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Small particle size lipid emulsions, satiety and energy intake in lean men

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HIGHLIGHTS

- Lipid emulsions have been proposed to suppress hunger and food intake
- Lipids delivered direct to the ileum *via* tube feeding trigger the ileal brake
- Dietary lipid emulsions are hypothesised to also trigger the brake mechanism
- We investigated whether a high phospholipid, small droplet emulsion can alter VAS-assessed appetite and *ad libitum* energy intake
- No evidence that a high phospholipid emulsion can alter eating behaviour in lean men

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ABSTRACT

Lipid emulsions have been proposed to suppress hunger and food intake. Whilst there is no consensus on optimal structural properties or mechanism of action, small particle size (small-PS) stable emulsions may have greatest efficacy. Fabuless®, a commercial lipid emulsion reported in some studies to decrease energy intake (EI), is a small-PS, 'hard' fat emulsion comprising highly saturated palm oil base (PS, 82 nm). To determine whether small-PS dairy lipid emulsions can enhance satiety, firstly, we investigated 2 'soft' fat dairy emulsions generated using dairy and soy emulsifying agents (PS, 114 nm and 121 nm) and a non-emulsified dairy control. Secondly, we investigated a small-PS palmolein based 'hard' fat emulsion (fractionated palm oil, PS, 104 nm) and non-emulsified control. This was a 6 arm, randomized, cross-over study in 18 lean men, with test lipids delivered in a breakfast meal: (i) Fabuless® emulsion (F_{EM}); (ii) dairy emulsion with dairy emulsifier (DE_{DE}); (iii) dairy emulsion with soy lecithin emulsifier (DE_{SE}); (iv) dairy control (DC_{ON}); (v) palmolein emulsion with dairy emulsifier (PE_{DE}); (vi) palmolein control (PC_{ON}). Participants rated postprandial appetite sensations using visual analogue scales (VAS), and *ad libitum* energy intake (EI) was measured at a lunch meal 3.5 h later. Dairy lipid emulsions did not significantly alter satiety ratings or change EI relative to dairy control (DE_{DE}, 4035 kJ; DE_{SE}, 3904 kJ; DC_{ON}, 3985 kJ; $P > 0.05$) nor did palm oil based emulsion relative to non-emulsified control ($PE_{DE,}$ 3902 kJ; $PC_{ON,}$ 3973 kJ; $P > 0.05$). There was no evidence that small-PS dairy lipid emulsions or commercial Fabuless altered short-term appetite or food intake in lean adults.

1. Introduction

Several studies have shown the small particle size (small-PS), 'hard fat' palm and oat oil based emulsion [2]Fabuless® (Olibra) to decrease short and medium term energy intake (EI) [1–4], although others have failed to show efficacy [5–12]. The mechanism proposed is the ileal brake [13,14]. To activate the brake emulsified oils are hypothesised to remain intact, bypassing the stomach and uptake by the proximal duodenum [15]. The delay of lipolysis and fat absorption then leads to increased exposure of fat in the distal ileum which in turn stimulates a proximal feedback loop to slow gastric emptying and small bowel motility, promotes secretion of gastrointestinal (GI) peptides, and alters hunger, fullness and food intake [13,14]. The brake mechanism is well supported by early [16–18] and more recent enteral feeding studies where small amounts of fat infused directly into the ileum altered appetite response [14,19,20]. Whether this can be

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[2] Fabuless trademark.

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achieved when protected lipid/water emulsions are consumed in the diet has yet to be convincingly demonstrated. Notably there is also evidence of a duodenal/jejunal brake [21], although effects may be less potent [22].

Emulsion structure directs response of the GI tract and in turn digestion rate and site [23,24], and is highly likely to alter the brake effect. The principal of oro-ileal delivery by a high phospholipid (PL) emulsion hinges on protection of central core lipids by the surrounding surface polar lipids, which may alter the rate at which core lipids are hydrolysed [25]. Hence composition and modification of both core and polar lipids may alter activation of the brake. PLs in particular have been shown to slow lipid breakdown by inhibiting the activity of lipases at the lipid/water interface by a decrease in physical contact between the lipid and the enzyme [26]. Whilst findings from Fabuless trials remain unconvincing, a recent dietary trial investigating a different high polar lipid fractionated oat oil as emulsifying agent reported increased short-term satiety [27] with evidence of increased circulation of satiety-related GI peptides. In addition, a number of recent trials have now shown small-PS emulsions to alter various aspects of satiety and food intake [19,28,29] with some evidence of greater efficacy than large-PS lipid emulsions [19,28], although again effects can be inconsistent [29]. Maljaars showed that small-PS lipid droplets significantly altered gastric emptying and satiety *versus* large-PS droplets [19], although effects were observed during infusion into the duodenum rather than ileum. Similarly, in a sophisticated magnetic resonance imaging (MRI) feeding study, Hussein and colleagues showed that decreasing fat droplet size of a plant-based emulsion could also slow gastric emptying, increase water content within the small intestine (SI), and in turn decrease short term energy intake [28]. Increased intragastric stability, which was achieved by adding a locust bean gum as a 'thickener' to prevent lipid layering, creaming and coalescence within the stomach, also suppressed food intake even when a large droplet emulsion was consumed.

