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Emulsion oil droplet size significantly affects satiety: A pre-ingestive approach

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Abstract

Previous research has demonstrated that the manipulation of oil droplet size within oil-in-water emulsions significantly affects sensory characteristics, hedonics and expectations of food intake, independently of energy content. Smaller oil droplets enhanced perceived creaminess, increased Liking and generated greater expectations of satiation and satiety, indicating that creaminess is a satiety-relevant sensory cue within these systems. This paper extends these findings by investigating the effect of oil droplet size ($d_{4,3}$: 2 and 50 µm) on food intake and appetite. Male participants (n = 34 aged 18 – 37; BMI of 22.7 ± 1.6 kg/m^2; DEBQ restricted eating score of 1.8 ± 0.1.) completed two test days, where they visited the laboratory to consume a fixed-portion breakfast, returning three hours later for a "drink", which was the emulsion preload containing either 2 or 50 µm oil droplets. This was followed 20 minutes later with an *ad libitum* pasta lunch. Participants consumed significantly less at the *ad libitum* lunch after the preload containing 2 µm oil droplets than after the 50 µm preload, with an average reduction of 12% (62.4 kcal). Despite the significant differences in intake, no significant differences in sensory characteristics were noted. The findings show that the impact that an emulsion has on satiety can be enhanced without producing significantly perceivable differences in sensory properties. Therefore, by introducing a processing step which results in a smaller droplets, emulsion based liquid food products can be produced that enhance satiety, allowing covert functional redesign. Future work should consider the mechanism responsible for this effect.

Keywords: Emulsions, Microstructure, Oil droplet size, Preload, Satiety, Food Intake

Introduction

Fat is the most energy dense macronutrient at 9 kcal per gram (Atwater and Woods, 1896) and consequently is of interest in the redesign of food products to tackle the "obesogenic" food environment. Reducing fat content within foods has been a commonly proposed method to reduce consumers' energy intake. However, this is typically detrimental to the food product's sensory properties (Norton, Moore and Fryer, 2007; Roller and Jones, 2001).

Increasing the functionality of the fat to reduce intake could be a novel alternative to produce inherently "healthier" fat based foods (Himaya *et al.,* 1997). Increasing a food product's impact on satiety may lead to a reduction in overall energy intake through inhibition of appetite after consumption (Chambers, McCrickerd and Yeomans, 2014; Hetherington *et al.,* 2013).

Designing food structures for functional benefits is a growing area of interest. Redesigning foods that are high in fat (such as emulsions) to impact on appetite has added importance because fat is considered to be the least satiating macronutrient (Blundell, Green, and Burley, 1994; Blundell and Macdiarmid, 1997; Blundell and Tremblay, 1995). Emulsions are common fat based food structures that are found within a variety of commercially available food products, such as sauces, condiments, spreads, dressings and desserts. Emulsions are formed by mixing two immiscible liquids, such as oil and water, so one liquid is dispersed within the other as droplets stabilised by an emulsifier.

Previous research considering emulsion structures has predominantly considered gastro-intestinal structuring, in an attempt to achieve satiety via post-ingestive and post-absorptive mechanisms, with emulsion oil droplet size and emulsifier type being the two main properties investigated (Armand *et al.,* 1999; Maljaars *et al.,* 2012; Mun, Decker and McClements, 2007; Golding and Wooster, 2010; Lundin, Golding and Wooster, 2008; Peters *et al.,* 2014; Seimon *et al.,* 2009; Singh, Ye and Horne, 2009; van Aken *et al.,* 2011). However, structuring emulsions to achieve satiety via

pre-ingestive approaches (i.e. considering sensory mechanisms) has recently been considered and highlighted as potentially effective (Lett *et al.,* 2015). In that study, decreasing the oil droplet size within an oil-in-water emulsion model drink, increased creaminess, which in turn increased liking and expectations of satiation and satiety, independent of energy content (Lett *et al.,* 2015). creaminess within emulsions was therefore highlighted as a hedonic sensory cue, and a potential satiety-relevant sensory cue, which agrees with other findings that high-energy beverages are more satiating when creamy sensory characteristics are present (McCrickerd, Chambers and Yeomans, 2014; Yeomans and Chamber, 2011). The mechanism by which satiety-relevant sensory cues appear to work suggests that people learn to associate sensory characteristics with the subsequent experience of satiety post-consumption (Brunstrom, Shakeshaft and Scott-Samuel, 2008; Yeomans *et al*., 2014). As such, it is thought that creaminess, which is typically associated with high fat content (de Wijk, Rasing and Wilkinson, 2003; Frost and Janhoj, 2007), generates expectations of satiety typically achieved after the consumption of fat containing energy dense foods, with the intensity of creaminess being a predictive marker of energy content.

