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SPECIAL ISSUE ARTICLE

Controlled Release of Food Lipids Using Monoglyceride Gel Phases Regulates Lipid and Insulin Metabolism in Humans

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Abstract We describe a solid vegetable oil–water gel structure which is stabilized through the use of low concentrations of monoglycerides, containing no added *trans* fats or saturated fats. The resulting structure consists of oil droplets encapsulated in self-assembled crystalline monoglyceride multilayers, surrounded by a continuous water phase. Acute ingestion human feeding trials indicated that the serum triglyceride loading was significantly lower after ingestion of the structured gel rather than a simple oil–water mixture, resulting in an attenuated increase in serum insulin. This indicates the effectiveness of encapsulation in modulating blood lipid and insulin response in humans, and suggests a strategy that can be employed for the controlled release of food macronutrients.

Keywords Food lipids · Oil–water gel · Monoglycerides · Insulin · Serum triglycerides · Free fatty acids · Controlled release

Public concerns about the consumption of foods containing *trans* fatty acids used in the solidification of edible oils have recently reached new heights. New methodologies are therefore required to produce solid fat replacements. Ideally, these replacements should incorporate unsaturated vegetable oils and be low in *trans* and saturated fats to be widely accepted. Over 15 years of clinical and epidemiological research suggest that there is a relationship between *trans* fatty acid intake and a *decrease* in serum high-density lipid (HDL or "good" cholesterol) combined with an *increase* in serum low-density lipid (LDL or "bad" cholesterol).[1–6] Fats containing high amounts of *trans* and saturated fatty acids have been shown to increase the risk of coronary heart disease. Both the Institute of Medicine and the American Heart Association recommend a reduction in the intake of saturated and effectively an elimination of *trans* fatty acids from the diet.[7–9] However, this would have been very difficult to achieve without proper labeling of the foods we consume. Thus, showing strong leadership on this issue, the US Food and Drug Administration (FDA) decreed that as of January of 2006, food manufacturers must include the *trans* fatty acid content in product labels.[10, 11]

This FDA ruling is a very important milestone for public health; however, it creates serious technological hurdles for the food manufacturing industry—it is difficult to eliminate *trans* fats from a food formulation. At the core of the problem is the ability to transform oil, which is liquid at room temperature to a fat, which is solid at room temperature. The difference between oil and fat is subtle. Oils and fats are mostly made up of triacylglycerol molecules: 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 and physical properties of the constituent fatty acids.

Solid fats are structured using either saturated animal or vegetable fats such as lard, milk fat, coconut oil or palm oil, or by using liquid oils which have been subjected to

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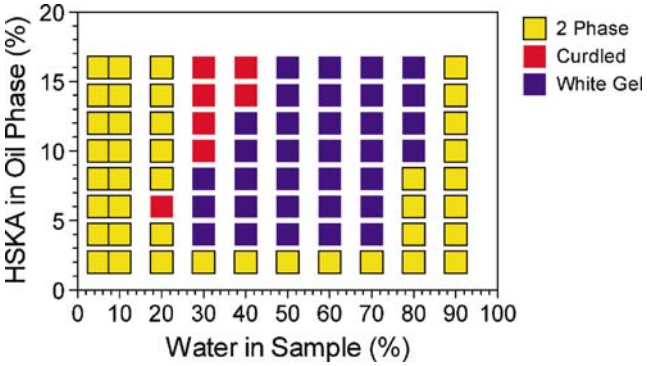

Fig. 1. Composition of the monoglyceride gel. Plot of the phase diagram of binary mixtures of monoglyceride HSKA in oil (given in weight percent) and water

hydrogenation, which leads to the creation of *trans* isomers of the constituent fatty acids. Hydrogenation has been commercially used for almost 100 years to transform oils into fats. Three reactions take place during hydrogenation—the saturation of carbon–carbon double bonds, the conversion of *cis* geometric isomers into more stable *trans* isomers, and the creation of new positional isomers, where double bonds are shifted to new positions along the fatty acid chain. Both the saturation of double bonds as well as the *cis* to *trans* isomerization of double bonds will result in an increase in the melting point of a fat. Thus, cooling of this hydrogenated fat below the melting point of the newly created triacylglycerol species containing saturated and *trans* fatty acids will lead to the partial crystallization of the material. This semisolid fat matrix will therefore be structured as a network of fat crystal aggregates with liquid oil trapped within.[12] Without this network of crystallized fat, the material would be oil. Traditionally, the only way to provide structure to oil, and thus convert it into a solid fat, is by the addition of high-melting point saturated or *trans* fats. To satisfy the growing health concerns associated with current fats, new strategies for structuring edible oils is thus required, ideally providing additional health benefits over traditional fats used now.

