Encapsulation-stucturing of edible oil attenuates acute elevation of blood lipids and insulin in humans†

Alejandro G. Marangoni,*[ad] Stefan H. J. Idziak,[b] Cesar Vega,[a] Heidi Batte,[a] Michel Ollivon,[d] Pamela S. Jantzi[c] and James W. E. Rush[c]

Received 21st August 2006, Accepted 13th November 2006
First published as an Advance Article on the web 22nd November 2006
DOI: 10.1039/b611985a

Blood triglyceride, free fatty acid and insulin levels are lower after acute intake of an oil–water–monoglyceride gel *versus* an oil–water mixture, demonstrating that food matrix nanostructure and microstructure can be engineered to modulate the physiological response. Oil emulsification by the monoglyceride L_α liquid-crystalline lamellar phase, followed by droplet wall crystallization, encapsulates oil and creates a material with the functionality and properties of a fat. This novel phase is devoid of *trans* fatty acids and can be manufactured with as little as 4% added saturated monoglyceride.

Obesity has reached epidemic proportions throughout the world. The problem is not restricted to developed nations, with the incidence of obesity increasing at alarming rates throughout the developing world as well.[1] Obesity affects all ages and socio-economic groups, and is mainly a consequence of reduced physical activity in combination with increased consumption of energy-rich, nutrient-poor foods. Increases in obesity and overweight conditions in adults, and particularly children, are alarming since they are strongly correlated with an increased incidence of cardiovascular disease, type-2 diabetes, hypertension and stroke, and some forms of cancer.[1] As part of a strategy to decrease risk factors for these diseases in the population, two important diet-related recommendations have been made. The first is the achievement of energy balance and a healthy weight. The second recommendation is to limit energy intake from fats while also shifting fat consumption from saturated fats to unsaturated oils, and eliminating *trans*-fatty acids from the diet completely. Consumption of fats containing high amounts of *trans* and saturated fatty acids have been clearly shown to increase the risk of coronary heart disease.[2–6]

One way of contributing towards these goals, and to make a significant difference to the health of a population, is to improve on the nutritional profile of *manufactured* food products. This, however, represents a technological challenge to the food industry. Many of the food products that we traditionally consume contain fat as a structuring material, while providing the texture, flavor and mouthfeel that we have evolved to associate with survival. At the core of the problem is the ability to transform oil, which is liquid at room temperature, to fat, which is solid at room temperature. Oils and fats are mostly composed of triacylglycerol molecules (Fig. 1A): three fatty acids esterified onto a glycerol backbone. Whether such material is solid or liquid at a particular temperature will depend on the chemical nature of the constituent

