Detailed methods for preparation of emulsions

Each fat emulsion meal was prepared individually in a separate 350 g batch using the equipment and preparation methods specified below. Each meal was assigned a unique identifier label and was subsequently stored in a fridge in order to later dispense 300g for the participants to ingest, within a week from preparation. The whole process had been checked for microbiology and droplet size.

Equipment used
- Scale: Model LC4800P, Sartorius UK Ltd, Epsom, UK
- Silverson: Model L5R, Silverson Machines Ltd, Waterside, UK
- Ultra-Turrax: Model IKA T25BS2, IKA®-Werke GmbH & Co. KG, Staufen, Germany
- Microfluidizer: Model M-110S, Microfluidics, Westwood, MA

Ingredients used
- Sunflower Oil: food ingredient; KTC Edibles supplied by Anglian Oils Ltd, Bexwell, Norfolk, U.K.
- Water; de-ionised with Elga water purification system
- Tween20: E432 emulsifier; Sigma Aldrich, Gillingham, U.K.
- Locust bean gum: food thickener; GENU® GUM type RL-200Z locust bean gum, CP Kelco, Leatherhead, U.K.
- Sweetener: Hermesetas gold (Sodium saccharin) (E 954), Tesco supermarkets, U.K.
- 13C Octanoic acid: in vivo breath test label; Euriso-Top, Parc des Algorithmes, Bâtiment Homère, Route de l’Orme, F-91194 Saint Aubin cedex, France.

Preparation of Coarse emulsion with no LBG (Coarse):

3.5 g of Tween 20 was added to 276.5 g water. Using a dedicated precision pipette 117 mg 13C-octanoic acid was added to 70g of sun flower oil (SFO) and mixed gently. The beaker was covered with a foil and the mixture was ultra-turraxed for 20 minutes at speed 3. The emulsion was then transferred to a labelled 500 mL glass bottle. 3.5g of coffee flavour and 5 sweetener tablets were added and mixed well by shaking. The emulsion was then stored in a fridge.

Preparation of 1% LBG stock Solution:

16 g LBG powder was dispersed in 1584g boiling water whilst stirring using the Silverson at full speed. The beaker was covered with aluminium foil whilst using the Silverson. Any aggregates were manually dissolved using clean spatula and dispersed whilst stirring again for 10 minutes with the Silverson. The final solution concentration was 1% w/w LBG/water. The glass bottle was cooled down rapidly by placing in an ice bath and then stored in a fridge.

Preparation of the Coarse emulsion with 0.5% LBG (Coarse +LBG):

3.5g Tween 20, 241.5g water and 175g 1% LBG stock solution were placed in a 600 mL glass beaker and mixed well using a spatula. Using a precision pipette, 117 mg 13C-octanoic acid was added to 70g sun flower oil (SFO) and the bottle was swirled gently to ensure the label was well mixed with the SFO. The beaker was covered with a foil and the mixture is ultra-turraxed for 5 minutes at speed 2. The emulsion was transferred to a labelled 500 mL new glass bottle. 3.5g of coffee flavour and 5 sweetener tablets were added to the emulsion and mixed well by shaking. The emulsion was then stored in a fridge.

Preparation of the Fine emulsion with 0.5% LBG (Fine+LBG):

A Coarse emulsion was prepared as above. The emulsion was then passed through the Microfluidizer once, with a pressure of 2.6 bars at the inlet, which corresponds to 605.8 bars in the interaction chamber. After this the Fine emulsion was then transferred to a labelled 500 mL glass bottle. 3.5g of coffee flavour and 5 sweetener tablets were added and mixed well by shaking. The emulsion was then stored in a fridge.
In vitro physical measurements

_Droplet size measurements:_ Droplet size analysis was carried out using a Malvern Mastersizer 2000 (Malvern, Worcestershire, UK). This uses laser diffraction to measure the size of particles/droplets by measuring the intensity of light scattered as the laser beam passes through the sample. The refractive index of sunflower oil and water were selected to model the data. The emulsion sample was dispersed into the sample chamber in re-circulating water in the ‘Hydro SM’ measuring cell until an obscuration rate of 12–14% was obtained. The dispersed sample was stirred until homogeneous dispersion without agglomeration was achieved. The sample was then pumped through the laser beam measuring zone. The measurement was carried out in triplicate on each emulsion. **Supplemental Fig. 1** shows examples of droplet size distribution for the three fat emulsion meals used in this study.