Pernetti et al.[13] have recently comprehensively reviewed the structuring of edible oils by alternatives to crystalline fat. Strategies have focused on finding molecules that self-assemble in oil and form networks at very low concentrations. For this strategy to be useful, these networks must form at relatively low concentrations (<5%), and the molecules used must be relatively inexpensive, widely available and have GRAS status. Also of great importance is that these novel "fats" have some of the functionality of fats in a food. If these novel structured oils have none of the functionality associated with fat, then their use as alternatives to conventional fats structured by networks of crystalline fat (saturated or *trans*) will be very limited, if any at all. Of particular interest would be to find such molecules with a specific health benefit as well. Bearing in mind that any solution must be food-grade, some very promising strategies used to solidify oils include (not exhaustive) mixtures of phytosterols and oryzanol,[14] mixtures of long-chain fatty acids and fatty alcohols,[15] hydroxylated fatty acids,[16,17] food-grade waxes,[18] and cellular solids structured with protein walls[19] or monoglyceride walls.[20] In this report, we will concentrate on cellular solids with monoglyceride walls.

In early 2007, we reported on the discovery of a new oil–water gel structure stabilized through the use of multilamellar monoglyceride multilayers, encapsulating the liquid oil drops in a water matrix.[20] Much of the information reported in this study was previously reported in that publication.

The gel is produced by vigorously mixing a hot oil–monoglyceride solution with alkaline deionized water in a

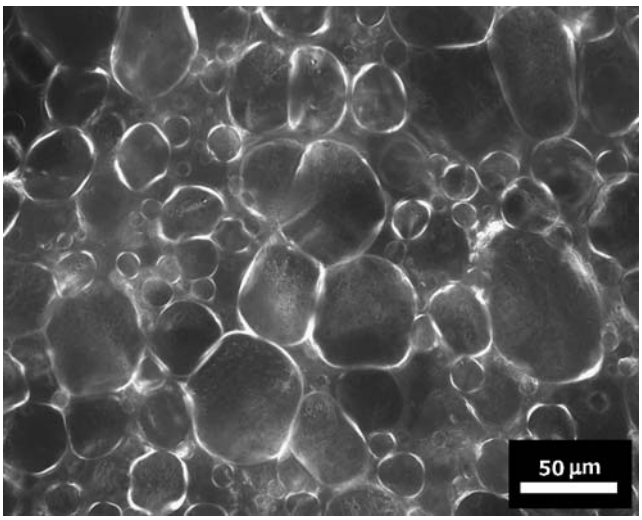

Fig. 2. Microstructure of the MAG gels: polarized light micrograph of the monoglyceride gels showing the cellular solid-like structure of the material. Gels with very large cell diameters were prepared expressly for this type of imaging

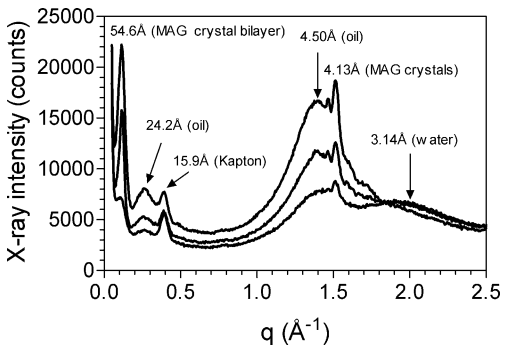

Fig. 3. Solid state structure of the monoglyceride gels. Plot of the powder X-ray diffraction intensities of the MAG gel taken for three different water concentrations (27%, 50%, and 70%) and constant MAG–oil ratio of 10% plotted as a function of reciprocal lattice spacing q, where $q = 2\pi/d = (4\pi/\lambda)\sin\theta$ 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

temperature above the Krafft temperature of the monoglyceride (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 monoglycerides used are a mixture of the commercially available distilled HSKA (10% monopalmitin, 90% monostearin) and sodium stearoyl lactylate (SSL) in a 20:1 (*w*/*w*) ratio of monglyceride to SSL. The mixture had to contain at least 4% (*w*/*w*) distilled monoglyceride for proper gel formation and stability.

The gel is stable for water contents ranging from 27 to 70% (*v*/*v*), corresponding to oil content of ~66 to 27% by volume (Figure 1). 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. Two other phases were also 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 monoglyceride 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 (not shown) suggested that the material is a gel over a wide range of compositions, 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.

