Delivery of Lipophilic Bioactives: Assembly, Disassembly, and Reassembly of Lipid Nanoparticles

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Abstract

The oral bioavailability of lipophilic bioactive molecules can be greatly increased by encapsulating them within engineered lipid nanoparticles (ELNs), such as micelles, microemulsions, nanoemulsions, or solid lipid nanoparticles (SLNs). After ingestion, these ELNs are disassembled in the gastrointestinal tract (GIT) and then reassembled into biological lipid nanoparticles (mixed micelles) in the small intestine. These mixed micelles solubilize and transport lipophilic bioactive components to the epithelial cells. The mixed micelles are then disassembled and reassembled into yet another form of biological lipid nanoparticle [chylomicrons (CMs)] within the enterocyte cells. The CMs carry the bioactive components into the systemic (blood) circulation via the lymphatic system, thereby avoiding first-pass metabolism. This article provides an overview of the various physicochemical and physiological processes responsible for the assembly and disassembly of lipid nanoparticles outside and inside the GIT. This knowledge can be used to design food-grade delivery systems to improve the oral bioavailability of encapsulated lipophilic bioactive components.

INTRODUCTION

Lipid Nanoparticles Inside and Outside the Body

Numerous lipophilic constituents within foods have been identified as having health benefits over and above their normal roles as nutrients (**Table 1**), such as bioactive lipids (e.g., ω-3 fatty acids and conjugated linoleic acids), oil-soluble vitamins (e.g., A, D, E and K), carotenoids, flavonoids, and phytosterols (Gonnet et al. 2010, Porter et al. 2007, Weiss et al. 2008). These bioactive components are often highly hydrophobic molecules that have low water solubility and relatively poor oral bioavailability (Jingling Tang 2007, Porter et al. 2007). Consequently, they are difficult to incorporate into many types of aqueous-based foods and beverages, and their potential beneficial health effects are not fully realized because they are poorly absorbed by the human body (During et al. 2002; McClements et al. 2009a,b). The oral bioavailability of lipophilic bioactive molecules can be greatly enhanced by encapsulating them within colloidal delivery systems, such as micelles, microemulsions, emulsions, and suspensions (Humberstone & Charman 1997, Kuentz 2012, Patel & Velikov 2011, Porter & Charman 2001b, Porter et al. 2007, Pouton 2000, Pouton & Porter 2008, Vors et al. 2013). These delivery systems contain engineered lipid nanoparticles (ELNs) that are normally fabricated using man-made processes outside of the human body, e.g., various physicochemical and mechanical methods (McClements et al. 2009b, McClements & Li 2010). Typically, ELNs consist of a hydrophobic core surrounded by a hydrophilic shell (**Figure 1**). However, their precise composition and structure can be tailored for specific delivery applications. In particular, they can be designed to incorporate bioactive components into particular food and beverage matrices, protect them from degradation, and then release them at an appropriate location within the human gastrointestinal tract (GIT) after ingestion.

There have been numerous, fairly recent reviews on the biological fate of ingested lipids and lipophilic bioactive components (Chakraborty et al. 2009; Humberstone & Charman 1997; Kohli et al. 2010; McClements et al. 2009a,b; Pouton & Porter 2008; Wilde & Chu 2011) and on the

Table 1 Examples of poorly water-soluble bioactive components whose bioavailability may be improved by delivery using engineered lipid nanoparticles

Compound	Molar mass (g mol^{-1})	T_m (°C)	Log*P*
Curcumin	368.4	183	3.07
Capsaicin	305.4	64	3.20
Genistein	270.2	300	3.11
Resveratrol	228.2	255	3.02
β-Carotene	536.9	180	14.76
Lycopene	536.9	175	14.5
Zeaxanthin	568.9	205	10.9
Astaxanthin	596.8	210	8.24
Lutein	568.9	180	11.5
β-Sitosterol	414.7	138	10.5
Campesterol	400.7	155	9.97
Stigmasterol	412.7	150	10.07
α-Tocopherol	430.7	3	10.96
Tocopherol acetate	472.7	−27.5	10.69
Coenzyme Q	863.3	49	19.12

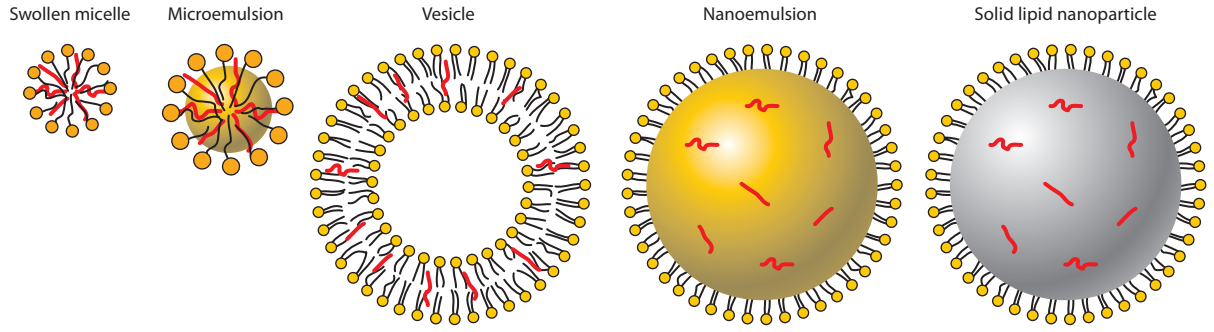

Figure 1

A variety of engineered lipid nanoparticles that are suitable for encapsulating and releasing lipophilic bioactive agents can be fabricated from food-grade ingredients.

development of nanoparticle-based delivery systems for lipophilic bioactive components (Acosta 2009, McClements 2010, Sagalowicz & Leser 2010, Velikov & Pelan 2008). In the current review, we focus on a novel nanotechnology aspect of the behavior of colloidal delivery systems within the GIT: the disassembly and assembly of lipid nanoparticles. After ingestion, an ELN may be disassembled and reassembled into various kinds of biological lipid nanoparticles as it passes through the GIT, e.g., mixed micelles and chylomicrons (CMs). An improved understanding of how these various kinds of lipid nanoparticles are assembled and disassembled could be used to rationally design functional foods with specific health effects. For example, controlling the initial composition and structure of the ELNs in a delivery system could impact the type and amounts of mixed micelles and CMs formed in the GIT. In turn, the nature of the biological lipid nanoparticles formed can determine the amount of bioactive components absorbed and their subsequent biological fate. For example, when lipophilic bioactive components are incorporated into CMs in the enterocyte cells, they are transported into the systemic circulation via the lymphatic system (rather than the portal vein); thus, first-pass metabolism within the liver is avoided (Trevaskis et al. 2008). Hence, the bioactivity of lipophilic components that are susceptible to hepatic metabolism can be improved by designing delivery systems that stimulate CM secretion and lymphatic transport. Although the focus of this article is on lipid nanoparticles, much of the discussion is also relevant to delivery systems containing larger particles ($d > 200$ nm), such as conventional emulsions, suspensions, and liposomes.

Bioavailability of Lipophilic Bioactive Components

The term bioavailability is normally defined as the fraction of an ingested bioactive component that eventually reaches the systemic (blood) circulation in an active form (Versantvoort et al. 2004). There are numerous factors influencing the overall oral bioavailability (F) of an ingested lipophilic bioactive component: $F = F_M \times F_B \times F_A$. Here, F_M is the fraction of the bioactive component that is not metabolized into an inactive state as its passes through the GIT. F_B is the fraction of the bioactive component that is released from the food matrix into the gastrointestinal fluids to become bioaccessible (Fernandez-Garcia et al. 2012). F_A is the fraction of the bioaccessible bioactive component that is absorbed by the GIT and ends up in the systemic circulation. The major physicochemical and physiological processes that influence these parameters are highlighted below (Bauer et al. 2005, Bermudez et al. 2004, Fave et al. 2004, Mohanty et al. 2012) (**Figure 2**):

- Metabolism (F_M): A bioactive component may undergo various types of biochemical or chemical transformation (metabolism) after it is ingested, which may alter its subsequent

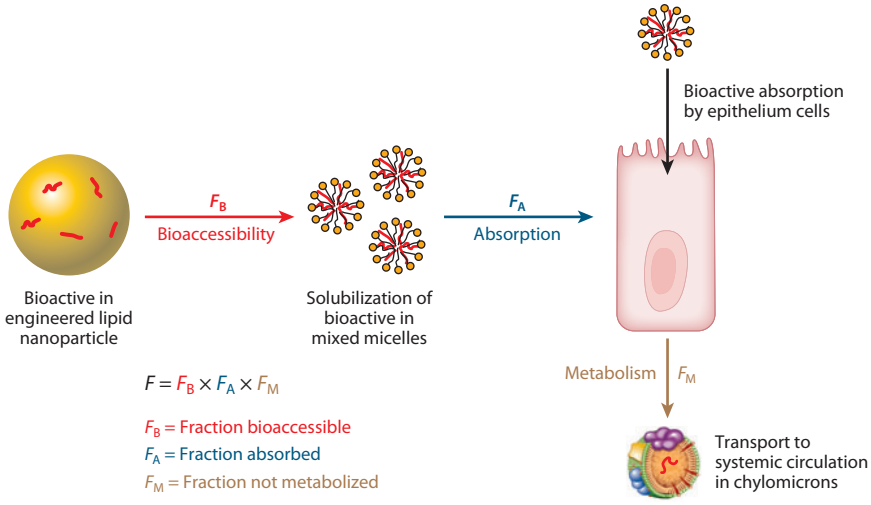

Figure 2

The overall oral bioavailability of a bioactive component in an engineered lipid nanoparticle depends on numerous processes that occur within the gastrointestinal tract, such as solubilization, absorption, and metabolism.

bioaccessibility, absorption, and bioactivity. These transformations may occur due to chemical reactions (e.g., hydrolysis by acid) or enzyme activities (e.g., lipolysis by lipases) in various regions of the GIT and other tissues (such as the mouth, stomach, small intestine, colon, enterocyte cells, lymphatic system, blood, liver, etc.).

