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*Research Paper*

# Use of a Dynamic *in Vitro* Lipolysis Model to Rationalize Oral Formulation Development for Poor Water Soluble Drugs: Correlation with *in Vivo* Data and the Relationship to Intra-Enterocyte Processes in Rats

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***Purpose.*** To examine the correlation between the *in vitro* solubilization process of lipophilic compounds from different lipid solutions and the corresponding *in vivo* oral bioavailability data. In particular, to assess the influence of intra-enterocyte processes (metabolism and lymphatic absorption) on this correlation.

***Materials and Methods.*** The dissolution of progesterone and vitamin $D_3$ in long (LCT), medium (MCT) and short (SCT) chain triglyceride solutions were tested in a dynamic *in vitro* lipolysis model. The absolute oral bioavailability of the drugs from the tested formulations was investigated in rats. Vitamin $D_3$ bioavailability was also examined following lymphatic transport blockage induced by cycloheximide (3 mg/kg).

***Results.*** The dynamic *in vitro* lipolysis experiments indicated a rank order of MCT > LCT > SCT for both progesterone and vitamin $D_3$. The bioavailability of progesterone correlated with the *in vitro* data, despite its significant pre-systemic metabolism. For vitamin $D_3$, an *in vivo* performance rank order of LCT > MCT > SCT was obtained. However, when the lymphatic transport was blocked the bioavailability of vitamin $D_3$ correlated with *in vitro* data.

***Conclusions.*** The *in vitro* lipolysis model is useful for optimization of oral lipid formulations even in the case of pre-systemic metabolism in the gut. However, when lymphatic transport is a significant route of absorption, the *in vitro* lipolysis data may not be predictive for actual *in vivo* absorption.

**KEY WORDS:** bioavailability; *in vitro* lipolysis; intestinal absorption; lipid based formulations; lipophilic drugs; lymphatic transport; pre-systemic metabolism.

## INTRODUCTION

Modern drug discovery techniques, including high-throughput *in vitro* screening methods of receptor binding and the introduction of combinatorial chemistry, are yielding an increasing number of poor water soluble pharmacologically active compounds. These molecules are generally characterized by very low oral bioavailability, and therefore fail to proceed to advanced stages of research and development (1).

The barriers that a lipophilic drug has to traverse along the intestinal absorption cascade already begin at pre-enterocyte processes, including poor solubility in the milieu of the gastrointestinal tract (GIT), the unstirred water layer adjacent to the absorptive membrane of the enterocyte and the limited transit time in the small intestine where the solubilization and absorption of the lipophilic molecule occurs. Once a drug molecule enters the enterocyte, it is exposed to metabolizing enzymes (e.g., cytochrome *P*-450 3A4 (CYP3A4)), and can also be secreted from the enterocyte back to the GI lumen by P-glycoprotein (P-gp) efflux pumps. After escaping the intra-enterocyte biochemical barriers the drug molecules are transported by the portal blood stream to the liver (unless they undergo lymphatic absorption), and thus are exposed to additional metabolic degradation. These consecutive barriers significantly limit the oral bioavailability of lipophilic molecules.

The most popular approach to improve the oral bioavailability of lipophilic drugs is the utilization of lipid based formulations (2,3). The mechanisms behind this methodology include enhanced dissolution and solubilization of the co-administered lipophilic drug by stimulation of biliary and pancreatic secretions, prolongation of GIT residence time, stimulation of lymphatic transport, increased intestinal wall permeability and by reduced metabolism and efflux activity (4).

Lipid based formulations offer a large variety of optional systems. These can be made as solutions, suspensions, emulsions, self emulsifying systems and microemulsions. Moreover, it is possible to form blends that are composed of several excipients. These can be pure triglyceride (TG) oils or a mixture of different TG, diglyceride (DG) and monoglyceride (MG). In addition, different types of surfactants

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(lipophilic and hydrophilic) can be added, as well as hydrophilic co-solvents. The development strategies in the area of lipid formulations are mostly empirical, demand large number of animal experiments, and consume time and money.

In most cases, the intestinal absorption of lipophilic drugs is dissolution rate limited, and they can be described as class 2 compounds according to the biopharmaceutical classification system (BCS) (5). In order to augment bioavailability, the formulation of such a molecule needs to maximize the rate and extent of drug dissolution and to maintain the drug in solution during its transit along the GI tract. Various *in vitro* methods for tracking the degree of solubilization of the drug in the GIT milieu have been proposed in the literature, and marked progress has been achieved in this field (6). In recent years, dynamic lipolysis models have been proposed, which mimic the GIT environment and better reflect the intestinal conditions in terms of maintaining constant pH, the presence of lipase/co-lipase and bile salts (BS) and phospholipids (PL) concentration. These models have also been shown to simulate other dynamic changes, including the formation of mixed micellar species that are generated throughout the interaction of the vehicle with the GIT environment (7–9). However, the ability of these lipolysis models to predict actual oral bioavailability of certain drug–vehicle combinations is not clear, and studies that evaluate the *in vitro–in vivo* correlation (IVIVC) are needed. The investigation of such IVIVC is of specific interest because it is not clear whether intra-enterocyte processes, such as lymphatic transport of the drug and pre-systemic metabolism in the gut wall, influence the ability of the *in vitro* lipolysis model, which mimics the pre-enterocyte processes, to predict overall *in vivo* bioavailability.