This led us in the current trial to investigate whether specific features of small-PS lipid emulsions promote changes in postprandial appetite response and eating behaviour when consumed within a meal. Since lipid characteristics such as fatty acid (FA) composition, physical structure properties (*e.g.,* solid/liquid at room temperature) and stability may alter appetite-related outcomes, we compared 2 small-PS emulsions with differing physical characteristics. The test emulsions were small-PS animal-origin 'soft' fat (dairy) and plant-origin 'hard' fat (palm) lipids, emulsified using dairy or soy PLs.

2. Participants and methods

2.1. Participants

Lean male volunteers (BMI 18–25 kg/m^2), aged 18–55 years and healthy by self-report were enrolled into this intervention trial. Recruitment was carried out in Auckland through news paper and electronic advertisement. For screening, participants came fasted (overnight) to the appetite research centre at the University of Auckland Human Nutrition Unit (HNU) where body weight, height, waist circumference and blood pressure were measured. Key exclusion criteria included self-reported history of obesity or eating disorders, current cigarette smoker or restriction diet, diabetes, cardiovascular disease including hypertension, and any other significant metabolic, endocrine or GI disease. Eligible participants were free of medications known to influence appetite or weight regulation. Written consent was obtained from each of the participants, and ethical approval for this study was obtained from the Northern Regional Ethics Committee, Auckland, New Zealand. The trial was registered on the Australia New Zealand Clinical Trial Registry, international trial #ACTRN12609000853246.

2.2. Study design

Dairy derived lipid emulsions and matched controls were administered at breakfast to assess short-term appetite responses and *ad libitum* EI at a subsequent lunch meal. All participants attended the HNU on 6 separate occasions and were randomly allocated to study treatment. Between each visit they returned home for a washout period of at least 7 days during which time they were free to resume usual diet and exercise patterns. Twenty four hours prior to each study day, participants were asked to abstain from alcohol, avoid significant change in habitual diet and strenuous physical activity. To ensure compliance on pre-treatment days, participants recorded 24 h dietary intake and exercise level (time spent sitting, standing, screen activities, mild-moderate activity or vigorous/strenuous activity).

2.3. Study procedures

Standardised protocols were applied based on recommended methods of Blundell et al., [30] and previous appetite trials conducted at HNU [6,31]. On each study day participants were asked to avoid morning exercise and to fast (water only) from 8:00 pm on evening prior. Upon arrival, body weight was measured with the participant lightly clad (Seca, Model 708, Germany) and 250 mL of water was consumed. A diet and activity questionnaire was completed and adverse events (AEs) during the washout period recorded. Height was measured on a single occasion at the screening visit (Seca, model 222, Germany). Baseline VAS (visual analogue scales) were completed to rate feelings of hunger, fullness, satisfaction and current thoughts of food (TOF) prior to breakfast. The test breakfast was served at 08:30 am and participants were asked to consume the meal in full but at their own pace within 15 min. No further foods were consumed throughout the morning and the participants remained within the HNU, during which time repeat VAS ratings were measured periodically prior to lunch. 250 mL of water was served at 10:30 am. The lunch meal was served at 12:15 pm, with participants seated within individual dining booths. The timing of the lunch meal was based on previous Fabuless trials which showed effects on appetite and/or food intake at 3.5 h [6] and 4 h [1–3] following the preload. Participants were asked to eat until they felt comfortably full. No distractions were allowed during the 45 min lunch period. VAS ratings were measured over a further 2 h after completion of the lunch meal, with 150 mL of water served at 2:00 pm. Immediately after the breakfast and the lunch meal participants rated pleasantness, visual appeal, smell, taste, aftertaste and overall palatability of the meals on separate 100-mm VAS. Participants remained at the HNU throughout each study day and were allowed to use laptop computers, read or undertake other similar sedentary activities but were not allowed to sleep. The daily study protocol showing the timing of the breakfast and the *ad lib* lunch is shown in Fig. 1.

2.4. Lipid treatments

The 6 lipid treatments comprised 4 lipid plus water emulsions (Fabuless®, 2 dairy lipid; 1 palm oil lipid) and 2 matched non-emulsified controls. Dairy PL (PC700, phospholipid concentrate 700; Fonterra Co-operative Group, New Zealand, 20% of total lipid) and soya bean lecithin (American Lecithin Company, CT, USA; 20% of total lipid) were used to emulsify the lipid and water (ratio 30:70) treatments. PS of all emulsions was matched as closely as possible to the commercial lipid emulsion Fabuless®, (Table 1), and all were very small-PS lipid and water emulsions. The 6 breakfast treatments were (i) Fabuless® emulsion (F_{EM}); (ii) dairy emulsion with dairy emulsifier (DE_{DE}) matched for PS to Fabuless®; (iii) dairy emulsion with soy lecithin emulsifier (DE_{SE}) matched for PS to Fabuless®; (iv) dairy control (DC_{ON}), not emulsified; (v) palmolein emulsion with dairy emulsifier (PE_{DE}) matched for PS and FA composition to Fabuless®; (vi) palmolein control (PC_{ON}) not emulsified, matched for FA composition to Fabuless®. Since

