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# Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products

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**Abstract**

An evaluation of the in-vitro digestion profile and phase behaviour of the common formulation lipids Miglyol 812 (medium chain triglyceride, MCT), Capmul MCM ($C_8/C_{10}$ monoglyceride/diglyceride mixture), soybean oil (long chain triglyceride, LCT) and Maisine 35-1 ($C_{18}$ monoglyceride/diglyceride mixture), is described. Experiments were conducted using titrimetric, high-performance thin-layer chromatographic (HPTLC) and ultracentrifugational techniques under model fasted and post-prandial intestinal conditions. The rate and extent of digestion of the medium chain lipids was greater than the corresponding long chain lipids, and independent of bile salt concentration, with complete conversion to monoglyceride and fatty acid occurring after 30 min digestion. The long chain lipid digests separated into an oily phase (containing undigested triglyceride and diglyceride), an aqueous phase (containing bile salt, fatty acid and monoglyceride) and a pellet phase (containing approximately 5 mM of fatty acid, presumably as an insoluble soap) after ultracentrifugation. Higher proportions of long chain fatty acid and monoglyceride were dispersed into the aqueous phase with increasing bile salt concentrations. In contrast, medium chain lipolytic products separated only into an aqueous phase and a pellet fraction in a bile-salt-independent manner. The digestion of both the $C_8/C_{10}$ and $C_{18}$ monoglyceride/diglyceride lipid mixtures was more rapid than the corresponding triglyceride, especially at early time points. This investigation provides insight into the relative digestion kinetics of medium chain and long chain lipids and provides information regarding the phase behaviour of their lipolytic products under conditions modelled on those expected after oral administration. The data also provide a background for improved understanding of the potential utility of long chain and medium chain lipid-based formulations.

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## Introduction

Lipid-based oral delivery systems have been utilised as a strategy to enhance the bioavailability of poorly water-soluble, lipophilic drugs (Humberstone & Charman 1997). Lipids may enhance the absorption of poorly water-soluble, lipophilic drugs by their actions on gastric transit, intestinal permeability or drug metabolism, but are perhaps most widely used to enhance drug solubilisation and dissolution in the gastrointestinal tract (Constantinides 1995; Charman et al 1997). Triglyceride lipids and their digestion products may enhance drug solubilisation and dissolution by stimulating bile salt and phospholipid secretion into the gastrointestinal lumen (Hernell et al 1990) and by enhancing the solubilisation capacity of endogenous bile

salt/phospholipid mixed micelles by intercalation into the micellar structure (Laher & Barrowman 1983; Vetter et al 1985).

A diverse range of lipids has been utilised for inclusion in lipid-based formulations including triglycerides and mixed monoglycerides and diglycerides consisting of medium chain ($C_8$–$C_{12}$) and long chain ($C_{16}$–$C_{22}$) fatty acids. Hydrolysis of triglycerides by lingual and gastric lipase and most importantly by the pancreatic lipase/colipase complex, proceeds as a two-step reaction. Firstly, hydrolysis of triglyceride yields a single fatty acid and diglyceride. Secondly, the diglyceride is hydrolysed to produce a second fatty acid and the corresponding 2-monoglyceride. Although 2-monoglyceride may undergo slow isomerisation to 1-monoglyceride, which may then be hydrolysed to yield a third fatty acid and glycerol, this process is generally believed to be limited in-vivo (Mattson & Volpenhein 1964). The physical phase behaviour of these lipolytic products, during lipid digestion and in the presence of endogenous amphipaths such as bile salt, lecithin and lysolecithin, is complex. While numerous studies of the physicochemical characteristics of lipid digestion have been conducted in humans following a test meal (Ricour & Rey 1970; Porter & Saunders 1971; Hofmann & Roda 1984; Hernell et al 1990; Staggers et al 1990; Armand et al 1996), the digestion and phase behaviour of typical formulation lipids has not been addressed in detail. Importantly, the intestinal phase behaviour of formulation-derived lipids and their digestion products are expected to play a significant role in the solubilisation profile of co-administered drugs.

The design of lipid-based formulations is often empirically based, principally as a consequence of the limited understanding of the mechanisms by which lipids improve absorption, and the lack of rapid in-vitro models that are predictive of bioavailability. Design strategies for lipid-based formulations have generally focused on optimising the solubility of the drug in the lipid vehicle, improving the in-vitro dispersion of the formulation (Constantinides 1995; Khoo et al 1998), or enhancing the digestibility of the lipid vehicle (MacGregor et al 1997). However, the fate of the co-administered drug as the formulation is exposed to, and is digested and dispersed by the gastrointestinal environment, remain to be elucidated. Greater understanding of the interaction between a co-administered drug and the digesting oily vehicle during pre-absorptive intraluminal processing may lead to a more guided selection of the ideal lipids for incorporation into a lipid-based formulation. To identify the key characteristics of lipid–drug combinations, a methodology for probing the digestion characteristics of different formulation lipids, the phase behaviour of their lipolytic products (fatty acids, monoglycerides and diglycerides) and the fate of a co-administered drug has been developed. The aim of the current study was to evaluate the digestion profiles of both long and medium chain triglycerides and their corresponding monoglyceride/diglyceride variants and the phase behaviour of their lipolytic products, integrating titrimetric techniques, high-performance thin-layer chromatography (HPTLC) and ultracentrifugation methods. The data obtained will provide a platform for the subsequent identification of optimal combinations of formulation lipids and lipophilic drugs to maximise oral absorption.

## Materials and Methods

### Materials

Sodium taurodeoxycholate (NaTDC), tributyrin, trizma maleate and type X-E L-$\alpha$-lecithin (L-$\alpha$-phosphatidylcholine, approximately 60% pure phosphatidylcholine, from dried egg yolk) were obtained from Sigma Chemical Co. (MO). Miglyol 812 (Hüls, UK), Capmul MCM (Abitec Corporation, Janesville, WI), soybean oil (Sigma Chemical Co.), and Maisine 35-1 (Gattefossé s.a., Saint-Priest, France) were employed as a source of $C_8/C_{10}$ triglyceride, $C_8/C_{10}$ monoglyceride/diglyceride blend, $C_{18}$ triglyceride and $C_{18}$ monoglyceride/diglyceride blend, respectively. Calcium chloride dihydrate (analytical grade) was purchased from BDH Chemicals (Melbourne, Australia). 1 M sodium hydroxide (Titrisol, Merck) was used to prepare 0.2 and 0.6 M NaOH titration solutions. Pancreatin (Sigma Chemical Co., MO) from porcine pancreas (activity equivalent to 8 × USP specification) was used as a source of pancreatic lipase and colipase. Polyallomer centrifuge tubes (11 × 60 mm) were purchased from Beckman Instruments Inc. (Palo Alto, CA). All solvents were of HPLC grade (Mallinckrodt, KY). All material was used as received. Water was obtained from a Milli-Q (Millipore, MA) water purification system.

