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Influence of Lipid Physical State on the in Vitro Digestibility of Emulsified Lipids

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The objective of this study was to investigate the influence of the physical state of emulsified lipids on their in vitro digestibility by pancreatic lipase. A 10 wt % tripalmitin oil-in-water emulsion stabilized by sodium dodecyl sulfate (0.9 wt % SDS) was prepared at a temperature (>70 °C) above the melting point of the lipid phase ($T_m \approx 60$ °C). A portion of this emulsion was cooled to a temperature (0 °C for 15 min) well below the crystallization temperature of the emulsified lipid ($T_c \approx 22$ °C) and then warmed to 37 °C so as to have completely solid lipid particles. Another portion of the emulsion was directly cooled from 70 to 37 °C (which is above the T_c) to have completely liquid (supercooled) lipid particles. Pancreatic lipase (8 mg/mL) and bile extract (5.0 mg/mL) were then added to each emulsion at 37 °C, and the evolution of the particle charge, particle size, appearance, and free fatty acid release were measured over a period of 2 h. It was found that the rate and extent of lipid digestion were higher in the emulsion containing liquid particles but that appreciable lipid digestion still occurred in the emulsion containing solid particles (i.e., >35% lipid digestion after 2 h). These results may have important consequences for controlling the digestion rate of lipids or for developing solid lipid particle delivery systems for lipophilic functional components.

KEYWORDS: Emulsions; lipase; lipid digestion; solid fat content; crystallization; solid lipid particles

INTRODUCTION

The lipids in food may be consumed in a variety of different forms including as bulk fats, as structural fats, or as emulsified fats (*1, 2*). Nevertheless, most fatty foods are broken down into oil-in-water emulsions within the mouth during mastication (*3*) and/or within the stomach and small intestine during processing, digestion, and absorption (*4, 5*). The rate and extent to which a particular lipid is digested within the human gastrointestinal tract determine its overall bioavailability, as well as the time dependence of the lipid profile within the blood and other organs (*6–8*). Ingested food lipids vary considerably in their solid fat content versus temperature profiles because of differences in their natural origin (e.g., marine, animal, or plant) and their subsequent processing (e.g., hydrogenization, winterization, interesterification) (*9*). The melting points of triacylglycerols tend to increase with increasing molecular weight and saturation (*9*). Consequently, it is important to understand the role of solid fat content on the digestibility of emulsified lipids. In addition, solid lipid particles are being used increasingly to encapsulate, protect, and deliver nonpolar functional agents, such as pharmaceuticals and bioactive lipids, via the human gastrointestinal (GI) tract (*10–14*). In these applications it is important that the solid lipid particles remain stable and retain any encapsulated components prior to consumption, but that they release their payload at the desired site of action after consumption. Consequently, it is important to ensure that solid lipid particles do not adversely affect the bioavailability of nonpolar functional agents.

Lipid digestion involves several sequential steps that include various physicochemical and enzymatic events (*1, 4, 5, 15*). In the mouth, an ingested food is mixed with saliva (pH ≈7), undergoes temperature changes ($T \approx 37$ °C), and is subjected to mechanical forces, which may alter the structural organization, physical state, and interfacial properties of the lipid phase (*3*). In the stomach, the lipids in the bolus are mixed with a highly acidic aqueous solution that contains minerals, biopolymers, surface active lipids, and enzymes (*1*). The lipid phase may undergo further changes in structural organization due to droplet disruption and coalescence processes, as well as changes in the nature and composition of the surface active materials adsorbed at the lipid−water interface. In particular, gastric lipase adsorbs to the lipid−water interface and initiates the lipid digestion process, converting some of the triacylglycerols (TAGS) to diacylglycerols (DAGS), monoacylglycerols (MAGS), and free fatty acids (*4, 16, 17*). In the small intestine, the emulsified lipids in the chyme are mixed with digestive juices that contain pancreatic lipase, colipase, bile salts, and phospholipids. The

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bile salts and phospholipids compete with and displace any surface active material originally present at the lipid−water interface, and the lipase/colipase complex binds to the lipid droplet surfaces. The pancreatic lipase converts TAGS into DAGS, MAGS, and free fatty acids. These lipolysis products leave the surface of the lipid droplets and are incorporated into mixed micelle structures consisting of phospholipids and bile salts, which then transport them to the enterocytes, where they are absorbed (*4*). Potentially, the physical state of the lipids may affect many of the above physicochemical and enzymatic processes, for example, droplet disruption, droplet coalescence, interfacial composition, lipid digestion, and micelle solubilization.