- Bioaccessibility (F_B): A highly lipophilic bioactive component needs to be released from the food matrix and solubilized within mixed micelles present in the small intestine before it can be absorbed. These mixed micelles consist of bile salts and phospholipids secreted by the body, as well as any lipid digestion products [i.e., monoacylglycerols (MAGs) and free fatty acids (FFAs)].
- Absorption (F_A): The mixed micelles transport the solubilized lipophilic components across the intestinal lumen, through the mucus layer, and to the surface of the intestinal epithelial cells (Cone 2009, Ensign et al. 2012). The lipophilic components are then incorporated into the epithelial cells through various passive and/or active transport mechanisms (Singh et al. 2009).

The overall bioavailability of ingested lipophilic components can therefore be controlled by designing ELNs and food matrices that increase the fraction that is nonmetabolized (F_M), bioaccessible (F_B), and absorbed (F_A). In principle, this goal can be achieved by manipulating the composition and structure of the ELNs. However, improved knowledge of the influence of specific nanoparticle properties on the bioavailability of specific bioactive components is still needed.

ASSEMBLY OF LIPID NANOPARTICLES OUTSIDE THE BODY: ENGINEERED LIPID NANOPARTICLES

ELNs with different compositions, structures, and physicochemical properties may be present within the colloidal delivery systems designed to encapsulate lipophilic bioactives (Acosta 2009; McClements et al. 2007a,b; McClements & Li 2010; Porter & Charman 2001a,b,c; Velikov & Pelan 2008) (**Figure 1**). These ELNs can be fabricated from food-grade ingredients, such as those

Table 2 Examples of structural components used to fabricate edible engineered lipid nanoparticles suitable for delivering lipophilic bioactive components

Structural component	Examples of ingredients used
Lipophilic substances	Triacylglycerol oils (e.g., canola, corn, fish, medium-chain triglycerides, palm, peanut, soybean, sunflower oils), essential oils (e.g., carvacrol, lemon grass, oregano, thyme, thymol oils), flavor oils (e.g., lemon, lime, orange, peppermint oils), indigestible oils (e.g., waxes, hydrocarbon, paraffin, mineral oils)
Surface-active substances	Small-molecule surfactants (e.g., monoglycerides, diglycerides, Tweens, Spans, sugar esters), phospholipids (e.g., lecithin and lysolecithin), proteins (e.g., casein, gelatin, soy, and whey), polysaccharides (e.g., gum arabic and modified starch), solid particles (e.g., silica or titanium)

shown in **Table 2**. A brief overview of the most important types of ELNs found in food-based colloidal delivery systems is given below.

Fabrication of Engineered Lipid Nanoparticles

Micelles. The lipid nanoparticles in micelle solutions are thermodynamically stable structures normally formed from surfactants that have a polar head and a nonpolar tail (Brodskaya 2012, Torchilin 2007). Surfactants spontaneously self-assemble into micelles when they are dispersed in water above a certain concentration—the critical micelle concentration (CMC) (Leser et al. 2006). The nonpolar surfactant tails form the hydrophobic core of the micelle, whereas the polar headgroups form the hydrophilic shell that faces the water (**Figure 1**). Combinations of surfactants with different molecular characteristics are often used to improve the formation, stability, or performance of micelles. Lipophilic bioactive components can be incorporated into the hydrophobic interiors of micelles; the resulting system is referred to as a swollen micelle (Huang et al. 2010, Marze 2013). Typically, swollen micelles have diameters within the range of 5 to 20 nm depending on their composition and the prevailing environmental conditions (such as temperature, pH, and salt concentration).

Micelle solutions containing lipophilic bioactive components are relatively straightforward to fabricate. Typically, the surfactant and bioactive components are mixed together and then added to an aqueous solution, which should lead to the spontaneous formation of swollen micelles. Alternatively, the surfactant and water are mixed together first to form a micelle solution, and then the lipophilic bioactive component is added. Often, it is necessary to heat and/or mechanically agitate the final solutions to ensure that all of the components are successfully incorporated into the micelles.

Microemulsions. In general, the term microemulsion refers to thermodynamically stable structures that contain surfactant, oil, and water molecules [such as oil-in-water (O/W), water-in-oil, and bicontinuous microemulsions] (Israelachvili 2011). O/W microemulsions are the most commonly used type for the delivery of lipophilic bioactive agents (Flanagan & Singh 2006, Israelachvili 2011, Spernath & Aserin 2006, Spernath et al. 2002). These systems have similar compositions, structures, and properties as swollen micelles, and sometimes the terms swollen micelles and microemulsions can be used interchangeably. The two systems may be considered identical when the lipophilic bioactive component acts as the only oil phase. However, the oil phase in microemulsions may also consist of a mixture of a bioactive component and a carrier oil. O/W microemulsions spontaneously form when certain combinations of surfactant, oil phase, and water are mixed together (Israelachvili 2011). Sometimes, additional ingredients are needed to facilitate their formation and stability, such as cosurfactants and cosolvents (Garti et al. 2005, Israelachvili 2011, Yaghmur et al. 2002). As do swollen micelles, microemulsions consist of a hydrophobic core comprised of

oil molecules and nonpolar surfactant tails, and a hydrophilic shell comprised of polar surfactant head-groups (**Figure 1**). Lipophilic bioactive components tend to be present predominately within the hydrophobic core of microemulsions, although any polar group may protrude into the hydrophilic shell and water phase. Typically, microemulsions have diameters in the range of 10 to 100 nm depending on their composition and environmental conditions. Microemulsions can be formed in a similar manner to swollen micelle solutions. Typically, the surfactant, bioactive, and any carrier oil components are mixed together and then added to the aqueous phase. The resulting solution may then need to be heated and/or mixed to ensure good dispersion of the different components (Rao & McClements 2011). Microemulsions are thermodynamically stable systems that should form spontaneously and remain stable indefinitely provided environmental conditions are not altered too much. Phase diagrams are often developed to determine the range of compositions and temperatures in which a microemulsion remains thermodynamically stable (Flanagan & Singh 2006, Garti et al. 2005).

Liposomes. Liposomes consist of one or more concentric shells of surfactant bilayers (**Figure 1**) (Sharma & Sharma 1997). A unilamellar liposome has a single bilayer shell, whereas a multilamellar liposome has multiple concentric bilayer shells (Maherani et al. 2011, Reineccius 1995, Taylor et al. 2005). The building blocks of liposomes are surface-active substances with intermediate hydrophilic-lipophilic balance (HLB) numbers and optimum curvatures close to zero, e.g., phospholipids (Israelachvili 2011). This kind of surface-active substance tends to spontaneously form bilayers when dispersed in aqueous solutions. Liposomes typically have diameters in the range of approximately 25 to greater than 1,000 nm.

There are numerous different approaches for forming liposomes, including solvent evaporation, surfactant depletion, and extrusion methods (Maherani et al. 2011). For example, in the solvent evaporation method, the phospholipids and bioactive component are dissolved in an organic solvent that is then placed within an appropriate container, e.g., a volumetric flask. The organic solvent is removed by evaporation, which leaves a thin film of phospholipids and bioactive components on the surface of the container. An aqueous solution is then added to the container, which leads to the spontaneous formation of liposomes due to peeling off of the surfactant bilayers from the containing surface after hydration. In the extrusion method, the phospholipids, bioactive, and aqueous solution are mixed together, and then the resulting mixture is passed through a high-pressure homogenizer. It may be necessary to optimize preparation conditions to produce stable liposomes with relatively small dimensions, such as temperature, pH, ionic strength, and homogenizer operation conditions.

Nanoemulsions. The lipid nanoparticles in nanoemulsions have somewhat similar structures to those found in microemulsions (McClements 2011, McClements & Rao 2011), consisting of a hydrophobic core comprised of oil molecules and a hydrophilic shell comprised of surface-active molecules (**Figure 1**). However, nanoemulsions are thermodynamically unstable colloidal dispersions and will therefore break down over time through a variety of destabilization mechanisms, such as flocculation, coalescence, gravitational separation, and Ostwald ripening (McClements 2012b). The long-term stability of nanoemulsions can be improved by incorporating stabilizers such as emulsifiers, weighting agents, ripening inhibitors, and texture modifiers (McClements 2011, McClements & Rao 2011).