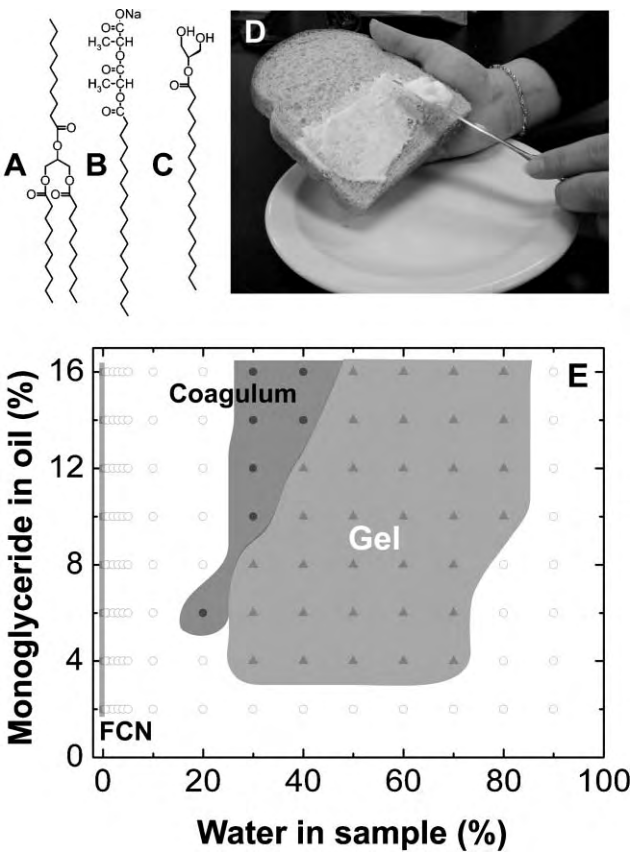

Fig. 1 Composition of the monoglyceride gel. Schematics of the chemical structure of a typical triglyceride (A), sodium stearoyl lactylate (B), and monostearin (C) which is the dominant component of HS K-A. (D) Photograph of the monoglyceride gel which had a texture and appearance similar to that of commercial spreads. (E) Phase diagram of binary mixtures of monoglyceride HS K-A in oil (given in weight percent) and water, where triangles represent compositions in the gel phase. The solid circles represent a coagulated, or curdled, phase and the vertical line at 0% water denotes a yellow colored phase composed of fat crystals in the vegetable oil (FCN).

[a]*Department of Food Science and Guelph-Waterloo Physics Institute, University of Guelph, Guelph, Canada. E-mail: amarango@uoguelph.ca*
[b]*Department of Physics and Astronomy, Guelph-Waterloo Physics Institute, University of Waterloo, Waterloo, Canada*
[c]*Dept. of Kinesiology, University of Waterloo, Waterloo, Canada*
[d]*Université Paris Sud, Centre d'Etudes Pharmaceutiques, Laboratoire de Physico-Chimie des Systèmes Polyphasés, UMR CNRS 8612, Châtenay-Malabry, France*

† Electronic supplementary information (ESI) available: Sample preparation and characterisation, details of human trials. See DOI: 10.1039/b611985a

fatty acids. Solid fats are structured by using either saturated animal or vegetable fats such as lard, milk fat, coconut oil or palm fat, or by using oils which have been subjected to hydrogenation. The "hydrogenation" process converts naturally occurring *cis* unsaturated fatty acids to their *trans* and/or saturated counterparts, resulting in an increase in the melting point of some of the constituent triacylglycerol molecules. An edible fat is therefore a semisolid plastic material structured as a gel-like network of fat crystal aggregates with liquid oil trapped within.[7–10] Traditionally, the only way to structure oil, and thus convert it to fat, is by the addition of high-melting point saturated or *trans* fats. Thus, in order to satisfy the growing health concerns associated with *trans* and saturated fats, a new strategy for structuring edible oils is required. These solid fat replacements should ideally be composed of unsaturated vegetable oils, be devoid of added *trans* and saturated fats, and also have a reduced fat content.

One of the most promising strategies to achieve the structuring of oil is by exploiting the properties of lyotropic liquid crystalline phases of long-chain saturated monoglycerides.[11,12] The phase behavior of saturated monoglyceride–water systems is well understood,[13–17] and the material is available commercially in relatively high purity (>98%). In the presence of an anionic co-surfactant, long-chain saturated monoglycerides form a highly hydrated lamellar L_α phase in the presence of water at temperatures above the Krafft (chain-melting) temperature of the monoglyceride.[16,17] When the material is cooled below this Krafft temperature, a highly hydrated L_β mesomorphic phase (the "alpha gel") appears, which eventually transforms to an anhydrous crystalline phase (the "coagel"). This dynamic is sensitive to environmental factors such as water–monoglyceride ratio, pH, type and amount of co-surfactant used, storage time and temperature, among others.[11] Even though the behavior of monoglyceride–water systems is relatively well-understood and several commercial applications have been proposed,[11,12] very-little to no information exists on the ternary phase behavior of oil–monoglyceride–water systems. Of particular interest to our work was to exploit the properties of these monoglyceride gel phases (alpha gel and coagel) to encapsulate liquid oil, create a mesoscopic network structure, and convert a simple oil–water mixture into a fat-like material with desirable functional properties. Here, we report on the discovery of a new oil–water gel structure stabilized by crystalline multilamellar monoglyceride multilayers which encapsulate high volume fractions of liquid oil within a water matrix.

