between the size of the emulsified droplets and the apparent viscosity of the fibre solutions used.

The overall effect of soluble fibres on the emulsification process was evaluated by calculating the total surface area provided by the emulsified droplets generated. At a given fibre concentration (0.3 %), the value given by the control (0.14 ± 0.04 m^2) was not changed in the presence of gum arabic (0.16 ± 0.02 m^2), NND pectin (0.09 ± 0.03 m^2) and LVG (0.09 ± 0.01 m^2) whereas significantly lower values were obtained with MVG (0.07 ± 0.01 m^2) and HVG (0.04 ± 0.03 m^2). As shown in Figure 2(B), the emulsion surface area generated in the presence of the fibre solutions was found to be log-linearly correlated ($S = 0.1073–0.0706 \log \eta$; $r = 0.69$; $P = 0.009$) with the zero-shear viscosity of the solution in the range 0–20 mPa·s. Under well-defined conditions of isotropic turbulence, which cannot strictly apply to the rather complex flow conditions occurring in our experiments during emulsification, a function of this type has been reported to relate minimal droplet size and viscosity [41]. Since the limiting zero-shear value might be to some extent an overestimation of the actual apparent viscosity of the solution during the emulsion process, correlation for conditions with apparent viscosity lower than 5 mPa·s was also determined. In this case, a linear function ($S = 0.32\eta + 0.149$; $r = -0.70$; $P = 0.01$) was fitted to the data.

Effects of fibres on emulsion hydrolysis catalysed by gastric lipase

As shown in Table 4, when excess amounts of a standard fine emulsion (4.5 μm) were used as the substrate, adding 0.3 % gum

Table 4 Effects of gum arabic and guar gums on lipase activity

For the initial-velocity measurements, a standard emulsion (5 μm) was used as substrate provided in excess and fibre solutions were added extemporaneously. Lipase activity is given as μmol of non-esterified fatty acid released/min. For time courses of triacylglycerol hydrolysis (15 and 30 min), the emulsions were made in the presence of fibres as described for emulsification measurements (for details, see the Experimental section). Values are means $\pm$ S.E.M. of three determinations. Different superscript letters (a, b) in the same column indicate significant differences (analysis of variance; $P < 0.05$).

	Initial lipase activity (μmol/min)	Triacylglycerol lipolysis (% of non-esterified fatty acid releasable)	
		15 min	30 min
Control	9.2 ± 0.9^{a}	5.4 ± 1.2^{a}	8.8 ± 1.2^{a}
Gum arabic	8.5 ± 0.7^{a}	2.9 ± 1.2^{b}	4.3 ± 1.1^{b}
LVG	9.5 ± 0.7^{a}	5.2 ± 1.1^{a}	8.5 ± 0.7^{a}
MVG	10.0 ± 1.4^{a}	5.2 ± 1.0^{a}	8.4 ± 0.8^{a}
HVG	10.5 ± 0.7^{a}	3.7 ± 1.2^{b}	6.0 ± 1.0^{b}

arabic, LVG or HVG to the medium did not modify the initial reaction velocity of human gastric lipase.

Incubating the control emulsion for 15 or 30 min with gastric lipase at pH 5.4 led to 5.4 and 8.8 % triacylglycerol lipolysis respectively. After incubation of emulsions of different droplet sizes (prepared in the presence or absence of fibres) with gastric lipase for 15 and 30 min (Table 4), the extent of triacylglycerol lipolysis catalysed by the enzyme was comparable for LVG, MVG and the control emulsions and significantly lower in the presence of the HVG emulsion and more markedly gum arabic.

