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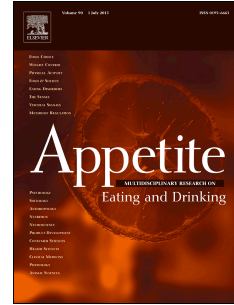
1 **Emulsion oil droplet size significantly affects satiety: A pre-ingestive approach**

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6 **Abstract**

7 Previous research has demonstrated that the manipulation of oil droplet size within oil-in-water

8 emulsions significantly affects sensory characteristics, hedonics and expectations of food intake,

9 independently of energy content. Smaller oil droplets enhanced perceived creaminess, increased

10 Liking and generated greater expectations of satiation and satiety, indicating that creaminess is a

11 satiety-relevant sensory cue within these systems. This paper extends these findings by investigating

12 the effect of oil droplet size ($d_{4,3}$: 2 and 50 μm) on food intake and appetite. Male participants (n =

13 34 aged 18 – 37; BMI of 22.7 ± 1.6 kg/m^2; DEBQ restricted eating score of 1.8 ± 0.1.) completed two

14 test days, where they visited the laboratory to consume a fixed-portion breakfast, returning three

15 hours later for a "drink", which was the emulsion preload containing either 2 or 50 μm oil droplets.

16 This was followed 20 minutes later with an *ad libitum* pasta lunch. Participants consumed

17 significantly less at the *ad libitum* lunch after the preload containing 2 μm oil droplets than after the

18 50 μm preload, with an average reduction of 12% (62.4 kcal). Despite the significant differences in

19 intake, no significant differences in sensory characteristics were noted. The findings show that the

20 impact that an emulsion has on satiety can be enhanced without producing significantly perceivable

21 differences in sensory properties. Therefore, by introducing a processing step which results in a

22 smaller droplets, emulsion based liquid food products can be produced that enhance satiety,

23 allowing covert functional redesign. Future work should consider the mechanism responsible for this

24 effect.

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

26   **Introduction**

27   Fat is the most energy dense macronutrient at 9 kcal per gram (Atwater and Woods, 1896) and

28   consequently is of interest in the redesign of food products to tackle the "obesogenic" food

29   environment. Reducing fat content within foods has been a commonly proposed method to reduce

30   consumers' energy intake. However, this is typically detrimental to the food product's sensory

31   properties (Norton, Moore and Fryer, 2007; Roller and Jones, 2001).

32   Increasing the functionality of the fat to reduce intake could be a novel alternative to produce

33   inherently "healthier" fat based foods (Himaya *et al.,* 1997). Increasing a food product's impact on

34   satiety may lead to a reduction in overall energy intake through inhibition of appetite after

35   consumption (Chambers, McCrickerd and Yeomans, 2014; Hetherington *et al.,* 2013).

36   Designing food structures for functional benefits is a growing area of interest. Redesigning foods that

37   are high in fat (such as emulsions) to impact on appetite has added importance because fat is

38   considered to be the least satiating macronutrient (Blundell, Green, and Burley, 1994; Blundell and

39   Macdiarmid, 1997; Blundell and Tremblay, 1995). Emulsions are common fat based food structures

40   that are found within a variety of commercially available food products, such as sauces, condiments,

41   spreads, dressings and desserts. Emulsions are formed by mixing two immiscible liquids, such as oil

42   and water, so one liquid is dispersed within the other as droplets stabilised by an emulsifier.

43   Previous research considering emulsion structures has predominantly considered gastro-intestinal

44   structuring, in an attempt to achieve satiety via post-ingestive and post-absorptive mechanisms,

45   with emulsion oil droplet size and emulsifier type being the two main properties investigated

46   (Armand *et al.,* 1999; Maljaars *et al.,* 2012; Mun, Decker and McClements, 2007; Golding and

47   Wooster, 2010; Lundin, Golding and Wooster, 2008; Peters *et al.,* 2014; Seimon *et al.,* 2009; Singh,

48   Ye and Horne, 2009; van Aken *et al.,* 2011). However, structuring emulsions to achieve satiety via

49   pre-ingestive approaches (i.e. considering sensory mechanisms) has recently been considered and

50   highlighted as potentially effective (Lett *et al.,* 2015). In that study, decreasing the oil droplet size

51   within an oil-in-water emulsion model drink, increased creaminess, which in turn increased liking

52   and expectations of satiation and satiety, independent of energy content (Lett *et al.,* 2015).