The first aim of the present study was to examine the impact of different lipid based solutions on the solubilization process of two lipophilic, poorly water soluble drugs, in a dynamic *in vitro* lipolysis model. Subsequently, to compare these *in vitro* results with *in vivo* oral bioavailability data obtained for these drug-vehicle combinations, in order to evaluate under what conditions the *in vitro* model can predict *in vivo* performance.

For this investigation we selected two model lipophilic drugs that undergo different intra-enterocyte processes. Progesterone reflects low oral bioavailability due to poor solubility and significant pre systemic metabolism, when more than 50% of progesterone dose undergo pre-systemic metabolism in the gut wall (10,11). Vitamin $D_3$ represents lipophilic compounds that are transported mainly via the lymphatic system (12). Three lipidic vehicles were tested for each molecule: peanut oil ($C_{18}$, long chain triglycerides (LCT)), captex 355 ($C_{8–10}$, medium chain triglycerides (MCT)) and triacetin ($C_2$, short chain triglycerides (SCT)) based solutions.

Based on the results of this part of the study, we hypothesized that the *in vitro* digestion model as a predictive tool has certain limitations associated with the absorption characteristics of the tested drug, and that for a molecule with significant lymphatic oral absorption, the *in vitro* data may not predict *in vivo* behavior. Therefore, in the second part of the study, we conducted a further series of *in vivo* experiments, to examine this hypothesis. In these experiments, we have utilized a pharmacological blockage of the chylomicron flow which inhibits the lymphatic transport pathway, without affecting other absorption pathways, as we previously shown (12).

## MATERIALS AND METHODS

### Materials

Progesterone, vitamin $D_3$ (cholecalciferol), testosterone, vitamin E (tocopherol), peanut oil (LCT), triacetin (SCT), taurocholic acid, pancreatin (8 × USP), L-α-phosphatidylchcline, tris maleate, cycloheximide and calcium chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Captex 355, triglycerides of caprylic/capric acid (MCT) was generously supplied by Abitec Corporation Co. (Janesville, WI). Saline was obtained from Teva Medical (Ashdod, Israel). Ethanol, methanol, acetonitrile, water, *n*-hexane and sodium chloride (J.T.Baker, Deventer, Holland) were HPLC grade. All other chemicals were of analytical reagent grade.

### *In Vitro* Dynamic Lipolysis Experimental Procedure

The procedure for the dynamic *in vitro* lipolysis experiment was based on a previous report by Sek *et al.* (13) with some modifications, in order to achieve maximum pseudo-physiological conditions. The experiment medium, which was comprised of 35.5 ml of digestion buffer (50 mM tris maleate, 150 mM NaCl, 5 mM $CaCl_2$, pH = 7.4) containing 5 mM taurocholic acid and 1.25 mM phosphatidylchcline (conditions mimicking fasted state GIT), was continuously stirred and maintained at 37°C. Either 1 g or 200 mg (regular and low lipid load, respectively) of the tested formulation (containing 5 mg of the drug) was then dispersed in the medium and stirred for 15 min. Fresh pancreatin extract was prepared by adding 1 g of porcine pancreatin powder to 5 ml digestion buffer, stirring for 15 min followed by centrifugation, as previously described in the literature (14). 3.5 ml of the pancreatin extract (1,000 IU/ml) was inserted into the medium and initiated the enzymatic digestion of the formulation. In order to achieve maximum pseudo-physiological conditions a pH-titrator unit was used (DL-50 Graphix, Mettler Toledo Inc., Columbus, OH) to maintain the pH at 7.4 throughout the experiment. This is important since during the lipolysis process of triglycerides, free fatty acids are liberated and consequently the pH decreases. The experiment was continued for 30 min, in which time the enzymatic digestion process was completed, as indicated by the completion of the pH titration. Subsequently, the medium was ultracentrifuged (L8-55 Ultracentrifuge, SW-41 rotor, Beckman Co., Palo Alto, CA) at 40 K rpm for 90 min, and a separation into three phases was received: an aqueous phase (containing bile salts, fatty acids and MG), a lipid phase (containing undigested TG and DG) and sediment (containing un-dissolved fatty acids). Each of the phases was analyzed for drug content. Following oral administration, dissolution of the drug molecule in the intestinal milieu is a prerequisite for the absorption process. Hence, drug molecules solubilized in the aqueous phase of the lipolysis medium is thought to be available for absorption, in contrast to drug in the sediment

which will not be available for absorption in *in vivo* conditions.

### Bioavailability Studies in Rats

All surgical and experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee of the Hebrew University Hadassah Medical School Jerusalem. Male Wistar rats (Harlan, Israel), 275–300 g in weight, were used for all surgical procedures.

