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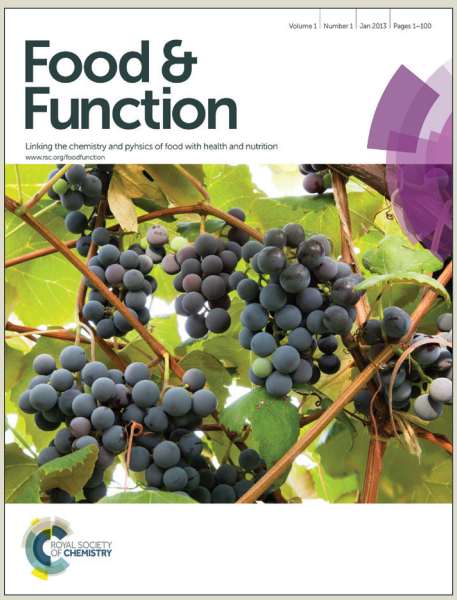

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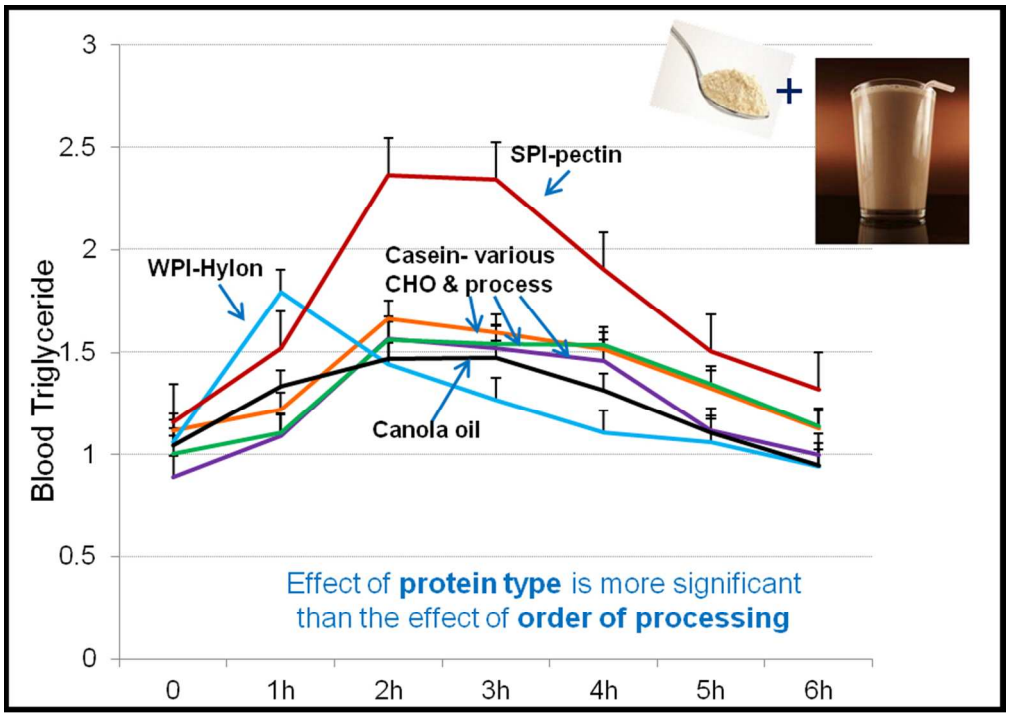

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Digestion of microencapsulated oil powders: *In vitro* lipolysis and *In vivo* absorption from a food matrix

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ABSTRACT

Microencapsulation provides a vehicle for the incorporation of fats and oils into powders that can be used as dry ingredients in an expanded variety of food products. Microencapsulated omega-3 oils in particular are commonly incorporated into a variety of foods and numerous *in vitro* studies have examined the impact of individual microencapsulation technologies on the simulated digestibility of the oil core. We have exposed twelve microencapsulated canola oil-in-water emulsions, formulated with different proteins (milk or soy) or mixtures of protein and carbohydrates (sugars, oligosaccharides, resistant starch) and processed into powders by spray drying, to simulated gastric and intestinal fluids *in vitro*. The extent of lipolysis of the canola oil in these products varied between 12-68 % suggesting that the *in vitro* digestibility of the oil within the powders was dependent on both the formulation and the processing steps used in their manufacture. Five microencapsulated powders with differing extents of *in vitro* lipolysis were then incorporated into a dairy beverage for an *in vivo* human trial with neat oil incorporation in the beverage used as the control. Measurement of triglyceride levels in blood revealed both enhanced peak height and area under the curve (AUC) *in vivo* for the microencapsulated oil groups when compared to the neat oil control. The range of lipolysis efficiencies observed with the different formulations *in vitro* was not reflected *in vivo*. These observations suggest that care needs to be taken when extrapolating data from *in vitro* lipolysis to bioavailability in humans.