The gel is produced by vigorously mixing a hot oil–monoglyceride solution with alkaline deionized water (0.05 N NaOH) at a temperature of 70 °C with an electric hand mixer until a macroscopically homogeneous white paste is obtained. Canola oil was used in these studies, although any edible oil is suitable. The monoglyceride used was commercially available HSK-A (10% monopalmitin, 90% monostearin, Fig. 1C) distilled monoglyceride, and cosurfactants such as sodium stearoyl lactylate (SSL, Fig. 1B), or stearic acid, in a 20 : 1 (w/w) ratio. The mixture had to contain at least 4% (w/w) distilled monoglyceride for proper gel formation and stability. The resulting gel was firm and spreadable, as shown in Fig. 1D.

The gel is stable for water contents ranging from 27 to 70% (v/v), corresponding to an oil content of ~66 to 27% by volume, as shown by the triangles in the phase diagram (Fig. 1E). The gel is characterized by a bright white color and a smooth texture. The gel continuously transforms into a pastier, drier substance, ultimately with the texture of a slightly wet paste at higher concentrations of monoglyceride in the oil. As seen in the Figure, two other phases were found, one consisting of a yellow jelly-like solid at very low water concentration, and the other resembling a curdled liquid. The resulting material was phase separated at all other concentrations.

The incorporation of the charged co-surfactant SSL into the material was necessary to ensure stability. The addition of salt destabilized the structure, indicating that the material is at least partially charge stabilized. The degree of mixing had an impact, with extreme mixing resulting in a stiffer, more stable gel. Dynamic rheological measurements shown in Fig. 2A indicate that the material is a gel over a wide range of water dilution, with the storage modulus (G') consistently larger than the loss modulus (G''). Moreover, a decrease in the elastic moduli and an increase in the G'' : G' ratio as a function of increasing water concentration indicated a loss in the solid-like character of the material.

Confocal laser scanning microscope images of the gel are shown in Fig. 2B and C, where a close-packed arrangement of globules can be seen in a structure that resembles a cellular network or foam. Fig. 2B represents the monoglyceride gel stained with the lipid soluble dye Nile Red, while Fig. 2C represents the gel stained with the water-soluble dye, coumarin. These micrographs clearly indicate that the structure consists of a dense distribution of oil globules in a water-continuous phase. More vigorous mixing yielded materials with smaller globules. To determine if this structure was similar to mayonnaise, which is simply emulsified oil in water, X-ray diffraction measurements were conducted using our in-house facility.[18] Broad diffraction peaks corresponding to bulk oil and water were detected as seen in Fig. 3A. In addition, there are several sharp diffraction peaks corresponding to a crystalline structure, which are not present in mayonnaise. These peaks, at q = 0.115 and 1.52 $Å^{-1}$ do not change position with increasing water content in the gel. In addition, the intensity of the crystalline diffraction peaks (and the bulk oil peaks) decrease with increasing water concentration (or decreasing oil and MAG fraction), while the water peak intensity grows. The gel structure thus consists of oil encapsulated within a crystalline multilamellar monoglyceride shells, with the shells incorporating the charged cosurfactant, as shown in Fig. 3B.

The monoglycerides used in the gel are amphiphilic, with a polar head group and an aliphatic tail. In general, this type of molecule can readily form a lyotropic liquid crystalline phase and can be expected to self-assemble in an oil–water mixture. In order to confirm that this was also the case in our system, and propose a possible mechanism for the formation of this oil–water–monoglyceride gel, synchrotron X-ray diffraction studies of monoglyceride–water mixtures in the presence of 0.05 N NaOH, both above and below the Krafft (chain melting) temperature were carried out as shown in Fig. 4A. The first peak indicated in the small-angle region of the spectrum corresponds to the (001) reflection of the phase, while subsequent diffraction peaks correspond to higher order reflections of the same repeating distance, indicating the formation of a lamellar phase. The large 001 repeating distance represents the width of the monoglyceride bilayers, which in this case are swollen with water. At wide angles, the single peak at q = 1.503 $Å^{-1}$ (4.18 Å) is indicative of in-plane chain ordering in the L_β phase which is absent in the L_α phase.