The purpose of the current study is to compare the digestion of solid and liquid lipid particles by pancreatic lipase using a simplified in vitro digestion model that simulates the small intestine. Previous workers have already studied the in vitro digestion of liquid and solid lipid nanoparticles by pancreatic lipase (*18–21*). They found that solid lipids were still digested by lipase, but that the rate of lipid digestion was slower than for liquid lipids. Nevertheless, the systems used in these previous studies did not allow the researchers to directly compare digestion of the same lipid in different physical states at similar temperatures. In the present study, we utilized a supercooling method that allows one to produce two emulsions at body temperature using the same lipid as the disperse phase, but in which the lipid particles are completely *liquid* in one system and completely *solid* in the other system. This method is based on the fact that the crystallization temperature (T_c) of an emulsified lipid is usually well below its melting temperature (T_m) because of the kinetic energy barrier to crystal formation associated with nuclei formation (*22, 23*). For example, for the emulsified tripalmitin used in this study$T_c \approx 20$ °C and $T_m \approx 60$ °C. In the supercooling method, an emulsion is prepared at a temperature well above the melting point ($T > T_m$) of the lipid phase using hot homogenization. A portion of this emulsion is then cooled to a temperature well below the crystallization temperature (T_c) of the emulsified lipid and then warmed to body temperature (37 °C) so as to have completely solid lipid particles. Another portion of the emulsion is directly cooled to 37 °C ($T > T_c$) so as to have completely liquid lipid particles. The influence of the physical state of the emulsified lipids on the rate of lipid digestion by lipase can then be determined directly.

MATERIALS AND METHODS

Materials. All chemicals were of analytical grade and obtained from either the Sigma-Aldrich Chemical Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Emulsions were prepared using tripalmitin (95% pure, Sigma) as the lipid phase, and sodium dodecyl sulfate (>98.5% pure, Sigma) as the surfactant. Phosphate buffer solutions were prepared from sodium phosphate monobasic (>99% pure, Sigma) and sodium phosphate dibasic (Fisher Scientific). The digestive juice was prepared using porcine bile extract (batch 106K0097, EC 232-369-0, Sigma) and porcine pancreatic lipase, type II (batch 115K0681, EC 232-619-9, Sigma). Free fatty acid titrations required ethanol (95% pure, Fisher Scientific), sodium hydroxide (Fisher Scientific), phenolphthalein (Fisher Scientific), and oleic acid (Sigma). Distilled and deionized water was used for the preparation of all solutions.

Emulsion Preparation. A surfactant solution was prepared by dispersing 1 wt % SDS in phosphate buffer (10 mM, pH 7). A primary emulsion was prepared by homogenizing 10 wt % tripalmitin with 90 wt % surfactant solution (1 wt % SDS dispersed in phosphate buffer, pH 7) in a high-speed blender for 2 min (Tissue Tearor, Biospec Product, Bartlesville, OK). The lipid (tripalmitin) and aqueous phase (SDS solution) were heated at 80 °C before mixing to maintain the tripalmitin in the liquid state. The resulting coarse emulsion was passed 10 times through a high-pressure homogenizer (Microfluidics, Newton, MA) at 9000 bar and 80 °C.

After this stage, a portion of the emulsion was cooled in ice–water for 15 min to promote lipid crystallization and then stored at 37 °C until used. This emulsion contained solidified tripalmitin as determined by differential scanning calorimetry (DSC) (see later). Another portion of hot emulsion was cooled directly to 37 °C. This emulsion contained liquid tripalmitin as determined by DSC (see later). For the following measurements, these emulsions were diluted to 1 wt % tripalmitin by mixing the original emulsions with phosphate buffer (10 mM, pH 7) at 37 °C, that is, a 1:10 dilution.

In Vitro Digestion Procedure. An in vitro digestion model, which simulated the small intestine, was used to study the hydrolysis of the emulsified lipids by lipase (*24*). All emulsions and solutions were heated to 37 °C prior to use. A model digestive juice was prepared by mixing 2 mg of pancreatic lipase and 1.25 mg of bile extract in 50 mL of phosphate buffer (10 mM, pH 7, 37 °C). The lipase and bile solution was stirred for 2 h and then stored at 37 °C for at least 15 min prior to use. Thirty milliliters of emulsion (1% tripalmitin) was mixed with 7.5 mL of digestive juice. The final concentrations of pancreatic lipase and bile extract in the reaction mixtures were 8 and 5 mg/mL, respectively. These values were selected to simulate those typically found in the human small intestine (*24*) and to give a measurable amount of digestion over 2 h. The lipid−digestive juice mixtures were stirred for 2 min and then stored at 37 °C with stirring (95 rpm) during 2 h to mimic conditions in the GI tract. During this time samples were periodically selected for analysis.