Animals were anesthetized for the period of surgery by intra-peritoneal injection of 1 ml/kg of ketamine-xylazine solution (9%:1%, respectively) and placed on a heated surface maintained at 37°C (Harvard Apparatus Inc., Holliston, MA). An indwelling cannula was placed in the right jugular vein of each animal for systemic blood sampling, by a method described before (15). The cannula was tunneled beneath the skin and exteriorized at the dorsal part of the neck. After completion of the surgical procedure, the animals were transferred to metabolic cages to recover overnight (12–18 h). During this recovery period and throughout the experiment, food, but not water, was deprived. Animals were randomly assigned to the different experimental groups.

In order to study the absorption of vitamin $D_3$, the vitamin (1 mg/kg), dissolved in either peanut oil (LCT), captex 355 (MCT) or triacetin (SCT) (1 mg/ml, w/v, freshly prepared 30 min before each experiment), was administered to the animals by oral gavage. Systemic blood samples (0.25 ml) were taken at 5 min pre-dose, 2, 4, 6, 8, 12, 18 and 24 h post-dose. To prevent dehydration equal volumes of physiological solution were administered to the rats following each withdrawal of blood sample.

The same procedure was used for the progesterone oral absorption study (15 mg/kg), where blood samples (0.45 ml) were taken at 5 min pre-dose, 0.5, 1, 2, 3, 4, 6, 8 and 12 h post-dose.

In order to determine absolute bioavailability of vitamin $D_3$ and progesterone, two additional groups of animals ($n$ = 4) received intravenous injection of vitamin $D_3$ (0.125 mg/kg) or progesterone (15 mg/kg), dissolved in ethanol:propylene glycol:saline (3:3:4, respectively).

### *In Vivo* Absorption in Chylomicron Flow Blocked Rats

The animals were treated with intraperitoneal injection of cycloheximide (3 mg/kg) dissolved in saline (3 mg/ml, w/v). One hour after the injection the animals ($n$ = 5 in each group) were given oral gavage of vitamin $D_3$ (1 mg/kg) dissolved in either LCT or MCT vehicle (1 mg/ml), and the pharmacokinetic experiment was conducted.

## ANALYTICAL METHODS

The amount of vitamin $D_3$ in plasma and in the *in vitro* digestion medium was determined using a high performance liquid chromatography (HPLC) system (Waters 2695 Separation Module) with a photodiode array UV detector (Waters 2996), by a method described before with some modifications (16). Plasma or medium aliquots of 100 μl were mixed with 200 μl of ethanol containing the internal standard (vitamin E, 5 μg/ml) and vortex-mixed for 1 min. Two milliliters of *n*-hexane were added, followed by 1 min vortex-mixing. After centrifugation at 1,500 $\times g$ for 10 min, the organic layer was transferred and the extraction process was repeated. The two organic layers were combined, evaporated (Vacuum Evaporation System, Labconco, Kansas City, MO) and reconstituted in 50 μl of ethanol. Twenty-five microliters of the resulted solution were injected into the HPLC system. The HPLC conditions were as follows: XTerra, $RP_{18}$, 3.5 μm, 4.6×100 mm column (Waters Co., Milford, MA), an isocratic mobile phase, methanol:water (93:7 v/v), flow at a rate of 0.5 ml/min in room temperature. Vitamin $D_3$ and vitamin E were detected at 265 and 292 nm, respectively.

A liquid chromatography mass spectra (LC-MS) system comprising of Waters pump (600 controller), Waters autosampler ($717_{plus}$ Autosampler) and Waters Micromass ZQ mass spectrometer (Waters Co., Milford, MA), was used to determine progesterone levels in plasma and in the *in vitro* digestion medium. Plasma or medium aliquots of 200 μl were mixed with 400 μl of ethanol containing the internal standard (testosterone, 10 ng/ml) and vortex-mixed for 1 min. After centrifugation at 1,500 × g for 10 min, the supernatant was

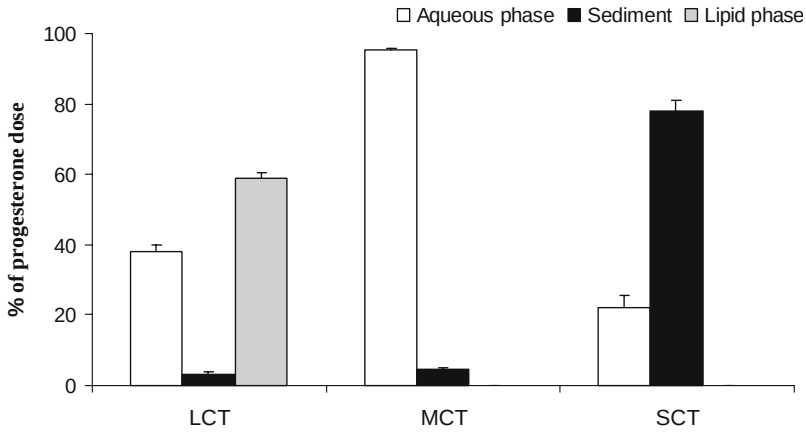


**Fig. 1.** Distribution of the progesterone molecules across the aqueous phase (□), sediment (■) and lipid phase (▩) of the dynamic *in vitro* lipolysis medium following LCT, MCT or SCT based solution. Data presented as mean ± standard deviation, $n$ = 6 for each formulation.