### In-vitro lipid digestion

The progress of in-vitro lipid digestion was monitored indirectly by pH-stat titration and directly by HPTLC analysis. Fasted and fed intestinal contents were simulated utilising 5 and 20 mM bile salt, respectively. By way of comparison, in duodenal contents, total bile salt con-

centrations of 5.9±1.8 mM in fasting samples (Armand et al 1996) and 14.5±9.4 mM (Fausa 1974) 30 min after a test meal have been reported. Phospholipids were included in the digestion mixture at a bile salt–phospholipid molar ratio of 4:1, which is the ratio secreted in bile (Schersten 1973). The pancreatic lipase and colipase activity (1000 IU $mL^{-1}$) employed was also within the physiological range (Borgström & Donnér 1975; Armand et al 1996). The pH of the digestion buffer selected was slightly higher than that reported in postprandial duodenal contents (pH range 6.0–7.0 (Armand et al 1996)) to ensure a high degree of fatty acid ionisation and therefore efficient titration (Patton & Carey 1981).

In-vitro lipolysis experiments were performed as described previously (Sek et al 2001), and utilised a pH-stat automatic titration unit (Radiometer, Copenhagen, Denmark). Standard digestion buffer comprised of (in mM): 50 Tris-maleate (pH 7.5); 150 NaCl and 5 $CaCl_2 \cdot 2H_2O$. Sodium taurodeoxycholate (NaTDC) was employed as a model bile salt and lecithin (type X-E; approximately 60% pure phosphatidylcholine) was utilised as a source of phospholipid for the preparation of phospholipid/bile salt mixed micelles. Phospholipid/bile salt mixed micelles were prepared as described previously (Sek et al 2001). Pancreatin extract was prepared by adding 1 g of porcine pancreatin powder (containing pancreatic lipase and colipase) to 5 mL of digestion buffer. This mixture was stirred for 15 min followed by centrifugation at approximately 1600 *g* and 5°C for 15 min. The supernatant was collected and stored on ice. Fresh pancreatin extracts were prepared each day. Pancreatic lipase activity determination was undertaken using 6 mL of tributyrin in 10 mL of digestion buffer at pH 7.5 and 37°C. The activity of pancreatic lipase was expressed in terms of tributyrin units (TBU), where 1 TBU is the amount of enzyme that can liberate 1 μmol of titratable fatty acid from tributyrin per min. The pancreatic lipase activity of the pancreatin employed was 50 TBU per mg of dry pancreatin powder. The NaOH concentrations employed during long and medium chain lipid digestion were 0.2 and 0.6 M, respectively.

The progress of lipolysis of soybean oil ($C_{18}$ triglyceride), Miglyol 812 ($C_8/C_{10}$ triglyceride), Maisine 35-1 ($C_{18}$ monoglyceride/diglyceride) and Capmul MCM ($C_8/C_{10}$ monoglyceride/diglyceride) was determined in separate experiments. For each digestion experiment, 250 mg of lipid was crudely emulsified in 9 mL of digestion buffer before enzyme addition by stirring continuously for 10 min in the jacketed glass reaction vessel, after which time the pH was re-adjusted with NaOH or HCl to 7.500±0.001. One millilitre of pancreatin extract (containing 10000 TBU of pancreatic lipase activity) was then added to initiate lipolysis. Lipolysis was allowed to continue for 30 min.

In addition to other unknown components, the lecithin used to prepare phospholipid/bile salt micelles contained small amounts of triglyceride (~ 1% w/w), diglyceride (~ 6% w/w) and fatty acid (< 1% w/w), which were digested by pancreatic lipase/colipase to produce fatty acid. Phosphatidylcholine was also hydrolysed by phospholipase $A_2$ (present in pancreatin) to produce fatty acid and lyso-phosphatidylcholine. To compensate for the additional fatty acid produced by these digestion processes (i.e. other than by digestion of added triglyceride), blank experiments were performed. Blank digestion experiments were performed in the same manner as the experimental run, except that triglyceride was not added to the phospholipid/bile salt mixed micellar solutions. Digestion of the lipids under study was corrected for background fatty acid production by subtraction of the data obtained in the blank digestion runs.

During blank and lipid digestion experiments, 100-μL samples were collected at time 0 (before enzyme addition), 1, 2, 3, 4, 5, 10 and 30 min. The 100-μL portions of digestion samples were acidified with 50 μL of 1 M HCl and diluted with 2850 μL of chloroform–methanol (2:1 v/v) in polypropylene tubes, before analysis of fatty acid, monoglyceride, diglyceride and triglyceride content using previously validated HPTLC techniques (Sek et al 2001).

### Separation and collection of phases in digestion samples

At the end of each digestion experiment, two samples of 4 mL of digestion mixture were transferred into polyallomer centrifuge tubes and ultracentrifuged (Model Optima XL-100K; Beckman, Palo Alta, CA) at 334000 *g* for 30 min at 37°C utilising an SW-60 swinging bucket rotor to separate the different digestion phases. During ultracentrifugation, the lipid digests separated into an oily layer, an aqueous phase and a precipitated pellet. Each fraction was collected from each tube and combined together in the following manner. The floating oily phase was aspirated using a disposable pipette and transferred into a 10-mL volumetric flask. The aqueous phase was aspirated into a 5-mL syringe by penetrating the side of the tube with a 23-guage needle (Terumo 0.65×25 mm) and transferred into 12-mL polypropylene tubes. Lastly, the pellet fraction was dissolved in chloroform–methanol (2:1 v/v) and transferred to a 10-

mL volumetric flask. Both the oily and pellet fractions were acidified with 100 $\mu$L of 1 M HCl and made up to volume with chloroform–methanol (2:1 v/v). Each 100-$\mu$L sample of the aqueous phase was acidified with 50 $\mu$L of 1 M HCl and diluted with 2850 $\mu$L of chloroform–methanol (2:1 v/v) in polypropylene tubes. All digestion samples dissolved in chloroform–methanol (2:1 v/v) were centrifuged at approximately 1600 *g* for 15 min at 25°C to remove any insoluble precipitates before analysis. Lipid classes (triglycerides, diglycerides, monoglycerides and fatty acids) were assayed using HPTLC, employing a previously published and validated HPTLC method (Sek et al 2001).