If the enhanced expectation of satiety through altering oil droplet size also impacts on the experience of post-ingestive satiety, this could confirm this type of restructuring as a valuable approach to product development. Early pre-ingestive satiety signals, such as sensory properties integrate with post-ingestive and post-absorptive signals (Blundell, Rogers, and Hill, 1987), and adjust digestive and absorptive mechanisms accordingly, at least partly through anticipatory physiological responses (Power and Schulkin, 2007; Smeets, Erkner and de Graaf, 2010).

The present study aimed to extend previous findings from Lett et al. (2015). We hypothesised that reducing the average oil droplet size of an oil-in-water emulsion will enhance satiety, through pre-ingestive sensory-mediated routes by increasing the perception of the identified satiety-relevant sensory cue, creaminess.

Materials and Methods

Design

A repeated-measures single-blind randomised cross-over design preload paradigm was used to investigate the satiating effects of two oil-in-water emulsion based drinks, varying in oil droplet size, but with equal energy content. Test meal intake and subjective ratings (Visual analogue scales: VAS) were used to assess food intake behaviour. Ethical approval for the study was obtained from the University of Birmingham ethics committee (ERN_14-0807, Approved: 14/08/2014).

Participants

Thirty-four healthy male adults participated in the study. Sample size was determined on the basis of the effect size needed to find a difference in satiety between two emulsions with different average oil droplet sizes (2 and 50 μm). These emulsions were produced in a preliminary study in which oil droplet size of an emulsion beverage had been manipulated changing sensory properties (Lett *et al*., 2015). To estimate participant numbers, we examined the outcome of previous preload studies where a difference in creaminess, similar in size to that in our recent emulsion study, was associated with a significant reduction in intake at a similar test meal. One such study where a difference in creaminess was associated with reduced intake was Yeomans and Chambers (2011), where less was consumed after a preload with higher rated creaminess (achieved primarily by varying viscosity) than after an isoenergetic less creamy preload. Based on the intake data in that study, one-tailed significance (P <0.05, predicted reduction with more creamy preload) and power = 0.8, indicated that a sample of 34 would be required. All participants were staff or students at the University of Birmingham, who had expressed an interest in participating in a research study investigating “The effect of mood on appetite”, as to mask any expectancy effects concerning the true nature of the investigation. Prospective participants were contacted by a recruitment email via an email database and were asked to reply if they were interested in participation and considered themselves to be a

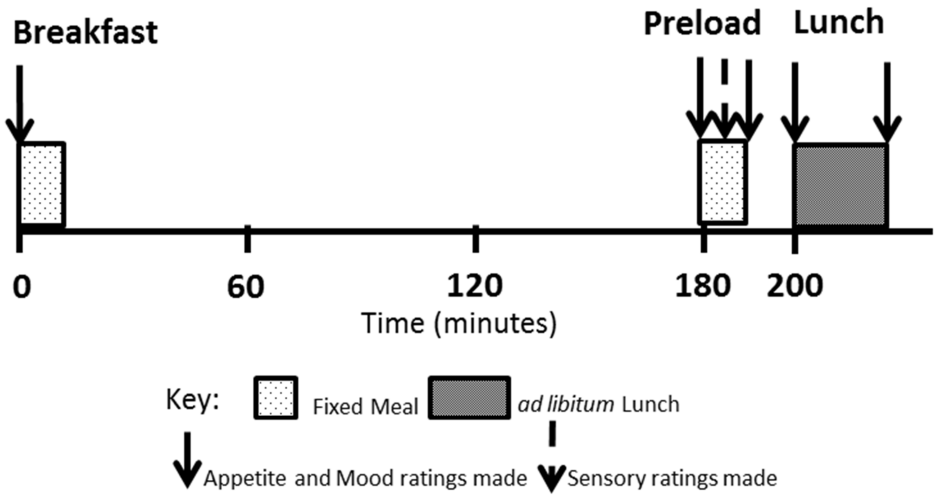
healthy, non-smoking, normal weight (BMI: 18.5 -25) male with no food allergies or intolerances. Females were excluded as they typically practice significantly higher levels of restricted eating and other eating behaviours than males (Arganini *et al.,* 2012; *Fortes et al.,* 2014; *Wardle*, 1987), and males who do not restrict their eating behaviour were chosen, as this cohort demonstrates the most accurate regulation of food intake (Rolls *et al.,* 1994). Respondents to the recruitment email were provided with an information sheet and enrolled in the study if they were still interested in participation. Prior to the start of a session, participants were screened for food allergies, smoking habits and current medical status via a health questionnaire, body mass index (BMI), calculated as kg/m² (with height and weight measurements being obtained with participants wearing light clothes and in a fasted state using a freestanding stadiometer (Seca 213, Birmingham, UK) and digital calibrated weighing scales (Seca 813, Birmingham, UK) and dietary restraint measured using the restraint scale from the Dutch eating behaviour questionnaire (DEBQ) (van Strien, *et al*.,1986). Potential participants were prevented from participating if they indicated any food allergies, history of smoking, had a BMI above 24.9 kg/m^2 or below 18.5 kg/m^2, were taking medication known to interfere with sensory perception or food intake or had a DEBQ restricted eating score of >2.4. One potential participant was prevented from participating, based on the recruitment criteria. Additionally, participants were given the opportunity to ask any questions about the study and its protocol to clarify issues or queries before the study began. The test cohort was made up of 34 men aged 18 - 37, with a mean BMI of 22.7 ± 1.6 Kg/m^2 and DEBQ restricted eating score of 1.8 ± 0.1. All participants gave written informed consent prior to participation.