53   creaminess within emulsions was therefore highlighted as a hedonic sensory cue, and a potential

54   satiety-relevant sensory cue, which agrees with other findings that high-energy beverages are more

55   satiating when creamy sensory characteristics are present (McCrickerd, Chambers and Yeomans,

56   2014; Yeomans and Chamber, 2011). The mechanism by which satiety-relevant sensory cues appear

57   to work suggests that people learn to associate sensory characteristics with the subsequent

58   experience of satiety post-consumption (Brunstrom, Shakeshaft and Scott-Samuel, 2008; Yeomans *et*

59   *al.*, 2014). As such, it is thought that creaminess, which is typically associated with high fat content

60   (de Wijk, Rasing and Wilkinson, 2003; Frost and Janhoj, 2007), generates expectations of satiety

61   typically achieved after the consumption of fat containing energy dense foods, with the intensity of

62   creaminess being a predictive marker of energy content.

63   If the enhanced expectation of satiety through altering oil droplet size also impacts on the

64   experience of post-ingestive satiety, this could confirm this type of restructuring as a valuable

65   approach to product development. Early pre-ingestive satiety signals, such as sensory properties

66   integrate with post-ingestive and post-absorptive signals (Blundell, Rogers, and Hill, 1987), and

67   adjust digestive and absorptive mechanisms accordingly, at least partly through anticipatory

68   physiological responses (Power and Schulkin, 2007; Smeets, Erkner and de Graaf, 2010).

69   The present study aimed to extend previous findings from Lett et al. (2015). We hypothesised that

70   reducing the average oil droplet size of an oil-in-water emulsion will enhance satiety, through pre-

71   ingestive sensory-mediated routes by increasing the perception of the identified satiety-relevant

72   sensory cue, creaminess.

73

74 **Materials and Methods**

75 *Design*

76 A repeated-measures single-blind randomised cross-over design preload paradigm was used to

77 investigate the satiating effects of two oil-in-water emulsion based drinks, varying in oil droplet size,

78 but with equal energy content. Test meal intake and subjective ratings (Visual analogue scales: VAS)

79 were used to assess food intake behaviour. Ethical approval for the study was obtained from the

80 University of Birmingham ethics committee (ERN_14-0807, Approved: 14/08/2014).

81 *Participants*

82 Thirty-four healthy male adults participated in the study. Sample size was determined on the basis of

83 the effect size needed to find a difference in satiety between two emulsions with different average

84 oil droplet sizes (2 and 50 µm). These emulsions were produced in a preliminary study in which oil

85 droplet size of an emulsion beverage had been manipulated changing sensory properties (Lett *et al.*,

86 2015). To estimate participant numbers, we examined the outcome of previous preload studies

87 where a difference in creaminess, similar in size to that in our recent emulsion study, was associated

88 with a significant reduction in intake at a similar test meal. One such study where a difference in

89 creaminess was associated with reduced intake was Yeomans and Chambers (2011), where less was

90 consumed after a preload with higher rated creaminess (achieved primarily by varying viscosity) than

91 after an isoenergetic less creamy preload. Based on the intake data in that study, one-tailed

92 significance (P <0.05, predicted reduction with more creamy preload) and power = 0.8, indicated

93 that a sample of 34 would be required. All participants were staff or students at the University of

94 Birmingham, who had expressed an interest in participating in a research study investigating "The

95 effect of mood on appetite", as to mask any expectancy effects concerning the true nature of the

96 investigation. Prospective participants were contacted by a recruitment email via an email database

97 and were asked to reply if they were interested in participation and considered themselves to be a

98   healthy, non-smoking, normal weight (BMI: 18.5 -25) male with no food allergies or intolerances.