Nanoemulsions may be formed using a variety of methods that can be classified as either high-energy or low-energy methods (McClements 2011, McClements & Rao 2011). High-energy methods use mechanical devices (homogenizers) capable of generating intense disruptive forces to break up and mingle the oil and aqueous phases leading to the formation of ultrafine oil droplets

in water, such as high-pressure valve homogenizers, microfluidizers, and sonicators (Koroleva & Yurtov 2012, McClements 2011, Silva et al. 2012). Low-energy methods rely on the spontaneous formation of ultrafine oil droplets in water when certain types of surfactant, oil, and water are mixed together under controlled conditions, such as phase inversion temperature, spontaneous emulsification, and emulsion inversion point methods (Solans & Sole 2012). The food-grade emulsifiers that can be used to form nanoemulsions include small-molecule surfactants, phospholipids, proteins, and polysaccharides, whereas those that can be used to form micelles, microemulsions, or liposomes are usually only certain types of small-molecule surfactants and/or phospholipids. The lipid nanoparticles in food-grade nanoemulsions typically have diameters in the range of 20 to 200 nm (Koroleva & Yurtov 2012, McClements 2011, Silva et al. 2012); however, larger lipid particles may be present if conventional emulsions are used rather than nanoemulsions (McClements 2005).

Solid lipid nanoparticles and nanostructured lipid carriers. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are similar in structure to nanoemulsions (**Figure 1**), but the lipid core is either fully or partially crystalline (Jores et al. 2004, Muller et al. 2000, Weiss et al. 2008). SLNs and NLCs are typically formed from high-melting-point lipids using a two-step process. First, a nanoemulsion is prepared at a temperature above the melting point of the lipid phase to ensure that it remains liquid throughout the homogenization process. Second, this nanoemulsion is cooled to a temperature below the melting point to promote crystallization of the lipid phase. SLNs and NLCs can also be formed using low-energy methods by spontaneously forming a nanoemulsion at high temperatures and then cooling it to induce lipid crystallization. The morphology of the lipid nanoparticles in SLNs and NLCs may change appreciably after the liquid-to-solid transition of the lipid phase has occurred due to the physical constraints associated with packing lipid molecules within crystals (Bunjes 2011, Jores et al. 2004). As do nanoemulsions, the particles in SLN and NLC suspensions typically have diameters in the 20-to-200-nm range, although larger particles may be present if conventional emulsions are used as templates rather than nanoemulsions. For convenience, the term SLN is used below to refer to both fully and partly crystalline ELNs.

Design of Engineered Lipid Nanoparticle Properties

The ELNs found in foods and beverages vary considerably in their compositions and structures depending on the type of ingredients and fabrication methods used in their production. The functional performance of colloidal delivery systems (such as stability, opacity, rheology, and biological fate) is ultimately determined by their particle characteristics (Lesmes & McClements 2009). Consequently, colloidal delivery systems with different functional performances can be designed by controlling the characteristics of the ELNs they contain, such as composition, structure, dimensions, interfacial properties, charge, and physical state. Methods of controlling the properties of various kinds of ELNs have been described elsewhere (Fathi et al. 2012; McClements 2010, 2011; Müller et al. 2002; Patel & Velikov 2011; Spernath & Aserin 2006); as such, they are not covered here.

DISASSEMBLY OF ENGINEERED LIPID NANOPARTICLES WITHIN THE GASTROINTESTINAL TRACT

After ingestion, ELNs may undergo numerous different disassembly processes during their passage through the various regions of the GIT; however, eventually they are usually reassembled into mixed micelles within the small intestine prior to absorption. The fraction of a lipophilic

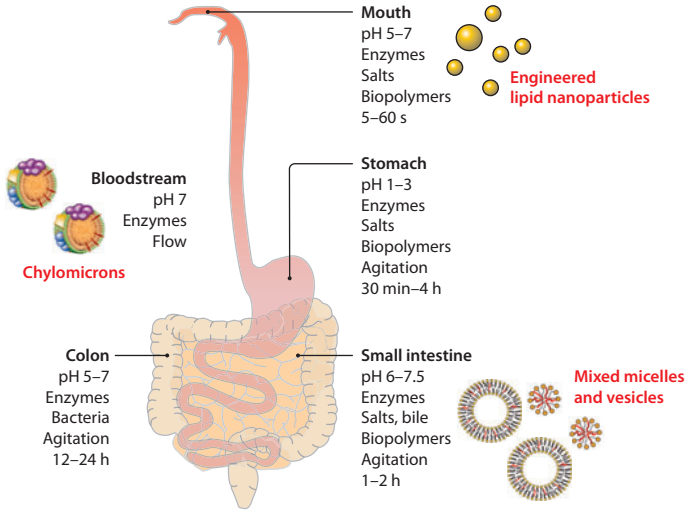

Figure 3

Schematic diagram of the physicochemical and physiological conditions in different regions of the human gastrointestinal tract and the different kinds of lipid nanoparticles.

bioactive component incorporated into these mixed micelles is usually taken as a measure of its bioaccessibility (F_B).

Characteristics of the Major Regions of the Gastrointestinal Tract

The initial structure and dimensions of an ingested ELN depend on the type of ingredients and processing operations used to assemble it (**Figure 1**). ELNs may be distributed in different matrices within different products: freely suspended in an aqueous solution (as in beverages and soft drinks), trapped within a biopolymer matrix (as in some desserts, sauces, and yogurts), or trapped within a solid matrix (as in some powders and cereal products). The initial environment of an ELN may have a major effect on its biological fate. For example, the food matrix may have to be disrupted or dissolved before the ELNs are released, which may determine the time and location where they interact with the gastrointestinal fluids (Matalanis et al. 2011; McClements et al. 2009a,b; McClements & Li 2010). After ingestion, ELNs experience a complex set of environmental conditions as they pass through the various regions of the GIT (**Figure 3**), which alters their composition and structure. The physiochemical and physiological characteristics of these different regions have been reviewed recently (McClements et al. 2009a,b; Singh et al. 2009; van Aken 2010); as such, we provide only a brief overview here.

- Mouth: After entering the oral cavity, foods containing ELNs may undergo a variety of changes: mixture with saliva; dissolution, dispersion, and/or dilution; alterations in pH, ionic strength and/or temperature; exposure to digestive enzymes; interactions with oral surfaces; and exposure to complex fluid flow/stress patterns.
- Stomach: After being processed within the oral cavity, the ELNs are swallowed, pass through the esophagus, and enter the stomach. They are then exposed to relatively extreme conditions that may further alter their properties: low pH (1 to 3), high ionic strength (e.g., calcium and sodium salts), enzyme activity (e.g., proteases, amylases, and lipases), surface-active substances (e.g., phospholipids and proteins), and complex fluid flow/stress patterns.

- Small intestine: After leaving the stomach, the resulting material (chyme) passes through a small valve (pylorus sphincter) and enters the small intestine, where it is further processed for absorption (Barrett 2006). Any ELNs remaining in the chyme are mixed with small-intestinal fluids that contain bile salts, phospholipids, lipases, proteases, bicarbonate, and other minerals. The presence of the bicarbonate causes the pH to rise to approximately neutral, which facilitates the action of the pancreatic enzymes, such as lipases, proteases, and amylases. These enzymes will break down any digestible components present, such as lipids, proteins, and starches. The hydrolysis of triacylglycerols (TAGs) leads to the formation of mixed micelles that can transport digested lipids and lipophilic bioactive components to the epithelial cells.
- Colon: If all the components used to fabricate an ELN were fully digestible, then one would expect it to be digested and absorbed largely within the stomach and small intestine. However, if an ELN is fabricated from indigestible components (such as indigestible oils or fibers), then it may pass through these regions and enter the colon. The colon contains a variety of different microorganisms capable of metabolizing and utilizing dietary components, such as fats, proteins, carbohydrates, and dietary fiber (Basit 2005).

Changes in Particle Properties in the Gastrointestinal Tract

After ingestion, the ELNs originally present within a food or beverage product usually change considerably as they pass through the different regions of the GIT as a result of their exposure to this complex physiological and physicochemical environment (Golding et al. 2011; Johnson 2001; McClements et al. 2007a, 2009b; Singh et al. 2009). There may be changes in the composition, structure, dimensions, charge, interfacial properties, and physical state of the lipid nanoparticles, all of which could greatly alter their biological fate (McClements & Xiao 2012). **Figure 4** shows an example of changes in the size and charge of ELNs initially stabilized by different kinds of emulsifiers. It is therefore important to understand how different kinds of ELNs respond to changes in GIT conditions. A colloidal delivery system can then be designed to alter the biological fate of an encapsulated bioactive component in a desired manner, e.g., to increase its bioaccessibility, reduce its metabolism, or release it within a particular location of the GIT. This section gives a brief overview of the major physicochemical mechanisms responsible for the disassembly of ELNs in the GIT.

Particle structure. The structure of ELNs may change appreciably as they pass through the different regions of the GIT due to alterations in their environment, such as dilution, pH, ionic strength, temperature, surface-active substances, polymers, biological surfaces, and enzymes. The ELNs originally present in a food or beverage may have a variety of different initial structures depending on the ingredients and method used to prepare them: swollen micelles, microemulsions, nanoemulsions, SLNs, or liposomes (**Figure 1**). The manner by which these structures are altered in the GIT depends on the initial organization of the components within the lipid nanoparticles. For example, micelles, microemulsions, or liposomes may easily dissociate in the mouth or stomach after ingestion due to dilution or interaction with specific substances in the GIT (such as phospholipids, bile salts, or minerals) (Hu et al. 2013). However, indigestible nanoemulsions or SLNs may pass through the GIT with their hydrophobic cores largely intact (although their surface characteristics may change appreciably).