Keywords: omega-3 oil, microencapsulation, *in-vitro*, *in-vivo*, food matrix

Introduction

Emulsions stabilised by low molecular weight emulsifiers, gums or proteins or combinations of these have been used for the delivery of oils into foods.[1-3] Emulsions may be converted into powdered ingredients to enable them to be delivered in a convenient format for food manufacturing applications. Proteins alone or in combination with carbohydrates have been typically used in the formulations for spray dried oil powders.[4] To improve the resistance of unsaturated oil powders to oxidation, proteins and carbohydrates used for encapsulation have been heat treated to form Maillard reaction products.[5] The heat treatment can be carried out on the aqueous protein-carbohydrate mixture prior to emulsion formation or the oil may be emulsified with a physical blend of protein and carbohydrate prior to heat treatment of the whole emulsion.[6]

Many studies using different *in vitro* models have been used to evaluate the bioaccessibility of oils and food bioactive components delivered in emulsions.[7] Factors affecting the *in vitro* digestion of the emulsions include *in vitro* test conditions (e.g. shear, temperature, pH, concentration and type of digestive enzymes, bile salts) and the physico-chemical properties of the emulsion formulation (e.g. the size of the oil droplets, their interfacial composition and structure, the degree of crystallisation of the fat phase) which affect the lipolysis of the oil.[8-11] The interfacial composition is altered after each digestion stage (i.e. mouth, stomach, small intestine) under simulated *in vitro* conditions as well as *in vivo,* and this has a significant effect on the digestibility of lipids. In addition, structuring of emulsions take place at each stage of the digestion process which influences the subsequent rate and extent of lipolysis as the emulsion transits through to other parts of the gastrointestinal tract.[12,13]

The extent to which *in vitro* test results can be used to predict the fate of the oil in the body needs to be further understood. The comparability of *in vitro* and *in vivo* results is

dependent on the *in vitro* model used, the nature of the carrier systems for the emulsified lipids and the animal model used for the *in vivo* studies. When different types of emulsion-based delivery systems (conventional emulsions, small microcluster emulsions, large microcluster emulsions and filled hydrogel beads) were compared, the data from *in vitro* digestion and *in vivo* rat trials had similar qualitative trends although there were quantitative differences.[14] However, a lack of correlation between some *in vitro* evaluations and *in vivo* data has also been observed. For example, although *in vitro* studies of emulsified lipid droplets coated by lecithin or lecithin-chitosan suggested that the additional chitosan layer produced using a layer-by-layer deposition technique inhibited the digestibility of the emulsified droplet, studies on mice showed that *in vivo* lipid digestibility of chitosan-encapsulated oil droplets was not inhibited.[15] *In vitro* studies showed that there was incomplete lipolysis of microencapsulated fish oil stabilised by heated protein-carbohydrate mixtures incorporated into foods after exposure to simulated gastric and intestinal fluids, with lipolysis of the oil in the cereal bar being lower than that in orange juice or yoghurt.[16] However, an ileostomy trial with the same formulations suggested higher levels of lipid digestion and absorption in humans (>98%) although there was some evidence of altered transit time with different food matrices.[17]

In this work, a direct comparison of a large number of formulations tested *in vitro,* from which formulations with very different extents of *in vitro* lipolysis were chosen to be tested in human trials. We determined the *in vitro* lipolysis of twelve microencapsulated canola oil powders stabilised with different proteins (milk or soy) or mixtures of protein and carbohydrates (sugars, oligosaccharides, resistant starch) and processed in different ways. We also compared the triglycerides in the blood of healthy humans who consumed dairy beverages enriched with either neat canola oil or selected microencapsulated oil powders. The aim was to determine whether microencapsulation altered the lipolyis of the oils *in vitro*

and the uptake of oil on digestion, and to determine if there is a relationship between *in vitro* lipolysis and the timing of the peak in triglycerides *in vivo*.

Materials and methods

Materials

Commercially available food grade ingredients were purchased from suppliers or from a local supermarket. Sodium caseinate (NaCas; Alanate 180®) and whey protein isolate (WPI; Alacen 895®) were from New Zealand Milk Products, Fonterra (Rowville, Victoria, Australia). Hydrolysed whey protein (HWP) was from Myopure (Petersham, New South Wales, Australia). Soy protein isolate (SPI; Supro® 760 IP) was from Solae Australia Pty Ltd (Chatswood, New South Wales, Australia). Dried glucose syrup (DGS, Fieldose 30® with dextrose equivalent of 30) was from Penford Australia Ltd (Lane Cove, New South Wales, Australia) and oligofructose (Oligo, Beneo P95®) was from Mandurah Australia Pty Ltd (Matraville, New South Wales, Australia). High amylose resistant starch (Hylon VII®) was from National Starch (Seven Hills, New South Wales, Australia). Pectin (Grinsted pectin SS200) was from Danisco Australia Pty Ltd (Botany, New South Wales, Australia). Canola oil (Crisco®) was from Goodman Fielder (Port Melbourne, Victoria, Australia). Powder drinking chocolate (Weight Watchers) and UHT fat free skim milk (Devondale) was purchased from Coles Supermarket (Werribee, Victoria, Australia).

Formulation and processing of microencapsulated oil powders

Twelve microencapsulated oil powder formulations containing 50% canola oil (dry basis), with protein alone (WPI) or protein (NaCas, WPI, HWP, SPI) in combination with one or more carbohydrates (processed Hylon VII (Hylon), oligofructose (Oligo), dried glucose syrup (DGS) or pectin) were manufactured on a pilot scale (Table 1). The processing of the Hylon

VII® involved heating a 20% total solids (TS) starch dispersion at 121°C for 60 min (FMC Surdry, Spain), cooling to room temperature, mashing the starch gel formed, diluting with deionised water to 10% TS and homogenising at 35MPa (Rannie Pilot Homogenizer, Denmark) prior to its use as an encapsulant.