DISCUSSION

We aimed to evaluate for the first time the effects of soluble fibres on dietary fat emulsification and lipolysis *in vitro*, mimicking conditions prevailing in the stomach. We selected lipid compositions and concentrations [2,6] relatively low in fibre (up to 2 %), a moderately acid pH (5.4) optimal for gastric lipase activity [1,3] and mild mechanical shear forces to achieve lipid emulsification. Thus the optimal conditions developed *in vitro* allowed emulsification of a proportion of the triacylglycerols present (about 25 %). Although the percentage of emulsified lipids was lower (25 compared with 63–85) than *in vivo* [6,8], the droplet sizes (range 1–100 μm) and the median diameter (range 7–46 μm) were comparable with those recently reported by our laboratory for the stomach contents of humans [6,8] and rats [9].

The data obtained clearly indicate that a slight increase in the medium viscosity is not sufficient to alter the process of triacylglycerol emulsification, as observed even with 2 % solutions of fibres with negligible apparent viscosity (gum arabic, LVG). In contrast, reaching a sufficiently high medium viscosity with various concentrations of moderately or highly viscous fibres [36,37] dramatically reduced the overall process of emulsification of dietary lipids. Data obtained with individual fibre sources indicate that an increased viscosity markedly increases the droplet size of emulsions and thus decreases the emulsion interface area generated. The key role of medium viscosity on dietary fat emulsification was more clearly demonstrated by the finding of linear correlations between droplet diameters (positive correlation) or emulsion surface areas (negative correlation) and apparent zero-shear viscosities in the range studied (0–5 mPa·s). An average 50 % reduction in the generated droplet surface area was obtained when a medium viscosity of about 4 mPa·s was reached *in vitro*. It is interesting to note that such levels of solution viscosity are reached in the stomach contents after ingestion of a fibre-enriched meal, as previously shown in the rat [23] and pig [22] with guar gums. Nevertheless, one should stress the fact that the flow conditions encountered during emulsification could be somewhat different from the steady laminar flow used to determine the apparent viscosity of fibre solutions. It is likely that the flow conditions present during the emulsification procedure used (shaking) are mostly turbulent with local flow velocity fluctuating in a chaotic way. Thus a possible explanation of the role played by fibre during emulsification is that increasing the apparent viscosity of the continuous phase depresses turbulence and removes the smallest eddies from the spectrum (Table 3). According to Walstra [35], this would cause an increase in the average size of the droplets. Moreover, in the case of shear-thinning behaviour, velocity could differ more from place to place than in the case of Newtonian liquid, leading to an expanded width of the droplet-size spectrum [35].

Of the fibre sources tested, gum arabic and the two pectins are charged polymers, with pK_a values in the range 3.2–3.7 [38,39]. Their overall behaviour in solution was not markedly different from that of the uncharged guar gum, at a comparable solution viscosity. It thus seems very likely that electric charges borne by the fibre polymers in mildly acidic conditions do not play a key role in hindering lipid emulsification. Moreover, gum arabic, which is composed of a carbohydrate as well as a protein moiety [40], is well known to display some emulsifying properties at neutral or mildly basic pH [40]; this property was not observed here, with LVG concentrations (maximum 2 %) and at acidic pH (5.4). Thus, in line with our basic knowledge on the mechanisms involved in emulsion formation [35,41], the data obtained here indicate that soluble dietary fibres that sufficiently increase the apparent viscosity of (reconstituted) gastric medium significantly reduce the extent of dietary lipid emulsification. The underlying mechanism is probably as follows: under a constant and low to moderate input of mechanical energy, as is probably found in the digestive tract [2], medium viscosity is a limiting factor for emulsification of dietary fat by reducing the energy efficiency of deforming and then disrupting the lipid mass to large and finally small droplets. Experiments are in progress in our laboratory to obtain data *in vivo*.

Although some studies have already reported the possible interference of dietary fibres in pancreatic lipase-catalysed triacylglycerol lipolysis [15–18], the mechanisms involved have not been investigated in detail and no data are available on gastric lipase which begins hydrolysis of dietary fat in the stomach [1–6]. Two pieces of information have been obtained in this study. First, we showed that guar gums solubilized in the medium do not modify the initial velocity of the lipase when excess triacylglycerol substrate is provided in the form of a preformed fine (4.5 μm) and stable emulsion, as conventionally used for lipase kinetic studies [11,30,32]. This indicates that the addition of viscous soluble fibres to a preformed emulsion does not alter the efficiency of the binding step of gastric lipase at the emulsion surface [11,32] and does not affect the catalytic reaction *per se*.