## Results and Discussion

### Hydrolysis of long chain lipids and phase behaviour of their lipolytic products

Figure 1A depicts the fatty acid concentrations determined by pH-stat titration during long chain triglyceride (LCT) digestion under simulated fasted and fed conditions. Immediately before enzyme addition (time 0), a crude emulsion of LCT and phospholipid/bile salt mixed micelles was evident as small oil droplets in a clear slightly yellow solution. After enzyme addition, the mixture became white and cloudy as lipolysis proceeded, presumably as a function of calcium fatty acid soap formation (Patton et al 1985). Figure 2A and 2B illustrates, in addition to changes in fatty acid concentration, the changes in monoglyceride, diglyceride and triglyceride levels during LCT digestion, as quantified by HPTLC (Sek et al 2001). LCT digestion mixtures in the presence of bile salts and phospholipids have been shown previously to be sufficiently homogeneous to allow reproducible and accurate sampling (Sek et al 2001), thereby facilitating accurate monitoring of the progress of lipid digestion by HPTLC analysis. The changes in lipolytic product concentration during the 30-min digestion of LCT (Figure 2A, 2B) were qualitatively in agreement with previous studies (Borgström 1954; Lykidis et al 1994, 1995). As lipolysis proceeded, there was a continuous decrease in triglyceride, a transient accumulation in diglyceride and an increase in fatty acid and monoglyceride. After the first 10 min, the rate of diglyceride and triglyceride hydrolysis decreased. The reduction in the rate of lipolysis may be due to the saturation of phospholipid/bile salt micelles with lipolytic products. Phospholipid concentrations also gradually decreased (data not shown) as hydrolysis by phospholipase $A_2$ (present in pancreatin extract) occurred, producing further fatty acid and lyso-phosphatidylcholine. During LCT digestion, the rate and extent of fatty acid production under fed-state conditions was approximately 2-fold higher than that observed under fasted-state conditions. In both cases, LCT was partially digested over 30 min with 57.9$\pm$6.0% and 39.4$\pm$5.0% triglyceride remaining under fasted- and fed-state conditions, respectively (Figure 2A, 2B). Maisine 35-1 ($C_{18}$ monoglyceride/diglyceride), which is comprised primarily of long chain monoglyceride and diglyceride and a small amount of triglyceride (38, 48, and 13% w/w of monoglyceride, diglyceride and triglyceride, respectively; Product information, Gattefossé, France), may be regarded as a partially digested variant of the LCT commonly used in lipid-based formulations. The digestion of $C_{18}$ monoglyceride/diglyceride (Figure 1B) was much more efficient than that of LCT, presumably reflecting the more amphiphilic nature of the monoglyceride and diglyceride, thereby facilitating improved dispersion into the phospholipid/bile salt micelles. Before digestion, a white emulsion was formed on dispersion of the monoglyceride and diglyceride in the phospholipid/bile salt mixed micellar media. The digestion of $C_{18}$ monoglyceride/diglyceride showed an initial rapid decline in monoglyceride and diglyceride in the first 10 min followed by a more gradual decrease (Figure 2C, 2D). Unlike LCT digestion where an accumulation of monoglyceride was noted during hydrolysis, in the case of $C_{18}$ monoglyceride/diglyceride blend, the monoglyceride was rapidly digested, which may reflect the differences in monoglyceride isomer present. Pancreatic lipase has a relatively higher specificity for the outer chain ester bonds of triglyceride and is more active against the 1-monoglyceride than the 2-monoglyceride isomer (Hofmann 1963). Consequently, during triglyceride hydrolysis, 1,2-diglycerides and 2-monoglycerides are more commonly formed, whereas in Maisine 35-1 approximately 90% of the monoglyceride is present as the 1-monoglyceride isomer (Product information, Gattefossé, France).

Similar to the LCT data, the rate and extent of digestion of $C_{18}$ monoglyceride/diglyceride was also higher under fed- rather than fasted-state conditions (Figure 1B). The higher rate and extent of long chain lipid digestion with greater bile salt concentrations is in agreement with previous studies (Borgström & Erlanson 1973; Alvarez & Stella 1989; MacGregor et al 1997). In the absence of colipase, bile salt is generally found to inhibit the ability of pancreatic lipase to digest LCT, and the mechanism of this inhibition has been suggested to be via bile-salt-mediated desorption of the lipase

