

1 **SUPPLEMENTARY MATERIAL**

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3 **Sample preparation**

4 Distilled monoacylglycerols (MAGs) were kindly provided by Danisco (Kansas,
5 MO), and included Dimodan® HS K-A (10% monopalmitin, 90% monostearin), with
6 fatty acids predominantly at positions sn-1 and sn-3. Sodium hydroxide (2N NaOH) was
7 obtained from Fischer (St. Louis, MO), canola oil (low-erucic rapeseed oil) was
8 purchased in a local supermarket. Deionized water was used in all experiments and was
9 of milliQ grade.

10 A typical 10% (w/w) stock of HS K-A in vegetable oil - all oils performed equally
11 well - was prepared by melting 10g of the solid MAG HS K-A (m.p. ~73°C) and 500mg
12 of sodium stearyl lactylate (SSL) or stearic acid powders in 89.5g of oil at 70°C for 30
13 minutes. Appropriate volume fractions of 70°C 0.2N NaOH, were then added to the
14 MAG-oil stock in a glass bowl heated to 70°C. The material was mixed with the aid of
15 an electric hand mixer until a macroscopically homogeneous white paste was obtained,
16 and then allowed to set undisturbed at room temperature (22°C). The material set almost
17 immediately. For 100mL batches, setting was complete within 30 minutes. The mixture
18 had to contain at least 4% (w/w) distilled monoglyceride for proper gel formation and
19 stability. Added salts and proteins generally decreased stability and/or prevented gel
20 formation.

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22 **Sample characterization**

23 Powder X-ray diffraction (XRD) measurements were conducted using an in-house
24 diffractometer with Cu source ($\lambda=1.54 \text{ \AA}$) and Position Sensitive Detectors. Very small

1 angle XRD measurements were carried out at the Austrian Small Angle X-ray Scattering
2 (SAXS) beamline (<http://www.ibr.oeaw.ac.at/beamline>) at Elettra (Sincrotrone Trieste,
3 Italy). Confocal laser scanning microscopy was performed using a Leica TCS SP II
4 confocal laser scanning microscope. For confocal microscopy, samples were stained
5 with Coumarin which is soluble in the aqueous phase and Nile Red for the oil phase.
6 Polarized light microscopy was carried out using a Leica DM RXA2 upright light
7 microscope (Leica Microsystems, Toronto, Canada) equipped with a digital monochrome
8 camera (Q Imaging Retiga® 1300, Vancouver, Canada). Dynamic shear loss (G'') and
9 storage (G') were obtained using a TA Instruments AR2000 rheometer at 20°C by stress sweeps
10 using a temperature controlled parallel plate geometry at a frequency of 1Hz.

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12 **Acute human ingestion trials**

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14 Five male and four female subjects were recruited for this study. Approval for the study
15 was received from the Research Ethics Committee of the University of Waterloo and
16 each participant provided written informed consent prior to participation. Questionnaires
17 were used to determine medical history and exercise habits for each participant. Height
18 and weight measurements were obtained and body mass index (BMI) calculated. The
19 average BMI of the subjects was $25.2 \pm 2.8 \text{ kg/m}^2$, while the average age was 24 ± 2
20 years (mean \pm standard deviation).

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22 The subjects completed two separate trials in which they consumed one of the two test
23 meals in randomized order. The test meals consisted of two pieces of 100% whole wheat
24 toast with either a canola oil-water mixture (CO) or a structured oil-water mixture (MAG

1 Gel). The subjects consumed 80g of the MAG Gel (4.8g monostearin, 43.2g of canola oil,
2 32g water), or 80g of the CO-water mixture (48g CO, 32g water), but no monoglycerides.
3 The CO was mixed with water in proportions equivalent to those of the MAG Gel. The
4 test meal was ingested within ten minutes. Each trial was six hours in duration during
5 which the subjects were permitted to drink only one liter of water. Subjects were
6 instructed to refrain from alcohol and strenuous exercise for 24 hours prior to each trial.
7 They were also not permitted to consume any food or caffeine after 9pm the night before.
8 The trials were separated by seven days and conducted in a random order.

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10 Blood samples were obtained throughout each trial using a forearm venous catheter. A
11 baseline sample was taken prior to administration of the test meal and at 30, 60, 90, 120,
12 150, 180, 240, 300 and 360 minutes after meal consumption. Serum was separated from
13 untreated whole blood by centrifugation (21°C, 10 minutes, 1800g), while heparin and
14 EDTA plasma was obtained by centrifugation (4°C, 10 minutes, 1100g) after adding
15 either 15µL of heparin (5U/µL) or 60µL of EDTA (0.05mg/µL) respectively to 1.5 ml of
16 whole blood. All serum and plasma samples were stored at -70°C until analysis.

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18 Serum triglyceride (TG), cholesterol (CHOL) and high-density lipoprotein (HDL) levels
19 were determined at a third party, accredited diagnostic laboratory (Toronto Medical
20 Laboratories; Toronto, Ontario, Canada) by automated methods employing a Bayer
21 Advia 1650 auto analyzer. The Friedewald equation was used to calculate the low-density
22 lipoprotein (LDL) levels. The serum TG responses over time are expressed as the
23 difference from baseline, which was established using t=0 and t=30 measures. Free fatty

1 acids (FFA) were measured in quadruplicate from EDTA plasma by using the NEFA
2 ACS-ACOD method with a kit provided by Wako Chemicals GmbH (Neuss, Germany).
3 The plasma FFA responses over time are expressed as the difference from baseline,
4 which was measured at 0 hours. Glucose concentration was determined from heparinized
5 plasma samples in triplicate using a spectrophotometric enzymatic assay. The assay was
6 based on the coupled glucose oxidase/oxidase enzymatic reactions and all reagents
7 were obtained from Sigma (St. Louis, Missouri, USA). Insulin was determined in
8 duplicates from heparinized plasma using a Coat-A-Count Radioimmunoassay Kit
9 (Diagnostic Product Corporation: Los Angeles, California, USA).

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11 The trapezoidal method was used to calculate the specified net AUC for each of the
12 variables (3-6h: TG; 0-6h: FFA, glucose, insulin). Differences between the two trials
13 were assessed for significance using a paired t-test ($p < 0.05$). Analyses were completed
14 using GraphPad Prism Version 4.03 for Windows, GraphPad Software: San Diego,
15 California, USA.

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