The outline of the processes used for the manufacture of the microencapsulated oil powders is given in Fig 1. Briefly the protein was dispersed in water (60°C) using a high shear mixer with stirring for 30 min. For Processes A and B (Fig 1), carbohydrates were added to the protein solution and stirred for a further 5-10 min and the pH of the protein-carbohydrate mixtures was then adjusted to 7.5. This aqueous protein-carbohydrate mixture is referred to as the aqueous phase. For formulations where heating of the aqueous phase was carried out (Fig 1, Process A), the aqueous phase was heat-treated in a retort at 100°C for 50 mins (FMC Surdry, Spain). Canola oil preheated to 60°C, was then added into the aqueous phase whilst stirring using a high shear mixer. This pre-emulsion was then homogenised using two-stage homogenization pressures at 350/100 bar. The emulsions were spray dried at 180°C and 80°C inlet and outlet temperature, respectively. For formulations where the emulsion was heated (Fig 1, Process B), canola oil, preheated to 70°C for 30 min, was added into the aqueous phase using a high shear mixer and the pre-emulsion was homogenised using two-stage homogenization pressures at 350/100 bar. The homogenised emulsions were heated in a retort at 100°C for 50 mins (FMC Surdry, Spain), cooled down to 60°C and spray dried (Niro Production Minor, GEA process Engineering A/S, Sørborg, Denmark) at 180°C and 80°C inlet and outlet temperature, respectively.

For the heat-denatured WPI stabilised emulsion (Fig 1, Process C), the WPI powder was initially dispersed in water at 60°C, pH adjusted to 7.0 and heated at 90°C for 30 mins prior to oil emulsification (350/100 bar) and spray drying. For the SPI-pectin stabilised emulsion (Fig 1, Process D), an aqueous dispersions of SPI solution prepared at 60°C was mixed with a

pectin dispersion prepared separately at 80°C, then canola oil was added and the combined mixture then emulsified (350/100 bar) and spray dried. Spray drying was carried out as outlined above.

Characterisation of microencapsulated oil powders

The total oil content of powders was determined using an acid extraction method.[18] The total oil content was expressed as g oil/100 g powder (dry basis). The estimation of 'free oil' in powder was based on the method by Pisecky[18] except that petroleum ether was used in place of carbon tetrachloride.[5] The results were expressed as g/100g powder (dry basis).

***In-vitro* digestion and analysis of microencapsulated oil powders**

The simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) with bile extract were prepared as described by Oliver et al.[19] Sequential digestion in SGF and SIF, hereafter termed SGF-SIF, were carried out as follows: 1 g of microencapsulated oil powder or 1 g oil was dispersed in SGF (10 g). The mixture was incubated in a shaker water-bath (100 rpm) at 37°C for 2 h. After exposure to SGF, the pH of the sample was adjusted to pH 6.8. Then SIF (8 g) was added and the mixture was incubated at 37°C/100 rpm for 20 min. After this time, 2 ml of 50 mM $CaCl_2$ solution was added and the resulting mixture was further incubated at 37°C/100 rpm for 160 min. The final concentration of bile salt and calcium chloride in the mixture was 12 mM and 10 mM, respectively.

Extraction of oil after *in vitro* digestion in SGF-SIF. Oil was extracted from the samples after *in vitro* digestion using the ammonia extraction method specified in AS 2300.1.3.[20] The solvent was evaporated from the extracts using a rotary evaporator (60°C) then further dried in a vacuum oven at 48°C overnight. The amount of the oil extracted after SGF-SIF was

160 expressed as g/100 g powder (dry basis). The oil recovered (as % of the total oil) was
161 calculated from the amount of oil extracted after SGF-SIF divided by the total oil content of
162 powders or the neat oil added. The extracted oil was used for analysis of fatty acids by GC.
163 **Quantification of total free fatty acids.** The major individual fatty acids both in free
164 and glycerol bound forms (namely palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic
165 (C18:2) and linolenic (18:3)) were determined after acid methylation. The fatty acids in
166 glycerol bound forms were quantified after alkaline methylation. The levels of individual free
167 fatty acids, which indicate the extent of lipolysis, were obtained by difference. The total free
168 fatty acids were the sum of the individual free fatty acids.
169 For the acid methylation, 50 (±0.01) mg of the extracted oil, 3 ml aliquot of internal
170 standard solution (1.83 mg/ml C23:0 methyl ester /ml isooctane), 2 ml tetrahydrofuran and 2
171 ml 2M sulphuric acid in methanol were added into a culture tube. The mixture solutions were
172 blanketed with argon, capped tightly and agitated (200 rpm) at 70°C for 2.5 h in a water bath
173 shaker. Every 30 min, the tubes were taken out from the shaker water bath and vortexed for
174 one minute. Water (5 ml) containing sodium chloride (5% w/v) was added to stop the
175 methylation and the methyl esters were extracted with 3 ml hexane. The top organic phase
176 was washed with water (4 ml) containing potassium bicarbonate (5% w/v) and dried over
177 anhydrous sodium sulphate. The methyl ester solution was subjected to GC injection without
178 further concentration.
179 For the alkaline methylation**,** 50 (±0.01) mg of extracted oil, 3 ml aliquot of internal
180 standard solution (1.83 mg C23:0 methyl ester /ml isooctane), 3 ml of hexane and 300 µl of
181 2M KOH/methanol solution were added into a culture tube. The mixture solutions were
182 blanketed with argon, capped tightly and vortexed (2000 rpm) at room temperature for 10
183 min. The methyl ester solution was neutralized by 300 µl of 2M HCL-methyl orange water.
184 After 30 min settling, the top layer was injected into GC without further concentration.