99   Females were excluded as they typically practice significantly higher levels of restricted eating and

100   other eating behaviours than males (Arganini *et al.,* 2012; *Fortes et al.,* 2014; *Wardle*, 1987), and

101   males who do not restrict their eating behaviour were chosen, as this cohort demonstrates the most

102   accurate regulation of food intake (Rolls *et al.,* 1994). Respondents to the recruitment email were

103   provided with an information sheet and enrolled in the study if they were still interested in

104   participation. Prior to the start of a session, participants were screened for food allergies, smoking

105   habits and current medical status via a health questionnaire, body mass index (BMI), calculated as

106   kg/m² (with height and weight measurements being obtained with participants wearing light clothes

107   and in a fasted state using a freestanding stadiometer (Seca 213, Birmingham, UK) and digital

108   calibrated weighing scales (Seca 813, Birmingham, UK) and dietary restraint measured using the

109   restraint scale from the Dutch eating behaviour questionnaire (DEBQ) (van Strien, *et al.,*1986).

110   Potential participants were prevented from participating if they indicated any food allergies, history

111   of smoking, had a BMI above 24.9 kg/m^2 or below 18.5 kg/m^2, were taking medication known to

112   interfere with sensory perception or food intake or had a DEBQ restricted eating score of >2.4. One

113   potential participant was prevented from participating, based on the recruitment criteria.

114   Additionally, participants were given the opportunity to ask any questions about the study and its

115   protocol to clarify issues or queries before the study began. The test cohort was made up of 34 men

116   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

117   participants gave written informed consent prior to participation.

118   *Procedure*

119   Participants attended 2 sessions on non-consecutive days. Study protocol was identical on each test

120   day, with only the preload varying (See Fig.1). Participants arrived at a scheduled date and time

121   between 08.30 - 10.30 am, Monday to Friday. Participants arrived having consumed only water from

122   11.00pm the night before. All testing was carried out in an individual booth containing a PC

123   computer running Sussex Ingestion Pattern Monitor (SIPM). SIPM was used to collect VAS scores of

124   all mood and appetite questions throughout the study and preload sensory scores, and monitor food

125   intake at lunch with a digital balance concealed by a placemat (Sartorius BP 4100). All VASs used

126   within the study, collecting data on mood, appetite and preload sensory ratings were randomised

127   differently for all participants. SIPM equipment and software were developed at the University of

128   Sussex (Yeomans, 2000), based on a modification of the Universal Eating Monitor developed by

129   Kissileff, Kilngsberg, and Van Italie (1980), and has been used extensively in studies of human

130   appetite (Yeomans and Bertenshaw, 2008). After successful screening, the participant sat within an

131   individual booth to begin the breakfast session and consumed the test breakfast (see Standard

132   Breakfast section) within 15 minutes. To begin participants completed a set of the mood and

133   appetite questions. The mood ratings (Alert, Anxious, Calm, Clearheaded, Happy and Tired) and

134   appetite ratings (Hunger and Fullness), were presented as 100-point computerised VASs anchored

135   with "not at all [mood or appetite]" and "extremely [mood or appetite]". Mood questions were

136   included as distracters and to be consistent with the premise that the study was investigating "The

137   effect of mood on appetite". The participant was then instructed to return exactly 3 hours later for

138   the preload session. During the inter breakfast-preload period participants were not allowed to

139   participate in exercise or consume any food or drink, apart from a 250 ml bottle of still water, which

140   was provided and had to be fully consumed upon their return. Upon the participants return, they

141   began the preload session. Participants completed the standard mood and appetite questions and

142   then were presented with 200 ml of one of the two preloads (see Drink Preload section). 17

143   participants received the 2 μm droplet preload on their first session and the other 17 participants

144   received the 50 μm droplet preload on their first session, with the other preload being consumed on

145   the second session. SIPM instructed the participant to take a mouthful and then carry out a number

146   of VAS to assess the samples sensory characteristics. The preload was evaluated for thickness,

147   slipperiness, smoothness, creamy mouthfeel, overall creaminess, liking, expectation of hunger in 1

148   hours time (Satiety) and expectation of fullness immediately (Satiation). Both questions determining

149   expectations of food intake were in reference to if they consumed the full portion presented.