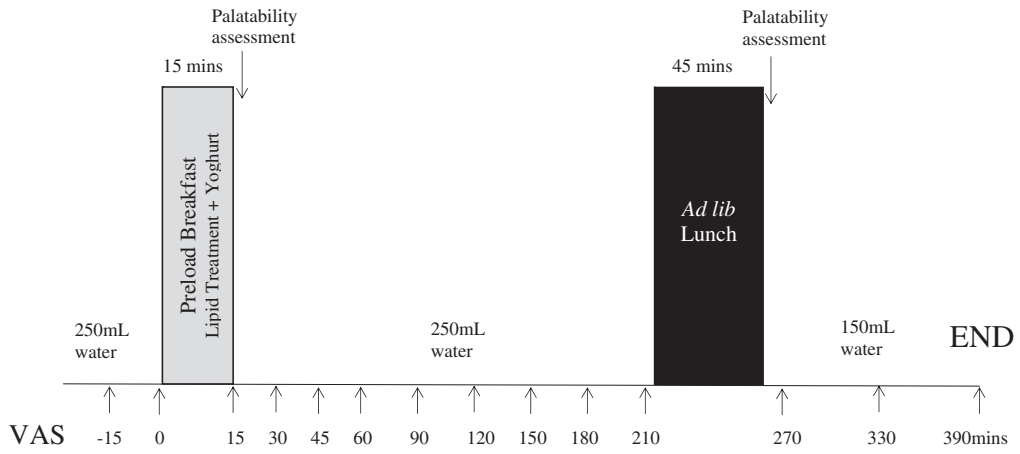

Fig. 1. Daily protocol.

FA composition and lipid physical structure ('hard'/'soft' fat at room temperature) may also be important functional parameters, FA/physical structure matched (non-dairy 'hard' fat palmolein) emulsion and control preloads were also included in the study. The FA profiles are shown in Table 2, with the dairy emulsions that of a typical dairy product (DE_{DE}/DE_{SE}: C16:0 27%; C18:0 11%; other saturated FA 26%; C18:1 29%; other unsaturated FA 7%) whilst the palmolein emulsion (PE_{DE}: C16:0 40%; C18:1 43%; C18: 2 12%; other FA 5%) was closely matched to Fabuless® (F_{EM}: C16:0 42%; C18:1 40%; C18:2 10%; other FA 8%). All lipids were administered at breakfast and served within fermented dairy yoghurt, based on a previous study in our laboratory which showed greater feelings of fullness with Fabuless® stirred into yoghurt than either alone or with other food formats [6]. Lipids were stirred into the semi-liquid yoghurt immediately before the breakfast was served and there was no further processing of the preload.

The lipid and water emulsions were developed and prepared within the Department of Food Science at the University of Auckland. A prolonged development process was undertaken prior to commencing the clinical intervention in order to obtain the required small-PS products, to characterise, and also to ensure stability of each of the test emulsions. During preparation the lipid and water components were initially warmed and mixed by hand, and then pre-homogenized (APV 2000, SPX FLOW Inc., NC, USA; setting 5 for DE_{DE} and PE_{DE}; setting 1 for DE_{SE}) for 3 min. Following a rest period of 30 min at room temperature, pre-homogenization was repeated (setting 5 for DE_{DE} and PE_{DE}; setting 1 for DE_{SE}) for 1.5 min. All samples were then passed through a microfluidizer (M-110 L Microfluidizer Processor, MFIC Corporation, MA, USA) at 12000 psi, which was then repeated for 5 cycles. Following preparation, lipid emulsions were stored overnight and administered to the participants the following morning. PS was measured by Zetasizer (Nano ZS series, Malvern, United Kingdom) using dynamic light scattering (DLS) or photon correlation spectroscopy (PCS), where rate of intensity fluctuation was used to calculate the size of the emulsified high PL lipid particles. Polydispersity index (PdI) was also assessed which provides an indication of the aggregation in the particles, *i.e.,* the width of the overall distribution. The greater the value the more it shows a polydisperse system and the closer to zero a monodisperse system. Further measures of viscosity and refractive index were assessed using an RVDV-III+ model rheometer (Brookfield Engineering, Middleboro, MA, USA). Testing was conducted to also ensure emulsion stability, absence of aggregation/sedimentation and non-uniform particle distribution, reflective index and viscosity measurements. Each sample was measured in triplicate at 20°C. The commercial emulsion ('Slim Shots' Fabuless®) was purchased from a retail outlet in Auckland, New Zealand. The energy and macronutrient composition of the 6 test breakfasts was calculated using the dietary software FoodWorks (Professional Edition, Version 5, 1998–2007; Xyris, Brisbane, AUS; see Table 2).

Table 1
Particle size (PS) and polydispersity index (PdI) of the 4 lipid emulsions.