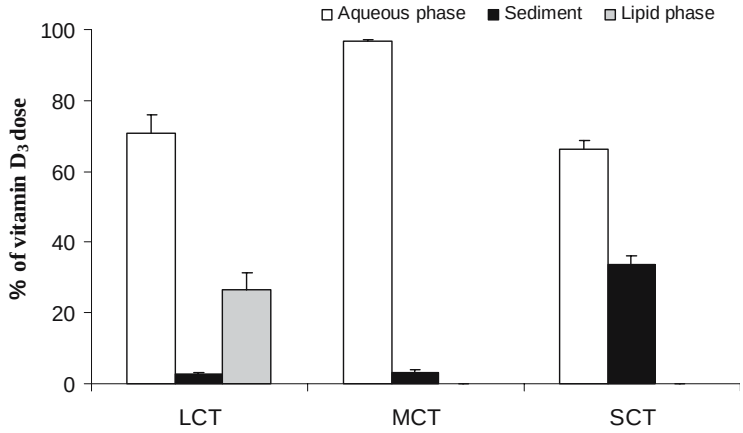


**Fig. 2.** Distribution of the vitamin $D_3$ molecules across the aqueous phase (□), sediment (■) and lipid phase (■) of the dynamic *in vitro* lipolysis medium following LCT, MCT or SCT based solution. Data presented as mean ± standard deviation, $n = 6$ for each formulation.

evaporated, reconstituted in 50 µl of ethanol and 20 µl of the resulted solution were injected into the LC-MS system. Chromatography conditions were as follows: XTerra MS, $RP_{18}$, 3.5 ìm, 2.1 × 100 mm column (Waters Co., Milford, MA), an isocratic mobile phase, methanol:acetonitrile:water (50:23:27 v/v) containing 0.1% formic acid and 0.05% TFA at a flow of 0.2 ml/min, column temperature was 35°C, and the detection masses were 315 and 289 g/mole for progesterone and testosterone, respectively.

Separate standard curves were used for plasma and the *in vitro* digestion medium samples ($R^2 < 0.999$). The minimum quantifiable concentrations for vitamin $D_3$ and for progesterone were 80 and 1 ng/ml, respectively. The inter- and intra-day coefficients of variation were <1.0 and 0.5%, respectively.

### Pharmacokinetic Analysis

Plasma concentrations *vs.* time data obtained for progesterone and vitamin $D_3$ in individual rats were analyzed using WinNonlin® Professional software version 4.0.1, by means of the noncompartmental analysis model. The absolute bioavailability of the drugs was calculated from the ratio of the AUC values normalized by dose after oral and intravenous administration.

### Statistical Analysis

All values are expressed as mean ± standard deviation (S.D.). To determine statistical significantly differences among the experimental groups, the non-parametric Kruskal-Wallis test was used for multiple comparisons, and the two-tailed non-parametric Mann-Whitney *U* test for two-group comparison when appropriate. A $p$ value of less than 0.05 was termed significant.

## RESULTS

### *In Vitro* Dynamic Lipolysis Model Results

The distribution and solubilization pattern of progesterone and vitamin $D_3$ across the different phases of the digestion medium resulting from the LCT, MCT and SCT solutions are presented in Figs. 1 and 2, respectively, and summarized in Table I. The data demonstrate, for both progesterone and vitamin $D_3$, that formulating with MCT, rather than LCT or SCT, leads to extremely high amounts of solubilized drug in the aqueous phase (>95% of the drug dose).

For progesterone, the SCT vehicle caused the lowest aqueous phase concentration, and the highest amount of drug in the sediment (77.7%). As denoted above, drug molecules that precipitated during the lipolysis process in this biorelevant medium are not expect to be available for absorption in *in vivo* conditions. Hence, formulating with SCT is expected to yield the poorest *in vivo* bioavailability among the three tested formulations.

Regarding the LCT formulation, only about 50% from the vehicle was digested throughout the experiment, as

**Table I.** Distribution (% of dose) of the Progesterone and the Vitamin $D_3$ Molecules Across the Aqueous Phase, Sediment and Lipid Phase of the Dynamic *in vitro* Lipolysis Medium Following LCT, MCT or SCT Based Solution

| TG type in the formulation | Progesterone (percent of dose) | | | Vitamin $D_3$ (percent of dose) | | |
|---|---|---|---|---|---|---|
| | Aqueous phase | Sediment | Lipid phase | Aqueous phase | Sediment | Lipid phase |
| LCT | 38.0 ± 1.9 | 3.2 ± 0.6 | 58.7 ± 1.5 | 70.7 ± 5 | 2.6 ± 0.5 | 26.5 ± 4.6 |
| MCT | 95.3 ± 0.4 | 4.6 ± 0.4 | No lipids | 96.6 ± 0.4 | 3.6 ± 0.5 | No lipids |
| SCT | 22.2 ± 3 | 77.7 ± 3.1 | No lipids | 66.1 ± 2.3 | 33.3 ± 3 | No lipids |

Data presented as mean ± standard deviation, $n = 6$ for each formulation.