A polarized light image of the gel is shown in Figure 2, where a closely packed arrangement of globules can be seen in a structure that resembles a cellular network or foam. Former studies[20] indicated that the structure consists of a dense distribution of oil globules in a water continuous

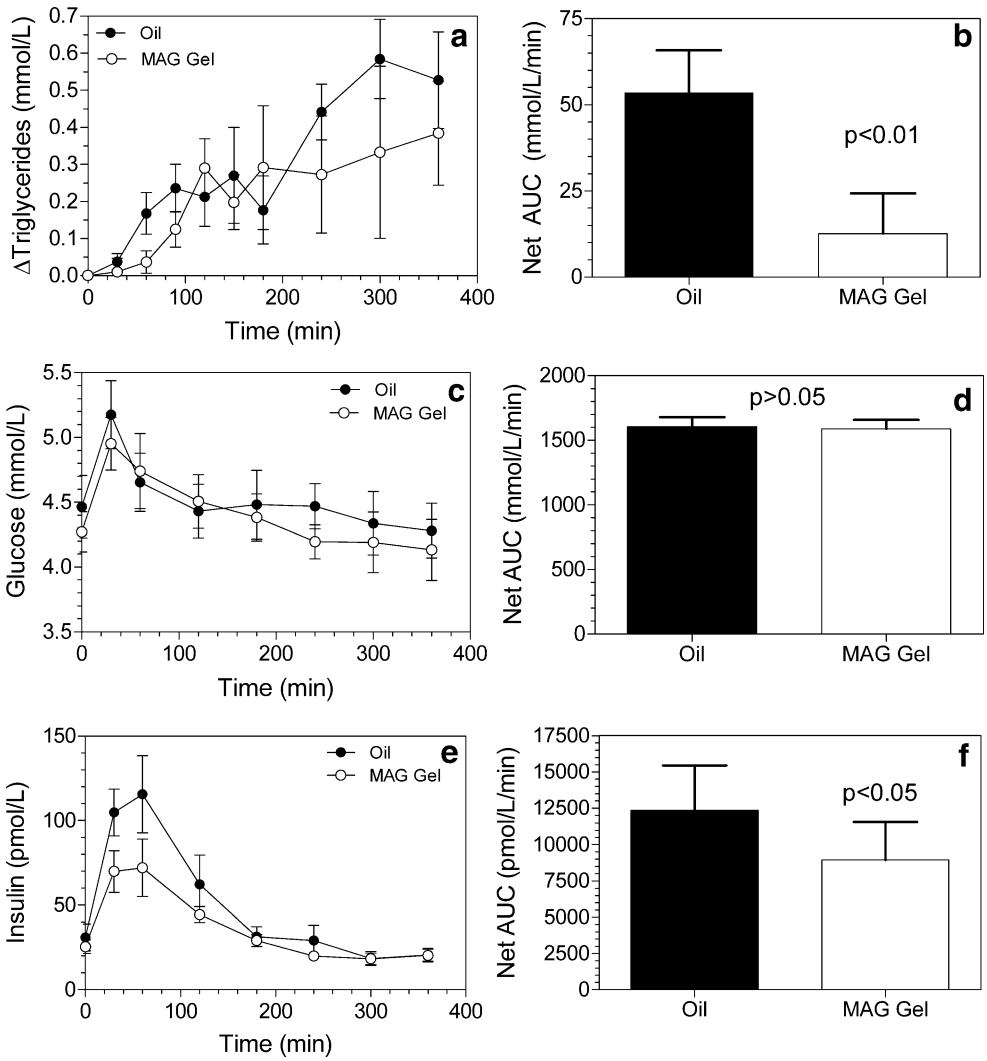

Fig. 4. Comparison of serum triglyceride levels (**a** and **b**), glucose levels (**c** and **d**), and insulin levels (**e** and **f**) in human subjects after acute ingestion of the MAG gel and a compositionally equivalent (without MAG) oil–water mixture. Responses are for the five male and six female, where the meal was taken with toast. The net area under the curve (AUC) for the increase in metabolite levels was determined between 3 and 6 h after ingestion for triglycerides, and between 0 and 6 h for glucose and insulin