Particle composition. The composition of ELNs may also change appreciably as they pass through the GIT, e.g., due to the exchange of molecules with the surrounding media or due to

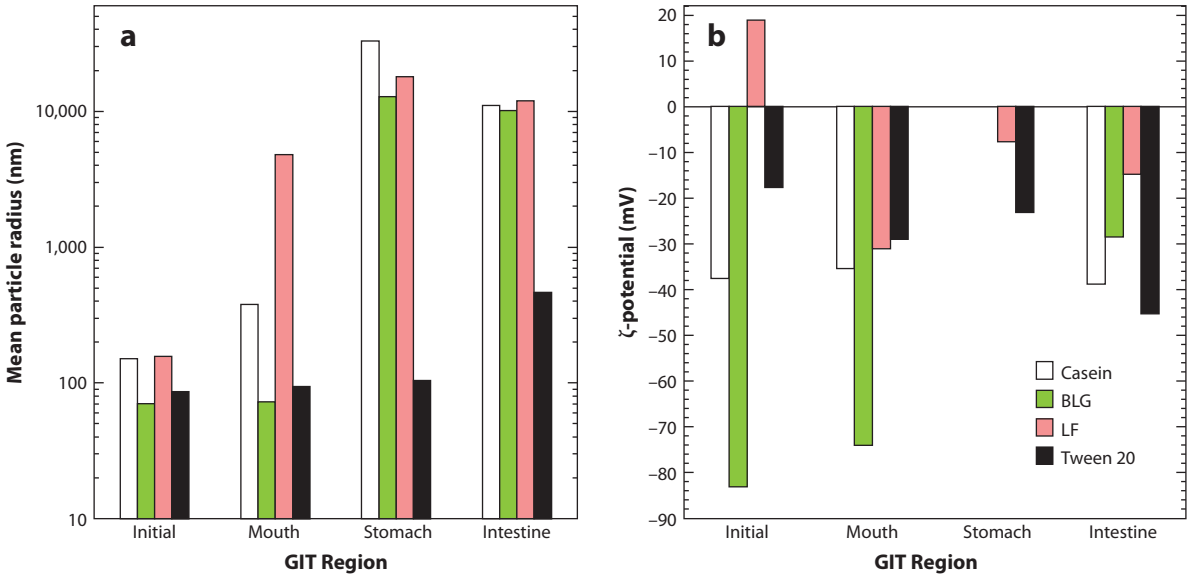

Figure 4

(*a*) Changes in the mean particle radius of colloidal dispersions initially containing lipid nanoparticles as they pass through different stages of the gastrointestinal tract (GIT): β-lactoglobulin (BLG), casein, Tween 20, lactoferrin (LF). (*b*) Changes in the particle charge (ζ-potential) of colloidal dispersions initially containing lipid nanoparticles as they pass through different stages of the GIT: β-lactoglobulin (BLG), casein, Tween 20, lactoferrin (LF).

chemical or enzymatic degradation reactions. For example, TAG molecules within the hydrophobic core of a nanoemulsion droplet or SLN may be converted to FFAs, MAGs, and diacylglycerols by gastric and pancreatic lipases in the stomach and small intestine; then they leave the droplet. Surface-active substances in the shell of ELNs (such as proteins, phospholipids, or surfactants) may be hydrolyzed by digestive enzymes, or they may be displaced by endogenous surface-active molecules in the surrounding digestive fluids.

Particle dimensions. A variety of different physicochemical mechanisms can change the dimensions of the particles (Augustin et al. 2011; Li et al. 2012a,b; Liu et al. 2012; Singh & Sarkar 2011; Tokle et al. 2012; van Aken 2010):

- Flocculation: Two or more particles associate with each other but keep their individual integrities.
- Coalescence: Two or more particles merge with each other and form a larger particle.
- Partial coalescence: Two or more partly crystalline particles fuse together to form a clump.
- Ostwald ripening: Large particles grow and small ones shrink due to molecular diffusion of oil molecules through the aqueous phase.
- Digestion: Particles shrink due to enzymatic degradation of their components and the subsequent release of digestion products (such as FFAs, MAGs, or amino acids).
- Solubilization: Particles shrink due to transfer of some of the lipophilic bioactive components or lipid molecules into the surrounding aqueous phase (**Figure 5**).

Interfacial properties. The interfacial characteristics of ELNs may also be altered as they pass through the GIT due to the following:

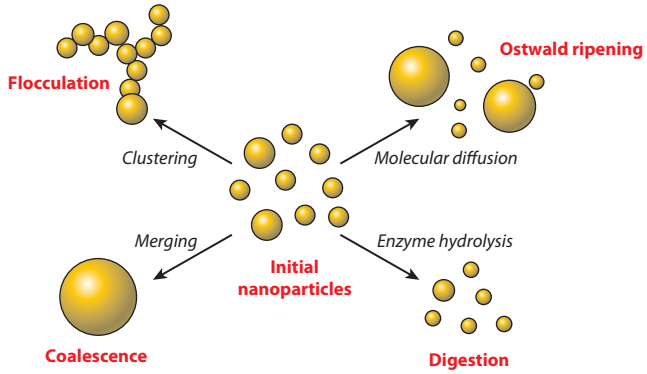

Figure 5

The dimensions and structure of lipid nanoparticles may change considerably as they pass through the gastrointestinal tract due to several different physicochemical processes.

- Competitive adsorption: Surface-active components within the gastrointestinal fluids may adsorb to the nanoparticle surfaces and displace some of the original surface-active substances.
- Coadsorption: Surface-active components within the intestinal fluids may adsorb to the nanoparticle surfaces and penetrate between the original surface-active substances.
- Multilayer formation: Substances within the gastrointestinal fluids may adsorb on top of the original interfacial coating (e.g., charged polymers onto oppositely charged particles).
- Digestion or degradation: Surface-active substances at the particle surfaces may be fully or partially digested or degraded due to the highly acidic conditions in the stomach or due to the presence of specific enzymes (Mackie & Macierzanka 2010).

Numerous studies have shown that the interfacial characteristics of lipid nanoparticles are altered as they pass through different regions of the GIT (Gallier et al. 2012; Liu et al. 2012; Mun et al. 2007; Nik et al. 2012; Troncoso et al. 2012a,b; Wilde & Chu 2011; Ye et al. 2011).

Physical state. The physical state of one or more of the components used to assemble an ELN may change as it passes through the GIT. The hydrophobic core of ELNs may be solid within a freezer or refrigerator during storage but may melt when it is exposed to elevated body temperatures. Conversely, the hydrophobic core may be liquid within a hot food but crystallize when it is exposed to cooler body temperatures. Lipophilic bioactives that are fully dissolved within the TAG core of an ELN may precipitate when the TAG is digested and the bioactives are released into the gastrointestinal juices (McClements 2012a). Conversely, lipophilic bioactives that are initially crystalline within a food may dissolve when they are exposed to gastrointestinal juices. Studies have shown that changes in the physical state of ingested components may alter their biological fate (Bonnaire et al. 2008, Golding et al. 2011, Li et al. 2012a, Nik et al. 2012).

Controlled Disassembly of Engineered Lipid Nanoparticles

The disassembly of ELNs due to these different mechanisms can be controlled by altering their initial composition and structure. For example, fabricating the hydrophobic core from a digestible lipid may cause the lipid nanoparticles to be completely dissembled in the small intestine, whereas fabricating it from an indigestible liquid may leave them partly intact.

ASSEMBLY OF LIPID NANOPARTICLES WITHIN THE GASTROINTESTINAL TRACT: MIXED MICELLES

One of the key events determining the bioavailability of lipophilic bioactive components within the GIT is the formation of mixed micelles (Madenci & Egelhaaf 2010, Wilde & Chu 2011). Mixed micelles solubilize lipophilic bioactive components and transport them to the epithelial cells, where they can be absorbed (Porter & Charman 2001a,b,c; Porter et al. 2007). In the absence of digestible lipids, simple mixed micelles are formed by the endogenous bile salts and phospholipids in the intestinal secretions (Di Maio & Carrier 2011). In the presence of digestible lipids, complex mixed micelles are formed from lipid digestion products (MAGs and FFAs), bile salts, and phospholipids (McClements et al. 2009a,b; Porter & Charman 2001a,b,c; Shen et al. 2001). The term mixed micelles actually refers to a complex mixture of colloidal structures whose properties change throughout digestion, such as micelles, vesicles, and liquid crystals (Müllertz et al. 2012). Many of these structures have dimensions within the nanometer range and can therefore be considered to be biological lipid nanoparticles. For example, micelles typically have diameters in the 3–20-nm range, whereas vesicles have diameters from 20 nm to greater than 1,000 nm (Müllertz et al. 2012). An electron microscopy study of the colloidal structures formed in the small intestine after lipid digestion (**Figure 6*a***) suggests an interesting mechanism for the formation of mixed micelles (Müllertz et al. 2012). The FFAs and MAGs generated at the surface of the lipid droplets during the digestion of TAGs form liquid crystalline bilayers that bud off and form vesicles or micelles that move into the aqueous phase (**Figure 6*b***). Presumably, lipophilic bioactive components are trapped within these bilayers and therefore end up within the mixed micelle phase. In practice, the precise nature of the colloidal structures formed is likely to depend