	Z-average (d·nm)	PdI
F_{EM}: Fabuless® emulsion	81.80	0.611
DE_{DE}: Dairy lipid + PC700 emulsion[$] *FA composition **not matched** to Fabuless®*	114.7 ± 1.2	0.4 ± 0.03
DE_{SE}: Dairy lipid + soy lecithin emulsion[$] *FA composition **not matched** to Fabuless®*	121.7 ± 3.1	0.5 ± 0.01
PE_{DE}: Palmolein lipid + PC700 emulsion[$] *FA composition **matched** to Fabuless®*	104.4 ± 2.9	0.5 ± 0.02

Lipid:water (ratio 30:70) emulsions measured in triplicate, reported as mean ± SD. Prepared by mixing, pre-homogenisation, plus 5 repeat microfluidiser cycles at 12000 psi. Particle size measured by Zetasizer using dynamic light scattering (DLS), where *Z*-average particle diameter (d·nm) is the intensity weighted mean hydrodynamic size. Polydispersity index (PdI) provides an indication of the aggregation in the particles, *i.e.,* the width of the overall distribution. FA, fatty acid; F_{EM}: Fabuless® emulsion; DE_{DE}, dairy emulsion + dairy emulsifier; DE_{SE}, dairy emulsion + soy emulsifier; PE_{DE}, palmolein + dairy emulsifier; PC700, phospholipid concentrate 700 (dairy emulsifier); [$]oil:emulsifier ratio 80:20.

2.5. Visual analogue scales (VAS)

Throughout each study day participants rated their hunger, fullness, satisfaction and TOF using validated VAS ratings. The standard methodology used has been previously described [6,9,30,32]. The questions asked were "How hungry do you feel?", "How full do you feel?", "How satisfied do you feel?" and "How much do you think you can eat now?". Subjective measurements were recorded by the participant on paper questionnaires by placing a vertical line across 100 mm scales, anchored at either end by statements; "I am not hungry at all/I am not full at all/I am completely empty/nothing at all" on the left and "I am as hungry I have ever been/ I am totally full/ I cannot eat another bite/ a large amount" on the right. Further VAS ratings included how thirsty, energetic and relaxed the participants felt. Nausea was also rated throughout each study day. VAS was completed prior to the test breakfast (t − 15 min), and then at t = 15 (end of test breakfast), 30, 45, 60, 90, 120, 150, 180, 210 (*ad lib* lunch), 270, 330 and 390 min. Immediately after breakfast participants also rated the pleasantness, visual appeal, smell, taste, aftertaste and overall palatability of the breakfast on separate 100-mm VAS. These questions were anchored on the left by the statements "not at all pleasant (pleasantness)/ bad (visual appeal, smell, taste, palatability)/ none (aftertaste)" and on the right by the statements "as pleasant as I have ever tasted (pleasantness)/good (visual

Table 2
Composition of the 6 lipid + yoghurt breakfast preloads.

	Emulsion lipid/water (g)	Yoghurt/ polycal (g)	Water (g)	Total weight (g)	Energy (kJ)	ED (kJ/g)	C16:0 SFA %	C18:0 SFA %	Other SFA %	C18:1 MUFA %	C18:2 PUFA %	Other USFA %	Minor FA* %
(i) F_{EM}: Fabuless® (commercial) emulsion	10	157.5	32.5	200	786	4	42	0	–	40	10	–	8
(ii) DE_{DE}: dairy lipid + PC700 emulsion. *FA composition **not matched** to Fabuless®*	10	157.5	32.5	200	790	4	27	11	26	29	0	7	–
(iii) DE_{SE}: dairy lipid + soy lecithin emulsion. *FA composition **not matched** to Fabuless®*	10	157.5	32.5	200	786	4	27	11	26	29	0	7	–
(iv) DC_{ON}: dairy lipid + PC700 non-emulsion. *FA composition **not matched** to Fabuless®*	$10	157.5	32.5	200	790	4	27	11	26	29	0	7	–
(v) PE_{DE}: palmolein + PC700 emulsion. *FA composition **matched** to Fabuless®*	10	157.5	32.5	200	790	4	40	0	–	43	12	–	5
(vi) PC_{ON}: palmolein + PC700 non-emulsion. *FA composition **matched** to Fabuless®*	$10	157.5	32.5	200	790	4	40	0	–	43	12	–	5

Breakfast preloads (lipid stirred into a semi-liquid dairy yoghurt) contained 5.7 g fat, 27.4 g CHO, 6.5 g protein. Emulsifying agents were added to all test products, including non-emulsions. DE_{DE}, dairy emulsion + dairy emulsifier; DE_{SE}, dairy emulsion + soy emulsifier; DC_{ON}, dairy control non-emulsion; PE_{DE}, palmolein + dairy emulsifier; PC_{ON}, palmolein control non-emulsion; PC700, phospholipid concentrate 700 (dairy emulsifier); polycal, carbohydrate supplement; ED, energy density; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; USFA, unsaturated fatty acid; %, percentage of total FA content; *unspecified minor components; $control, did not undergo emulsification treatment.

appeal, smell, taste, palatability)/much (aftertaste)". All VAS ratings were completed independently and there was no consulting or interaction with other participants during questionnaire completion.