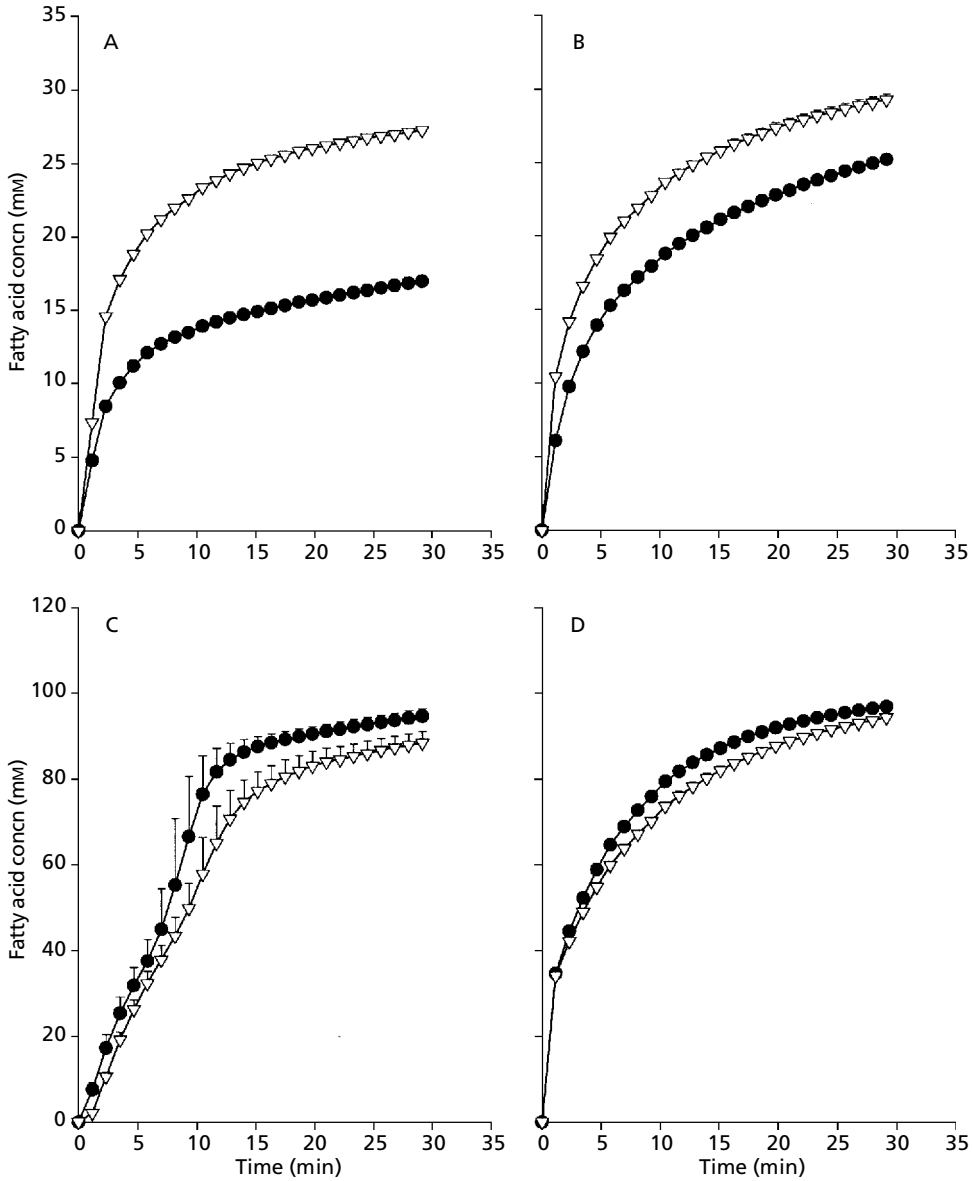


**Figure 1** Fatty acid concentrations as determined by pH-stat titration techniques during a 30-min digestion period for long chain triglyceride (LCT) (A), $C_{18}$ monoglyceride/diglyceride (B), medium chain triglyceride (MCT) (C) and $C_8/C_{10}$ monoglyceride/diglyceride (D). Digests were conducted under simulated fasted (●) (1.25 mM phosphatidylcholine, 5 mM sodium taurodeoxycholate (NaTDC)) and fed (▽) (5 mM phosphatidylcholine, 20 mM NaTDC) conditions. Data are mean±s.d., n = 3. In some cases, the error bars are within the size of data points.

from the oil/water interface (Vandermeers et al 1976). In contrast, colipase binds avidly to the oil/water interface and acts to anchor pancreatic lipase to the interface, precluding pancreatic lipase desorption, even in the presence of bile salt. Indeed, in the presence of colipase, bile salts increase the initial rate of fatty acid release during LCT digestion (Borgström & Erlanson 1973), most likely by reducing the emulsion droplet size, thereby increasing the surface area available for pancreatic lipase/colipase binding. Bile salts also play a role in the removal of the poorly water-soluble lipolytic products from the oil/water interface by solubilisation (Carey et al 1983; MacGregor et al 1997). In contrast, phospholipids appear to inhibit pancreatic lipase/colipase activity (Larsson & Erlanson-Albertsson 1986). However, the inhibitory effect of phospholipids in the presence of bile salts is dependent on a number of factors including substrate concentrations, the ratio of each amphiphile

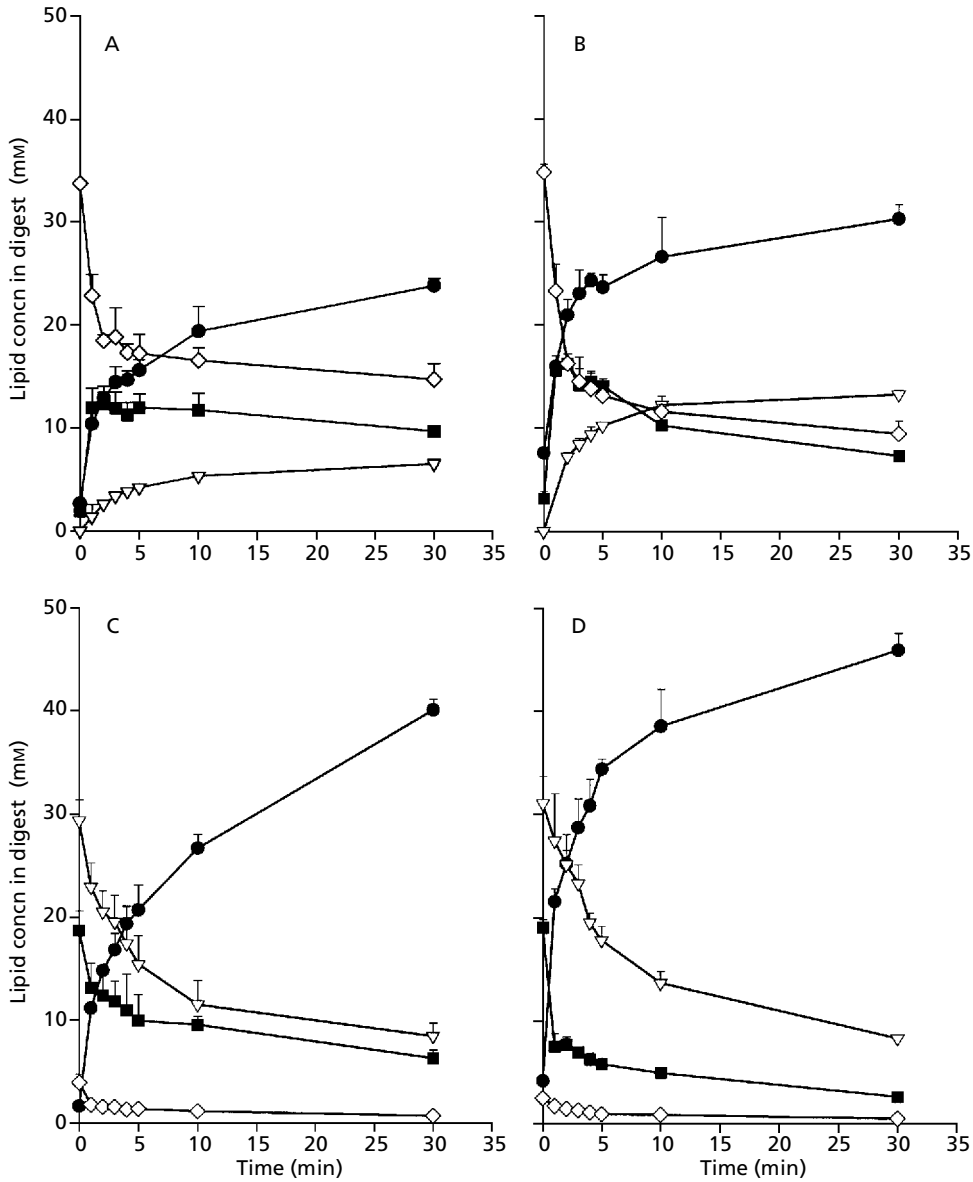


**Figure 2** The time course of hydrolysis of long chain triglyceride (LCT) under fasted (1.25 mM phosphatidylcholine, 5 mM sodium taurodeoxycholate (NaTDC)) (A) and fed (5 mM, phosphatidylcholine, 20 mM NaTDC) (B) conditions and $C_{18}$ monoglyceride/diglyceride under fasted (C) and fed (D) conditions during a 30-min digestion period. Fatty acids (●); monoglycerides (▽); diglycerides (■); triglycerides (◇). Data are mean±s.d., n = 3.

and the relative concentrations of colipase and inhibitor present (Patton & Carey 1981; Lykidis et al 1997). Two alternate mechanisms have been suggested to explain this inhibition. Firstly, that mixed phospholipid/bile salt micelles may form an alternate binding site for colipase, leading to reduced interfacial colipase concentrations and diminished binding of pancreatic lipase to the oil/water interface (Patton & Carey 1981). Secondly, that the presence of phospholipid at the interface may interfere with the binding of the lipase/colipase complex (Carey et al 1983). The former explanation appears more likely since removal of phospholipid from the aqueous phase (and not the droplet surface) has been shown to attenuate the phospholipid-mediated inhibition (Larsson & Erlanson-Albertsson 1986). In the current study, as in previous studies (Alvarez & Stella