Procedure

Participants attended 2 sessions on non-consecutive days. Study protocol was identical on each test day, with only the preload varying (See Fig.1). Participants arrived at a scheduled date and time between 08.30 - 10.30 am, Monday to Friday. Participants arrived having consumed only water from 11.00pm the night before. All testing was carried out in an individual booth containing a PC

computer running Sussex Ingestion Pattern Monitor (SIPM). SIPM was used to collect VAS scores of all mood and appetite questions throughout the study and preload sensory scores, and monitor food intake at lunch with a digital balance concealed by a placemat (Sartorius BP 4100). All VASs used within the study, collecting data on mood, appetite and preload sensory ratings were randomised differently for all participants. SIPM equipment and software were developed at the University of Sussex (Yeomans, 2000), based on a modification of the Universal Eating Monitor developed by Kissileff, Kilngsberg, and Van Italie (1980), and has been used extensively in studies of human appetite (Yeomans and Bertenshaw, 2008). After successful screening, the participant sat within an individual booth to begin the breakfast session and consumed the test breakfast (see Standard Breakfast section) within 15 minutes. To begin participants completed a set of the mood and appetite questions. The mood ratings (Alert, Anxious, Calm, Clearheaded, Happy and Tired) and appetite ratings (Hunger and Fullness), were presented as 100-point computerised VASs anchored with “not at all [mood or appetite]” and “extremely [mood or appetite]”. Mood questions were included as distracters and to be consistent with the premise that the study was investigating “The effect of mood on appetite”. The participant was then instructed to return exactly 3 hours later for the preload session. During the inter breakfast-preload period participants were not allowed to participate in exercise or consume any food or drink, apart from a 250 ml bottle of still water, which was provided and had to be fully consumed upon their return. Upon the participants return, they began the preload session. Participants completed the standard mood and appetite questions and then were presented with 200 ml of one of the two preloads (see Drink Preload section). 17 participants received the 2 µm droplet preload on their first session and the other 17 participants received the 50 µm droplet preload on their first session, with the other preload being consumed on the second session. SIPM instructed the participant to take a mouthful and then carry out a number of VAS to assess the samples sensory characteristics. The preload was evaluated for thickness, slipperiness, smoothness, creamy mouthfeel, overall creaminess, liking, expectation of hunger in 1 hours time (Satiety) and expectation of fullness immediately (Satiation). Both questions determining

expectations of food intake were in reference to if they consumed the full portion presented. Sensory VAS questions were headed “How [target rating] is the drink?” and end-anchored with “not at all [target rating]” (scored as zero) and “extremely [target rating]” (scored as 100); wording may have slightly differed to be grammatically correct. Upon completion of the sensory VAS questions, SIPM instructed the participant to consume the rest of the preload within 5 minutes, before another series of standard mood and appetite questions were presented to finish the preload session. Participants then remained within the laboratory until the lunch session. Results from our previous work showed that expectations of food intake are significantly different due to sensory differences between the emulsions. As such, a 20 minute delay between the preload being presented and the lunch session was used. This fits within the optimal time period for detecting oro-sensory effects on satiety (<30 minutes) (Livingstone *et al*., 2000), and allowed enough time for participants to comfortably consume the preload and complete all mood, appetite and sensory VASs. During the lunch session, participants first completed a set of standard mood and appetite ratings in the absence of any food cues (pre-lunch ratings). Next, 500 g white penne pasta with tomato and herb sauce (see Lunch section) was served by an experimenter who explained that the participant could eat as little or as much as he liked. A hidden digital balance secured under a placemat and linked to SIPM, which recorded the weight of food being eaten. If the participant consumed 300 g of the lunch, an onscreen alert message prompted the participant to call the experimenter. The experimenter then served the participant another 500 g pasta in a new bowl, with the consumed bowl of pasta being removed; no limit was placed on the number of refills permitted. To reduce the influence of habit and portion-size effects on intake, participants were encouraged not to use the refill prompt as a cue to end the lunch session. When participants had confirmed that they had finished eating, the participants then completed a final set of standard mood and appetite ratings (post-lunch ratings) before the lunch session and test day was completed. On the final test day, the participants were given a £20 Amazon voucher as compensation for participating in the study.