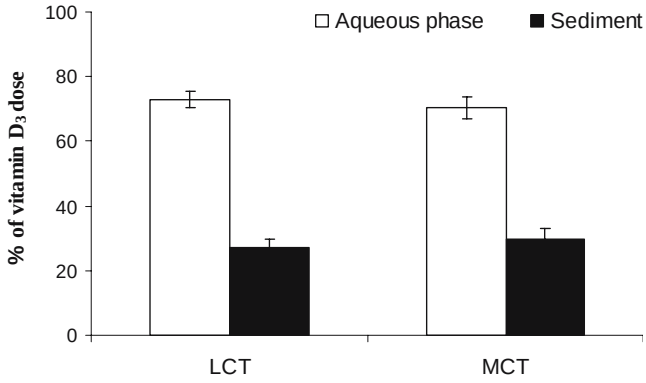


**Fig. 3.** Distribution of the vitamin $D_3$ molecules across the aqueous phase (□) and the sediment (■) of the dynamic *in vitro* lipolysis medium, following low lipid load (5 mg/ml) of LCT or MCT solution. No lipid phase was obtained in these experiments. Data presented as mean ± standard deviation, $n = 6$ for each formulation.

indicated by the stoichiometric ratio of NaOH employed by the pH titrator unit to maintain constant pH. Hence, an undigested lipid phase was obtained at the end point of the lipolysis process. This lipid phase has very high affinity for the drug molecules, and the fate of drug molecules that are retained in this lipid phase under *in vivo* conditions is not clear, and cannot be interpreted unequivocally. In any case, since the LCT yielded a two fold greater aqueous phase drug concentration and much less drug was found in the sediment in comparison to the SCT vehicle Table I, the performance rank order of these three vehicles in *in vivo* conditions by this dynamic *in vitro* lipolysis model is MCT > LCT > SCT.

A similar trend was obtained for the vitamin $D_3$ as well. The MCT vehicle provided a very high drug concentration solubilized in the aqueous phase (96.6%), with a very small amount in the sediment. The LCT solution resulted in a lesser amount of the vitamin in the aqueous phase in comparison to the MCT, and the SCT vehicle provided the poorest results among the three formulations. Again, an undigested lipid phase was present at the end point of the lipolysis process of the LCT vehicle, with a significant concentration of drug retained in it. The overall indication, therefore, from these dynamic *in vitro* lipolysis experiments, was that for both progesterone and vitamin $D_3$, a performance rank order of MCT > LCT > SCT is expected in *in vivo* conditions.

Since the amount of lipid used in the lipolysis experiment has been proposed to influence the reliability of this experiment (17), we conducted a further *in vitro* lipolysis study with reduced lipid load. The distribution pattern of vitamin $D_3$ across the different phases of the *in vitro* lipolysis experiment following the insertion of 200 mg LCT or MCT vitamin $D_3$ solution (5 mg/ml medium) are shown in Fig. 3. It can be seen that no significant difference between the distribution of the vitamin $D_3$ molecules across the aqueous phase and the sediment was observed following the *in vitro* lipolysis of the LCT or the MCT solutions.

## *In Vivo* Absorption of Progesterone and Vitamin $D_3$ in Rats

The plasma concentration time profiles for progesterone and vitamin $D_3$ following oral administration of the different solutions are shown in Figs. 4 and 5, respectively. The corresponding pharmacokinetic parameters obtained in these *in vivo* experiments for both progesterone and vitamin $D_3$ are listed in Table II.

The bioavailability of progesterone from the MCT based solution was four-fold greater in comparison to the LCT vehicle, and 11-fold greater than the SCT formulation. These results correlate well with the data obtained from the dynamic *in vitro* lipolysis experiment, and the expected performance rank order of MCT > LCT > SCT proved to be correct in *in vivo* conditions. Moreover, the correlation between the percent of progesterone dose that was solubilized in the aqueous phase of the *in vitro* lipolysis medium and the *in vivo* AUC values following oral administration of the corresponding vehicles is presented in Fig. 6. It can be seen that a strong correlation ($R^2 > 0.99$) was obtained between these parameters. It should be noted that this correlation only reflects the state for the three tested formulations. On the other hand, in the case of vitamin $D_3$, no correlation was obtained between the outcomes of the dynamic *in vitro* lipolysis model and the *in vivo* bioavailability