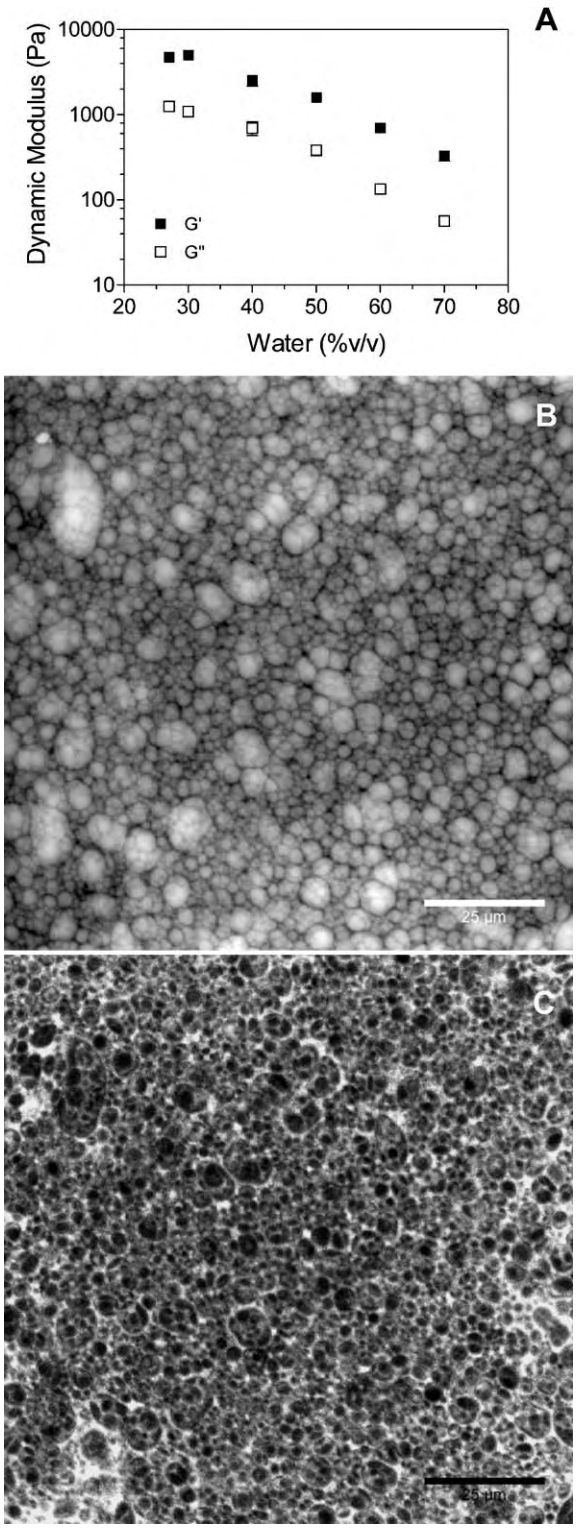

Fig. 2 (A) Effect of water content on the storage and loss dynamic shear moduli of the monoglyceride gels. (B) Confocal laser scanning (CLS) micrograph of the gels stained with the lipid soluble dye Nile Red. (C) CLS micrograph of the gels stained with the water soluble dye coumarin blue. A high fluorescence intensity is shown here as the bright areas. The scale bars represents 25 microns.

In the case of oil–water–monglyceride gels (MAG gels), as stated above, only a single small-angle reflection was detected,