Particle Size Measurement. The mean particle diameter and particle size distribution of emulsions were measured using static light scattering (Mastersizer X, Malvern, Malvern Instruments Ltd., Westborough, MA). The initial 1 wt % tripalmitin oil-in-water emulsions were diluted with buffer solution (1:42) prior to analysis to avoid multiple scattering effects. These measurements were done before and after digestion. A refractive index of 1.54 was used for the solid lipid phase of the emulsions and 1.437 was used for the liquid lipid phase of the emulsions to calculate the particles size distribution (*25*). The particle size measurements were reported as volume-weighted mean diameters (d_{43}), surface-weighted mean diameters (d_{32}), and full particle size distributions (*26*). The particle size distribution of three freshly prepared samples was measured, and means and standard deviations were calculated from these data.

ζ-Potential Measurements. The 1 wt % tripalmitin oil-in-water emulsions were diluted in buffer solution (1:80) before being injected into the measurement chamber of a particle electrophoresis instrument (Zetamaster, Malvern, Malvern Instruments Ltd.) to avoid multiple scattering effects. The ζ-potential was then determined by measuring the direction and velocity that the droplets moved in the applied electric field. The ζ-potential was calculated from five measurements made on each emulsion placed within the instrument. The ζ-potential of three freshly prepared samples was measured, and means and standard deviations were calculated from these data.

Differential Scanning Calorimetry. Emulsion samples (≈5 mg) were accurately weighed in hermetic aluminum pans (TA Instruments, New Castle, DE) and then placed into the measurement chamber of the DSC instrument, which was initially set at 37 °C (Q 1000, TA Instruments). DSC scans were made as the samples were either heated to 75 °C or cooled to 5 °C at scan rates of 10 °C/min. Melting points and crystallization points correspond to maxima and minima observed in the DSC curves, respectively. We made at least two scans on each sample, which were highly reproducible. A representative scan of heat flow versus temperature is shown for each sample. The melting and crystallization points were reproducible to better than 1 °C.

Free Fatty Acid Release. The digestion activity was measured by determining the release of free fatty acids from emulsions during 2 h of storage using a titration method (*27*). Samples (5 mL) were collected from the emulsions at different times during digestion, and then 10 mL of ethanol (95%) was added to quench the enzyme activity and 3 drops of 1% (w/v) phenolphthalein was added as an indicator. The amount of free fatty acids released during the reaction was then determined by direct titration with 0.05 N NaOH using a burette to a phenolphthalein end point.

A calibration curve was prepared to correlate the measured results in NaOH (milliliters) to a FFA concentration. Oleic acid solutions were prepared at different concentrations (0, 50, 100, and 200 mM), and the above titration procedure was carried out. These data were then used to prepare a calibration curve of NaOH (milliliters) versus free fatty acid concentration (millimolar). The amount of free fatty acids in a sample was then calculated from the amount of NaOH required to reach the end point using this calibration curve. To determine the endogenous level of fatty acids present prior to the action of lipase, the amount of free fatty acid of each emulsion with only bile extract was also measured. This value was then subtracted from the value measured in each sample during digestion to determine the amount of free fatty acids liberated by hydrolysis. The results are reported as the percentage of free fatty acids released compared to the total amount that would be released if all of the triacylglycerol molecules present were converted to one monoacylglycerol and two free fatty acid molecules. FFA measurements were carried out on three freshly prepared samples, and means and standard deviations were calculated from these data.

Statistical Analysis. All experiments described above were carried out using three freshly prepared samples (replicates). The results presented are the means and standard deviations of the experiments. Statistical analysis was performed using either a *t* test (Excel, Microsoft Corp., Redmon, WA) or regression analysis (SigmaPlot, version 10, Systat Software, San Jose, CA) when appropriate.

RESULTS AND DISCUSSION

Characterization of Emulsions Containing Solid and Lipid Particles. Initially, we established appropriate conditions for preparing tripalmitin oil-in-water emulsions that contained either completely liquid or completely solid particles. An oil-in-water emulsion was prepared using the hot homogenization technique described above. A portion of this emulsion was cooled to 0 °C to induce lipid crystallization, whereas another portion was held at 37 °C to maintain the lipid phase in the liquid state. The melting and crystallization behavior of these two emulsions was then characterized by DSC as they were heated and cooled (**Figure 1**). When the emulsion held at 37 °C was heated from 37 to 70 °C, there was no evidence of an endothermic transition corresponding to the melting of fat crystals, which indicated that all of the droplets in this emulsion had remained in a liquid state (**Figure 1a**). On the other hand, when the emulsion that had been held at 0 °C was heated from 37 to 70 °C, there was evidence of a series of endothermic transitions corresponding to fat crystal melting (**Figure 1b**). The profile of the endothermic peaks was characteristic of melting of primarily the β-polymorphic crystal form (*28*). Hence, we could infer that the particles in this emulsion were in the solid state. We carried out an additional DSC experiment to show that all of the droplets were indeed solid by cooling the emulsion that had been held at 0 °C from 37 to 5 °C (data not shown). No crystallization peaks were observed upon cooling, which indicated that all of the droplets were already solidified.