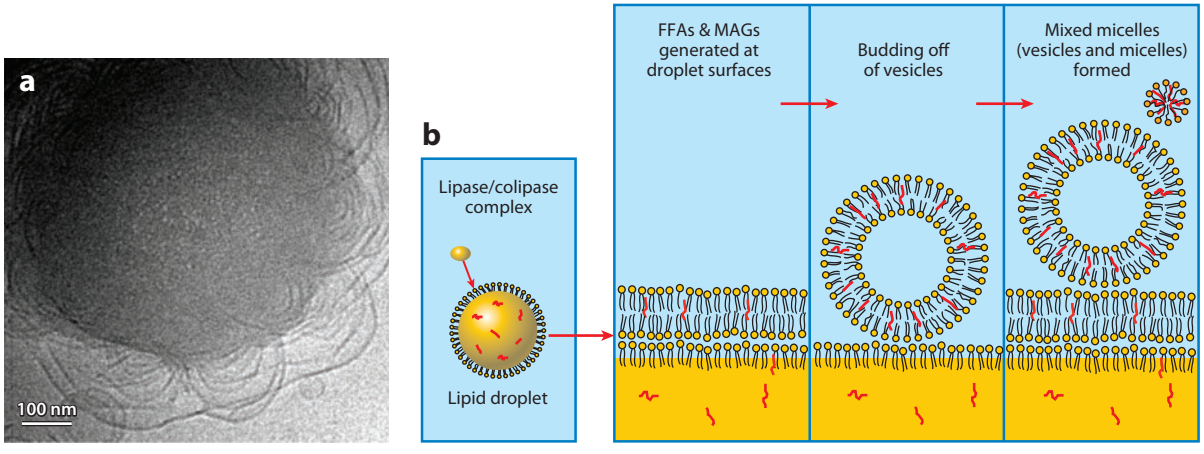

Figure 6

(*a*) Cryo-TEM image of an aspirate collected from human small-intestinal fluids after ingestion of a fatty meal. The image shows lipid bilayers forming at the surface of a fat droplet during digestion, which are believed to mix with bile salts and phospholipids to form mixed micelles. The images were kindly supplied by Professor Dimitris Fatouros from his article "Insights into Intermediate Phases of Human Intestinal Fluids Visualized by Atomic Force Microscopy and Cryo-Transmission Electron Microscopy ex Vivo" (Müllertz et al. 2012), with permission. (*b*) Highly schematic proposed mechanism for the formation of mixed micelles during lipid digestion. Surface-active substances may consist of a mixture of phospholipids, bile salts, FFAs, and MAGs and not just a single substance (as shown here). The structures formed may interact with other molecules to form mixed micelles. Abbreviations: FFA, free fatty acid; MAG, monoacylglycerol; TEM, transmission electron microscopy.

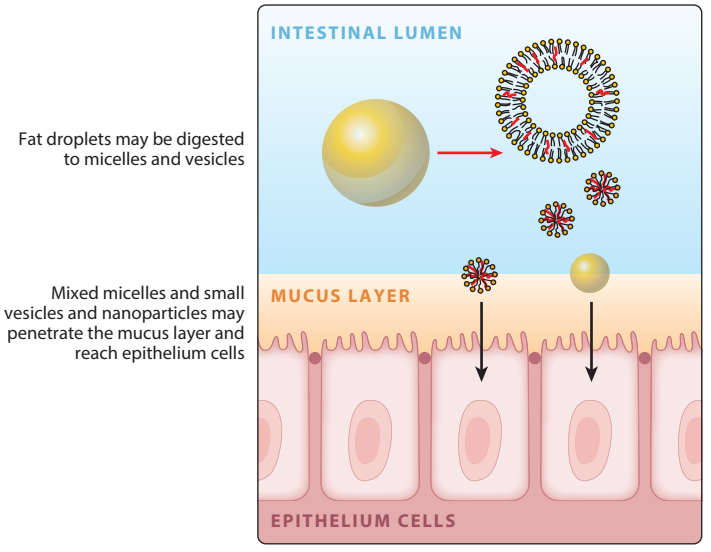

Figure 7

Lipid nanoparticles may penetrate into the mucus layer and become trapped (mucoadhesion) or transported across it. Nanoparticles that are not digested and that travel through the mucus layer may be directly adsorbed by the epithelial cells.

on the nature of the delivery system and food matrix ingested, as well as the physiological conditions within the person. After they are formed, the mixed micelles transport FFAs, MAGs, and bioactive lipophilic compounds from the lumen of the small intestine, across the mucus layer, and to the surface of the enterocyte cells (**Figure 7**). Once they reach the apical side of the epithelial cells the FFAs, MAGs, and lipophilic bioactive components may be absorbed through various passive or active transport mechanisms (Darwich et al. 2010).

In the absence of lipid digestion products, the bile salts and phospholipids present within the intestinal fluids form simple mixed micelles (Madenci & Egelhaaf 2010). Bile salts are anionic surface-active substances that have fairly rigid plate-like structures, with one side being hydrophilic and the other side being hydrophobic (Madenci & Egelhaaf 2010). Phospholipids are ionic surface-active substances that have a polar head-group and two nonpolar hydrocarbon tails. The structure of these two biological surface-active substances is therefore quite different from that of most conventional food-grade surfactants that have a hydrophilic head-group and a single hydrophobic tail. The main driving force for the formation of simple mixed micelles is the hydrophobic effect, but other interactions may also be important, such as hydrogen bonding (Madenci & Egelhaaf 2010). It is proposed that simple mixed micelles have a disk-like shape with hydrodynamic diameters ranging from approximately 3.6 to 60 nm depending on their composition (Mazer et al. 1980). At relatively low ratios of lecithin to bile salt, relatively small micelles are formed ($\delta \approx 3.6$ to 7 nm); however, at high ratios, relatively large micelles are formed ($\delta \approx 4.0$ to 60 nm). Simple mixed micelles tend to be present in the GIT under conditions where lipophilic bioactive components are ingested in the absence of fat or in the presence of indigestible fats. Simple mixed micelles can solubilize certain types of lipophilic bioactive components in foods, but they tend to be much less efficient than complex mixed micelles (Charman et al. 1997, Porter et al. 2007, Schwebel et al. 2011).

In the presence of lipid digestion products, the bile salts and phospholipids in the intestinal fluids interact with the FFAs and MAGs to form complex mixed micelles, which may adopt a variety

of different structural organizations, such as micelles and vesicles, depending on the composition of the system (Müllertz et al. 2012). Micelles found in human intestinal fluids after ingestion of digestible fats have been reported to be relatively small ($d \approx 20$ nm), whereas vesicles were reported to be larger ($d \approx 20$ to 150 nm). The main driving force for the formation of these structures is the hydrophobic effect, i.e., the tendency to reduce the contact area between nonpolar groups and water (Israelachvili 2011).

In this section, we discuss some of the major factors that influence the composition, structure, and properties of this type of biological lipid nanoparticle, with an emphasis on how these factors can be controlled to improve the oral bioavailability of ingested lipophilic bioactive components.

Influence of Molecular Characteristics of Ingested Lipids on Mixed Micelle Properties

A major way that the properties of mixed micelles can be manipulated is through altering the composition of the lipid phase that is ingested. Indeed, the solubilization capacity of mixed micelles is highly dependent on the type and amount of digestible lipids consumed with lipophilic bioactive components (Xiao & Lewis 2012). The simple mixed micelles formed in the absence of digestible lipids have relatively low solubilization capacities, whereas the complex mixed micelles formed in their presence have much higher solubilization capacities (Porter & Charman 2001a,b). The structure, dimensions, and solubilization capacities of complex mixed micelles depend on the total amount of lipids consumed, as well as on the molecular characteristics of the fatty acid chains, such as chain length and degree of unsaturation (Porter & Charman 2001a,b).

The bioaccessibility of carotenoids has been reported to be higher for lipids containing long-chain fatty acids (LCFAs) than those containing medium-chain fatty acids (MCFAs), presumably because of differences in the solubilization capacity of the mixed micelles formed (Qian et al. 2012). The large hydrophobic carotenoid molecules may be able to fit into the large hydrophobic cores of mixed micelles formed by LCFAs but not the smaller hydrophobic cores formed by MCFAs (**Figure 8**). However, for other types of lipophilic bioactive molecule (e.g., curcumin), the solubilization capacities of mixed micelles formed from MCFAs and LCFAs were found to be fairly similar (Ahmed et al. 2012), which may be because the bioactive molecules were small enough to be accommodated within both types of micelle.