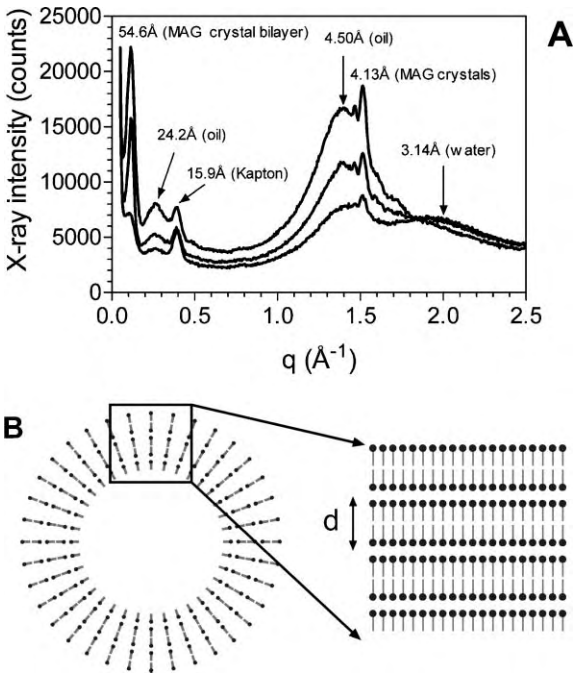

Fig. 3 (A) Powder X-ray diffraction spectra of the MAG gels taken for three different water concentrations (30, 50 and 70% from highest to lowest scattering intensity profile) and constant MAG–oil ratio of 10% plotted as a function of reciprocal lattice spacing q, where $q = 2\pi/d = (4\pi/\lambda)\sin\theta$ and d is the lattice spacing, λ is the X-ray wavelength and 2θ is the Bragg scattering angle. The Kapton peak comes from the windows enclosing the gel. (B) Schematic of the cell walls surrounding the oil phase demonstrating that they are composed of stacked crystalline monoglyceride and water bilayers.

which was indistinguishable from the MAG crystalline powder shown in Fig. 4B. Thus, the monoglyceride bilayers in the MAG gel are dehydrated. In the wide-angle region (inset) of the MAG gel, we noticed the presence of both the characteristic $q = 1.503$ Å^{-1} peak from the L_β phase of hydrated MAGs (black), as well as several other peaks arising from the crystalline MAG phase (black). Thus, both the wide-angle patterns from the MAG–water L_β and MAG crystal phases can be observed in the MAG gel pattern (grey). This provides evidence for the initial dehydration of the L_β phase, which then slowly transforms to the crystalline phase.

This evidence allows us to propose a mechanism for MAG gel formation, which is depicted in Fig. 5: upon addition of water and vigorous mixing, the monoglycerides and charged cosurfactant dissolved in the oil phase will emulsify the oil and self-assemble to form a highly hydrated, multilamellar L_α phase at temperatures slightly above the Krafft (chain melting) temperature of the monoglycerides. Upon cooling, the monoglyceride–cosurfactant bilayers transform to the L_β phase. The L_β lamellae dehydrate and eventually crystallize, forming crystalline multilayer shells around the oil droplets. This rapid dehydration process upon formation of the L_β phase in the presence of oil explains the apparent lack of sensitivity of the X-ray spectra (reflections at $q = 0.115$ and 1.52 Å^{-1}) to water content in the MAG gels. Any evidence of lamellar swelling induced by higher water contents would disappear upon gelation and crystallization of the bilayers.

Upon initial emulsification, the droplets interact and form a network which causes the material to gel, and upon monoglyceride

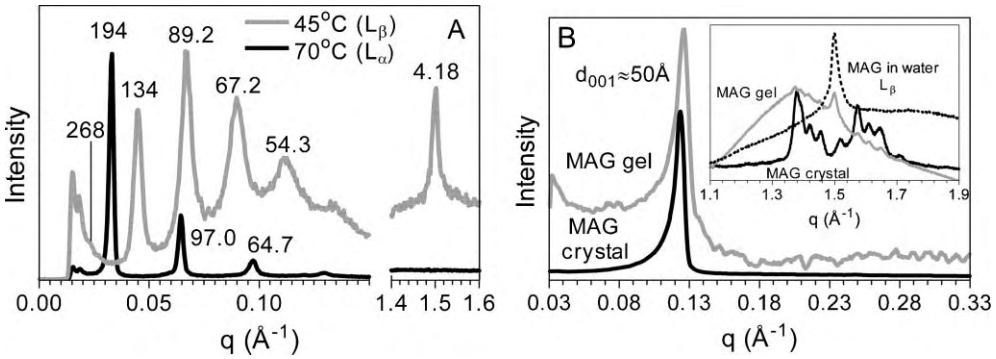

Fig. 4 (A) Synchrotron powder X-ray diffraction pattern of 20% monoglyceride in water mixture in the L_α phase at 70 °C, and in the L_β phase at 45 °C both in the presence of 0.05 N NaOH. (B) Powder X-ray diffraction patterns of the HSKA powder, the MAG in water and the MAG gel (containing oil) at small and wide angles (inset).