When both emulsions were cooled from 70 °C (at which the droplets were completely liquid) to 10 °C, we observed two exothermic peaks, a small one around 26 °C and a larger one around 22 °C, which can be attributed to lipid crystallization (**Figures 1**). The crystallization peaks in the emulsions occurred at much lower temperatures than observed for crystallization of bulk tripalmitin at the same cooling rate ($T_c \approx 35$ °C). This indicated that the lipid phase remained as small droplets, rather than coalescing and oiling off, that is, that the lipid particles were stable to a melt−crystallization cycle (*29, 30*). When bulk tripalmitin was cooled, we observed only a single exothermic peak at 35 °C, whereas smaller and larger exothermic peaks were observed for the emulsified tripalmitin at 26 and 22 °C upon cooling (**Figure 1**). We postulate that the smaller peak

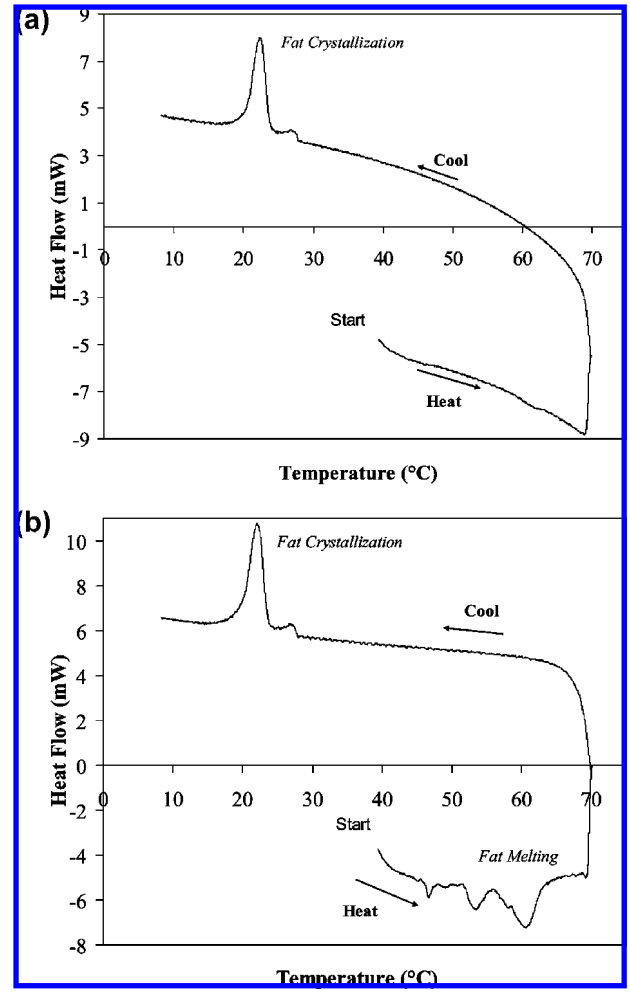

Figure 1. DSC thermograms of 10 wt % tripalmitin oil-in-water emulsions initially containing (**a**) liquid or (**b**) solid lipid particles at 37 °C. The emulsions were heated from 37 to 70 °C at 10 °C/min and then cooled from 70 to 10 °C at −10 °C/min.

that occurred at the higher temperature was due to crystallization of the nonpolar tails of the SDS molecules adsorbed to the lipid droplet surfaces. These crystallized surfactant tails may have acted as a template that induced surface nucleation of the tripalmitin within the droplets, as has been previously reported for hydrocarbon oil-in-water emulsions (*31*).

Light scattering measurements at 37 °C indicated that the emulsion containing solid particles had mean diameters ($d_{32} = 0.21 \pm 0.02$ μm; $d_{43} = 0.27 \pm 0.01$ μm) similar to those in the emulsion containing liquid particles ($d_{32} = 0.19 \pm 0.02$ μm; $d_{43} = 0.25 \pm 0.02$ μm). One might expect the mean particle diameter to decrease slightly upon fat crystallization because the density of solid fat is higher than that of liquid oil; therefore, the droplet volume should decrease. Nevertheless, recent microstructure studies have shown that there is a change in shape of lipid triacylglycerol particles when they transform from the liquid state (spherical) to the β-form polymorphic solid state (disk-like) (*32*). These changes in particle shape may account for the fact that the measured mean particle diameters were similar.