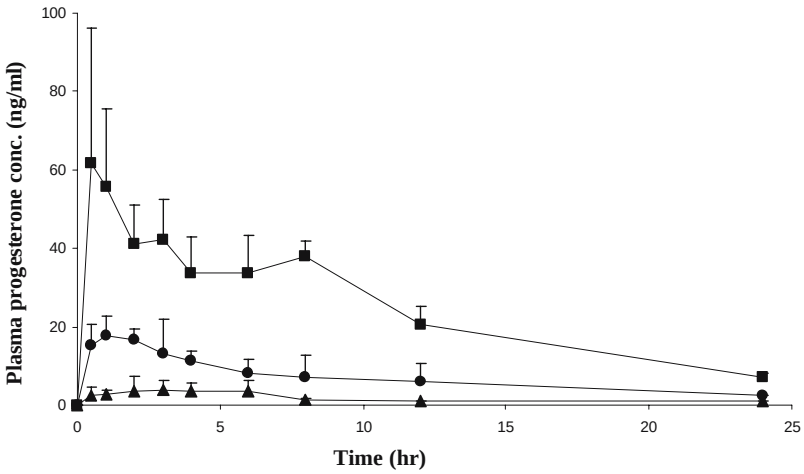


**Fig. 4.** Plasma progesterone concentration-time profiles (mean ± S.D.), following oral administration of 15 mg/kg progesterone LCT (●), MCT (■) or SCT (▲) solution ($n = 5$ for each formulation).

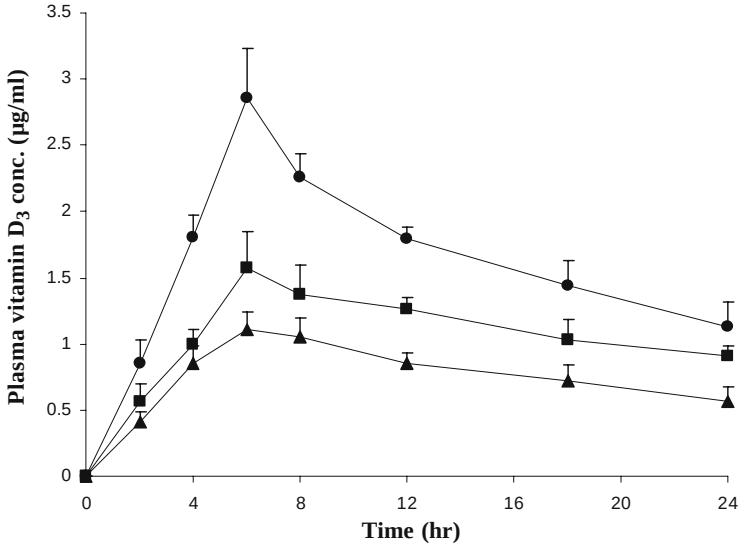


**Fig. 5.** Plasma vitamin $D_3$ concentration-time profiles (mean ± S.D.), following oral administration of 1 mg/kg vitamin $D_3$ LCT (●), MCT (■) or SCT (▲) solution ($n$ = 5 for each formulation).

data. While the *in vitro* experiments indicated that the MCT based vitamin $D_3$ solution was expected to yield the highest *in vivo* absorption among the three tested formulations, *in vivo* assessment following oral administration of the vitamin revealed that the LCT solution was a better vehicle, and the actual *in vivo* performance rank order for the vitamin was LCT > MCT > SCT.

The MCT solution, which caused higher aqueous phase vitamin $D_3$ concentration in comparison to the SCT vehicle (96.6 *vs.* 66.1% of the dose) in the lipolysis experiment, showed also better *in vivo* performance (52.5 and 34.3 h·µg/ml AUC values, respectively).

### *In Vivo* Absorption of Vitamin $D_3$ in Chylomicron Flow Blocked Rats

We have showed in a previous publication that the absorption of vitamin $D_3$ in animals pretreated with cycloheximide, a protein synthesis inhibitor known to inhibit the secretion of chylomicrons from the enterocyte (18,19) is similar to the absorption in mesenteric lymph duct cannulated animals (12). Thus, comparison between the different formulations in cycloheximide treated rats discard the lymphatic transport component included in the overall bioavailability and enables an assessment of the vehicles impact on the absorption regardless its effect on the lymphatic transport pathway.

Vitamin $D_3$ plasma concentration *vs.* time profiles following oral administration of LCT or MCT solution to cycloheximide treated animals is presented in Fig. 7. The data demonstrate that under conditions that do not allow lymphatic transport the administration of the vitamin in MCT solution proved to be better than in LCT solution, and a correlation between the *in vitro* lipolysis model outcomes and these *in vivo* results was obtained.