2.6. Ad libitum lunch

The *ad libitum* lunch consisted of beef casserole and rice, and was served hot (Table 3). Both food items were provided in moderate excess to ensure that participants would not consume the entirety of either item. Participants were asked to eat until they were comfortably full, to eat as much or little as they chose, and to remain in their individual dining booth throughout the lunch period without interaction with other participants. Each meal item was weighed before and after service to the nearest 0.5 g (Sartorius AG, Goettingen, Germany) and the energy and macronutrient content of the foods calculated using the dietary software FoodWorks (Professional Edition, Version 5, 1998–2007; Xyris, Brisbane, Australia).

2.7. Statistical analysis

Participant information at baseline is presented as mean and standard deviation (SD). Efficacy outcomes for VAS and EI are presented as mean and standard error of the mean (SEM). VAS data were analysed using a repeated measures Linear Mixed Model ANOVA (SAS: PROC MIXED, SAS version 8.0, SAS Institute Inc., Cary, NC, 2001). Participant, test meal (condition), study day (visit number) and study period (time) were included in the procedure, with baseline VAS as a covariate, as was the diet/time interaction which addressed whether the trajectory over time following the test meal differed between diets (diet*time). Three sets of pre-defined comparisons were considered which included comparison of 'soft' fat dairy and 'hard' fat palmolein emulsions with pair matched non-emulsified controls, and comparison of commercial emulsion Fabuless with both dairy and palmolein non-emulsified control lipids, as follows: DE_{DE}, DE_{SE} *vs* D_{CON}, PE_{DE} *vs* P_{CON}, F_{EM} *vs* D_{CON} *vs* P_{CON}. Repeat measures analyses of VAS were performed over the 210 min (3.5 h; $VAS_{0–3.5\ h}$) period between the test breakfast and the *ad lib* lunch meal, and over the full study day (390 min, 6.5 h; $VAS_{0–6.5\ h}$). VAS data were also analysed as incremental area under the curve (iAUC) over 210 min between the test breakfast and lunch meal. *Ad libitum* lunch data was also analysed using a repeated measures ANOVA which takes into account the correlation between repeated measures from each individual (SAS: PROC MIXED, SAS version 8.0, SAS Institute Inc., Cary, NC, 2001). Power calculations performed using data from previous studies in our laboratory in order to provide estimates of variance components for EI, showed a difference between treatment arms of 400 kJ likely to be significant for pre-specified paired analyses. Statistical significance was based on 95% limits ($P < 0.05$).

3. Results

3.1. Participants

Eighteen young, healthy, male participants completed all 6 dietary treatments. No individuals withdrew from the study and there was no missing data. The mean age of the participants was 20 years (3.1 SD), and the mean BMI was 21.7 kg/m^2 (1.8 SD).

3.2. Visual analogue scales

Breakfast preloads were rated for pleasantness, visual appeal, aroma, taste, aftertaste and palatability immediately after the meal (Fig. 2). Overall the pleasantness, visual appeal, aroma, taste and palatability ratings were high and did not differ between preloads (ANOVA, $P > 0.05$).

Table 3
Energy and macronutrient composition of *ad libitum* lunch meal.

	Weight (g)	Energy (kJ)	ED (kJ/g)	Fat (g)	Fat (en%)	CHO (g)	CHO (en%)	Protein (g)	Protein (en%)
Beef casserole	1646	4204	2.6	41	37	59	22	96	38
Rice, white, boiled	933	5880	6.3	4	3	314	84	26	8
Water, bottled	1500	0	0	0	0	0	0	0	0

ED, energy density; CHO, carbohydrate; en%, percentage of energy.

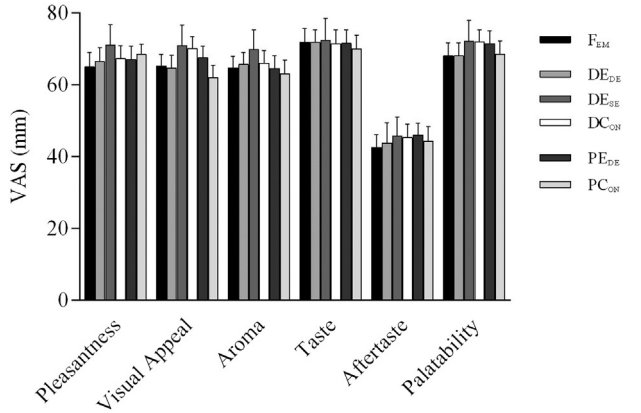

Fig. 2. Palatability ratings did not differ between the 6 preload breakfasts (ANOVA, $P > 0.05$). (i) Fabuless emulsion (F_{EM}); (ii) dairy emulsion with dairy emulsifier (DE_{DE}); (iii) dairy emulsion with soy lecithin emulsifier (DE_{SE}); (iv) dairy control (DC_{ON}); (v) palmolein emulsion with dairy emulsifier (PE_{DE}); (vi) palmolein control (PC_{ON}). Visual analogue scale, VAS. Mean, SEM.