Particle electrophoresis measurements indicated that the electrical charge on the particles were highly negative ($\zeta = -65.9 \pm 3.4$ mV for the solid particles and $\zeta = -71.1$ mV $\pm$ 5.4 for the liquid particles), which can be attributed to adsorption

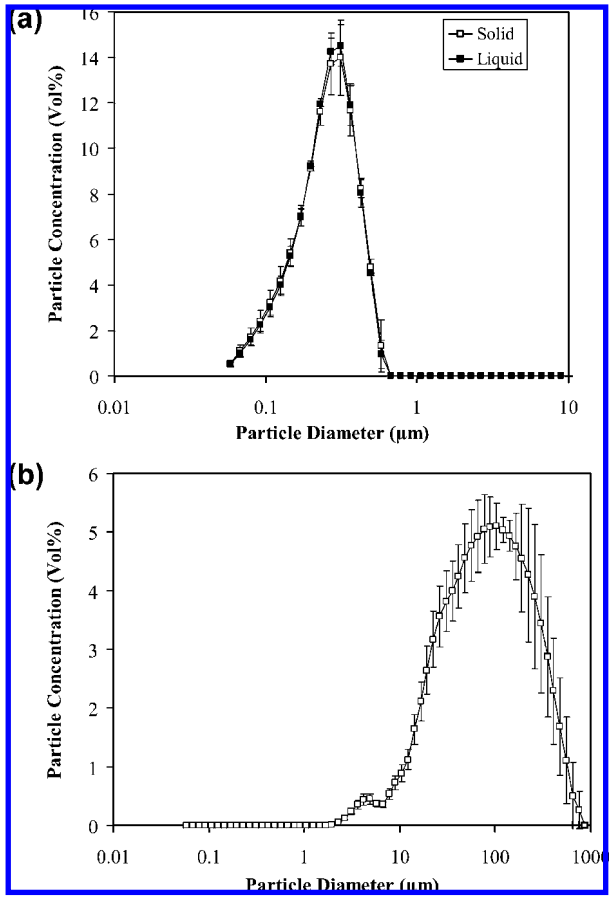

Figure 2. Particle size distributions of (**a**) diluted tripalmitin oil-in-water emulsions initially containing either liquid or solid lipid particles at 37 °C and (**b**) artificial digestive juices.

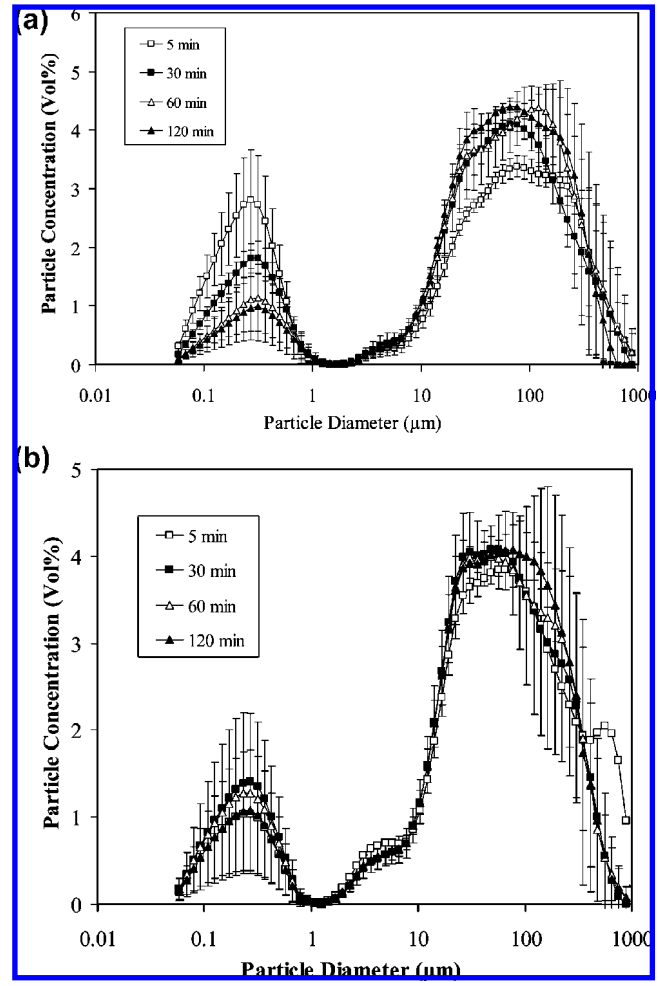

Figure 3. Time dependence of the particle size distribution of mixed systems containing tripalmitin particles and artificial digestive juices at 37 °C: (**a**) liquid particles; (**b**) solid particles.