150   Sensory VAS questions were headed "How [target rating] is the drink?" and end-anchored with "not

151   at all [target rating]" (scored as zero) and "extremely [target rating]" (scored as 100); wording may

152   have slightly differed to be grammatically correct. Upon completion of the sensory VAS questions,

153   SIPM instructed the participant to consume the rest of the preload within 5 minutes, before another

154   series of standard mood and appetite questions were presented to finish the preload session.

155   Participants then remained within the laboratory until the lunch session. Results from our previous

156   work showed that expectations of food intake are significantly different due to sensory differences

157   between the emulsions. As such, a 20 minute delay between the preload being presented and the

158   lunch session was used. This fits within the optimal time period for detecting oro-sensory effects on

159   satiety (<30 minutes) (Livingstone *et al*., 2000), and allowed enough time for participants to

160   comfortably consume the preload and complete all mood, appetite and sensory VASs. During the

161   lunch session, participants first completed a set of standard mood and appetite ratings in the

162   absence of any food cues (pre-lunch ratings). Next, 500 g white penne pasta with tomato and herb

163   sauce (see Lunch section) was served by an experimenter who explained that the participant could

164   eat as little or as much as he liked. A hidden digital balance secured under a placemat and linked to

165   SIPM, which recorded the weight of food being eaten. If the participant consumed 300 g of the

166   lunch, an onscreen alert message prompted the participant to call the experimenter. The

167   experimenter then served the participant another 500 g pasta in a new bowl, with the consumed

168   bowl of pasta being removed; no limit was placed on the number of refills permitted. To reduce the

169   influence of habit and portion-size effects on intake, participants were encouraged not to use the

170   refill prompt as a cue to end the lunch session. When participants had confirmed that they had

171   finished eating, the participants then completed a final set of standard mood and appetite ratings

172   (post-lunch ratings) before the lunch session and test day was completed. On the final test day, the

173   participants were given a £20 Amazon voucher as compensation for participating in the study.
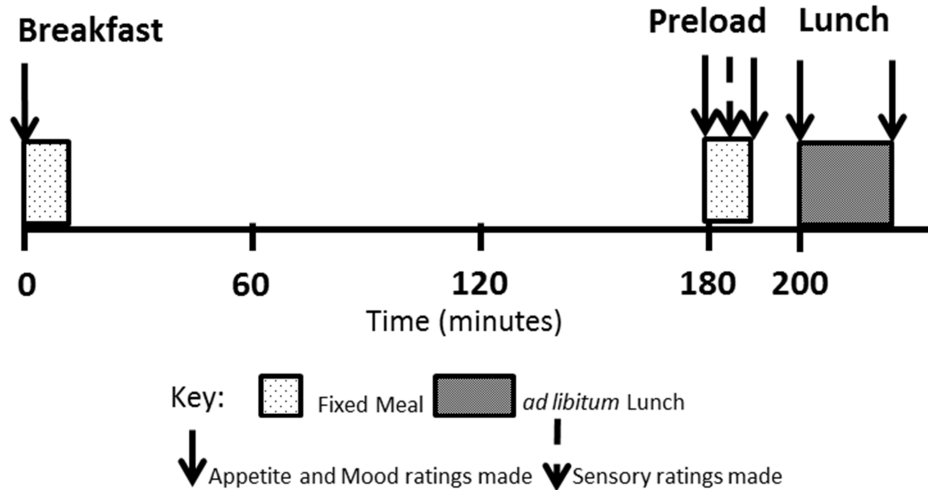
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175   *Fig. 1* Schematic representation of the timing of the fixed and test meals and the sets of appetite and
176   mood ratings and sensory ratings on a test day.