185 Total free fatty acids were calculated using the following formula:

186 $$\text{Individual FA (X) (mg/g oil)} = \frac{\text{GC area for X} \times \text{IS (mg)} \times \text{CF}}{\text{GC area for IS} \times \text{oil mass (g)}} \quad (1)$$

187 $$\text{Individual FFA} = (\text{free} + \text{glycerol bound})\ \text{FA} - \text{glycerol bound FA} \quad (2)$$

188 $$\text{Total FFA} = \text{Sum of individual free fatty acids} \quad (3)$$

189 Where: FA = fatty acid; FFA = free fatty acid; X = individual fatty acid; IS = internal

190 standard added; CF = ratio of theoretical flame ionization detector correction factor of X/IS

191 The above method was validated with fresh canola oil and the less than 0.01% total free

192 fatty acids was obtained.[16]

193

194 **Clinical Trial**

195 For the clinical trial, 6 healthy normotriglyceridemic volunteers (three men and three women)

196 aged 20-65 years with BMI 25-30 kg/m^2 were recruited. The inclusion criteria were no recent

197 history (past 3 months) of weight loss or changes to diet or physical activity routine. The

198 exclusion criteria were persons with one or more of the following conditions: Type 1 or 2

199 Diabetes, active liver and kidney disease, current gastrointestinal disease, past history of

200 gastrointestinal surgery which may affect study outcomes, intolerance to fat or on

201 medications which affect gastrointestinal tract motility or hunger /appetite. The volunteers

202 fasted from midnight, the night prior to attending the clinic in the morning. All experimental

203 procedures were approved by Human Ethics Committees of the Commonwealth Scientific

204 and Industrial Research Organization and participants provided written informed consent.

205 All research was conducted following institutional guidelines.

206 **Preparation of test samples for consumption.** The dairy-based beverages containing

207 either neat canola oil or microencapsulated canola oil powder were prepared. Five

208 microencapsulated canola oil powders, displaying different extents of lipolysis *in vitro*, were

209 chosen after completion of the *in vitro* trials and incorporated into beverages for the human

trial. The dairy based beverages were made up with water (78 g), skim milk (200 g) and drinking chocolate powder (Weight Watchers) (12 g) using a hand-held mixer for 45 s. Either a microencapsulated oil powder (60 g powder containing 30 g oil) or neat oil (30 g oil plus 30 g water) was added and mixed for a further 30 s. Each volunteer consumed the entire 350 g chilled beverage formulation on 6 different occasions and at least three days apart to assess the time line of fat digestion from the pre-load (dairy based beverage).

Conduct of clinical study. Six healthy normotriglyceridemic volunteers (three men and three women) attended the clinic on 6 separate occasions with a minimum 3 days apart for each visit. On each occasion the volunteers consumed 350 g of an isocaloric chilled beverage containing 30 g canola oil added as neat oil (control) or oil encapsulated in selected protein or protein-carbohydrate powdered formulations. They were allowed to sit or stand but not to lie down during their time in the clinic. Finger prick samples were taken after fasting and then every hour (over 6 h) for whole blood triglyceride levels.

Measurement of triglycerides. Fingerprick triglycerides (TG) was measured using the Cardiochek Triglyceride system (Polymer Technology, Systems Indianapolis, Indiana, USA).

Power calculation. With 6 people there was 84% power, $p<0.05$ to see a 0.35 mmol/L difference in peak TG values and a 79% power, $p<0.05$ to see a 1.1 mmol/l/h difference in AUC above baseline. Plasma values are obviously a summary of absorption and clearance rates. We know nothing of the latter but given the fact that the fat is the same in all preparations there should no differences. Absorption rates will be dictated primarily by lipolysis rates with no contribution likely from differences in intestinal cell metabolism.

Data analysis. Data was analysed using SPSS v19 (IBM, Australia). The primary analysis was repeated measures ANOVA with 6 treatments repeated across 7 time points. Post hoc contrast were performed if p was <0.05 for time by treatment. No adjustments were

made for the number of post hoc contrasts and $p<0.05$ was deemed to be significant. Time by treatment reflects both a change in peak height as well as a change in peak timing.

Results and Discussion

Total oil content and 'free oil' of microencapsulated oil powders

The total oil and 'free oil' of the microencapsulated oil powders are given in Table 2. The total oil content of most microencapsulated oil powders was 46-54 % (dry basis). This compares well with the formulated amount of 50 % (dry basis) in most cases.

The 'free oil' of the powders was variable and dependent on the formulation and process conditions (Table 2). The protein only (WPI Process C), protein-pectin (SPI-pectin Process D) and NaCas or WPI formulations containing Hylon (both Process A and Process B) had high 'free oil' (13-25 % of the powder) (Table 2). The addition of Oligo and further replacement of Hylon with DGS to the NaCas-Hylon formulation resulted in a decrease in 'free oil' from 21.6 % (NaCas-Hylon Process A) to 13.4 % (NaCas-Oligo-Hylon Process A) and 0.5 % (NaCas-Oligo-DGS Process A) (Table 2). The presence of small sugars is known to aid the efficiency of the encapsulation process during drying[4]. The presence of low molecular weight sugars in powdered oil-containing formulations reduces the accessibility by the extracting solvent.[21] However, the exception was observed with the addition of sugars to the WPI-Hylon formulations which had little effect on the 'free oil' (Compare WPI-Oligo-Hylon with WPI-Hylon where the same process was used) (Table 2). This is possibly because the globular whey proteins by themselves form a more robust interfacial layer than the caseins. Studies on the adsorption of milk proteins at interfaces have suggested that globular whey proteins such as β-lactoglobulin form a more cohesive gel-like layer at the interface than the more randomly ordered caseins.[22]