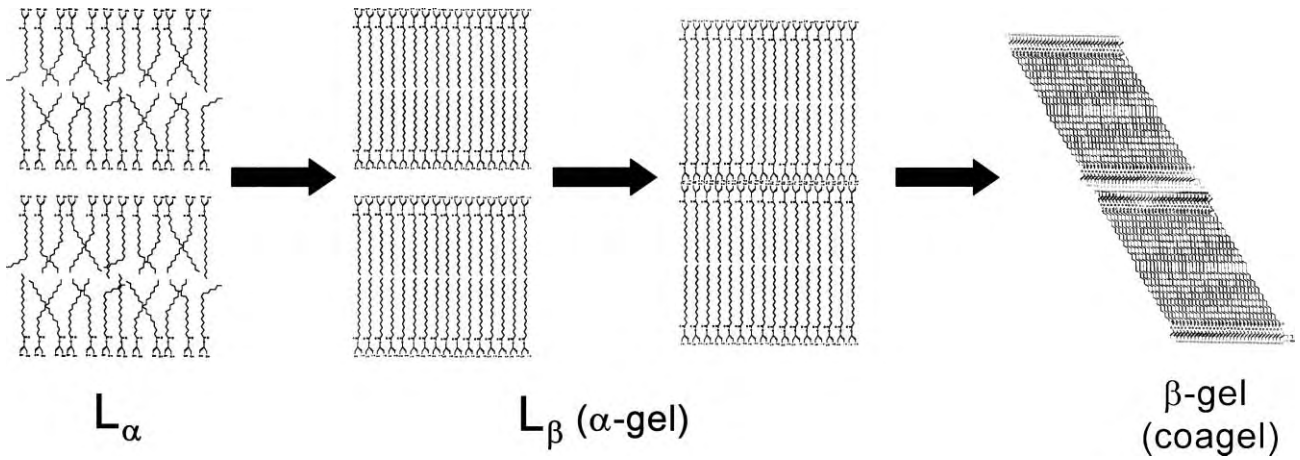

Fig. 5 Mechanism of formation of the crystalline monoglyceride emulsion droplet walls, from the hydrated lamellar (L_α) phase above the Krafft (*i.e.*, chain-melting) temperature of the monoglyceride, to an L_β phase (the "α-gel"), to a dehydrated L_β phase, and eventually to an anhydrous crystalline phase (β-gel or coagel). For the MAG gel to form, the monoglyceride must crystallize from the liquid crystalline L_α phase. The transition to the crystalline phase is enhanced by the presence of oil.

gelation and crystallization then adopts the consistency of a fat. Melting studies of the gel using optical microscopy indicate that the globules initially separate, before fusing into larger globules with increasing temperature, indicating the structure is stabilized through relatively weak inter-globular forces.

Saturated monglycerides have been previously used to structure vegetable oils at relatively high concentrations of 10% (w/w) or higher.[19] In this case, however, the crystalline monoglyceride is used as a traditional 'hardstock' in the absence of water. The structure of these non-aqueous monoglyceride–oil mixtures is similar to that of traditional edible fats, and bears no resemblance to the structure described in this study.

From a nutritional point of view, besides the obvious potential of novel materials such as the one described in this report to reduce the amount of saturated and *trans* fats in our diets, very interesting additional physiological effects were evident upon consumption of this MAG–oil–water gel (Fig. 6). The blood lipid response after acute ingestion of the gels included attenuated increases in triglyceride ($P = 0.0052$) and free fatty acid ($P = 0.0379$) levels (Fig. 6A and 6B) relative to the acute response to compositionally equivalent oil–water mixtures in the absence of the monoglycerides. Interestingly, plasma insulin levels were also lower after consumption of the MAG–oil–water gel ($P = 0.0216$), while no significant differences ($P > 0.05$) were detected in glucose concentration between the accumulated responses to oil–water mixture *vs.* MAG gel ingestion (Fig. 6C and 6D). Baseline/fasting triglyceride levels for the MAG gel *vs.* oil–water mixture trials were 1.28 (SEM = 0.25) and 1.49 (SEM = 0.39) mmol L^{-1}, respectively, while baseline/fasting free fatty acid levels for the MAG gel *vs.* oil–water mixture trials were 0.51 (SEM = 0.060) and 0.34 (SEM = 0.055) mmol L^{-1}, respectively.