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175 *Fig. 1* Schematic representation of the timing of the fixed and test meals and the sets of appetite and
176 mood ratings and sensory ratings on a test day.

177 *Test Foods*

178 *Standard Breakfast*

179 On the morning of each test day, participants consumed a breakfast of 60 g of a proprietary
180 breakfast cereal (Crunchy Nut Cornflakes; Kellogg Co) plus 160 mL semi-skimmed milk (Tesco) and
181 200 mL orange juice (Tesco). The breakfast provided 420 kcal, 6.3 g fat, 10.8 g protein, and 79.1 g
182 carbohydrate. The breakfast provided approximately 17% of a male adults daily average
183 recommended energy intake.

184 *Lunch*

185 For the ad libitum lunch, each 500 g serving of pasta consisted of 300 g cooked weight of white pasta
186 (Penne; Aldi) plus 200 g of a prepared pasta sauce (tomato and herb; Aldi) served hot. The pasta
187 lunch was cooked on the test day as per packaging instructions. The test meal provided 96 Kcal
188 energy (3.2 g protein; 19.5 g carbohydrate and 0.58 g fat) per 100 g.

189 *Drink Preloads*

The preload drinks were 200 ml of emulsions containing either 2 or 50 µm droplets; these were *No Flavour* versions of emulsion samples described in a previous study (Lett *et al.,* 2015). These emulsions were chosen as, based on our previous work, emulsions containing 2 µm droplets gained significantly greater ratings for creaminess (P = 0.003) and Liking (P = 0.01) and resulted in reduced expectations of Hunger in 1 hours time (Satiety) than the emulsion containing 50 µm droplets (P = 0.017). Rheological and lubrication properties of these systems have been investigated in other work by the authors, and it was shown that 2 and 50 µm emulsions were also comparable in these properties. Samples consisted of an oil-in-distilled water emulsion (1 wt.% sodium caseinate (Excellion EM7, DMV International, The Netherlands); 2 wt.% sucrose (Silverspoon granulated, British Sugar Plc, UK) and 15 wt.% sunflower oil (Tesco Plc, UK)). Emulsions were produced using two different methods dependent upon the required mean droplet size of the emulsion being produced: a high shear mixer (Silverson L5M, Silverson machines Ltd, UK) or a high-pressure homogeniser (GEA Niro Soavi Panda Plus 2000, GEA Niro Soavi, Italy). In a 600 ml beaker, 15 wt.% sunflower oil was added to 85 wt.% aqueous phase (1 wt.% NaCas, 2 wt.% sucrose, 97 wt.% distilled water solution). The whole sample was then emulsified for 5 minutes using the high shear mixer. Dependent on oil droplet size being produced the sample was subjected to a different rotational speed (rpm) and emulsor screen. 50 µm oil droplet samples were subject to high shear mixing at 2500 rpm with a 1.6 mm pore emulsor screen. 2 µm oil droplet samples were subject to high shear mixing at maximum rpm with a 0.8 mm pore emulsor screen to produce a pre-emulsion, the pre-emulsion was then homogenised at 100 Bar with 2 passes. All samples were produced in 400 g batches, under clean and hygienic conditions on the day of evaluation and stored under refrigerated conditions at 2-5 ^{o}C. The 200 ml emulsion preload provided approximately 282 kcal, 30 g fat, 2 g protein, and 4 g carbohydrate.