In a second set of experiments, emulsions prepared in the presence or absence of 0.3 % solutions of guar gums and gum arabic were provided as substrate to excess gastric lipase for a 30 min incubation in order to mimic intragastric digestion in terms of duration and maximum extent of lipolysis, i.e. about 10 % [4–6]. In this case, the extent of lipolysis was significantly decreased by emulsions prepared in the presence of the HVG, compared with those obtained without fibre or with LVG or MVG. This indicates that viscous gum that sufficiently alters droplet size and possibly droplet interface properties can reduce

the rate of triacylglycerol lipolysis catalysed by gastric lipase. This was also observed with pancreatic lipase (B. Pasquier and D. Lairon, unpublished work) and agrees well with our recent *in vitro* observations [11,12] that the smaller the droplet diameter, the higher the maximum velocity of the lipase. This could result from changes in some interface properties linked to surface curvature or area. Given the lack of a short-term effect (1 min) of solution viscosity on lipase initial velocity on a preformed emulsion, the most likely explanation seems to be as follows. As lipolysis proceeds, long-chain oleic acid moieties generated ($pK_a = 7.2–7.6$) are only poorly dissociated and solubilized in the aqueous medium at acidic pH (5.4) [42]: thus the non-esterified generated fatty acids tend to accumulate within the droplet surface monolayer as already observed [43], with a surface concentration inversely related to the emulsion surface area provided. It is well known that gastric lipase is very sensitive to interface properties [11,31,32]. It is very efficiently inhibited by protonated non-esterified fatty acids [1,31,44,45], and we recently showed that this inhibition process is a surface-dependent one [11] which may explain the low extent (10–15 %) of intragastric lipolysis recorded in humans [4–6]. In fact, a strong linear correlation ($r = 0.95$; $P = 0.05$) was found here between the area of the emulsion provided and the amount of non-esterified fatty acid generated (30 min incubation) in the presence of guar gums. Thus it is likely that the smaller the droplet surface area generated in the presence of viscous fibre, the lower the extent of lipolysis. In addition, a possible mechanism could involve the formation of a gum monolayer [41,46] around the lipid droplets which could be more tightly bound or rigid as the medium viscosity increases thus hindering the release of the lipolytic products from the droplet interface to the aqueous phase and, in turn, reducing the rate of lipolysis.

The strong inhibition of lipolysis induced by gum arabic at pH 5.4 after 30 min cannot be attributed to the proposed size-related mechanism (as it was shown to be without any effect on emulsion droplet size under the present conditions) but probably to interactions at the droplet surface. In fact, in contrast with the other fibres tested, gum arabic is a carbohydrate polymer associated with a protein moiety (up to 5 %, w/w) [40,47] which confers on the gum tensioactive properties and thus a potential emulsifying capability. It has already been shown that tensioactive proteins inhibit gastric lipase activity unless supramicellar concentrations of bile salts are added [31,32] and that gum arabic can inhibit the activity of pancreatic lipase in reconstituted duodenal medium [10]. The most likely explanation is that the protein moiety of gum arabic can strongly interact with the droplet interface, possibly blocking the available interface or, more likely, preventing the release of the non-esterified fatty acids generated at the droplet surface.

The present *in vitro* data show that viscous fibres reduce the emulsification of dietary lipids and subsequently lower the extent of triacylglycerol lipolysis in gastric conditions. Because intragastric emulsification of dietary lipids and subsequent partial lipolysis are the first steps of fat digestion in mammals, the mechanism demonstrated here may help us to understand how viscous soluble fibres lower the hyperlipidaemic postprandial response to a meal [48,49].

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