The dairy lipid emulsion containing soy lecithin was rated higher, but not significantly, than the other treatments for pleasantness, visual appeal and aroma ($P > 0.05$). There was no evidence of adverse sensory effects caused by incorporation of the lipids into the yoghurt, nor any reports of nausea or adverse GI symptoms during the study day for any of the 6 treatments (data not shown). Palatability was also assessed following the outcome lunch meal for all participants, on each study day. No significant change was observed in any of the 6 palatability ratings ($P > 0.05$, data not shown), and there was no evidence of a decrease in palatability of the meal during the repeat study visits.

Fig. 3 shows the change from baseline of VAS-rated measures of hunger, fullness, TOF and satisfaction following each test breakfast. Fasting baseline was not different between treatments (ANOVA, $P > 0.05$) confirming matched appetite state prior to each breakfast preload. As expected, the 0.8 MJ preload rapidly decreased hunger/TOF and increased fullness/satisfaction relative to fasting baseline for all treatments by 15 min post-breakfast (all, $P < 0.05$), after which VAS ratings gradually rebounded to fasting levels. Whilst there was some variability between treatments, there was no significant difference between any of the emulsion and matched non-emulsion controls, or between dairy and non-dairy emulsions when measured both between breakfast and lunch (210 min) or throughout the full study day (time * treatment, all, $P > 0.05$). The incremental area under the curve ($iAUC_{0–210\ min}$) for hunger and fullness also confirmed no detectable effect of any of the emulsions on appetite ratings (see Fig. 3, histograms). In addition, Fabuless® did not significantly alter hunger or fullness when compared to the non-emulsion control lipids.

3.3. Ad libitum energy intake at lunch meal

EI and macronutrient intake at the outcome lunch meal is shown for all treatments in Fig. 4. Again, there was no significant difference between emulsion and control for any of the treatments (ANOVA, $P > 0.05$). Mean EI for the 3 emulsions PE_{DE}, DE_{DE} and DE_{SE} was 3902 kJ (217 SEM), 4035 kJ (250 SEM) and 3904 kJ (312 SEM) respectively, whilst mean EI for the 2 non-emulsions, PC_{ON} and DC_{ON}, was 3973 kJ (252 SEM) and 3983 kJ (307 SEM) respectively. F_{EM} did not significantly suppress EI compared to any other treatments (4038 kJ, SEM 284), including when compared to the non-emulsion controls. For all lipid treatments, EI was similar for each of the 2 savoury items that comprised the single course lunch meal ($P > 0.05$; beef casserole, range: 53.0–54.4% of total EI; boiled rice, range: 45.9–47.0% of total EI).

4. Discussion

There was no evidence from this study that very small-PS lipid emulsions which differed in FA composition and physical characteristics, including 'hard' fat (palmolein) or 'soft' fat (dairy) structure and therefore likely to also differ in gastric stability, could promote postprandial satiety or alter short-term eating behaviour. The emulsions and pair matched non-emulsified lipid controls were presented within a dairy yoghurt as a breakfast meal, and appetite-related feelings and EI were assessed over the following 4.5 h period. We hypothesised that delivery