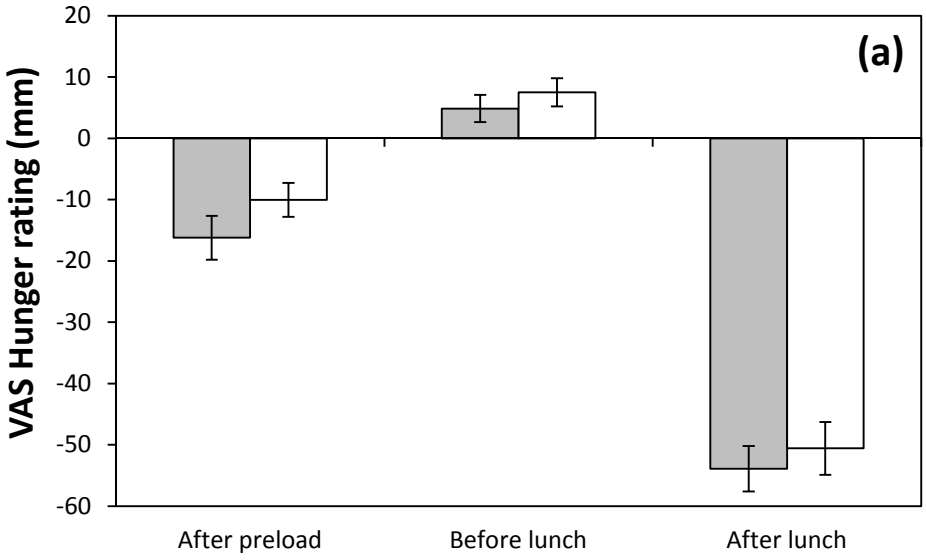
Data Analysis

The aim of the study was to investigate whether altering the oil droplet size of an emulsion altered subsequent food intake behaviour. Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 21 (SPSS Inc., USA). VAS scores for hunger and fullness throughout the study are reported from baseline (pre-preload) data, and were analysed using 2-way ANOVA based on the three post-preload time points (immediately post-preload, pre-lunch and post-lunch) and two oil droplet sizes. Nutrient and energy composition of the breakfast and lunch was calculated using compositional data provided by the manufacturers. The energy density of the preload drink was calculated using Atwater factors (Atwater and Woods, 1896).

Results

Mood and appetite ratings

Protected contrasts of baseline evaluations of mood and appetite (hunger and fullness) ratings before preload ingestion (at breakfast and just before preload consumption) did not differ significantly, and so effects of preload oil droplet size on appetite were assessed using change from baseline data. As can be seen (Figure 2a), hunger decreased immediately after the preload, recovered prior to lunch and then fell markedly after lunch, reflected in an overall effect of rating time on hunger ($F(2,66) = 182.68$, $p<0.001$, ETA = 0.85), but the change in hunger was consistently lower after the preload with 2 µm than 50 µm oil droplet size ($F(1,33 = 9.66$, $p=0.004$, ETA = 0.23), with no significant time x droplet interaction ($F(2,66) = 1.04$, $p=0.36$, ETA = 0.03). Fullness ratings showed the reverse pattern over time to hunger (Figure 2b: effect of time $F(2,66) = 82.45$, $p<0.001$, ETA = 0.71), but there was no significant effect of droplet size ($F(1,33 = 0.07$, $p=0.80$, ETA = 0.01) or time x droplet interaction ($F(2,66) = 0.71$, $p=0.50$, ETA = 0.02).



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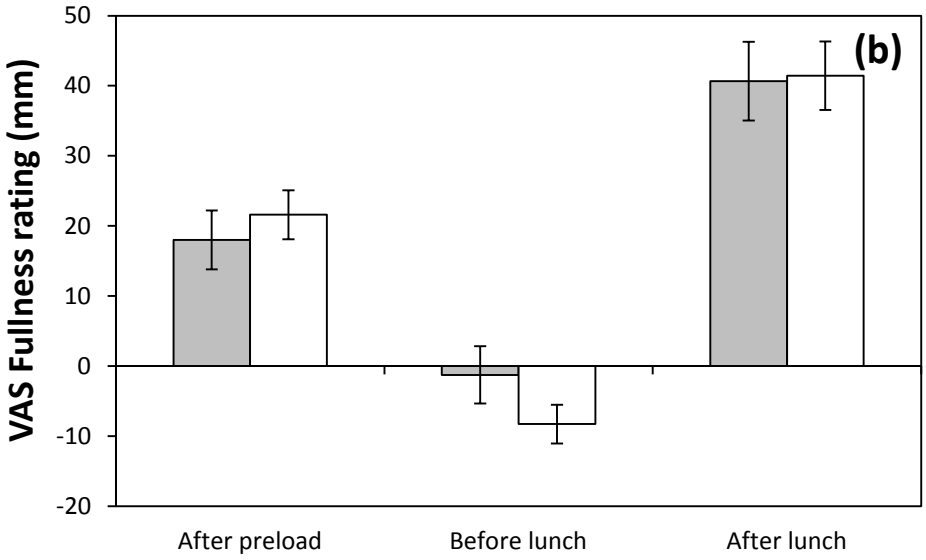

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239 *Fig. 2* Mean (± SEM) changes in ratings of Hunger (a) and Fullness (b) across the course of the test
240 session for both 2 µm (filled bars) and 50 µm (open bars) emulsion preloads.

241

242 *Preload sensory and hedonic ratings*

243 There were no significant differences in the scores of any sensory attributes, hedonics and
244 expectations of food intake for the 2 µm and 50 µm emulsion preloads (P >0.05: See Table 1). This
245 finding contradicts previous results (Lett *et al.,* 2015: see Table 1) and is discussed further in section
246 4.

Table. 1 Mean (± SEM) of attribute ratings of 2 µm and 50 µm samples used in current study and Lett *et al.,* 2015 (N = 24). Filled cells represent significance ($P < 0.05$) between 2 µm and 50 µm.