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*

190 The preload drinks were 200 ml of emulsions containing either 2 or 50 μm droplets; these were *No*

191 *Flavour* versions of emulsion samples described in a previous study (Lett *et al.,* 2015). These

192 emulsions were chosen as, based on our previous work, emulsions containing 2 μm droplets gained

193 significantly greater ratings for creaminess ($P = 0.003$) and Liking ($P = 0.01$) and resulted in reduced

194 expectations of Hunger in 1 hours time (Satiety) than the emulsion containing 50 μm droplets ($P =$

195 0.017). Rheological and lubrication properties of these systems have been investigated in other work

196 by the authors, and it was shown that 2 and 50 μm emulsions were also comparable in these

197 properties. Samples consisted of an oil-in-distilled water emulsion (1 wt.% sodium caseinate

198 (Excellion EM7, DMV International, The Netherlands); 2 wt.% sucrose (Silverspoon granulated,

199 British Sugar Plc, UK) and 15 wt.% sunflower oil (Tesco Plc, UK)). Emulsions were produced using two

200 different methods dependent upon the required mean droplet size of the emulsion being produced:

201 a high shear mixer (Silverson L5M, Silverson machines Ltd, UK) or a high-pressure homogeniser (GEA

202 Niro Soavi Panda Plus 2000, GEA Niro Soavi, Italy). In a 600 ml beaker, 15 wt.% sunflower oil was

203 added to 85 wt.% aqueous phase (1 wt.% NaCas, 2 wt.% sucrose, 97 wt.% distilled water solution).

204 The whole sample was then emulsified for 5 minutes using the high shear mixer. Dependent on oil

205 droplet size being produced the sample was subjected to a different rotational speed (rpm) and

206 emulsor screen. 50 μm oil droplet samples were subject to high shear mixing at 2500 rpm with a 1.6

207 mm pore emulsor screen. 2 μm oil droplet samples were subject to high shear mixing at maximum

208 rpm with a 0.8 mm pore emulsor screen to produce a pre-emulsion, the pre-emulsion was then

209 homogenised at 100 Bar with 2 passes. All samples were produced in 400 g batches, under clean and

210 hygienic conditions on the day of evaluation and stored under refrigerated conditions at 2-5 °C. The

211 200 ml emulsion preload provided approximately 282 kcal, 30 g fat, 2 g protein, and 4 g

212 carbohydrate.

213 *Data Analysis*

214 The aim of the study was to investigate whether altering the oil droplet size of an emulsion altered

215 subsequent food intake behaviour. Data were analysed using the Statistical Package for the Social

216 Sciences (SPSS) version 21 (SPSS Inc., USA). VAS scores for hunger and fullness throughout the study

217 are reported from baseline (pre-preload) data, and were analysed using 2-way ANOVA based on the

218 three post-preload time points (immediately post-preload, pre-lunch and post-lunch) and two oil

219 droplet sizes. Nutrient and energy composition of the breakfast and lunch was calculated using

220 compositional data provided by the manufacturers. The energy density of the preload drink was

221 calculated using Atwater factors (Atwater and Woods, 1896).

222

223 **Results**

224 *Mood and appetite ratings*

225 Protected contrasts of baseline evaluations of mood and appetite (hunger and fullness) ratings

226 before preload ingestion (at breakfast and just before preload consumption) did not differ

227 significantly, and so effects of preload oil droplet size on appetite were assessed using change from

228 baseline data. As can be seen (Figure 2a), hunger decreased immediately after the preload,

229 recovered prior to lunch and then fell markedly after lunch, reflected in an overall effect of rating

230 time on hunger (F(2,66) = 182.68, p<0.001, ETA = 0.85), but the change in hunger was consistently

231 lower after the preload with 2 μm than 50 μm oil droplet size (F(1,33 = 9.66, p=0.004, ETA = 0.23),

232 with no significant time x droplet interaction (F(2,66) = 1.04, p=0.36, ETA = 0.03). Fullness ratings

233 showed the reverse pattern over time to hunger (Figure 2b: effect of time F(2,66) = 82.45, p<0.001,

234 ETA = 0.71), but there was no significant effect of droplet size (F(1,33 = 0.07, p=0.80, ETA = 0.01) or

235 time x droplet interaction (F(2,66) = 0.71, p=0.50, ETA = 0.02).