The heat treatment of emulsion resulted in a marked reduction in solvent extractable 'free oil' when WPI was used as an encapsulant but not when NaCas or HWP was used as the protein source. This was evident when formulations with the same gross formulation made using Process A, were compared to those made using Process B (Table 2). It may have been expected that the interface of the droplets formed would be different depending on whether the aqueous protein-carbohydrate mixture was heated prior to emulsification (Process A) or whether the emulsion was formed prior to heating (Process B). This is because in systems where the unheated mixtures of proteins and carbohydrates are emulsified with the oil, only the proteinaceous species in the aqueous phase will accumulate at the interface as carbohydrates are not surface active. When these emulsions are heated, the proteins at the interface may be decorated by carbohydrates which conjugate with available sites on the protein. In systems where the oil is emulsified after heating the protein carbohydrate mixture (Process A), the composition of the interface is dependent on the type of protein encapsulant or hydrolysed protein and protein-carbohydrate conjugate formed by heating of the aqueous mixture of protein and carbohydrate and that are present at the time of emulsification.[23] The observation that the encapsulation efficiency was only different for heat-treatment of WPI-based formulations but not when NaCas or HWP was used as the protein source maybe related to the greater ability of WPI (a globular protein which is also prone to denaturation), to unfold at the interface compared to the caseins and hydrolysed whey protein which are less structured.[22]

***In vitro* digestibility**

The *in vitro* digestibility of the microencapsulated oil powders was examined by measuring the free fatty acid released after exposure to SGF-SIF. The amount of oil that was extractable from the sample after SGF-SIF, the calculated oil extraction efficiency, the extent of lipolysis

283 measured from extracted oil and the calculated extent of lipolysis after SGF-SIF are given in
284 Table 2.

285 Although the total oil extractable from the powders before SGF-SIF (Table 2) was close
286 to the formulated oil content of the powders (Table 1), not all the oil could be recovered by
287 extraction after SGF-SIF for some formulations (Table 2). The oil recovered after exposure to
288 SGF-SIF (expressed as % of the total oil) was 53 – 115 % (Table 2). Most of the samples had
289 >80 % of total oil recovered by solvent extraction. The exceptions were formulations
290 containing NaCas-Hylon made using Process B (52.6 % of total oil) and HWP-Oligo-Pectin
291 made using Process B (54.4 % of total oil) (Table 2).

292 The calculated extent of lipolysis assumes the amount of oil that is not extracted from the
293 samples after *in vitro* digestion, is undigested oil in triglyceride form (Table 2). This
294 calculated extent of lipolysis was taken as the % of the total oil that is bioaccessible.
295 Quantitation of the measured amounts of free fatty acids by gas chromatography combined
296 with the data on oil recovered suggested that 45 % - 68 % of oil was bioaccessible after SGF-
297 SIF except for powders made from NaCas-Hylon Process B (12.1 %) and HWP-Oligo-Pectin
298 process B (12.5 %) (Table 2). These latter two microencapsulated oil powders with the lowest
299 amounts of (calculated) lipolysed oil (~12 % of total oil) also had the lowest amounts of
300 recoverable extractable oil after SGF-SIF exposure (~50 %). In comparison, when neat
301 canola oil is subject to *in vitro* digestion, there was an extremely low extent of lipolyis
302 (1.08±0.42%) as under the conditions of the *in vitro* system the oil is not in the form of fine
303 emulsion droplets. This is in contrast to when microencapsulated formulations are used and
304 the emulsions are homogenised prior to drying. The particle size (volume median diameter)
305 of all formulated emulsions (measured by laser light scattering) used was d(0.5) 0.30 – 11.46
306 μm depending on the formulation and order of processing (data not shown).

Where the same emulsion composition was used with different processing steps, there was a consistent trend in the relative extents of bioaccessibility of the oils. Systems where microencapsulated oil powders were made from heat treated emulsions (Process B) were less bioaccessible than corresponding powders made from emulsions stabilised by heat treated aqueous mixtures of protein and carbohydrate (Process A) (Table 2). The cross-linking of interfaces of heated emulsions may have contributed to the lower bioaccessibility of oil in powders made from heat treated emulsions (Process B).

Selection of microencapsulated oil powders for *in vivo* human trials

The *in vitro* analysis of the microencapsulated oil powders exposed to SGF-SIF indicated differences in the extent of lipolysis (Table 2). Based on the *in vitro* analyses, five samples with different levels of *in vitro* lipolysis were chosen for a human study in which the bioavailability of microencapsulated oil powders and neat oil (control) when incorporated into a dairy based average were compared. The powders chosen were NaCas-Hylon Process B, NaCas-Hylon Process A, NaCas-Oligo-DGS Process A, WPI-Hylon Process A and SPI-Pectin process D. NaCas-Hylon Process B (12.1 % lipolysed) was one of the least digested microcapsules based on *in vitro* analysis. NaCas-Hylon Process A (53.4 % lipolysed) and SPI-Pectin Process D (54.6 % lipolysed) were in the middle range for extents of lipolysis. WPI-Hylon Process A (64.2 % lipolysed) and NaCas-Oligo-DGS Process A (67.6 % lipolysed) were two of the most extensively lipolysed samples *in vitro* amongst the powders screened (Table 2). Although HWP-Oligo-Pectin Process B (12.5 % lipolysed) had a very low extent of digestion comparable to NaCas-Hylon Process B (12.1 %), this system was not used in the clinical trial due to its unacceptable flavour profile, which was attributed to the hydrolysed protein component.

Clinical trial

There was an enhanced peak height and area under curve (AUC) for most subjects (individual results not shown) when the dairy beverage with microencapsulated oil powders were consumed, compared to that for the dairy beverage made by incorporation of the neat oil (Fig 2). As the fat used was the same in all preparations no differences in the handling of the triglyceride after digestion and absorption should occur. Therefore the enhanced peak and AUC may be attributed in part to smaller oil droplet size of all the microencapsulated formulations compared to neat oil. Others have shown that decreasing particle size of oil droplets increases the rate of lipolysis[24] and β-carotene bioaccessibility in emulsions[25]. This was considered to be due to the increase in lipid surface area exposed to pancreatic lipase.