**Table 1** Distribution of long chain lipid digestion products across the different physical phases formed following 30 min digestion of long chain triglycerides (LCT) and $C_{18}$ monoglyceride/diglyceride.

| Lipid | LCT | | | $C_{18}$ monoglyceride/diglyceride | | |
|---|---|---|---|---|---|---|
| | Control | Fasted | Fed | Control | Fasted | Fed |
| Oil phase | | | | | | |
| Fatty acid | 14.9±0.1 | 2.3±0.5 | 2.3±0.1 | 24.4±6.0 | 10.3±0.7 | 1.4±0.4 |
| Monoglyceride | 6.5±0.1 | 0.8±0.1 | 1.5±0.1 | 18.9±7.8 | 3.2±0.6 | 0.3±0.1 |
| Diglyceride | 6.8±0.1 | 6.9±0.4 | 5.3±0.2 | 0.9±0.3 | 2.8±0.3 | 0.9±0.2 |
| Triglyceride | 11.2±0.1 | 11.0±2.2 | 6.5±0.1 | – | 0.6±0.4 | 0.5±0.1 |
| Aqueous phase | | | | | | |
| Fatty acid | 2.5±0.0 | 21.3±2.8 | 35.0±2.5 | 1.8±0.5 | 26.2±1.6 | 49.0±1.2 |
| Monoglyceride | – | 4.2±0.4 | 11.2±0.6 | – | 3.7±0.5 | 5.3±0.2 |
| Diglyceride | – | 0.8±0.7 | 2.0±0.2 | – | 0.7±0.1 | 1.3±0.2 |
| Triglyceride | – | – | – | – | – | – |
| Pellet phase | | | | | | |
| Fatty acid | 7.9±0.1 | 5.9±0.7 | 4.8±0.2 | 3.1±0.2 | 5.1±0.5 | 5.3±0.9 |
| Monoglyceride | – | 0.1±0.1 | 0.2±0.1 | – | – | – |
| Diglyceride | – | 0.1±0.1 | 0.1±0.0 | – | – | – |
| Triglyceride | – | – | – | – | – | – |

Digests were performed under control (without phosphatidylcholine and sodium taurodeoxycholate (NaTDC)), fasted (1.25 mM phosphatidylcholine, 5 mM NaTDC), and fed (5 mM phosphatidylcholine, 20 mM NaTDC) conditions. The numeric data in the table refer to the amount of each particular lipid (fatty acid, monoglyceride, diglyceride or triglyceride) ($\mu$mol) in each phase (oil phase, aqueous phase or pellet) per 1 mL of digest, mean±s.d., n = 3. Dashes indicate values below the limit of quantification (0.04, 0.03, 0.02 and 0.01 mM for fatty acids, monoglycerides, diglycerides and triglycerides, respectively).

**Table 2** Visual appearance of the aqueous phase post ultracentrifugation of medium chain triglyceride (MCT), $C_8/C_{10}$ monoglyceride/diglyceride, long chain triglyceride (LCT) and $C_{18}$ monoglyceride/diglyceride digests under control (without phosphatidylcholine and sodium taurodeoxycholate (NaTDC)), fasted (1.25 mM phosphatidylcholine, 5 mM NaTDC), and fed (5 mM phosphatidylcholine, 20 mM NaTDC) conditions.

| Lipid | Visual turbidity | | |
|---|---|---|---|
| | Control | Fasted | Fed |
| LCT | +/− | ++ | +/− |
| $C_{18}$ monoglyceride/diglyceride | +/− | +++ | + |
| MCT | + | +/− | − |
| $C_8/C_{10}$ monoglyceride/diglyceride | − | − | − |

− clear, +/− clear to slightly turbid, + low turbidity, ++ medium turbidity, +++ high turbidity.

1989; MacGregor et al 1997), the net effect of phospholipid/bile salt addition in a fixed ratio appears to stimulate the digestion of long chain lipids.

The distribution of the long chain lipolytic products across the digestion phases obtained after 30 min of lipid digestion is presented in Table 1. Post ultracentrifugation, LCT and $C_{18}$ monoglyceride/diglyceride digests separated into an oily phase, an aqueous phase and a pellet phase under fasted- and fed-state conditions. An additional diffuse layer of a white paste-like material (composed of fatty acid and monoglyceride) was present between the oily phase and aqueous phase of $C_{18}$ monoglyceride/diglyceride digests under fasted conditions, which for the purpose of these experiments were combined with the oily phase for analysis. The presence of a similar white pasty phase has been noted previously in potassium oleate systems in the absence of bile salt over a pH range of approximately 7.0–8.0, which was thought to be a metastable cubic phase (water in oil lattice) (Cistola et al 1988). The oily phase in these studies comprised primarily of triglyceride and diglyceride with small amounts of monoglyceride and fatty acid. The white precipitated pellet contained fatty acid only and was not examined further. The aqueous phase consisted primarily of fatty acid and monoglyceride (Table 1). All bile salts distributed into the aqueous phase (data not shown).

Interestingly, even in the absence of bile salt, digestion proceeded to a similar extent to that seen under fasted conditions, although the distribution profile of the lipolytic products was notably different. These findings