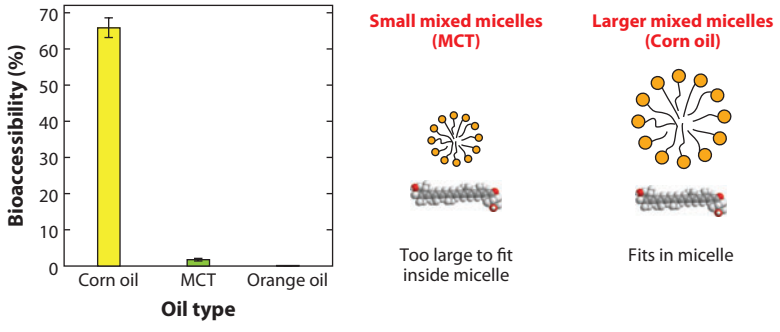

Figure 8

The bioaccessibility of a lipophilic compound is often influenced by the nature of the carrier oil. In this study, we examined the bioaccessibility of β-carotene encapsulated within nanoemulsions made from long-chain triglycerides (corn oil), medium-chain triglycerides (MCT), or an indigestible oil (orange oil). Data from Qian et al. (2012).

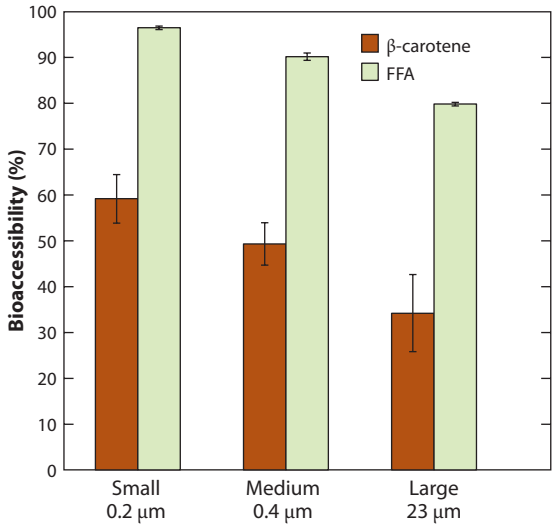

Figure 9

Influence of the diameter of initial engineered lipid nanoparticles in corn oil-in-water nanoemulsions on the extent of fatty acid release and bioaccessibility of β-carotene after 2 h of digestion by lipases under simulated small intestine conditions. The initial mean droplet diameters were large ($d_{43} \approx 23$ μm), medium ($d_{43} \approx 0.4$ μm), and small ($d_{43} \approx 0.2$ μm). Abbreviation: FFA, free fatty acid. From Silvia-Trujillo et al. (2013).

Influence of Structural Properties of Engineered Lipid Nanoparticles on Mixed Micelle Properties

Recent in vitro studies in our laboratory using corn O/W emulsions showed that the bioaccessibility of β-carotene increased with decreasing particle size, which was attributed to the faster and more extensive digestion of the triglycerides in the smaller droplets due to their greater surface areas (**Figure 9**). Another recent study using soybean O/W emulsions also reported that the bioaccessibility of β-carotene increased with decreasing droplet size after in vitro digestion (Wang et al. 2012).

Numerous researchers have also found that the rate and amount of fatty acid production during lipid digestion (and therefore the rate and extent of mixed micelle formation) increases as the droplet size decreases, which was attributed to an increase in the surface area of lipids exposed to the digestive enzymes (Golding et al. 2011, Li et al. 2011, Li & McClements 2010, Reis et al. 2009). Nevertheless, other factors may also contribute to the influence of initial particle size on digestion, such as the concentration of free surfactant remaining in the aqueous phase (given that this can inhibit lipase absorption) (Li & McClements 2011) and alterations in interfacial structure (given that this can affect the ability of lipase to reach the lipid phase) (Troncoso et al. 2012a,b). The kinetics of fatty acid production and mixed micelle formation has also been shown to depend on the physical state of the lipid phase in ELNs; for example, lipid digestion was faster for liquid than for solid tripalmitin particles (Bonnaire et al. 2008).

Influence of Molecular Characteristics of Bioactives on Mixed Micelle Properties

The ability of mixed micelles to incorporate lipophilic bioactive components is characterized by their solubilization capacity, which can be expressed as the mass of the bioactive component

solubilized per unit mass of mixed micelles. The solubilization capacity of mixed micelles depends on the type and amount of digestible lipids consumed, as well as the molecular characteristics of the bioactive component. The colloidal structures present within mixed micelles have regions capable of solubilizing lipophilic bioactive molecules, e.g., the hydrophobic cores of micelles or bilayers of vesicles. If the bioactive molecules have dimensions smaller than these regions, then they are easily incorporated into the mixed micelle structure (**Figure 8**). However, if they have dimensions considerably larger than these regions, then they will not be solubilized easily and will have low bioaccessibility. Recent studies have shown that curcumin can be incorporated easily into mixed micelles formed from MCFAs and LCFAs, which was attributed to its relatively small molecular dimensions (Ahmed et al. 2012). However, β-carotene (which has considerably larger molecular dimensions than curcumin) can only be incorporated into mixed micelles formed from LCFAs (**Figure 8**) (Qian et al. 2012).

Other recent studies have also reported that the transfer of lipophilic bioactive components from oil droplets to mixed micelles depends on the bioactives' molecular characteristics (Nik et al. 2011). In the absence of lipolysis, the fraction of bioactives transferred into simple mixed micelles was relatively low: ≈2% for β-carotene, ≈1.5% for Coenzyme Q, ≈24% for vitamin D, and ≈31% for phytosterols. Conversely, in the presence of lipolysis, the fraction of bioactive components transferred into complex mixed micelles was much higher: ≈80% for β-carotene, ≈90% for Coenzyme Q, ≈87% for vitamin D, and ≈93% for phytosterols. An in vitro study also showed that the bioaccessibility of carotenoids dispersed in bulk oils after lipase digestion was highly dependent on their molecular structure (Sy et al. 2012): ≈89% for lutein, ≈49% for β-carotene, ≈37% for astaxanthin, and ≈3% for lycopene.

Controlled Assembly of Mixed Micelles

These studies clearly highlight the importance of the structure of the bioactive components and mixed micelles in determining bioaccessibility. This knowledge can be used to tailor the design of ELNs so that they have specific characteristics (compositions, structures, and dimensions) that will form mixed micelles that will increase the bioavailability of specific lipophilic bioactive components.

DISASSEMBLY OF MIXED MICELLES WITHIN THE GASTROINTESTINAL TRACT AND ABSORPTION OF BIOACTIVES

After they have been formed within the GIT, there are numerous ways in which mixed micelles may be broken down or their properties altered. In this section, we give a brief outline of some of the most important physicochemical or physiological mechanisms responsible for the disassembly of mixed micelles within the GIT and their ability to transport encapsulated components into the enterocytes.

Interactions with Enterocytes

After being solubilized into mixed micelles and transported through the mucus layer, the bioactive components and lipid digestion products are transferred into the enterocyte cells through various passive and active transport mechanisms (Darwich et al. 2010, Hussain et al. 2001, Iqbal & Hussain 2009, Porter & Charman 1997). The precise mechanisms are still not fully understood, but it has been proposed recently that there are two major absorption mechanisms depending on the total amount of digested lipids present: (*a*) At low concentrations, FFA uptake is by a carrier-dependent (active) process; (*b*) at high concentrations, FFA uptake is primarily by a carrier-independent

(passive) process (Kindel et al. 2010). It is believed that the lipophilic components within a mixed micelle (FFAs, MAGs, bioactives) are first released into the aqueous phase and then transported to the epithelial cells by molecular diffusion. Individual lipophilic molecules interact with the apical side of the enterocyte cell membrane, then undergo a flip-flop mechanism within the membrane, and then are transported into the interior of the cell (Iqbal & Hussain 2009). However, in principle, an entire micelle or vesicle containing bioactive components could be absorbed by an epithelial cell or may fuse with the cell membrane (Hussain et al. 2001, Powell et al. 2010). At present, the precise fate of mixed micelles at the epithelial cell surface is unknown (Iqbal & Hussain 2009, Sugano 2009). Overall, the fraction of a lipophilic bioactive component absorbed by the enterocytes is usually taken as a measure of its absorption (F_A).

The molecular characteristics of the initial lipid phase in a delivery system may play an important role in determining the rate and extent of the transfer of lipid digestion products and bioactives to enterocyte cells. In particular, using cell culture models, the chain length and degree of unsaturation of fatty acids have been shown to have an important influence on their absorption (Duraisamy et al. 2007, Jewell & Cashman 2003, Usami et al. 2003). A recent study of the influence of mixed micelle composition on the uptake of carotenoids by Caco-2 cells found that the absorption of β-carotene depended on the amount of FFAs, MAGs, phospholipids, and cholesterol present (Kotake-Nara & Nagao 2012). Another cell culture study using oily solutions as delivery systems showed that the absorption of carotenoids by Caco-2 cells after digestion in a simulated small intestine model depended on their molecular structure (Sy et al. 2012). The fraction of carotenoids absorbed by the Caco-2 cells was ≈7.3% for lutein, ≈10.6% for β-carotene, ≈7.7% for astaxanthin, and ≈0% for lycopene.