These results suggest that ingestion of this microencapsulated oil resulted in lower post-prandial triglyceride and free fatty acid levels in the blood, which in turn resulted in a lower insulin levels, at similar glucose levels, in healthy young men and women. Moreover, this effect, which was strictly achieved by modulation of the physical structure of the food matrix, is interesting since it has been previously shown that a chronic high fat intake induces insulin resistance in humans, which has been associated with cardiovascular risk factors such as hypertension, abnormal blood coagulation, fibrinolysis and dyslipidaemia *via* activation of pro-inflammatory and pro-atherogenic pathways.[20–24] There were no significant differences in the cholesterol LDL, HDL, or cholesterol–HDL ratio responses between the two trials.

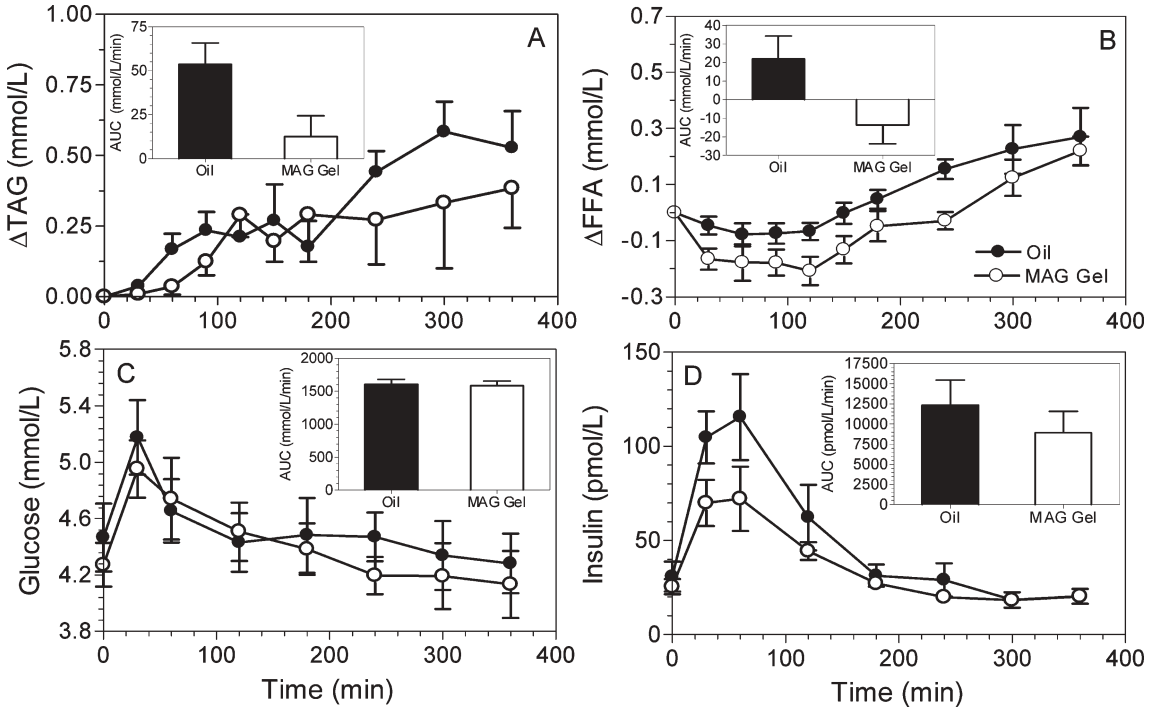

Fig. 6 Average physiological response of five male and four female subjects (mean age, 24 years; mean body mass index, 25.2 kg m^{-2}) to acute ingestion of an oil–water mixture or the microencapsulated oil water–mixture (MAG gel). For all panels, the main graph shows the metabolite dynamic in time, while the inset represents the accumulated response (net area under the curve). (A) Change in serum triglyceride levels, (B) change in plasma free fatty acid levels, (C) plasma glucose levels, (D) plasma insulin levels. All values represent the averages and standard errors of the subjects' responses.