Attribute	When Rated	2 µm	50 µm
Thickness	Current Study	43.4 ± 3.1	47 ± 3.6
	Lett *et al.,* 2015	40.2 ± 3.4	32.8 ± 2.9
Creamy Mouthfeel	Current Study	58.8 ± 2.9	60.3 ±3.2
	Lett *et al.,* 2015	58.7 ± 3.7	44.6 ± 3.6
Creaminess	Current Study	56.9 ± 3.2	59.4 ± 3.4
	Lett *et al.,* 2015	59.2 ± 4.1	43 ± 3.5
Slipperiness	Current Study	61.6 ± 2.8	62.3 ± 2.4
	Lett *et al.,* 2015	58 ± 3.7	58.1 ± 3
Smoothness	Current Study	65.9 ± 2.9	68.2 ± 2.8
	Lett *et al.,* 2015	63.4 ± 2.9	60.1 ± 3.8
Liking	Current Study	40.9 ± 3.6	39.4 ± 3.6
	Lett *et al.,* 2015	47.8 ± 3.4	40.4 ± 3.7
Expected Fullness	Current Study	54.3 ± 3.3	52.8 ± 4
	Lett *et al.,* 2015	61.1 ± 3.7	50.8 ± 4
Expected Hunger	Current Study	62.6 ± 4.2	62.9 ± 4
	Lett *et al.,* 2015	44.9 ± 5.1	57.4 ± 4

Lunch intake

Total lunch intake was significantly different dependent on oil droplet size preload consumed, with participants consuming significantly less after consumption of the 2 µm preload ($P = 0.027$). Total consumption was 67.7g or 62.4 Kcal less, which is a 12.3 % reduction in total food intake (g) and a 12.2 % reduction in energy intake (Kcal) (see Fig. 3a and b). No significant effect of preload session order on intake, for both droplet sizes ($P > 0.05$), was also shown, highlighting participant fatigue of the protocol did not factor in ratings or *ad libitum* food intake.

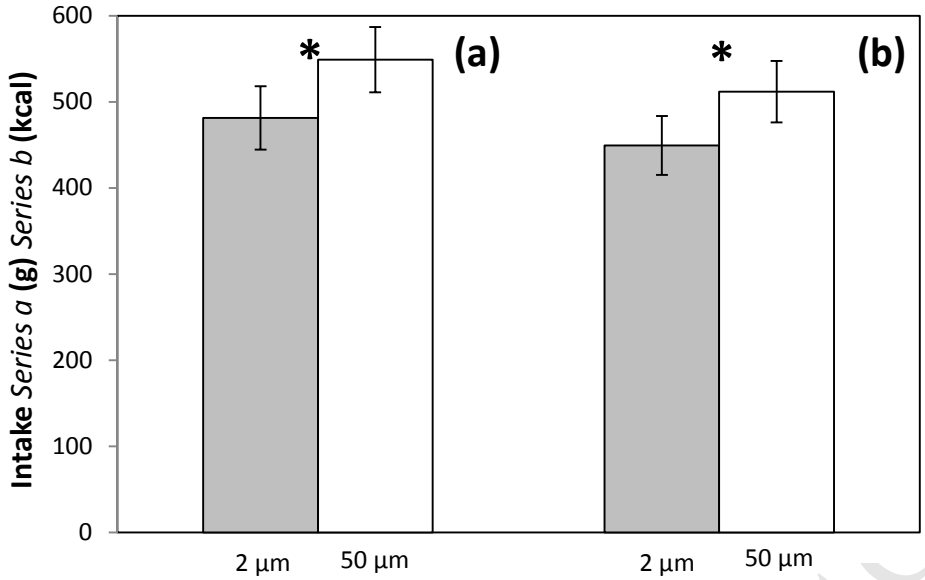

Fig. 3 Mean overall intake at the test meal (± SEM) in grams (a) and kilocalories (b). Filled bars represent preloads containing 2 µm droplets and Open bars represent preloads containing 50 µm. * represents significance at $P < 0.05$.

Discussion

The main finding from this study was that by decreasing the oil droplet size of an oil-in-water emulsion, the degree to which an emulsion impacts on satiety can be significantly increased, independent of energy content. Participants consumed 12.2 % (Kcal) less at the test meal after consuming an oil-in-water emulsion preload containing 2 µm droplets, than they did following consumption of a preload containing 50 µm oil droplets (See Fig.3a and b).

In earlier work, Lett and co-authors (2015) looked to identify satiety-relevant oro-sensory cues within model oil-in-water emulsions, with the intention of designing emulsion structures to promote these cues, therefore increasing an emulsion based food or beverages capacity to generate satiety. Using the same model emulsion systems as used within in this study, the authors showed that on decreasing the oil droplet size of the emulsion, creaminess perception significantly increases (see Table 1). Reducing oil droplet size also significantly increased hedonic appeal, in addition to significantly decreasing expectations of Hunger in 1 hour's time (an indication of satiety). As such, it is thought that, creaminess is a potential satiety-relevant oro-sensory cue.