Interactions with Food Components

The properties of mixed micelles may be altered due to their interactions with other components in the partially digested food matrix. Mixed micelles may interact with surfactants, which change their structures, compositions, and solubilization capacities (Delorme et al. 2011, Rozner et al. 2010), and may therefore impact the bioavailability of any bioactive lipophilic components. Cationic biopolymers (such as chitosan, polylysine, or proteins) may bind anionic mixed micelles through electrostatic attraction, causing them to precipitate, thereby trapping any encapsulated bioactive components (Helgason et al. 2008, Sarkar et al. 2010, Thongngam & McClements 2005, Tsujita et al. 2006). Cationic mineral ions (such as calcium) present in the intestine may also bind anionic mixed micelles and cause them to precipitate as calcium soaps, thereby reducing the absorption of lipids and lipophilic bioactive agents (Bendsen et al. 2008, Devraj et al. 2013, Hu et al. 2010). The precise mechanisms that are important in a given situation will depend on the nature of the initial ELNs and the food matrix ingested. The dependence of mixed micelle properties on the presence of specific food components may prove to be a valuable means of controlling the bioavailability of lipophilic bioactive components.

ASSEMBLY OF LIPID NANOPARTICLES WITHIN ENTEROCYTES: CHYLOMICRONS

Chylomicron Formation

After absorption, the biological fate of bioactive compounds depends on their molecular characteristics, as well as the nature of any coabsorbed lipid digestion products (Porter et al. 1996, Sun et al. 2011, Trevaskis et al. 2008, Yanez et al. 2011). More hydrophilic bioactives travel across the enterocytes and are released into the portal vein, which conducts blood containing the digested

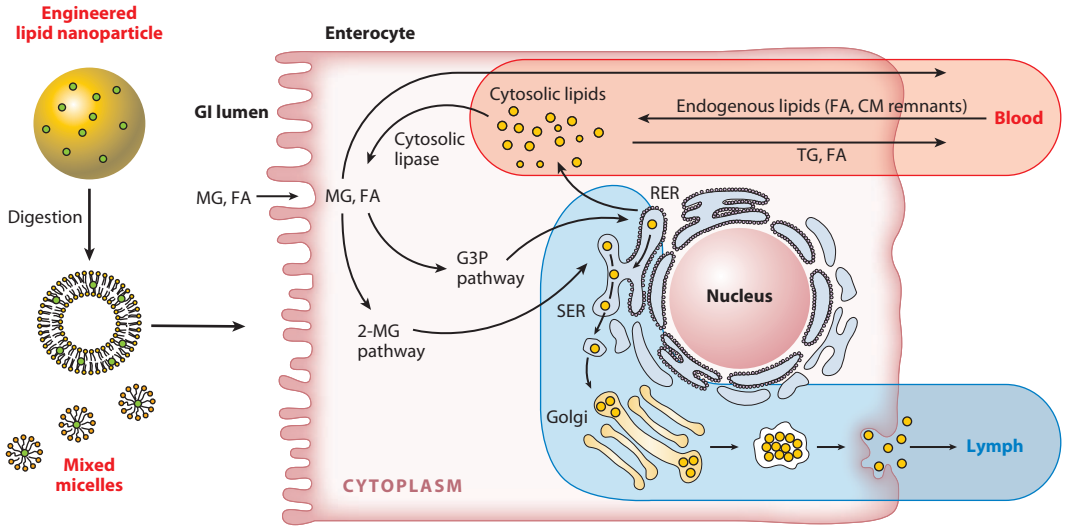

Figure 10

Lipophilic components may be transported by different pathways within the enterocyte depending on their molecular characteristics. After being transported across the apical membrane of the enterocyte, lipid digestion products (monoacylglycerides and fatty acids) can either diffuse across the enterocyte and enter the portal vein blood or be resynthesized into triglycerides. Part of figure (enterocyte cell) kindly provided by Professor Porter from his article "Lipids and Lipid-Based Formulations: Optimizing the Oral Delivery of Lipophilic Drugs" (Porter et al. 2007), with permission. Abbreviations: FA, fatty acid; MG, monoacylglyceride; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; TG, triglyceride.

nutrients and bioactive agents to the liver for processing. These hydrophilic molecules may be transported as individual molecules, or they may be bound to other components (such as carrier proteins). Within the liver, bioactive components usually undergo substantial metabolism before entering the systemic circulation, which may cause them to lose some of their bioactivity before reaching their site of action (Porter & Charman 2001c, Sun et al. 2011, Yanez et al. 2011). Conversely, highly lipophilic bioactive molecules are packaged into lipoprotein particles within the enterocyte cells, which then move into the lymphatic system, where they are released directly into the systemic circulation without experiencing first-pass metabolism in the liver (Abumrad & Davidson 2012, Iqbal & Hussain 2009, Lehner et al. 2012, Pan & Hussain 2012). The bioactivity of labile bioactive components therefore may be increased by targeting their transport to the lymphatic route rather than the portal blood route (**Figure 10**). The transport route therefore has a major impact on the metabolism of many lipophilic bioactive components (F_M).

The nature of the lipoprotein particles formed within the enterocytes depends on the type and amount of lipid digestion products present (Lairon 2008, Xiao & Lewis 2012). In the fed state (which is most important for food-based delivery systems), the predominant lipoprotein particles are CMs (Kindel et al. 2010). CMs consist of a lipophilic core (mainly TAGs, cholesterol, and bioactive components) and a hydrophilic shell (mainly proteins and phospholipids). CMs can be distinguished from other lipoproteins [such as very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs)] based on their physicochemical characteristics, such as density, size, and composition (Hamidi et al. 2006). CMs have been reported to have densities of less than 960 kg m^{-3}; mean diameters between 72 and 1,200 nm; and compositions of 80–95% TAG, 3–6% phospholipid, 2–4% cholesterol esters, 1–3% cholesterol, and 1–2% protein (Hamidi et al. 2006). In addition, they may contain lipophilic bioactive

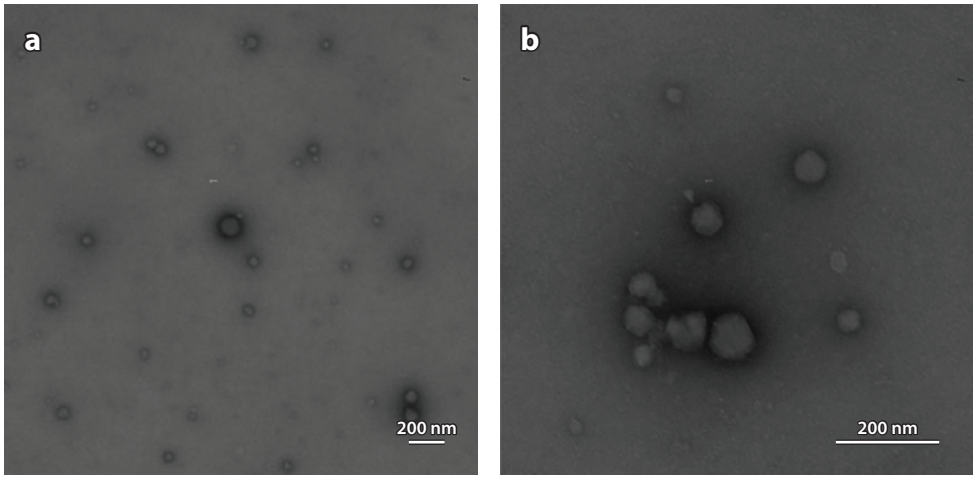

Figure 11

TEM images of lipoproteins (CMs and VLDLs) secreted by Caco-2 cell monolayers after incubation with oleic acid (*a*) and taurocholate acid (*b*) mixed micelles (1.6:0.5 mM) for 24 h (from authors' laboratory). Abbreviations: CM, chylomicron TEM, transmission electron microscopy; VLDL, very low-density lipoprotein.

molecules taken up by the enterocyte cells. CMs are too large to enter directly into the portal vein; thus, they are transported via the lymphatic route (O'Driscoll 2002, Porter & Charman 2001c). **Figure 11** shows electron microscopy images of lipoprotein particles (presumably CMs and VLDLs) produced by Caco-2 cells in response to exposure to lipids (fatty acids and bile salt).

The assembly of CMs within the enterocyte cells is not fully understood, but considerable progress has been made recently due to the important role they play in numerous chronic diseases (Abumrad & Davidson 2012, Iqbal & Hussain 2009, Kindel et al. 2010, Lehner et al. 2012, Xiao & Lewis 2012). A highly simplified schematic diagram of the process is shown in **Figure 10**. Absorbed FFAs and MAGs are transported from the apical side of the enterocytes to the endoplasmic reticulum (ER), which may be mediated by the presence of various binding proteins (Abumrad & Davidson 2012). After reaching the ER, the FFAs and MAGs are reassembled into TAGs by specific enzymes (Abumrad & Davidson 2012). The TAGs formed accumulate within the phospholipid membranes of the ER, eventually causing them to split and release TAG droplets (Kindel et al. 2010). The subsequent formation of lipoproteins requires at least two major structural proteins: microsomal triglyceride transfer protein (MTTP) and apo-lipoprotein B48 (apoB) (Abumrad & Davidson 2012). MTTP facilitates the transfer of TAGs from their initial location within the ER bilayer to the apoB molecule. ApoB is a very hydrophobic protein that is packaged into the TAG droplets to form lipoprotein particles. After formation, these primordial lipoprotein particles undergo further changes within the enterocyte cells to form CMs, e.g., incorporation of additional lipids and proteins (Abumrad & Davidson 2012).