## DISCUSSION

*In vitro* tests to help predict the fate of a poorly water soluble drug in a lipid based delivery system upon its dilution and digestion in the GI milieu are highly needed, and have received increasing attention in recent years (7–9). In this study we have utilized a dynamic *in vitro* lipolysis model, based on previously published studies (7,9,13). This experimental strategy enables high reflection of the dynamic

**Table II.** Progesterone and Vitamin $D_3$ Pharmacokinetic Parameters Obtained Following Oral Administration of LCT, MCT or SCT Solution

| TG type in the formulation | Progesterone | | | | Vitamin $D_3$ | | | |
|---|---|---|---|---|---|---|---|---|
| | AUC (h ng/ml) | k ($h^{-1}$) | $t_{1/2}$ (hr) | F(%)$^{\alpha}$ | AUC (h µg/ml) | k($h^{-1}$) | $t_{1/2}$ (hr) | F(%)$^{\alpha}$ |
| LCT | 181.3 ± 48.8 | 0.05 ± 0.01 | 12.7 ± 3.5 | 1.1 ± 0.2 | 67.9 ± 6.2 | 0.038 ± 0.006 | 18.1 ± 2.9 | 40.7 ± 3.7 |
| MCT | 675.8 ± 160.5 | 0.06 ± 0.02 | 13.2 ± 6.1 | 4.1 ± 1 | 52.5 ± 6.7 | 0.03 ± 0.006 | 20.5 ± 3.8 | 30.3 ± 4.1 |
| SCT | 55.4 ± 21.8 | 0.06 ± 0.02 | 12.6 ± 5.2 | 0.33 ± 0.13 | 34.3 ± 4.9 | 0.035 ± 0.006 | 19.7 ± 3.5 | 20 ± 2.5 |

$n$ = 5 rats in each group. Values presented as mean ± standard deviation.
$^{\alpha}$ Absolute bioavailability. AUC values following intravenous administration of 15 mg/kg progesterone and 0.125 mg/kg vitamin $D_3$ were 16,656 ± 2,050 (h·ng/ml) and 20.95 ± 2.2 (h·µg/ml), respectively.

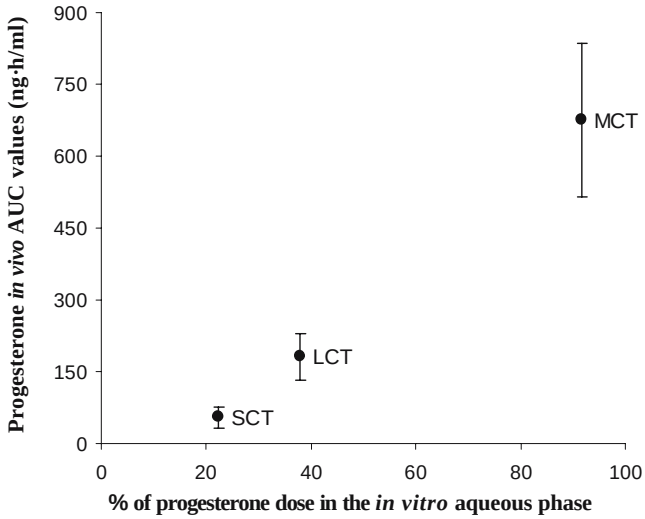


**Fig. 6.** Correlation between the percent of progesterone dose that solubilized in the aqueous phase of the *in vitro* lipolysis medium and the *in vivo* AUC values following oral administration of the corresponding vehicle ($R^2 > 0.99$).

formation of mixed micellar species throughout the lipolysis process, and simulates intestinal fast state conditions in terms of maintaining constant pH, the presence of lipase/co-lipase and having proper BS and PL concentrations. However, further work is needed in order to asses the advantages and limitations of this *in vitro* model in predicting the *in vivo* performance of different drug–vehicle combinations. In particular, there is an interesting question about the impact of various intra-enterocyte processes on the IVIVC. In the current investigation we have focused on two key intra-enterocyte processes: oxidative metabolism and association with chylomicrons that initiate the lymphatic absorption route.

In this study, progesterone was selected as a model lipophilic compound (log $P = 4.0$) with low oral bioavailability (less than 5%) due to poor water solubility and significant pre-systemic oxidative metabolism, where more than 50% of progesterone dose have been shown to undergo pre-systemic metabolism in the gut wall (10,11). It has been shown before that certain lipid based formulations may improve the oral bioavailability of this hormone (20,21). The data presented in this paper clearly demonstrates that the nature of the lipid in the progesterone formulation directly influences the magnitude of progesterone absorption. The notable finding is that the *in vitro* lipolysis experiment can predict the *in vivo* performance of different vehicles, regardless of the massive intra-enterocyte metabolism that this compound goes through. The strong positive correlation ($R^2 > 0.99$) obtained between the percent of progesterone dose that solubilized in the aqueous phase of the *in vitro* lipolysis medium and the *in vivo* AUC values following oral administration of the corresponding vehicle (Fig. 6) highlights two key points. On one hand it illustrates the ability of this dynamic *in vitro* lipolysis model to mimic the conditions of the *in vivo* dissolution process within the gut. On the other hand it clarifies that progesterone metabolism is a first order kinetic process, which is linearly related to the available drug concentration within the enterocyte, and thus does not affect the ability of the *in vitro* lipolysis model to predict the *in vivo* performance of different progesterone vehicles.