![Droplet size distribution](image)

**SUPPLEMENTAL FIGURE 1:** Example laser diffractometry droplet size distributions for the three, 20% sunflower oil in water emulsion meals used in this study: Coarse (larger droplet size), Coarse+LBG (larger droplet size with added 0.5% locust bean gum, LBG) and Fine+LBG (smaller droplet size with added 0.5% LBG).
**Rheology measurements:** During meal development the viscosity of the 3 fat emulsion meals was measured in duplicate using a Physica MCR 501 rheometer (Anton Paar Ltd, St. Albans, UK). All samples were measured at 37°C using a cup and bob geometry with smooth surfaces and 17mm diameter. Examples of rheometry data from the 3 emulsion meals used in this study are shown in **Supplemental Fig. 2**. The duplicates and the data show the good reproducibility. Due to sensitivity issues with the low viscosity Coarse (no LBG) sample, reliable data was not obtained until a shear rate of $10\,\text{s}^{-1}$ was reached. Thereafter it was determined to be a low viscosity fluid and Newtonian in nature. The Fine + LBG showed a higher viscosity at the low shear rates and was more shear-thinning compared to the Coarse + LBG sample. The Coarse + LBG had slightly higher viscosity at high shear rates compared to the Fine + LBG.

**SUPPLEMENTAL FIGURE 2:** Example of rheology profiles for the three, 20% sunflower oil in water emulsion meals, in duplicate.
SUPPLEMENTAL FIGURE 3: MRI image showing creaming of a 20% sunflower oil in water emulsion Coarse meal in the stomach of a healthy young adult and typical upper and lower voxel positioning for the MRS acquisitions.

SUPPLEMENTAL FIGURE 4: % gallbladder contraction with time for healthy young adults after they consumed the 20% sunflower oil in water emulsion meals on the three separate study days: Coarse (larger droplet size), Coarse+LBG (larger droplet size with added 0.5% locust bean gum, LBG) and Fine+LBG (smaller droplet size with added 0.5% LBG). Values are mean (±SEM), n=11. The arrow indicates the meal time.
SUPPLEMENTAL FIGURE 5: Cholecystokinin (CCK) plasma concentration with time for healthy young adults after they consumed the three, 20% sunflower oil in water emulsion meals on the three separate study days: Coarse (larger droplet size), Coarse+LBG (larger droplet size with added 0.5% locust bean gum, LBG) and Fine+LBG (smaller droplet size with added 0.5% LBG). Values are mean (±SEM), n=11. The arrow indicates the meal time.
SUPPLEMENTAL FIGURE 6: Changes from baseline (delta over baseline, DOB) of the ratio of $^{13}$C to $^{12}$C in the breath samples with time for healthy young adults after they consumed the three, 20% sunflower oil in water emulsion meals on the three separate study days: Coarse (larger droplet size), Coarse+LBG (larger droplet size with added 0.5% locust bean gum, LBG) and Fine+LBG (smaller droplet size with added 0.5% LBG). Values are mean ($\pm$SEM), $n=11$. The arrow indicates the meal time.
SUPPLEMENTAL FIGURE 7: Visual analogue scale (VAS) scores for fullness (A) hunger (B) and prospective food consumption (C) for healthy young adults after they consumed the three, 20% sunflower oil in water emulsion meals on the three separate study days: Coarse (larger droplet size), Coarse+LBG (larger droplet size with added 0.5% locust bean gum, LBG) and Fine+LBG (smaller droplet size with added 0.5% LBG). Values are mean (±SEM), n=11. The arrow indicates the meal time.