Influence of Molecular Characteristics of Bioactives on Their Lymphatic Transport

Researchers in the pharmaceutical industry have identified many of the key characteristics of lipophilic molecules that determine their ability to be transported via the lymphatic system (Hussain et al. 2001, O'Driscoll 2002, Porter & Charman 2001c, Tso & Simmonds 1984,

Yanez et al. 2011). Two of the most important physicochemical characteristics are the logarithm of the oil-water partition coefficient (log*P*) and the oil solubility (Lipinski et al. 2001, 2012; Lu et al. 2011). The log*P* value provides a measure of the relative affinity of a bioactive component for oil and water phases: the higher the log*P*, the greater the affinity for the oil phase. The oil solubility is the maximum concentration of bioactive that can be dissolved in a specific oil phase at equilibrium. To a first approximation, bioactives with a log$P > 5$ and an oil solubility >50 mg/L are considered to be highly lipophilic compounds that tend to be transported by the lymphatic rather than portal blood route (Yanez et al. 2011). In reality, these two parameters do not accurately predict the behavior of many bioactives; thus, more comprehensive systems have been developed to classify them (Lipinski et al. 2001, 2012).

Influence of Dietary Components on Chylomicron Formation and Properties

Different kinds of lipoproteins are assembled in the enterocytes depending on the nature of the food consumed. In the fed state (high fat), CMs are the major lipoproteins formed within the intestinal enterocytes, whereas in the fasted state (low fat), VLDLs are the predominant lipoproteins formed (Rifai et al. 1990). VLDL particles have smaller diameters (30 to 80 nm) than CM particles (75 to 1,200 nm) and contain lower amounts of TAG. Hence, the ability of VLDLs to carry lipids or bioactive lipophilic compounds is more limited. The assembly of intestinal CM and VLDL particles may occur by separate biochemical pathways (Tso et al. 1984, 1987).

The size and composition of CMs depend on the type and amount of digestible lipids within the digestion medium. Consequently, the biological fate of bioactive components (lymph versus portal vein) can be directed by controlling the composition of coingested lipids in colloidal delivery systems. The mean diameter of CMs separated from the lymph of animals has been reported to increase as the total amount of dietary fat consumed increased (Fraser et al. 1968). However, the total number of CMs produced remained relatively constant, suggesting that individual CMs grew rather than more CMs being produced (Hayashi et al. 1990). Transportation of a lipophilic bioactive compound to the lymph was increased more if coadministered with TAGs containing LCFAs ($C \geq 12$) than with those containing MCFAs ($8 \leq C < 12$) or short-chain fatty acids ($C < 8$) (Caliph et al. 2000). The fatty acids with shorter chain lengths were directly transported into the portal blood, while those with the longer chain lengths were re-esterified into triglycerides in the enterocyte and then incorporated into the core of CMs (Trevaskis et al. 2008, Wermuth 2008). Studies using Caco-2 cells have shown that coadministration of carotenoids with lipids that stimulate the formation of CMs led to a higher concentration of carotenoids on the basolateral side (Chitchumroonchokchai et al. 2004).

The degree of unsaturation of the fatty acids in digestible lipids also influences the nature of the CMs produced and the absorption of lipophilic components (**Table 3**) (Holm et al. 2001, Jackson et al. 2005, Sheehe et al. 1980, van Greevenbroek et al. 1996). For example, cell culture studies have shown that the secretion of CMs after incubation with fatty acids depended on their degree of unsaturation: C18:1 > C18:2 > C18:3 (Field et al. 1988, van Greevenbroek et al. 1996). Other studies suggest that the initial location of the fatty acid chains on the TAG molecule is important (Porsgaard et al. 2005). The presence of MCFAs in the sn-2 position and LCFAs in the sn-1 and sn-3 positions led to larger CMs (Porsgaard et al. 2005) and higher bioactive absorption (Porter et al. 1996) than having the opposite arrangement.

DISASSEMBLY OF CHYLOMICRONS WITHIN THE BODY

Ideally, one would like to know the complete biological fate of any ingested bioactive lipophilic component, such as where it ended up, how it got there, how its structure and activity changed

Table 3 Summary of effects of different fatty acids on CM[a] formation measured using Caco-2 cell models

Micelle solution	Lipoproteins found in basolateral side			Reference
	ApoB secretion[b]	TAG secretion	Lipoprotein density	
Oleic acid (C18:1)-TC	increased or decreased	increased	CM/VLDL <1.006 g/ml	(Luchoomun & Hussain 1999)
Palmitic acid (C16:0)-TC	unchanged	unchanged	IDL/LDL 1.009–1.068 g/ml	(Bateman et al. 2007)
Linoleic acid (18:2)-BSA	increased	increased	CM/VLDL <1.006 g/ml	(van Greevenbroek et al. 1995, 1996)
Linolenic acid (18:3)-BSA	increased, lower than C18:2	increased, lower than C18:2	CM/VLDL <1.006 g/ml	N/A
Stearic acid (18:0)-BSA	increased, but not significantly	unchanged	IDL/LDL, 1.009–1.068 g/ml	(van Greevenbroek et al. 1996)
Eicosapentaenoic acid (20:5, n-3)-TC	unchanged	decreased	CM/VLDL <1.006 g/ml	(Ranheim et al. 1994)

[a]Abbreviations: BSA, bovine serum albumin; CM, chylomicron; LDL, low-density lipoprotein; TAG, triacylglycerol; TC, taurocholate; VLDL, very low-density lipoprotein.
[b]ApoB and TAG secretion is compared to a no lipid treatment.

during transport, how long it remained there, and what biochemical pathways it altered. Some progress has been made in this area in the pharmaceutical industry, but knowledge of this area is relatively poor for lipophilic nutraceuticals. After CMs leave the enterocyte cells, they pass through the lymphatic system and are then released into the systemic blood circulation via the thoracic duct (near the neck) (Kindel et al. 2010, Xiao & Lewis 2012, Yanez et al. 2011). The properties of the CMs are then altered as they pass through the various regions of the body. Additional proteins adsorb to the surfaces of the CMs, whose role is believed to regulate their subsequent degradation by lipoprotein lipases in peripheral tissues (Lehner et al. 2012). TAGs in CMs undergo lipolysis in the systemic circulation, thereby delivering FFAs, MAGs, and possibly lipophilic bioactive components to the tissues (such as adipose tissue, mammary, heart, and skeletal muscle (Blanchette-Mackie & Scow 1971). TAG hydrolysis causes the CMs to shrink in size, leaving a CM remnant that is ultimately taken up by the liver (Cooper 1997, Lusis & Pajukanta 2008). A link has been suggested between elevated CM levels in the blood after consumption of a fatty meal and chronic diseases such as cardiovascular disease (Jackson et al. 2012, Kei et al. 2012, Vine et al. 2008). The nature of the TAGs within CMs may influence their ability to promote these diseases, e.g., fatty acid chain length and degree of unsaturation (Jagla & Schrezenmeir 2001). Replacement of saturated fats with unsaturated fats has been advocated as a means of reducing the risk of cardiovascular disease. However, polyunsaturated fats may promote lipid oxidation, thereby contributing to atherosclerosis, although this is still a matter of debate (Napolitano et al. 2004). It may therefore be important to carefully design delivery systems so that they increase the bioactivity of encapsulated lipophilic components but do not adversely affect health by promoting chronic diseases.

CONCLUSIONS

Nanotechnology has emerged as one of the most powerful tools for controlling the behavior of matter. It is finding increasing utilization within the food industry to improve food safety, quality, and healthfulness. Recently, there have been rapid advances in the fabrication and characterization

of ELNs suitable for utilization within the food industry. In particular, lipid nanoparticles have been identified as powerful building blocks for the creation of delivery systems designed to encapsulate, protect, and release lipophilic bioactive food components (nutraceuticals) and drugs (pharmaceuticals). This review article has shown that ingested ELNs may be disassembled within the human GIT and reassembled into biological lipid nanoparticles (mixed micelles and vesicles) in the small intestine, which are disassembled and reassembled into still other kinds of biological lipid nanoparticles in the enterocyte cells (CMs). Lipophilic bioactive components that are incorporated into CMs are transported into the systemic (blood) circulation via the lymphatic system and therefore avoid first-pass metabolism in the liver. The CM-lymphatic pathway plays an essential role in improving the oral bioavailability of many lipophilic drugs and may also be beneficial for the design of effective delivery systems for lipophilic nutraceuticals.

The extent of lymphatic transportation can be regulated by controlling the size, number, and composition of the CMs, which can in turn be regulated by controlling the properties of the ingested ELNs. Improved knowledge of the influence of the molecular characteristics of bioactive components and the composition and structure of ELNs may lead to the rational design of lipid-based delivery systems for food and beverage applications. These delivery systems may be used to incorporate bioactive lipids into functional food products designed to improve human health and wellness.

Some concern has been raised about the potentially adverse effects of using nanoparticles in foods, as the biological fate of nanoparticles may be different from that of larger particles. In particular, nanoparticles may greatly increase the amount of an encapsulated bioactive substance that is absorbed by the body, or they may be absorbed themselves with unintentional health effects. The potential biological fate and toxicity of nanoparticles have been reviewed recently (McClements & Xiao 2012, McClements 2013) and as such have not been considered further here.

DISCLOSURE STATEMENT

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Errata

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