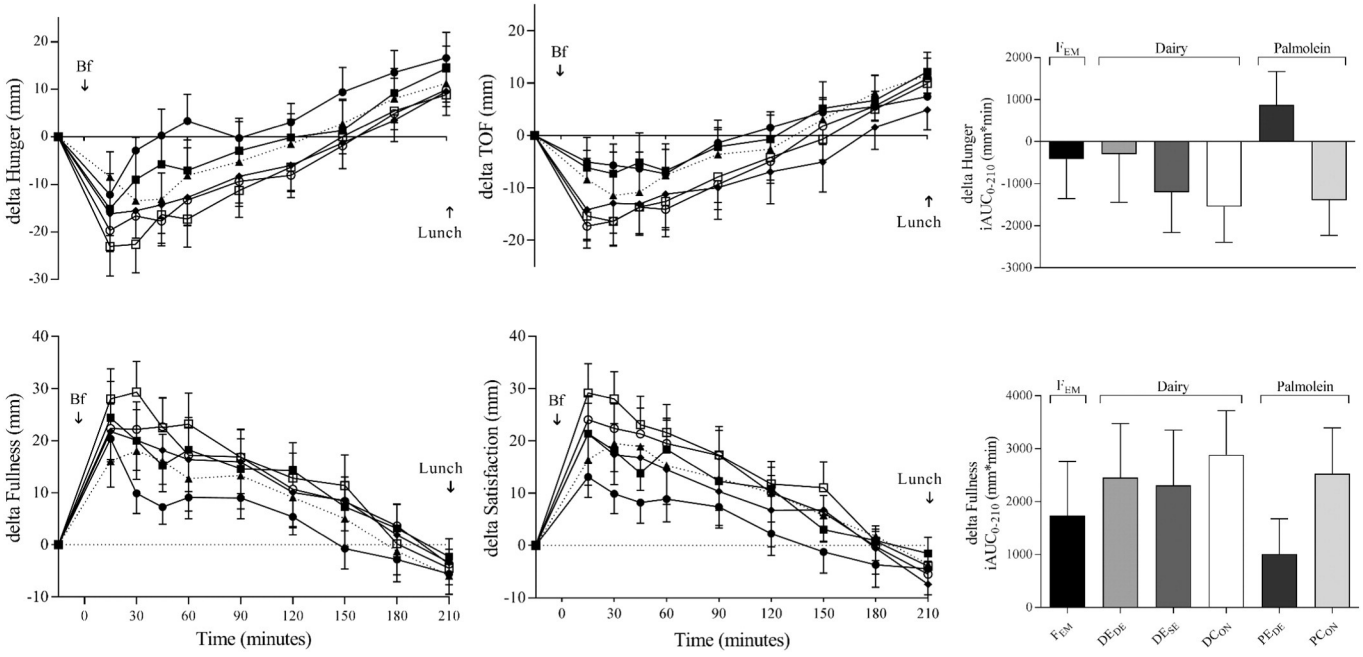

Fig. 3. Change from baseline in hunger, fullness, satisfaction and current thoughts of food (TOF) following the 6 test breakfasts (Bf). The *ad libitum* lunch was served 210 min after the breakfast. (i) Fabuless emulsion (F_{EM}, ▲); (ii) dairy emulsion with dairy emulsifier (DE_{DE}, ■); (iii) dairy emulsion with soy lecithin emulsifier (DE_{SE}, ◆); (iv) dairy control (DC_{ON}, □); (v) palmolein emulsion with dairy emulsifier (PE_{DE}, ●) (vi) palmolein control (PC_{ON}, ○). Delta, change from pre-breakfast baseline. Incremental area under the curve of change from baseline over 210 min (delta $iAUC_{0–210}$) is shown as histograms. No significant difference between treatments ($P > 0.05$). Mean, SEM.

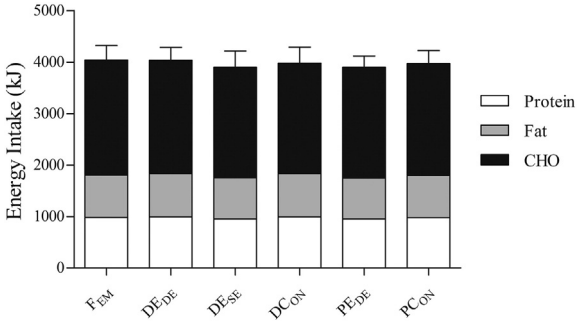

Fig. 4. Energy and macronutrient intake at the *ad libitum* lunch meal. (i) Fabuless emulsion (F_{EM}); (ii) dairy emulsion with dairy emulsifier (DE_{DE}); (iii) dairy emulsion with soy lecithin emulsifier (DE_{SE}); (iv) dairy control (DC_{ON}); (v) palmolein emulsion with dairy emulsifier (PE_{DE}); (vi) palmolein control (PC_{ON}). No significant difference between treatments ($P > 0.05$). CHO, carbohydrate. Mean, SEM.

of a small-PS, high PL emulsion into the SI would activate either the duodenal/jejunal or the ileal brake mechanism as a result of protection of the central core lipids from rapid lipolysis by the surrounding surface polar lipids. Notably, the trial also failed to show appetite suppression by the commercial small-PS lipid emulsion Fabuless® (diameter 81 nm) in line with a growing [5–12] but not conclusive [1–4] literature on appetite responses to consumption of this lipid emulsion. The reason for lack of consensus is not clear, but may be speculated to be result of variable trial design between studies including method of delivery and/or processing of the emulsion, the composition, choice and/or timing of the later outcome meal(s). We have previously reviewed these issues in detail elsewhere [6,9]. The majority of interventions have been small sample size, short term cross-over studies with between 18 and 30 participants in studies reporting both positive [1,2] and negative [6,8,9,11,12] findings. Interestingly, satiety effects have recently been reported from a dietary study which provided an alternate high PL, very small-PS (100 nm, diameter) fractionated oat oil lipid emulsion [27], although careful review of the trial shows that the authors have implied promotion of satiety based on changes in GI peptide concentrations despite a lack of statistically significant effects of the emulsion on VAS-rated appetite or EI. The liposome preparation in this recent study was a 10:1 water:lipid, high PL, very small-PS emulsion, and so similar to the emulsion treatments assessed in our current study.

When dietary lipids transit from the acid stomach into the proximal SI, GI responses may impact a wide range of events, including appetite and food intake. Delivery of fat into the small bowel is influenced by many things including surface area of lipid droplets, distribution of phase contents (*e.g.,* water, solid) within the stomach, and even the postural setting of the individual [33]. Whilst there is evidence from tube feeding studies of both a duodenal/jejunal and ileal brake [16–20,22,34] promoting a feedback loop to GI and satiety responses, a common mode of action that has been proposed for emulsified lipids is the ileal brake, acting more distally within the SI. Enteral studies show the brake to be activated by tube delivery of lipid to the ileum [14,18–20,35], hence if lipid emulsions can remain intact, bypassing uptake by the proximal duodenum [15,36], they may also successfully induce the brake. Delay of lipolysis, fat absorption and increased exposure of dietary fat in the distal ileum may then feedback to inhibit upper GI motility, slowing gastric emptying and intestinal transit, promoting ileal GI peptide secretion, and in turn promoting satiety. To date however, despite observations of suppression of EI by emulsions [1–4,37], there is little mechanistic evidence underpinning validity of this approach when lipids are consumed in the diet rather than administered by tube.