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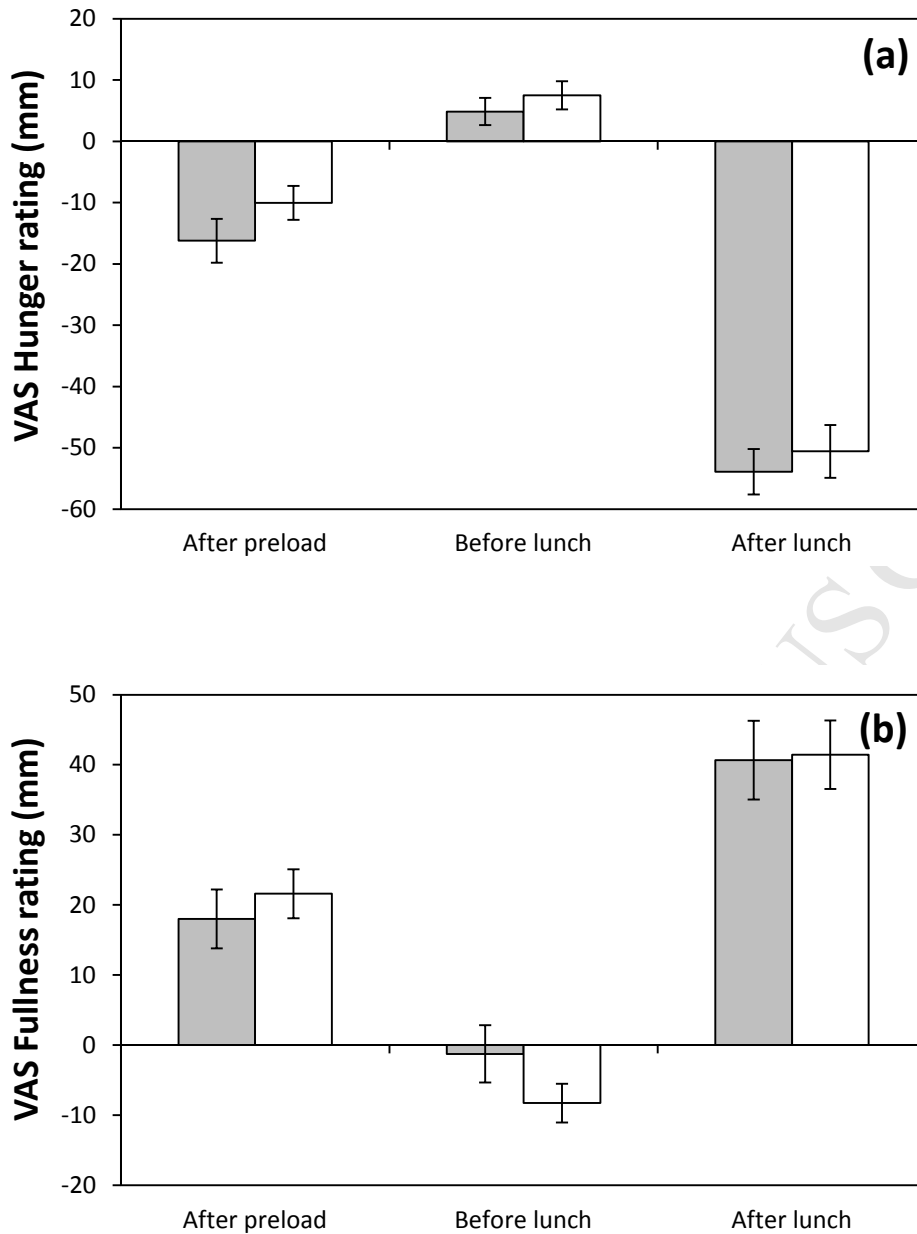
Fig. 2 Mean (± SEM) changes in ratings of Hunger (a) and Fullness (b) across the course of the test session for both 2 µm (filled bars) and 50 µm (open bars) emulsion preloads.