There were only small differences in the AUC for plasma triglyceride level between the canola oil mixed with the dairy beverage and four of the microencapsulated formulations (Cas-Hylon Process B, Casein-Hylon Process A, Casein-oligo-DGS Process A and WPI-Hylon Process A) in dairy beverages (Fig. 2). This suggests the accessibility of lipase to the interface of microencapsulated particles and the canola oil mixed with the dairy beverage was largely similar. Even if neat canola oil was added into beverage and was consumed, it will be emulsified when blended into the beverage because it is mixed with other components in the dairy beverage (e.g. dairy protein) that have emulsifying properties. After consumption there is the further combined action of the mixing in the stomach and the presence of other emulsifying food components that are residual in the stomach. Others have shown the bioequivalence of microencapsulated powders made by a coacervation process and the same oil delivered in gelatin capsules[1].

The highest peak and area under curve is most marked with SPI pectin Process D (e) compared to all other treatments. SPI pectin Process D (e) is significantly different ($p<0.05$) from neat canola oil (f), WPI-Hylon Process A (d) and Cas-Hylon Process B (a). In 3 out of 6

subjects, Cas-Oligo-DGS Process A (c) produced some prolongation or delay in the curves (individual results not shown) and is in fact significantly different ($p<0.05$) from WPI-Hylon Process A (d), SPI-pectin Process D (e) and neat canola oil (f) on time by treatment analysis.

The most noticeable difference was the shifting forward of the release of the WPI-Hylon Process A formulation compared to all other formulations. It was also noted that when the protein in the WPI-Hylon Process A formulation was substituted with casein to make the Cas-Hylon Process A formulation, a delayed release was obtained (Fig 2). This shows the important influence of the protein on the lipolysis *in vivo*, with a casein based interface being more resistant to early lipolyis.

A significant observation is the marked enhancement in the AUC for the SPI-pectin Process D formulation compared to all other formulations tested. Given that the same fat and the same amount of fat were used in all preparations any differences seen in postprandial triglyceride will reflect differences in the gut only i.e. gastric and intestinal emptying rates as well as differences in digestion. After digestion absorption of the free fatty acids and monoglycerides will be the same in all preparations. There are many factors that influence the lipolysis of oils. In encapsulated or emulsified systems, it is envisaged that lipolysis will depend on the access of the lipase to the encapsulated oil and the particle size of the oil droplet. These include the digestibility of the interface and the ease of displacement of protein from the interface and the subsequent attachment of lipase which then enables the lipase to act on the emulsified oil. It is well known that different proteins adopt different conformations at the interface and in addition the digestibility of different proteins is also different, whether this is *in vitro* or *in vivo*. A study which compared WPI and SPI based oil-in-water emulsions (10% w/w soybean oil, 1.5% w/w protein, homogenised at 40 MPa using a microfluidizer) suggested that there was more lipolysis of the SPI emulsions and also that the particle size of SPI emulsions were smaller than those of WPI emulsions[26]. In our work,

the % lipolysis in the WPI emulsions (45.2 – 64.9 % lipolyis, Table 2) depended on the formulation and process used and there was not a consistent trend in extent of lipolysis when compared to that of the SPI-pectin emulsion (54.6 lipolysis, % Table 2). Taken together, this highlights the sensitivity of the extent of lipolysis to the different interfacial structures formed with changed formulations and processes even *in vitro*. Although the particle size of the emulsions *in vivo* have not been measured, it is tempting to speculate that the increased digestibility of the SPI-pectin systems may be because the emulsified oil in this system *in vivo* remain more stable to coalescence compared to dairy protein stabilised emulsions and/or that the ease of displacement of the SPI pectin interface was greater, providing easier access to lipase and consequently a higher degree of lipolysis.

The data suggests that the SPI-pectin Process D formulation, which provided the highest concentration of triglycerides in the blood, is one that has the potential to be the most bioavailable and may provide an exceptional system for the delivery of fatty acids, when incorporated into a dairy beverage. It would be important to determine if this enhancement is also obtained with a range of other oils (e.g. omega- 3 fish oils) and extends to the transit of lipophilic bioactives (e.g. carotenes) and also when the SPI-pectin Process D formulation is delivered in different food matrices.

There is no direct correlation between lipolyis *in vitro* (12.2 – 67.6% lipolysis amongst formulations (a) – (e), Table 2) and the AUC *in vivo* for the formulations compared (Fig 2). A plot of AUC and the extent of lipolysis in vitro showed a poor correlation between these two parameters (n=5, r^2=0.011) (Figure not shown). One of the reasons for lack of correlation between the *in vitro* and *in vivo* data is likely to be the higher shear forces *in vivo* relative to that *in vitro.*

Conclusions

In vitro studies are useful to the extent that they give insights into how the structure of the interface around the oil droplets affects the accessibility of the oil within an oil droplet to digestive enzymes. In this work, there was no direct correlation between *in vitro* and *in vivo* digestibility for corresponding microencapsulated formulations. *In vivo* lipolysis is highly efficient in systems where oil is delivered in emulsions formulated with food proteins and carbohydrates.[17] When oil was delivered as microencapsulated oil powders incorporated into a dairy based beverage, there was enhanced peak height and AUC *in vivo* for the microencapsulated oil groups when compared to the neat oil control. *In vivo* human trials are essential to understand the bioequivalence of oil delivered in different food structures.