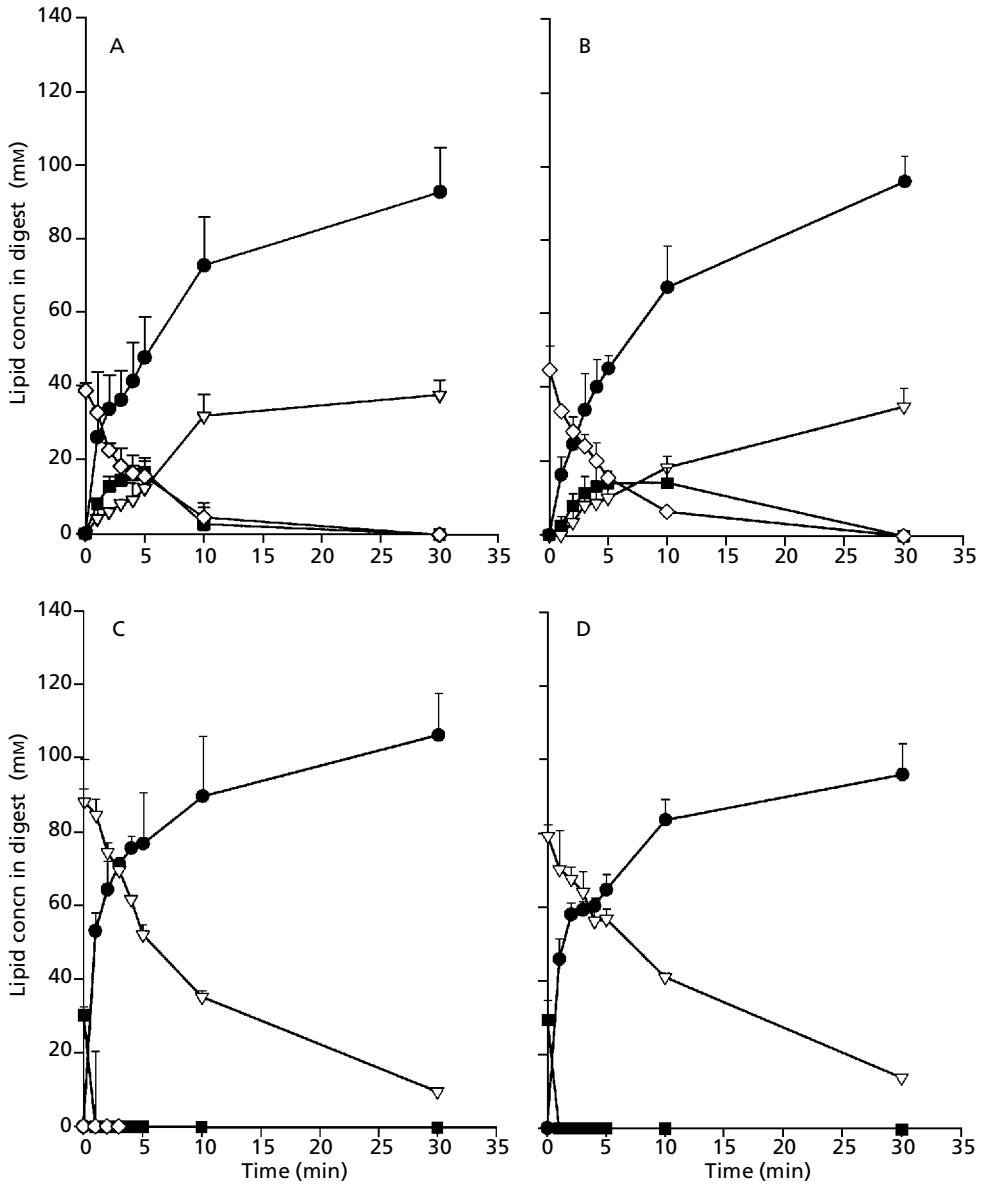


**Figure 3** The time course of hydrolysis of medium chain triglyceride (MCT) under fasted (1.25 mM phosphatidylcholine, 5 mM sodium taurodeoxycholate (NaTDC)) (A) and fed (5 mM phosphatidylcholine, 20 mM NaTDC) (B) conditions and $C_8/C_{10}$ monoglyceride/diglyceride under fasted (C) and fed (D) conditions during a 30-min digestion period. Fatty acids (●); monoglycerides (▽); diglycerides (■); triglycerides (◇). Data are mean±s.d., n = 3.

demonstrate the limitations of assessment of lipid digestion only in terms of fatty acid production (e.g. by titration methods) and further indicate the utility of more thorough techniques that include assessment of phase behaviour. In the absence of bile salt, lipid digestion products of LCT and $C_{18}$ monoglyceride/diglyceride formed a solid white plug on top of a predominantly clear aqueous phase (containing approximately 2.5 mM fatty acid), post ultracentrifugation (Table 1). In the presence of fasted bile salt concentrations, a larger proportion of the fatty acid and monoglyceride was distributed into the aqueous phase, but the relatively high lipid/bile salt mole ratios (range 2.4–6.2) resulted in the production of a turbid aqueous phase (Table 2), presumably indicating the presence of incompletely micellar-solubilised lipids, and the co-

existence of micellar, liquid crystalline and liposomal or vesicular structures (Cistola et al 1988; Staggers et al 1990). At higher (fed) bile salt levels the aqueous phase became less turbid, reflecting improved micellar solubilisation. In agreement with these data, substantial turbidity was also observed by Staggers et al (1990) in aqueous buffer (pH 6.5) systems containing lipids (oleic acid, monoolein and diolein) and mixed bile salt systems at lipid–bile salt molar ratios of greater than 0.68. Using light scattering techniques Staggers et al (1990) further demonstrated that the increase in turbidity at lipid–bile salt ratios above 0.68 was a function of a sharp increase (from 32 Å to 350–600 Å) in the mean hydrodynamic radii of the dispersed colloidal systems, and that this increase in particle size reflected the co-existence of unilamellar vesicles and micelles at these lipid–bile salt ratios.

Patton & Carey (1979) have also previously examined the distribution profile of the lipolytic products of LCT. In these studies olive oil was emulsified with gum arabic and digested for 12 min with 2 mM Tris-maleate (pH 6.5), 8 mM bile salt, 10 mM $CaCl_2$ and 24 TBU $mL^{-1}$ of lipase and colipase. After centrifugation at 50000 *g* for 15 min, the percentage of total lipids in the oil, aqueous and pellet fractions were $82.8 \pm 5.2$, $13.1 \pm 2.7$ and $4.0 \pm 1.7\%$, respectively. In this study, the higher proportions of lipolytic products dispersed into the aqueous phase in both fasted- and fed-state conditions (Table 1) may be attributed to the higher pH, temperature and higher (and more physiological) enzyme concentrations utilised, leading to an increase in the production of the more amphiphilic digestion products.

### Hydrolysis of medium chain lipids and phase behaviour of their lipolytic products

Figure 1C, 1D illustrates the fatty acid titration profile obtained during medium chain triglyceride (MCT) and $C_8/C_{10}$ monoglyceride/diglyceride digestion under simulated fasted- and fed-state conditions. Figure 3 shows the time course of hydrolysis of medium chain lipids determined by HPTLC. MCT hydrolysis (Figure 3A, 3B) was essentially complete after 30 min with only fatty acid and monoglyceride remaining. Diglyceride transiently accumulated in the first 5 min, but was further hydrolysed to monoglyceride and fatty acid. Hydrolysis of $C_8/C_{10}$ monoglyceride/diglyceride blend was even more rapid than MCT with almost complete conversion to fatty acid (Figure 3C, 3D). Unlike $C_8/C_{10}$ monoglyceride/diglyceride digestion, accumulation of monoglyceride occurs during MCT hydrolysis, which as described for the long chain system may be due to the production of the poorly digested 2-monoglyceride isomer, as opposed to the complete digestion of the 1,3-diglyceride isomer, principally present in Capmul MCM (Product information, Abitec Corporation, Janesville, WI). In contrast to long chain lipid digestion, the digestion profiles of medium chain lipids were similar under both fasted- and fed-state conditions (Figure 1C, D). In the absence of bile salt, the initial maximum rate of hydrolysis was slower than under fasted- and fed-state conditions (data not shown), although the extent of digestion after 30 min was not significantly different.