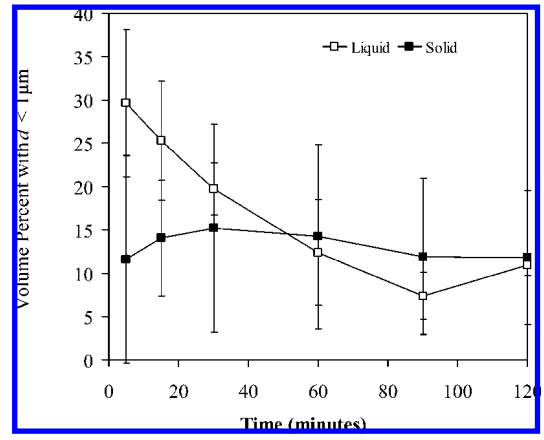

Figure 4. Percentage of particles with a droplet diameter of <1 μm versus digestion time at 37 °C for tripalmitin oil-in-water emulsions initially containing either liquid or solid lipid particles.

of anionic surfactant molecules (SDS) to lipid particle surfaces. The difference between the charge on the solid and liquid particles was not statistically significant ($p = 0.05$).

Change in Physicochemical Properties of Lipid Particles during Hydrolysis. In this section, we examine the change in the physicochemical properties of the solid and lipid particles during 2 h in vitro digestion by pancreatic lipase in the presence of bile salts (**Figures 2**−**4**). Light scattering measurements were used to monitor changes in the particle size distribution (PSD) of the lipid droplets during hydrolysis. Initially, we measured the PSD of the solid and lipid particles prior to adding lipase or bile salts (**Figure 2a**). Both emulsions had a monomodal PSD, with the majority of particles having diameters of less than about 0.7 μm (**Figure 2a**). The mean particle diameters (d_{32}) of the solid and lipid particles were 0.21 ± 0.02 μm and $d_{32} = 0.19 \pm 0.02$ μm, respectively. The artificial digestive juice that we prepared for the in vitro digestion model contained some insoluble matter (which came from the lipase rather than the bile extract); hence, we also measured its particle size distribution (**Figure 2b**). There was a broad peak in the PSD corresponding to particles with diameters of around 100 μm. The presence of these large particles would interfere with the measurement of the mean particle diameter of the lipid droplets during hydrolysis; hence, we plotted only the full particle size distributions (**Figure 3**). The relatively large standard deviations observed in the particle size distributions are typical of measurements made in highly aggregated emulsion systems and are usually attributed to changes in sample structure induced by dilution and stirring within the light scattering instrument (*26*).

The change in the PSD of systems containing liquid and solid lipid particles during in vitro digestion is shown in **Figure 3**. There was a bimodal distribution in the mixed systems containing lipid particles, bile extract, and lipase (**Figure 3**). The peak at low particle diameters corresponded to the lipid particles (**Figure 2a**), whereas the peak at high particle diameters corresponded to the insoluble particles from the artificial digestive juice (**Figure 2b**). There was a decrease in the area

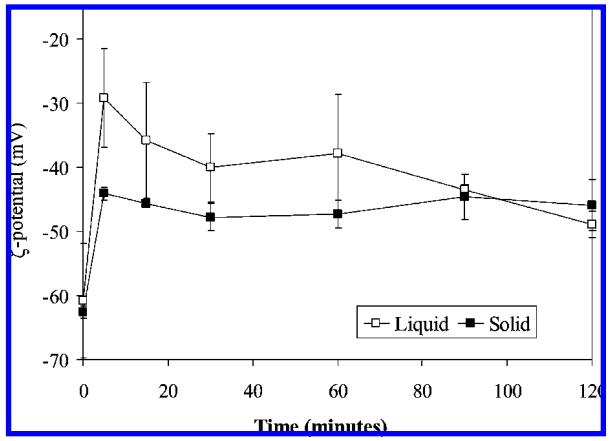

Figure 5. Time dependence of the ζ-potential of tripalmitin oil-in-water emulsions containing liquid or solid lipid particles at 37 °C during digestion by lipase.