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Table 1 Formulated compositions of the aqueous phase, emulsions and microencapsulated oil powders

Aqueous phase components[a]	ID Process	Heat-treatment		Aqueous phase composition (%, wet basis)		Emulsion composition (%, wet basis)			Powder composition (%, dry basis)		
		Aqueous	Emulsion	Protein	CHO[b]	Protein	CHO[b]	Oil	Protein	CHO[b]	Oil
NaCas-Hylon	B	No	Yes	6.0	6.0	5.4	5.4	10.7	25.0	25.0	50.0
NaCas-Hylon	A	Yes	No	6.0	6.0	5.4	5.4	10.7	25.0	25.0	50.0
NaCas-Oligo-Hylon	A	Yes	No	5.7	11.4	4.8	9.7	14.5	16.7	33.3	50.0
NaCas-Oligo-DGS	A	Yes	No	5.7	11.4	8.6	17.1	25.7	16.7	33.3	50.0
WPI-Hylon	A	Yes	No	5.0	5.0	4.6	4.6	9.1	25.0	25.0	50.0
WPI-Hylon	B	No	Yes	5.0	5.0	4.6	4.6	9.1	25.0	25.0	50.0
WPI-Oligo-Hylon	A	Yes	No	4.8	9.6	4.2	8.3	12.5	16.7	33.3	50.0
WPI-Oligo-Hylon	B	No	Yes	4.8	9.6	4.2	8.3	12.5	16.7	16.7	50.0
HWP-Oligo-Pectin	A	Yes	No	7.2	14.4	5.9	11.8	17.8	16.7	33.3	50.0
HWP-Oligo-Pectin	B	No	Yes	7.2	14.4	5.9	11.8	17.8	16.7	33.3	50.0
WPI	C	Yes	No	10.0	0	9.1	0	9.1	50.0	0	50.0
SPI-Pectin	D	No	No	9.0	0.5	8.3	0.4	8.7	47.6	2.4	50.0

[a]Hylon = Hylon where pre-processing involved homogenisation of a heated aqueous dispersion of the starch prior its use as an encapsulant
[b]CHO = carbohydrate

Table 2 Analyses of microencapsulated oil powders before and after sequential exposure to SGF and SIF[a]

Aqueous phase components[b]	ID	ID	Neat Powder (Before SGF-SIF)		Oil extractable (After SGF-SIF)		Extent of Lipolysis (After SGF-SIF)	
	Process	Clinical study sample	Total oil[c] (g/100g solids)	'Free oil' (g/100g solids)	Oil extracted (Measured) (g/100 g solids)	Oil recovered (Calculated) (% of total oil)	Lipolysis (Measured) (% in extracted oil)	Lipolysis (Calculated)[d] (% of total oil)
NaCas-Hylon	B	a	46.4±0.1	21.3±0.3	24.4±1.0	52.6	23.0±0.5	12.1±0.2
NaCas-Hylon	A	b	49.1±0.2	21.6±0.7	44.4±0.1	90.4	59.0±0.2	53.4±0.2
NaCas-Oligo-Hylon	A	-	51.4±0.3	13.4±0.1	52.4±1.1	101.9	61.0±0.3	62.2±0.3
NaCas-Oligo-DGS	A	c	50.3±0.5	0.5±0.0	57.6±0.7	114.5	59.0±0.2	67.6±0.2
WPI-Hylon	A	d	51.4±0.1	24.5±0.1	52.4±1.2	101.9	63.0±1.2	64.2±1.2
WPI-Hylon	B	-	47.2±0.2	14.2±0.3	44.9±0.4	95.1	59.0±0.8	56.1±0.7
WPI-Oligo-Hylon	A	-	52.6±0.5	25.2±0.1	56.9±0.8	108.2	60.0±1.1	64.9±1.1
WPI-Oligo-Hylon	B	-	53.9±0.2	14.5±0.3	43.5±0.6	80.7	56.0±0.5	45.2±0.4
HWP-Oligo-Pectin	A	-	51.3±0.5	0.6±0.0	51.2±0.2	99.8	63.0±0.1	62.9±0.1
HWP-Oligo-Pectin	B	-	52.9±0.1	0.7±0.0	28.8±0.8	54.4	23.0±0.3	12.5±0.2
WPI	C	-	52.9±0.3	19.4±0.3	50.9±1.4	96.2	56.0±0.8	53.9±0.8
SPI-Pectin	D	e	53.6±0.5	24.1±0.5	53.2±0.4	99.3	55.0±0.4	54.6±0.4

[a] Data is the mean±s.d. where oil extractions were carried out in duplicate and each oil extract was analysed by GC for fatty acids.
[b]Hylon = Pre-processed Hylon where pre-processing involved homogenisation of a heated aqueous dispersion of the starch.
[c]Total oil, Free oil, and oil extractable after SGF-SIF are calculated as % powder dry basis.
[d]The calculated extent of lipolysis assumes the amount of oil that is not recoverable after SGF-SIF is undigested oil in triglyceride form.