Since digestion was effectively complete, MCT and $C_8/C_{10}$ monoglyceride/diglyceride digests separated into an aqueous phase and pellet phase, without an oily phase, post ultracentrifugation (Table 3). Compared with the long chain lipolytic products, a significantly higher proportion of the medium chain lipolytic products (approximately 80%) dispersed into the aqueous phase, and this profile was independent of bile salt. The aqueous phase (comprising approximately 100 mM fatty acid and 20 mM monoglyceride) obtained after MCT digestion in the absence of bile salt was turbid (Table 2), suggesting the presence of larger colloidal structures, whereas, at increasing bile salt concentrations, the turbidity was reduced, indicating improved solubilisation of the lipolytic products into mixed micelles. In contrast to MCT digests, the aqueous phase (comprising 100 mM $C_8/C_{10}$ fatty acid only) obtained after $C_8/C_{10}$ monoglyceride/diglyceride digestion was clear even in the absence of bile salt. These data suggest that the turbidity present in the MCT digests results from the presence of monoglyceride (which was not present in the post-digestion aqueous phase of the $C_8/C_{10}$ monoglyceride/diglyceride digests) and is consistent with the relatively low aqueous solubility of monodecanoin in the absence of bile salt (approximately 4 mM in sodium phosphate buffer), but increased solubility in the presence of bile salt (11 mM in 4 mM NaTDC (Hofmann 1963)). The fatty acid concentrations obtained in both medium chain lipid digests were below the critical micellar concentrations (CMC) of sodium octanoate and sodium decanoate (approximately 400 and 100 mM, respectively (Mukerjee & Mysels 1970)), but within the range previously shown, at least for potassium decanoate, to form a lamellar fatty acid/fatty acid-soap phase in the absence of bile salt and over the pH range 7–9 (Cistola et al 1988). The turbidity present in the aqueous phase of the MCT digest may therefore also reflect the presence of aggregated $C_{10}$ fatty acid/fatty acid-soap lamellar phase systems. The clarity of the aqueous phase obtained after digestion of $C_8/C_{10}$ monoglyceride/diglyceride suggests that substantial quantities of lamellar phase

**Table 3** Distribution of medium chain lipid digestion products across the different physical phases formed following 30 min digestion of medium chain triglycerides (MCT) and $C_8/C_{10}$ monoglyceride/diglyceride.

| Lipid | MCT | | | $C_8/C_{10}$ monoglyceride/diglyceride | | |
|---|---|---|---|---|---|---|
| | Control | Fasted | Fed | Control | Fasted | Fed |
| Aqueous phase | | | | | | |
| Fatty acid | 108±8 | 99±11 | 98±12 | 100±11 | 92±3 | 98±6 |
| Monoglyceride | 19±1 | 20±4 | 16±4 | – | – | – |
| Pellet phase | | | | | | |
| Fatty acid | 22±0 | 21±5 | 18±3 | 18±2 | 20±1 | 18±2 |

Digests were performed under control (without phosphatidylcholine and sodium taurodeoxycholate (NaTDC)), fasted (1.25 mM phosphatidylcholine, 5 mM NaTDC), and fed (5 mM phosphatidylcholine, 20 mM NaTDC) conditions. All triglycerides and diglycerides were digested, producing fatty acids and monoglycerides and no oil phase was formed. The precipitated pellet comprised of fatty acid only. The numeric data in the table refer to the amount of each particular lipid (fatty acid or monoglyceride) ($\mu$mol) in each phase (aqueous phase or pellet) per 1 mL of digest, mean±s.d., n = 3. Dashes indicate values below the limit of quantification (0.2 and 0.1 mM for fatty acid and monoglyceride, respectively).

were not formed (or were pelleted out during centrifugation) under these circumstances and may reflect the relatively higher proportion of $C_8$ fatty acid resulting from the digestion of Capmul MCM (Capmul MCM glycerides contain 1.7% $C_6$, 80% $C_8$ and 18.3% $C_{10}$ fatty acids; Product information, Abitec Corporation, Janesville, WI) when compared with Miglyol 812 (comprising 0.23% $C_6$, 59.5% $C_8$, 39.8% $C_{10}$ and 0.24% $C_{12}$; Product information, Hüls, UK). The high salt concentrations employed in these digests has also previously been shown to enhance the solubilisation capacity of bile salt–fatty acid micellar systems by: lowering the apparent $pK_a$ of the solubilised fatty acid, thereby increasing the degree of ionisation at a set pH; decreasing the CMC of the bile salt system therefore allowing a greater proportion of the bile salt to be engaged in mixed micelle formation; and by changing the partition of the unionised products between the micelles and the intermicellar phase, in favour of the micelles (salting out) (Shankland 1970).

In comparison with long chain lipid digestion, the higher rate and extent of digestion, and greater aqueous phase distribution of medium chain lipolytic products may be attributed to several factors including solubility, hydrophilicity and phase behaviour. Medium chain fatty acids are ionised to a greater extent (apparent $pK_a$ 6.8 (Cistola et al 1988)) than long chain fatty acids (apparent $pK_a$ 8–8.5 (Cistola et al 1988)) at physiological pH, therefore increasing solubility and amphiphilicity and enhancing the potential for formation of sodium or calcium soaps, or incorporation into bile salt micellar systems. Furthermore, the aqueous solubility of medium chain digestion products is significantly higher than that of long chain digestion products. For example, the aqueous solubility of decanoic acid is approximately 25 $\mu$M whereas the solubility of oleic acid is approximately 1 $\mu$M (Bell 1973). Similarly the solubility of 1-monooctanoin in 4 mM NaTDC (in sodium phosphate buffer pH 6.3, $Na^+$ 150 mM, 37°C) is approximately 34 mM, and considerably higher than that of 1-monoolein (approximately 3 mM under the same conditions) (Hofmann & Borgström 1963). As described previously, partial ionisation of medium chain fatty acids may also lead to the production of stable fatty acid/fatty acid-soap lamellar phases (Cistola et al 1988), the formation of which may encourage aqueous dispersion of medium chain lipid digestion products (even in the absence of bile salt). Medium chain lipolytic products therefore rapidly dissociate from the digesting interface forming either a simple solution or colloidal dispersion, or may precipitate as soaps. This is in contrast to the long chain systems where removal of lipolytic products is limited by the concentration of bile salt, and the solubilisation capacity of the digestion media.