Our current work has shown that although expectations of food intake behaviour have been successfully realised in actual eating behaviour (See Fig.3), the mechanism mediating the effect has not been identified, as ratings of creaminess, or any other attribute, for the two preloads were not significantly different (see Table 1). Therefore, our findings do not fully agree with our hypothesis. Given that Lett and co-authors (2015) identified potential satiety-relevant sensory cues within these systems, and that the current study protocol was designed to maximise the influence of potential sensory effects of the preload on subsequent food intake (Blundell, 2010; Livingstone *et al*., 2000), it is unusual that a significant difference in satiety was identified (See Fig.3), but no significant differences in sensory perception were found.

Other studies have also shown differences between sensory properties of preloads in the "pilot" sensory study, but not in the "main" preload study, despite similarities in the studies cohort (Chambers, Ells and Yeomans, 2013; McCrickerd, Chambers and Yeomans, 2014; Yeomans and Chambers, 2011). Consequently, it seems sensible to suggest that the difference in protocol between this and our earlier study (Lett *et al.,* 2015), is the reason for the change in sensory results. The protocol in Lett et al. (2015) promoted sample assessment in a more analytical manner. Firstly, participants were recruited to participate in a "sensory analysis of emulsions" study, so would have approached the study consciously seeking sensory differences between samples. Although samples were unidentifiable and randomly ordered, the methodology used would not have controlled for the cross-comparison of sensory attributes between samples, as samples were analysed in a sequential manner in one session. Secondly, all sensory attributes investigated were defined via a description reference and not at the discretion of the individual participant, as was the case within the current protocol. Our previous study (Lett et al., 2015) also used 100mm paper-based VAS scale, compared to the use of 100-point computerised VAS using SIPM here. Although no published study has explicitly compared manual VAS ratings and computerised based VAS on SIPM, studies have shown VAS scores change, even subtlety, dependent on the protocol for collecting VAS based data used (Brunger *et al.,* 2015). Within the current study, participants were recruited to participant in a study

303 investigating “mood and appetite”, and so attention was not drawn specifically to the preload’s
304 sensory properties. Furthermore, and importantly, although preloads were also unidentifiable and
305 randomly ordered, they were assessed for sensory attributes at least 48 hours apart, with
306 participants’ practicing free-living behaviour between test days. The method would therefore have
307 hindered participant’s ability to draw cross-comparisons between sensory attributes of the preloads
308 as seen with the sensory protocol of the previous study. Consequently, results presented by Lett et
309 al. (2015) would be expected to highlight more pronounced sensory differences between samples
310 because of the comparative nature of the rating task used in the earlier study. Nevertheless, given
311 participants consumed commercially available foods at customary meal times, with at least a 48
312 hour free-living period in-between test days, our current study protocol is more replicable of “real
313 world” behaviour. As no significant differences in sensory properties between 2 µm and 50 µm
314 emulsion preloads were identified within this study (see Table 1), findings indicate that satiety can
315 be significantly enhanced without producing significantly perceivable differences in sensory
316 properties. Therefore, using the same formulation, by introducing a processing step which results in
317 a smaller average droplet size (for example, higher shear/pressure processing), emulsion based
318 liquid food products can be produced with enhanced effects on satiety, but with a very similar
319 sensory profile as the original product, allowing functional redesign unbeknown to the consumer.

320 A methodological issue with studies investigating satiety is the considerable overlap of physiological
321 and cognitive factors in satiety development (Livingstone *et al.,* 2000). The mechanism in which oil
322 droplet size changes satiety can, therefore, not be characterised simply according to one factor of
323 the “satiety cascade” (Blundell, Rodgers and Hill, 1987), especially as a lack of clarity exists
324 concerning the primary mechanism of our main finding (See Fig.3).

325 To the best of our knowledge this is the first paper to consider an emulsion structuring approach for
326 pre-ingestive mediated satiety. Previous work considering emulsion oil droplet size as a design
327 mechanism for satiety has only considered gastrointestinal structuring (Golding and Wooster, 2010;