phase. Vigorous mixing yielded materials with smaller globules. To determine if this structure was similar to mayonnaise, X-ray diffraction measurements were conducted using our in-house facility.[21] Broad diffraction peaks corresponding to bulk oil and water were detected as seen in Figure 3. In addition, there are several sharp diffraction peaks corresponding to a crystalline structure, which is 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 and do not correspond to diffraction peaks observed for any of the dry monoglycerides. In addition, it can be seen that 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 monoglycerides used in the gel are amphiphilic, with a polar head group and a hydrophobic tail. In general, this type of molecule can readily form a lamellar lyotropic liquid crystalline phase and can be expected to self-assemble in an oil–water mixture. In this case, the monoglycerides form a multilamellar crystalline shell around the oil globules, stabilizing them in the water matrix. The sharp X-ray diffraction peak at q=0.115 Å^{-1}, corresponds to an interlamellar spacing of 54.6 Å, while the sharp peak at q=1.52 Å^{-1}, d=4.13 Å indicates the presence of crystalline order within the lamellae. For a sample containing 30% water, 3% HSKA, 0.15% SSL, and 66.857% oil, we determined the domain size (ξ), a domain representing the size of the globule walls to be 22.9 nm ($\xi = 2\pi/\Delta q$, where Δq corresponds to the peak FWHM of the 001 reflection (centered at 52.1 Å), determined by fitting the spectral data to a Lorentzian peak shape). This corresponds to approximately 4.5 bilayers covering the globules. A simple calculation assuming a surfactant head group size of 35 Å^2 and a 2 μm globule diameter also yields an estimate of 4.5 bilayers per globule. This strongly suggests that the globules are covered by a monoglcyeride monolayer upon which four other bilayers stack.

Water must be present between the membrane bilayers to fully solubilize the charged SSL; however, the amount of water is saturated and does not depend on overall water concentration in the bulk material. The gel structure thus consists of oil encapsulated in a crystalline multilamellar monoglyceride shell, with the shells incorporating the charged SSL.

Melting studies of the gel using optical microscopy indicate that the globules initially separate before coalescing into larger globules with increasing temperature. This indicates that the structure is stabilized through relatively weak interglobular forces.

To examine the physiological effects of the oil encapsulation, the blood lipid response to ingestion of the gels was compared to that of a compositionally equivalent oil–water mixture without the monoglycerides in a human feeding trial. Blood serum triglyceride loading after ingesting the gelled structure was significantly lower than after ingesting a simple oil–water mixture (Figure 4a,b). What was also particularly interesting was that for the same glucose levels (Figure 4c,d), lower serum insulin levels were observed (Figure 4e,f). Related to this was the discovery that serum free fatty acid levels were lower after acute ingestion of the MAG Gel relative to unstructured oil (Figure 5). High levels of serum free fatty acids have emerged as a major link between obesity, insulin resistance, and type-2 diabetes.[22] This is particularly interesting since this would indicate that the encapsulation of the oil in the gel can influence the blood lipid and, indirectly, insulin response in humans. It is possible that encapsulation of oils/fats within a solid can be used to lessen serum lipid loading after consumption of fatty meals. There were no significant differences in the cholesterol LDL, HDL, or cholesterol/HDL ratio responses between the two trials. This suggests an interesting strategy for the manufacture of "next generation" food products where a judicious use of the food material's microstructure can be used to modulate blood lipid response in humans.

In this work, we have shown that it is possible to structure any edible oil using a monoglyceride-based gel material. The oil gel described in this report, in which oil is encapsulated in a crystalline monoglyceride shell, is a viable alternative to traditional hard stocks containing saturated or *trans* fats. Moreover, the blood lipid response after ingestion of these monoglyceride gels included a

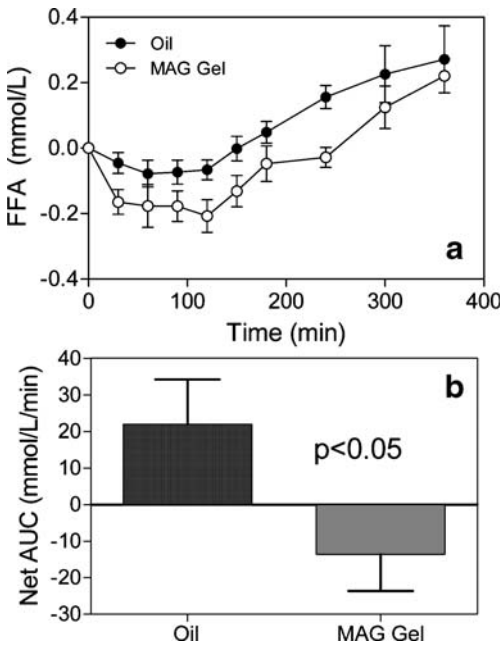

Fig. 5. Comparison of serum free fatty acid levels in human subjects after acute ingestion of the MAG gel and a compositionally equivalent (without MAG) oil–water mixture. Responses are for the five male and six female subjects, where the meal was taken with toast (**a**). The net area under the curve (AUC) for the increase in free fatty acids was determined between 0 and 6 h after ingestion (**b**)

lower serum triglyceride loading than compositionally equivalent oil–water mixtures. This work suggests a strategy that can be employed for the controlled release of food macronutrients.

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