under the peak corresponding to the lipid droplets with increasing digestion time in some of the emulsions. This change suggested that the concentration of small lipid droplets in the system decreased during hydrolysis, which may have occurred because of droplet coalescence (lipid particles grew) or because of conversion of triacylglycerols into monoacylglycerols and free fatty acids (particles disappeared). An estimate of this effect was obtained by plotting the time dependence of the percentage of particles remaining with diameters of <1 μm (*volume %*) (**Figure 4**). Regression analysis was carried out by fitting a quadratic equation to the data: $volume\ \% = At^2 + Bt + C$, where t is time. This analysis showed that there was a significant decrease ($p < 0.001$, $r^2 = 0.75$) in the percentage of small droplets in the emulsion containing lipid droplets with time, but no significant change in the emulsion containing solid droplets. These data suggested that the liquid droplets may have been digested more rapidly than the solid droplets. Nevertheless, we were unable to make measurements before 5 min of hydrolysis, and so it is difficult to say what happened during this time; for example, the droplets may have been already been appreciably digested in this period. In addition, it is difficult to quantitatively compare particle size data made on emulsions containing liquid droplets with those containing solid droplets because of the limitations of the light scattering method. In a system containing mixed particles (lipid droplets plus insoluble particles from digestive juices), one should use different refractive indices for the two different types of particles. Nevertheless, the light scattering instrument used in this study allowed us to input only a single refractive index for the particles. We used a different value for the solid lipid phase and the liquid lipid phase in the particle size analysis, but this did not take into account the contribution of the insoluble particles to the light scattering properties of the system. Consequently, the light scattering data should be used only to provide a qualitative insight into changes that occur in the two systems. A possible solution to this problem would be to centrifuge or filter out the large insoluble particles prior to analyzing the smaller lipid droplets—the dense insoluble particles should collect at the bottom of a sample, whereas the light lipid droplets should collect at the top.

The electrical charge (ζ-potential) on the lipid particles was also measured during the in vitro digestion process to gain some insight into changes in interfacial composition (**Figure 5**). The ζ-potential was measured for (i) the initial emulsion, (ii) the initial emulsion with bile (no lipase), and (iii) the emulsion with bile and lipase during the in vitrodigestion model. The initial ζ-potentials of the particles stabilized by SDS was -71 ± 3 mV for the liquid particles and -66 ± 3 for the solid particles, as discussed above. When bile extract was added to the emulsions, their electrical charge became less negative: going from -71 ± 3 to -54 ± 14 mV for the liquid particles and from -66 ± 3 to -62 ± 4 mV for the solid particles. Previously it has been reported that the ζ-potential of lipid droplets saturated with bile was -54 mV (*24*). Our data therefore suggest that bile acids were adsorbed to the particle surfaces in the system containing lipid particles and displaced the SDS molecules, but that bile adsorption and SDS displacement were less efficient in the system containing solid particles. Consequently, the physical state of the emulsified lipids seems to have caused a difference in the interfacial composition of the lipid particles, which would be expected to influence the ease of lipid hydrolysis because this is a surface-mediated reaction. The addition of bile extract alone to the emulsions had no effect on the measured particle size distributions, which indicated that bile extract did not promote droplet flocculation or coalescence.

When pancreatic lipase was added to the emulsions in combination with bile extract, we observed changes in the particle charge with digestion time (**Figure 5**). Within the first 5 min of hydrolysis the ζ-potential on the lipid particles became considerably less negative: $\Delta\zeta = +32$ mV for the liquid particles and $\Delta\zeta = +18$ mV for the solid particles. This change indicates that there was a change in interfacial composition, which could be attributed to adsorption of lipase/colipase complex to the lipid droplet surfaces. The change in particle charge in the initial stages of hydrolysis was larger for the liquid than the solid particles, which suggests that there may have been more adsorption of lipase to the liquid particle surfaces. Over time (5−120 min), the particle charge did not change appreciably in the system containing solid particles, but became somewhat more negative in the system containing liquid particles until it reached a value fairly similar to that as the solid particles (**Figure 5**). This latter change may have been due to accumulation of anionic free fatty acids at the particle surfaces. The particle charge measurements suggest that there were differences in the interfacial compositions of the solid and liquid particles at the earliest digestion time (5 min) and that bile and lipase could bind more effectively to the liquid particle surfaces than to the solid particle surfaces. Nevertheless, at later times ($\geq$15 min) there was no significant difference ($p < 0.05$) in the ζ-potentials of the solid and liquid particles when analyzed using Student's t test.

The change in the overall appearance of the emulsions was monitored during lipid hydrolysis (data not shown). Initially, all of the emulsions were optically opaque, having a milky whitish-yellow appearance. After 15 min of hydrolysis, all of the emulsions still looked fairly similar, but after 30 min, there were distinct differences in appearance. In particular, the emulsion containing liquid lipid droplets became much less cloudy and more yellowish, whereas the other emulsions remained optically opaque. There was no further noticeable change in the appearance of the emulsions up to 120 min of hydrolysis. The fact that the emulsion containing liquid lipid particles became much less turbid with time indicated that there was a reduction in the amount of small lipid droplets capable of scattering light, presumably because they had been digested. The reason that there was not a major change in the appearance of the emulsion containing solid lipid particles, even though a considerable amount of hydrolysis occurred (see below), may have been because the solid phase ($n = 1.54$) has a much higher