The phase behaviour of the colloidal species present in the intestinal lumen is an important determinant of the trafficking of dietary lipids to the absorptive surface, and their subsequent permeability across the enterocyte. This topic has been well reviewed recently by Phan & Tso (2001). While not the focus of the studies described here, the formation of different species such as vesicles in addition to micelles is also likely to impact on the solubilisation profile of co-administered lipophilic drugs, and may therefore prove critical in terms of the

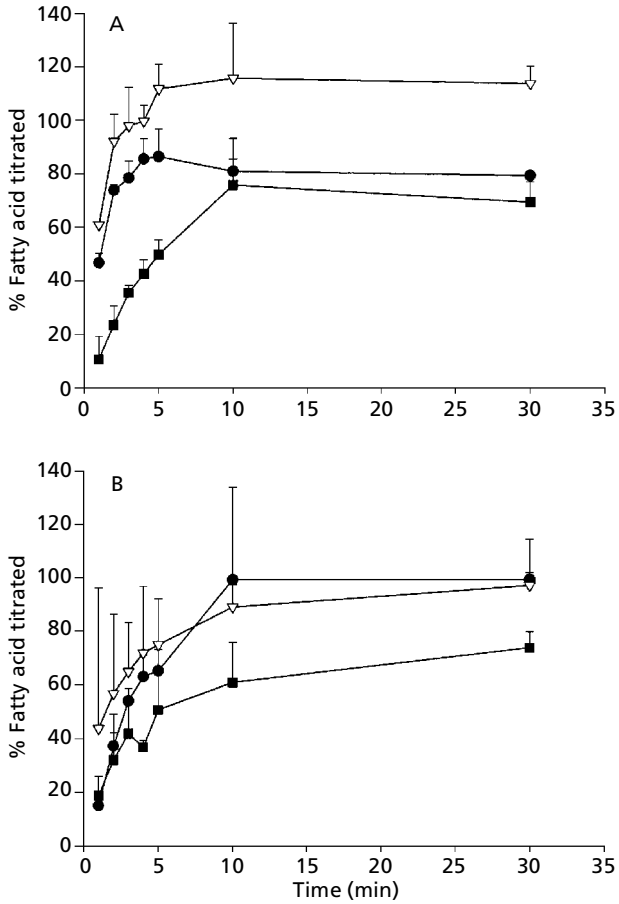


**Figure 4** Percentage ratio of analysed fatty acid concentration (pH-stat titrator/HPTLC assay) under control (■) (without phosphatidylcholine or sodium taurodeoxycholate (NaTDC)), fasted (●) (1.25 mM phosphatidylcholine, 5 mM NaTDC) and fed (▽) (5 mM phosphatidylcholine, 20 mM NaTDC) conditions for long chain triglycerides (A) and medium chain triglycerides (B). Data are mean±s.d., n = 3.

absorption enhancing capacity of lipidic formulations via both prevention of drug precipitation and efficient trafficking of drugs across the unstirred water layer and the absorptive membrane. These issues are the focus of ongoing studies in this laboratory.

### Comparison of fatty acid concentrations determined by pH-stat titration and HPTLC techniques

Figure 4 compares the fatty acid concentrations determined indirectly by the pH-stat and directly via HPTLC analysis over the course of a 30-min experiment. In all cases, as digestion progressed, the fatty acid concentration as assessed by pH-stat titration lagged behind the concentrations measured directly by HPTLC. By the end of the 30-min digestion period these differences were less evident. In general, the titration efficiency of the pH-stat was higher when assessing the digestion of medium chain systems when compared with long chain lipids and higher also in the presence of larger concentrations of bile salt. For example, after digestion of LCT for 30 min, the amount of fatty acid quantified by titration was 69.4±7.6, 79.3±2.1 and 113.6±6.4% of that determined by HPTLC under control (no bile salt or phospholipid), fasted and fed conditions, respectively, whereas for the MCT, in the presence of bile salt (fasted or fed) good correlation was seen between titration and HPTLC assay methodologies from 10 min onwards (Figure 4). The accuracy of the pH-stat titration technique in quantifying the fatty acid produced during lipid digestion is highly dependent on the ionisation of fatty acid, and the availability of titratable fatty acid, which is in turn dependent on several factors including the chain lengths of the fatty acid, the pH of the media, the bile salt and electrolyte concentrations and the phase behaviour of the fatty acid (Patton & Carey 1979; Patton et al 1985).

The apparent increase in effectiveness of the pH-stat in the quantification of medium chain fatty acid most likely reflects the lower $pK_a$ of medium chain fatty acid (apparent $pK_a$ 6.8) and therefore higher degree of ionisation, when compared with long chain fatty acid (apparent $pK_a$ 8–8.5) (Cistola et al 1988). Similarly fatty acid solubilisation in bile salt micelles has previously been shown to shift fatty acid titration profiles to lower pH values, suggesting an increase in ionisation of fatty acid in bile salt solubilised systems (Shankland 1970) at a set pH. Finally, the enhanced titration efficiencies seen when conducting digestion experiments in the presence of bile salt may also reflect improved trafficking of fatty acid to the aqueous phase of the digest as opposed to sequestration of the fatty acid in the oil phase. These data are consistent with the low titration efficiency (50%) described by Patton & Carey (1981) during digestion of LCT (olive oil) in relatively low concentrations (6 mM) of bile salt at pH 6.9, but improved efficiency at pH 8.0 (70%) where the long chain fatty acid was more efficiently ionised.

### Conclusions

In these studies an in-vitro lipid digestion model has been employed to provide a detailed and quantitative map of the chemical kinetics and phase behaviour of common formulation lipids during digestion, under simulated physiological conditions. This approach has

allowed comparison of the profiles obtained after digestion of medium and long chain triglycerides and the corresponding mono- and diglyceride blends. Monitoring lipid digestion indirectly by pH-stat titration is a simple and rapid technique; however, HPTLC methods were found to provide a more detailed and accurate reflection of digestion as each individual lipid species was quantified, and detection was not ionisation-state-(and therefore pH-) dependent.

The data showed that the rate and extent of digestion and the phase behaviour of the lipolytic products is markedly dependent on the fatty acid chain length. Thus, the rate and extent of medium chain lipid digestion, and dispersion of medium chain lipolytic products into the aqueous phase were significantly higher than the corresponding long chain lipid, and the extent of medium chain lipid digestion was effectively independent of bile salt concentration. Conversely, efficient digestion and dispersion of long chain lipids was much more dependent on bile salt concentration. The physical phases present during lipid digestion were highly dependent on the rate and extent of digestion. Medium chain glycerides were rapidly digested forming only an aqueous dispersed phase or a precipitated fraction, whereas long chain glycerides were relatively more slowly digested with the undigested oily phase persisting during digestion.

The differences in physical phase formed during lipid digestion may impact significantly on the fate of a co-administered drug during intraluminal pre-absorptive processing of a candidate lipid formulation, and may determine whether a drug will remain (and possibly concentrate) in the undigested oily phase, disperse into the aqueous phase by solubilisation, or precipitate. The data obtained in these studies will facilitate subsequent investigation of the dynamic interaction between the various physical phases formed during lipid digestion and the solubilisation and dissolution of a range of lipophilic compounds, and therefore assist in the more rational design of new lipid-based formulations.

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