In conclusion, this work suggests a strategy that can be employed for the gelation of liquid oils into a fat-like material in the absence of saturated and *trans* fats by encapsulation in multilamellar crystalline monoglyceride vesicles. This oil gel could be a viable alternative to traditional fats used in food manufacturing operations (shortening, spreads, icing), which contain high concentrations of saturated or *trans* fats. Moreover, this oil-structuring results in a controlled release of the lipids into the blood, which in turn attenuates the post-prandial increases in triglycerides, free fatty acid and insulin levels induced by acute ingestion of fat. This work suggests a strategy that can be employed for the controlled release of food macronutrients.

Acknowledgements

The authors acknowledge funding from the Ontario Ministry of Agriculture and Food and the Natural Sciences and Engineering Research Council of Canada.

Notes and references

1 *Global Strategy on Diet, Physical Activity and Health*, 57th World Health Assembly, World Health Organization, Geneva, 2004.
2 R. P. Mensink and M. B. Katan, *New Engl. J. Med.*, 1990, **323**, 439–445.
3 J. T. Judd, B. A. Clevidence, R. A. Muesing, J. Wittes, M. E. Sunkin and J. J. Podczasy, *Am. J. Clin. Nutr.*, 1994, **59**, 861–868.
4 M. B. Katan, P. L. Zock and R. P. Mensink, *Annu. Rev. Nutr.*, 1995, **15**, 473–493.
5 R. P. Mensink, P. L. Zock, A. D. M. Kester and M. B. Katan, *Am. J. Clin. Nutr.*, 2003, **77**, 1146–1155.
6 A. Ascherio, M. B. Katan, P. L. Zock, M. J. Stampfer and W. C. Willett, *New Engl. J. Med.*, 1999, **340**, 1994–1998.
7 S. S. Narine and A. G. Marangoni, *Phys. Rev. E*, 1999, **60**, 6991–7000.
8 S. S. Narine and A. G. Marangoni, *Phys. Rev. E*, 1999, **59**, 1908–1920.
9 A. G. Marangoni, *Phys. Rev. B*, 2000, **62**, 13951–13955.
10 A. G. Marangoni and M. A. Rogers, *Appl. Phys. Lett.*, 2003, **82**, 3239–3241.
11 L. Heertje, E. C. Roijers and H. A. C. M. Hendrickx, *Lebensm.-Wiss. Technol.*, 1998, **31**, 387–396.
12 I. Heertje, H. A. Hendricks, H. A. C. M. Knoops, E. C. Royers and H. Turksma, *Use of Mesomorphic Phases in Food Products*, *Eur. Pat.* 0 558 523 B2, 1991.
13 N. J. Krog and K. Larsson, *Chem. Phys. Lipids*, 1968, **2**, 129–143.
14 N. Krog and A. P. Borup, *J. Sci. Food Agric.*, 1973, **24**, 691–701.
15 K. Larsson, *Lipids – Molecular Organization, Physical Functions and Technical Applications*, The Oily Press, Dundee, Scotland, pp. 61–63, 1994.
16 N. J. Krog and F. Vang Sparso, in *Food Emulsions, 4th edn*, ed. S. Friberg, K. Larsson, J. Sjoblom, 2003, CRC Press, Boca Raton, FL, USA, pp. 45–92.
17 K. Larsson, in *Food Emulsions, 4th edn*, ed. S. Friberg, K. Larsson, J. Sjoblom, 2003, CRC Press, Boca Raton, FL, USA, pp. 93–106.
18 S. E. Guthrie, G. Mazzanti, T. N. Steer, M. R. Stetzer, S. P. Kautsky, H. Merz, S. H. J. Idziak and E. B. Sirota, *Rev. Sci. Instrum.*, 2004, **75**, 873–877.
19 N. K. O. Ojijo, I. Neeman, S. Eger and E. Shimoni, *J. Sci. Food Agric.*, 2004, **84**, 1585–1593.
20 G. Boden and L. H. Carnell, *Best Pract. Res. Clin. Endocrinol. Metab.*, 2003, **17**, 399–410.
21 G. Boden, *Diabetes*, 1997, **46**, 3–10.
22 G. M. Reaven, *Diabetes*, 1988, **37**, 1595–1607.
23 R. Ross, *New Engl. J. Med.*, 1999, **34**, 115–120.
24 H. M. Roche and M. J. Gibney, *J. Cardiovasc. Risk*, 2000, **7**, 317–324.