241

Preload sensory and hedonic ratings

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

247 *Table. 1* Mean (± SEM) of attribute ratings of 2 μm and 50 μm samples used in current study and Lett
248 *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

249

250 *Lunch intake*

251 Total lunch intake was significantly different dependent on oil droplet size preload consumed, with

252 participants consuming significantly less after consumption of the 2 μm preload (*P* = 0.027). Total

253 consumption was 67.7g or 62.4 Kcal less, which is a 12.3 % reduction in total food intake (g) and a

254 12.2 % reduction in energy intake (Kcal) (see Fig. 3a and b). No significant effect of preload session

255 order on intake, for both droplet sizes (*P* >0.05), was also shown, highlighting participant fatigue of

256 the protocol did not factor in ratings or *ad libitum* food intake.

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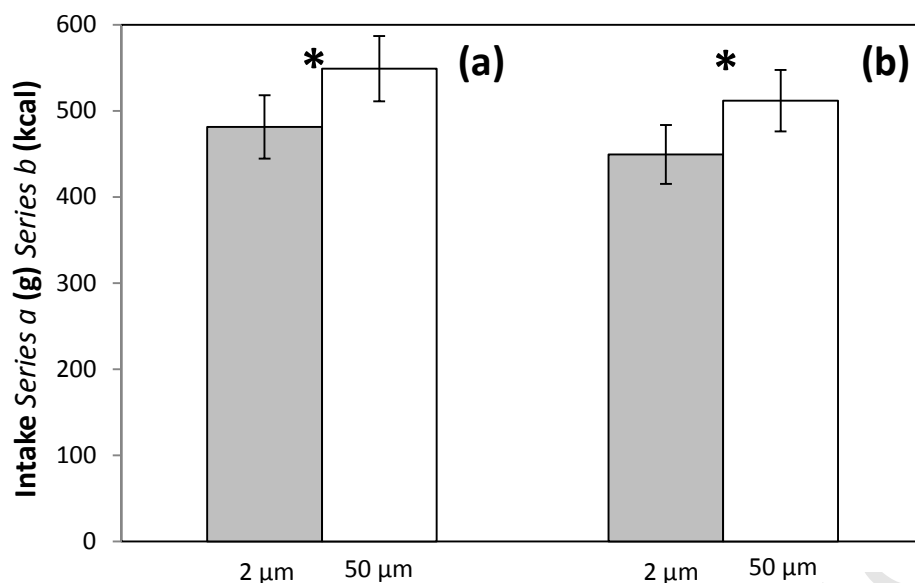
Fig. 3 Mean overall intake at the test meal (± SEM) in grams (a) and kilocalories (b). Filled bars
represent preloads containing 2 μm droplets and Open bars represent preloads containing 50 μm. *
represents significance at *P* < 0.05.

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.

277   Our current work has shown that although expectations of food intake behaviour have been

278   successfully realised in actual eating behaviour (See Fig.3), the mechanism mediating the effect has

279   not been identified, as ratings of creaminess, or any other attribute, for the two preloads were not

280   significantly different (see Table 1). Therefore, our findings do not fully agree with our hypothesis.

281   Given that Lett and co-authors (2015) identified potential satiety-relevant sensory cues within these

282   systems, and that the current study protocol was designed to maximise the influence of potential

283   sensory effects of the preload on subsequent food intake (Blundell, 2010; Livingstone *et al*., 2000), it

284   is unusual that a significant difference in satiety was identified (See Fig.3), but no significant

285   differences in sensory perception were found.

286   Other studies have also shown differences between sensory properties of preloads in the "pilot"

287   sensory study, but not in the "main" preload study, despite similarities in the studies cohort

288   (Chambers, Ells and Yeomans, 2013; McCrickerd, Chambers and Yeomans, 2014; Yeomans and

289   Chambers, 2011). Consequently, it seems sensible to suggest that the difference in protocol

290   between this and our earlier study (Lett *et al.,* 2015), is the reason for the change in sensory results.