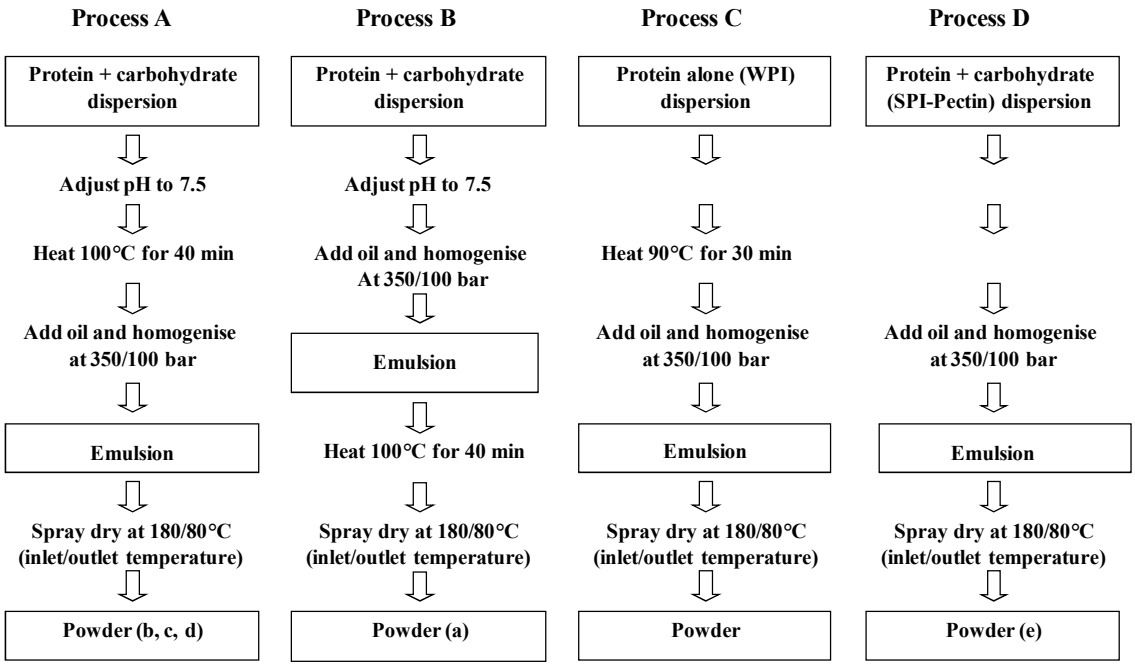

Fig 1 Process flow diagram for the manufacture of microencapsulated oil powders

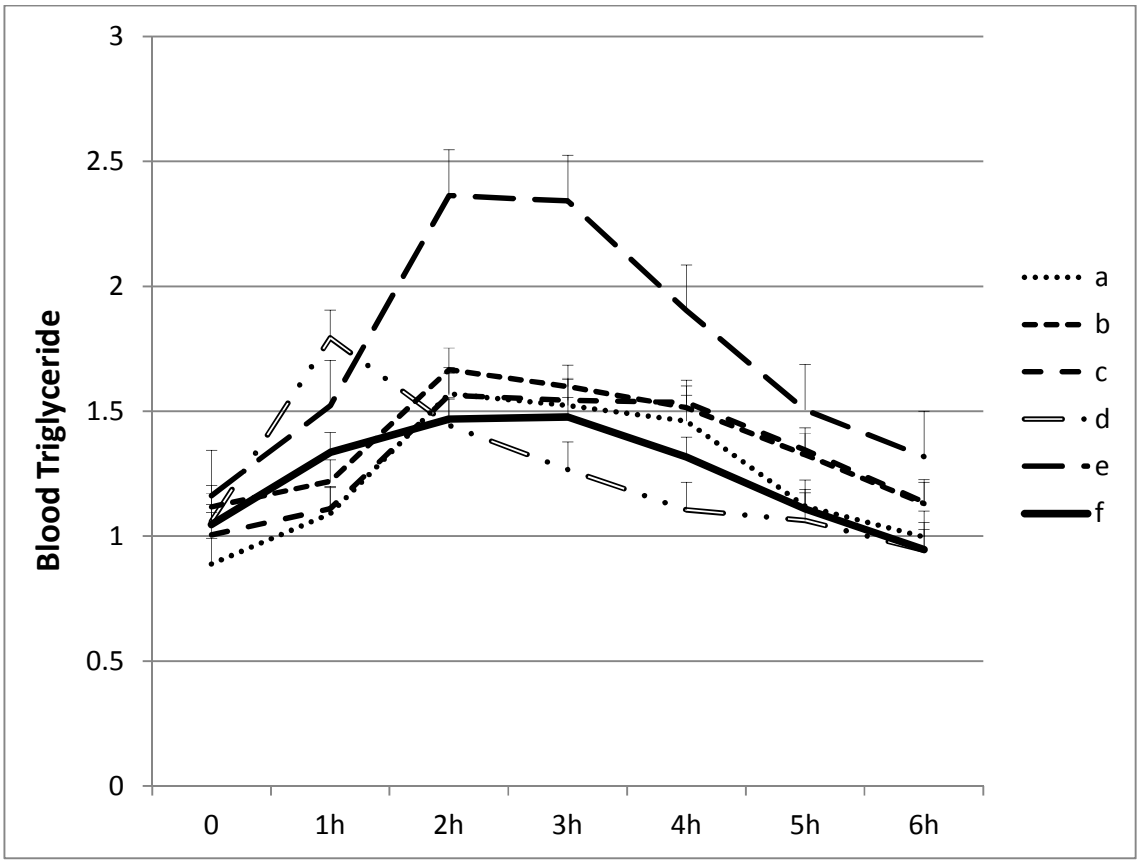

Fig 2 Average blood triglyceride values for 6 normotriglyceridemic subjects (3 Male, 3 Female) over 6 h after consumption of beverage. (a) Cas-Hylon Process B; (b) Cas-Hylon Process A; (c) Cas-oligo-DGS Process A; (d) WPI-Hylon Process A; (e) SPI-pectin Process D; (f) canola oil. Processes used are given in Fig 1. By repeated measures and using ANOVA, sample (e) is significantly different from all others ($p<0.05$). Sample(c) was significantly different from (d), (e) and (f) ($p<0.05$).