The small-PS lipid/water emulsions in this study were developed with reference to the small-PS Fabuless® [1–4] and other studies showing efficacy of small- *versus* large-PS emulsions [19,28]. Early tube feeding studies both from animal models [38] and clinical interventions [34] showed that decreasing droplet size of lipid/water emulsions delivered into the duodenum altered GI response and aspects of appetite related behaviours. Smaller-PS (260 nm) emulsions were shown to increase GI peptide response as well as suppress hunger and promote fullness relative to larger-PS (30 μm, 170 μm) lipids [34]. The Masclee team have conducted enteral studies where smaller-PS (880 nm) lipid particles were also shown to significantly decrease VAS- hunger/increase VAS-fullness when compared to larger-PS (15.5 μm) lipid particles [19], although again following duodenal rather than ileal infusion. Interestingly, a dietary study investigating lipid droplet size has now been conducted. GI outcomes were investigated using magnetic resonance imaging (MRI) and satiety assessed, in a small sample size intervention. The authors reported a significant decrease in EI of 11% at a meal consumed 5 h after a test breakfast meal when small-PS (400 nm, diameter) *versus* larger-PS (PS: 6 μm, diameter) emulsions were compared [28]. There was no effect on VAS-rated appetite. The lipid/water emulsion comprised sunflower oil (20%) and the emulsifier Tween 20 (1%). PS of the smaller emulsion was ~4 times greater than in our current trial, but resulted in differential effects *versus* the much larger 6000 nm droplet size emulsion. Interestingly, lipolysis has been shown to be higher for small-PS (700 nm) *versus* large-PS (10 μm) emulsions delivered to the duodenum [39], most likely due to the smaller droplet size providing a larger lipid surface area for lipase binding and subsequent hydrolysis of the triglyceride structure. Despite this observation, changes in food intake have been attributed to a duodenal/ileal brake but in the absence of supportive evidence.

There are a number of challenges when developing novel lipid:water emulsions, including ensuring stability of the emulsion, absence of aggregation/sedimentation and prevention of non-uniform particle distribution. Extensive pilot testing of both dairy emulsions investigated in our current study ensured these criteria were achieved, and that the emulsions were well matched to their paired controls. Although studies reporting that FA composition may alter appetite responses is limited, we ensured that lipid profile of the products was matched across pairs. There was however no evidence from our trial that FA composition (and accompanying change in lipid physical structure) significantly alters food intake, with no differential outcomes between either emulsified or non-emulsified lipids when FA profile was altered in favour of an increase in unsaturated acids. Inclusion of the palmolein-based highly saturated 'hard' fat (PE_{DE}) did not promote changes in any measured outcomes. This is in agreement with previous studies conducted in our [40] and other [41–43] laboratories where FA composition has been manipulated, but not all prior data [44,45]. It could be argued that changes in emulsion FA profile are most likely to drive changes in appetite or eating behaviour through alterations of factors such as emulsion physical structure and intragastric stability [28] rather than FA composition *per se*.

The absence of a detectable change in VAS-rated satiety or EI between emulsions and non-emulsified controls in our current study is unlikely to be due to inadequate design. The study adhered to published guidelines for acute assessment of food intake in a laboratory environment [30]. Eighteen participants completed the cross over, which was powered to detect a difference of 400 kJ, or approximately 10% of intake, at the outcome lunch meal in pre-specified analyses. Timing between the preload and outcome lunch was chosen based on previous lipid emulsion studies which have shown positive effects on appetite and EI at 4 h [1–3], whilst our laboratory have previously shown limited effects on VAS-appetite with Fabuless incorporated into dairy yoghurt assessed 3½ hours post preload [6]. In addition, the hypothesis that the ileal brake may play a role in suppression of food intake supports a longer delay between preload and outcome meal than has widely been used for other preload studies of similar design, *e.g.,* 30–90 min. Test products were delivered within a minimally processed fermented yoghurt format, as in prior successful Fabuless® [1–4] and recent oat liposome studies [27]. Our laboratory previously showed that food format and delivery medium is important when assessing effects of lipid emulsions

[6], as have other researchers [11,12]. Yoghurt is a highly specific product of acidic fermentation of dairy lactose, although with variable pH in the final product, so it was considered important to mimic the conditions of previous clinical studies where efficacy had been observed.

5. Conclusion

There was no evidence from this acute intervention that the small-PS (114–121 nm), high PL emulsions tested were able to significantly alter postprandial self-reported ratings of hunger, fullness or other appetite-related parameters, or to decrease *ad lib* food intake at a subsequent meal. There was also no evidence of any significant appetite-related effects by the commercial emulsion Fabuless® in this study of lean men.

Disclosures

SDP holds the Fonterra Chair in Human Nutrition at the University of Auckland.

AKM is a Fonterra Co-operative Group employee.

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