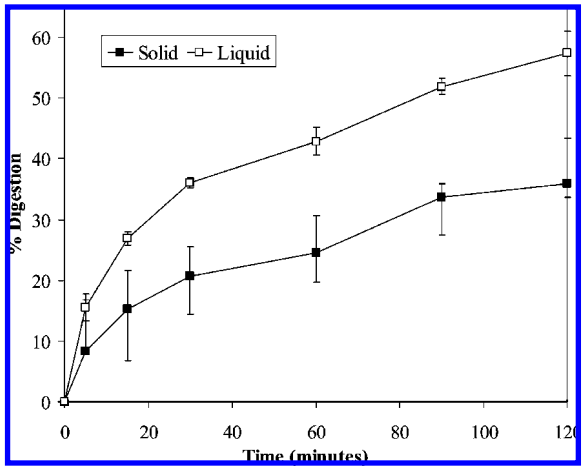

Figure 6. Time dependence of the percentage of free fatty acids released from tripalmitin oil-in-water emulsions containing either liquid or solid lipid particles at 37 °C during lipase digestion. One hundred percent digestion corresponds to the conversion of each triacylglycerol molecule to one monoacylglycerol and two free fatty acids.

refractive index than the liquid phase ($n = 1.437$), so that any remaining droplets scattered light more effectively. Hence, more lipid hydrolysis would be needed to decrease the lightness of the emulsions containing solid particles than those containing liquid particles.

Release of Free Fatty Acids. Finally, we examined the impact of the physical state of the lipid droplets on their in vitro digestibility by pancreatic lipase. In our study, the activity of pancreatic lipase was quantified in terms of the percentage of free fatty acids released from the emulsions containing solid and liquid lipid particles (**Figure 6**). One hundred percent total lipid hydrolysis corresponded to the conversion of each triacylglycerol (TG) molecule to two free fatty acids and one monoacylglycerol.

For all samples, there was a steep increase in the amount of free fatty acids produced during the first 15 min of lipid hydrolysis, followed by a more gradual increase at later times, indicating that lipase was able to hydrolyze the emulsified lipids (**Figure 6**). There was a statistically significant difference ($p < 0.05$) in the amount of hydrolysis of the liquid and solid lipid particles from 15 min onward. The liquid lipid particles were hydrolyzed more rapidly and to a greater extent after 120 min than the solid lipid particles. There are a number of possible reasons for this difference. First, the physical state of the lipid droplets may have influenced the tendency for lipase to adsorb to the droplet surfaces, a prerequisite for its proper functioning (*4*). The ζ-potential measurements indicated that there was a pronounced difference in the interfacial composition of lipid droplets in the solid and liquid states (**Figure 5**). Second, the differences in the spatial organization of the lipid molecules within the different physical states of the lipid phase may have altered the ability of lipase to hydrolyze the triacylglycerols. The β-form of triacylglycerol crystals is the densest polymorphic form, having a triclinic packing in which adjacent chains pack tightly together (*33*). This molecular organization of the lipids may have been less accessible to the lipase than the liquid state. Further work is required to identify the physicochemical basis for the different digestion rates of liquid and solid lipid particles.

The reason that a fairly constant value in the percentage of hydrolysis that occurred after a certain time was reached even though all of the lipids had not been digested may have been because of inhibition of lipase activity by the FFA release (*5*). Indeed, FFA are surface active molecules that will tend to absorb to the surface of the oil droplets and so displace lipase from the surface and prevent it from coming into close contact with the emulsified lipids. Alternatively, there may not have been sufficient mixed bile micelles present to solubilize all of the free fatty acids released from the digested lipid droplets.

Finally, it should be stated that a relatively simplistic in vitro digestion model that simulates only the small intestine was used in these studies to better understand the physicochemical changes occurring during the hydrolysis of emulsified lipids by lipase. This simple model does not take into account many of the complexities that occur in reality when an ingested food passes through a human GI tract: for example, interactions with other food components; interactions with biological surfaces; interactions with the various substances secreted by the human body, such as enzymes, acids, salts, and biopolymers; the motility of the mouth, stomach, and small intestine. Further studies should therefore be carried out using a more realistic in vitro digestion model, as well as using animal and human feeding studies.

This study may have important implications for the application of solid lipid particles as delivery systems for bioactive lipid components, such as ω-3 fatty acids, conjugated linoleic acid (CLA), phytosterols, or carotenoids. These substances may be protected from chemical or physical degradation in a solid lipid particle, but still be released when they pass through the digestive system, albeit at a somewhat slower rate than from a liquid droplet. It would be useful in future studies to examine the impact of surfactant type on the crystallization and digestion of emulsified lipids, because various types of surfactant are used in the food industry to stabilize lipid droplets, for example, nonionic surfactants, phospholipids, proteins, and polysaccharides.

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LITERATURE CITED

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