291   The protocol in Lett et al. (2015) promoted sample assessment in a more analytical manner. Firstly,

292   participants were recruited to participate in a "sensory analysis of emulsions" study, so would have

293   approached the study consciously seeking sensory differences between samples. Although samples

294   were unidentifiable and randomly ordered, the methodology used would not have controlled for the

295   cross-comparison of sensory attributes between samples, as samples were analysed in a sequential

296   manner in one session. Secondly, all sensory attributes investigated were defined via a description

297   reference and not at the discretion of the individual participant, as was the case within the current

298   protocol. Our previous study (Lett et al., 2015) also used 100mm paper-based VAS scale, compared

299   to the use of 100-point computerised VAS using SIPM here. Although no published study has

300   explicitly compared manual VAS ratings and computerised based VAS on SIPM, studies have shown

301   VAS scores change, even subtlety, dependent on the protocol for collecting VAS based data used

302   (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,

354 ghrelin decreased and subsequent *ad libitum* food intake was lower when participants believed a

355 beverage preload would gel in their stomach, even though it did not. This was also reflected in the

356 subjective comments made by participants after the consumption of the preloads. Additionally,

357 studies designed to bypass pre-ingestive signals have demonstrated weaker satiety responses than

358 studies also considering sensory and cognitive influences (Cecil *et al*, 1998; Cecil, Francis and Read,

359 1998; Lavin *et al.*, 2002). This evidence highlights the importance of pre-ingestive sensory signals in

360 subsequent satiety response through interaction with subsequent satiety mechanisms (anticipatory

361 physiological regulation interactions). Overall, this suggests that although no difference in sensory

362 properties was observed between preloads within this study (see Table.1), the difference in satiety

363 (See Fig.3) was clearly evident.

364 Having demonstrated a clear effect of manipulated droplet size on the behavioural expression of

365 satiety, a key question is how this effect was achieved, and there are a number of possible

366 explanations which would be valuable for future work to consider. One possibility is that the subtle

367 differences in orosensory experience of the emulsions (which were clearly evident in our earlier

368 study but less evident from the ratings made in the present study) differentially effect cephalic

369 phase responses (Smeets, Erkner and de Graff, 2010) , so altering the degree to which the gut was

370 primed to respond to the ingested nutrients. To test this, future studies should examine how the

371 pattern of release of key hormones implicated in cephalic phase responses (e.g. insulin and

372 pancreatic polypeptide:) and in broader satiety responses (e.g. CCK, PYY, GLP1) differ depending on

373 emulsion droplet size. Additionally, extensional work should look to assess whether such satiety

374 responses are reflected with repeated consumption of these preloads. Such findings would be

375 important in understating whether participants modify their satiety response, as a result of a

376 learning effect between the ingested energy content and preparatory cognitive and sensory

377 influences. This would highlight the effectiveness of the microstructural approach used within this

378 study in the longer term, and may highlight whether sensory differences between preloads are

379 detectable, if a modified satiety response occurs.

380 When considering the broader significance of the impact of manipulated oil droplet size on satiety, it

381 should be noted that both preloads in the present study were high in fat content, with more than

382 90% of energy likely to be derived from processing of the fat content. This high fat content is clearly

383 not representative of a normal diet and whether similar effects of droplet size manipulation would

384 be seen with stimuli with lower fat content needs to be explored. Additionally, to begin creating an

385 integrated approach, in microstructural engineering efforts for satiety using oil droplet size,

386 investigating the difference between the consumed and the oral/gastric/intestinal oil droplet size

387 would be beneficial, as anticipatory physiological regulation responses and gastric structuring

388 approaches can begin to be combined.

389

390 **Conclusion**

391 The present study has shown that smaller droplets within an emulsion preload result in a significant

392 reduction in food intake at a subsequent *ad libitum* meal, independent of formulation change,

393 energy content and perceivable changes in sensory characteristics. This outcome suggests that

394 emulsion based liquid food products can be produced to impact upon satiety, but with the same

395 sensory properties as the original product. Future studies should look to further understand the

396 relationship between emulsion droplet size in relation to satiety and the application of these results

397 in commercially available food systems.

398

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402

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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.