328 Lundin, Golding and Wooster, 2008; Singh, Ye and Horne, 2009). Although gastric colloidal
329 behaviour is largely governed by emulsifier type (Mun, Decker and McClements, 2007; van Aken *et*
330 *al.,* 2011), oil droplet size has been shown to effect digestive and absorptive behaviours, which
331 would impact on satiety through post-ingestive and post-absorptive effects and feedback
332 mechanisms. For example, a considerably greater rate of lipolysis (and therefore plasma triglyceride
333 concentration and CCK release) is observed with smaller oil droplet sizes, due to the greater
334 interfacial area available for digestive lipase binding. This behaviour has been observed within *in*
335 *vitro* (Armand *et al.,* 1992; Peters *et al.,* 2014) and *in vivo* studies (Armand *et al.,* 1999; Borel *et al,*
336 1994; Maljaars *et al.,* 2012; Peters *et al.,* 2014; Seimon *et al.,* 2009). In addition, the size of oil
337 droplets directly infused into the duodenum have been shown to have multiple effects on both
338 gastric behaviour and satiety (Seimon et al., 2009): larger oil droplets infused directly into the
339 duodenum were associated with less suppression of antral and duodenal pressure waves, reduced
340 release of CCK and PYY, and lower suppression of rated hunger and actual food intake. It is clearly
341 possible that differences in droplet size at the point of ingestion might also survive through to post-
342 gastric processing and so trigger these effects. However, it should be noted that the pre-prandial oil
343 droplet size may change substantially through all digestive mechanisms prior to gastric or intestinal
344 entrance (van Aken, Vingerhoeds and de Hoog, 2007). Apart from Peter's work (2014), all previous
345 work mentioned has bypassed oral processing, via infusion of the emulsion to specific sites of the
346 gastrointestinal tract. Moreover, even before the arrival of food in the gut, sensory and cognitive
347 signals, generated by the visual and sensory aspects of a food, are influencing food intake behaviour.
348 These early pre-ingestive satiety signals integrate with post-ingestive and post-absorptive signals to
349 determine the overall satiating capacity of a food, by influencing physiologically readiness for
350 effective digestion, absorption and metabolism, through mechanisms such as endocrine response
351 and gastric/intestinal secretions and motility. Cassady, Considine and Mattes (2012) demonstrated
352 the importance of pre-ingestive sensory and cognitive information on physiological satiety responses
353 as ratings of hunger were lower, gastric emptying was slower, insulin and GLP-1 release increased,

ghrelin decreased and subsequent *ad libitum* food intake was lower when participants believed a beverage preload would gel in their stomach, even though it did not. This was also reflected in the subjective comments made by participants after the consumption of the preloads. Additionally, studies designed to bypass pre-ingestive signals have demonstrated weaker satiety responses than studies also considering sensory and cognitive influences (Cecil *et al*, 1998; Cecil, Francis and Read, 1998; Lavin *et al.*, 2002). This evidence highlights the importance of pre-ingestive sensory signals in subsequent satiety response through interaction with subsequent satiety mechanisms (anticipatory physiological regulation interactions). Overall, this suggests that although no difference in sensory properties was observed between preloads within this study (see Table.1), the difference in satiety (See Fig.3) was clearly evident.

Having demonstrated a clear effect of manipulated droplet size on the behavioural expression of satiety, a key question is how this effect was achieved, and there are a number of possible explanations which would be valuable for future work to consider. One possibility is that the subtle differences in orosensory experience of the emulsions (which were clearly evident in our earlier study but less evident from the ratings made in the present study) differentially effect cephalic phase responses (Smeets, Erkner and de Graff, 2010) , so altering the degree to which the gut was primed to respond to the ingested nutrients. To test this, future studies should examine how the pattern of release of key hormones implicated in cephalic phase responses (e.g. insulin and pancreatic polypeptide:) and in broader satiety responses (e.g. CCK, PYY, GLP1) differ depending on emulsion droplet size. Additionally, extensional work should look to assess whether such satiety responses are reflected with repeated consumption of these preloads. Such findings would be important in understating whether participants modify their satiety response, as a result of a learning effect between the ingested energy content and preparatory cognitive and sensory influences. This would highlight the effectiveness of the microstructural approach used within this study in the longer term, and may highlight whether sensory differences between preloads are detectable, if a modified satiety response occurs.

When considering the broader significance of the impact of manipulated oil droplet size on satiety, it should be noted that both preloads in the present study were high in fat content, with more than 90% of energy likely to be derived from processing of the fat content. This high fat content is clearly not representative of a normal diet and whether similar effects of droplet size manipulation would be seen with stimuli with lower fat content needs to be explored. Additionally, to begin creating an integrated approach, in microstructural engineering efforts for satiety using oil droplet size, investigating the difference between the consumed and the oral/gastric/intestinal oil droplet size would be beneficial, as anticipatory physiological regulation responses and gastric structuring approaches can begin to be combined.

Conclusion

The present study has shown that smaller droplets within an emulsion preload result in a significant reduction in food intake at a subsequent *ad libitum* meal, independent of formulation change, energy content and perceivable changes in sensory characteristics. This outcome suggests that emulsion based liquid food products can be produced to impact upon satiety, but with the same sensory properties as the original product. Future studies should look to further understand the relationship between emulsion droplet size in relation to satiety and the application of these results in commercially available food systems.

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Highlights

- Emulsion oil droplet size ($d_{4,3}$ 2 and 50 µm) was investigated via a preload design.
- Food intake behaviour was explored, targeting pre-ingestive behaviours.
- Food intake significantly differed, however sensory scores did not.
- ↓ Oil droplet size = ↓ Intake at subsequent meal, independent of formulation.
- Emulsion designs identified which increase satiety but maintain sensory properties.