Vitamin $D_3$ is a highly lipophilic compound (log $P = 9.1$), demonstrated before to be significantly transported via the mesenteric lymph following oral administration (12). The dynamic *in vitro* lipolysis data for vitamin $D_3$ have indicated that MCT based formulation causes very high drug concentrations in the aqueous phase (>95% of the vitamin dose), with marked preference over the other tested formulations. Hence, the MCT formulation was expected to yield improved *in vivo* bioavailability in comparison to the LCT or SCT formulations. However, *in vivo* oral administration of the vitamin in MCT solution resulted in lower bioavailability in comparison to the LCT based formulation. This higher oral absorption of vitamin $D_3$ from LCT solution rather than from MCT is consistent with previously published data (22). Hence, the data clarify that the nature of the lipid does not only affect the pre-enterocyte processes but also may intervene in the intracellular events associated with lymphatic absorption (as following discussed). Thus, the *in vitro*

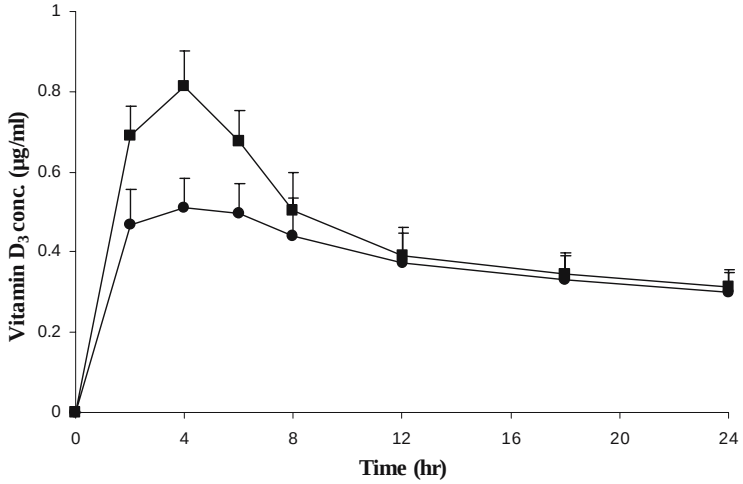


**Fig. 7.** Plasma vitamin $D_3$ concentration-time profiles (mean ± S.D, $n = 5$), following oral administration of 1 mg/kg vitamin $D_3$ LCT (●) or MCT (■) solution to cycloheximide treated rats.

lipolysis model, which reflects only pre-enterocyte processes, fails to predict the overall *in vivo* bioavailability.

### Low Lipid Load Effect

Porter *et al*. have encountered the same phenomenon of lack of IVIVC with the antimalarial lipophilic drug halofantrine (17). Whereas extremely high and preferred aqueous phase drug concentrations seen during the *in vitro* digestion of MCT solution formulation, the *in vivo* bioavailability of halofantrine was significantly higher following administration of the LCT formulation when compared with the MCT formulation. The authors attributed this contrast between the halofantrine *in vitro* and *in vivo* data to the amount of lipidic vehicle used in the *in vitro* digestion experiment, and suggested that five-fold lower lipid load (5 mg/ml medium) would better correlate with the *in vivo* data. Hence, to asses this possibility, we conducted a further *in vitro* lipolysis study with reduced amount of lipid. The reduction in lipid amount led to loss of solubilization capacity and increased drug precipitation (Fig. 3). The fact that the MCT vehicle was more dependent on the lipid load than the LCT could be anticipated since the MCT vehicle undergoes complete digestion throughout the experiment, and hence, when decreased amount of MCT (five-fold) was introduced into the lipolysis system, five-fold less lipolytic products were present in the medium at the end point of the lipolysis process, indicating that the solubilization capacity of the medium decreased. In contrast, in the case of the LCT vehicle only about 50% from the higher lipid load were digested throughout the experiment, as indicated by the stoichiometric ratio of NaOH solution required to maintain constant pH and by the presence of undigested lipid phase at the end point of the lipolysis process. On the other hand, in the low LCT load experiment complete lipolysis was observed, and no lipid phase remained at the end point of the digestion process (30 min). Hence, a five-fold reduction in the initial LCT load does not affect the solubilization capacity as dramatically as the same decrease in MCT load. Moreover, the presence of undigested lipid phase at the end point of the higher LCT load experiment reduced the amount of vitamin in the aqueous phase. This is because the drug molecules have very high affinity for this lipid phase, which retain the lipophilic vitamin molecules. The absence of this oily phase in the low LCT load experiment provided more molecules that became available for dissolution or precipitation.

Nevertheless, even with the low lipid load, no significant difference between the distribution of the vitamin $D_3$ molecules across the aqueous phase and the sediment was observed following the LCT or the MCT solution (Fig. 3). Thus, the amount of lipidic vehicle could not account for the lack of correlation between the *in vitro* and the *in vivo* data, and failed to adequately predict the *in vivo* performance of the tested formulation.

### The Role of Lymphatic Transport

Lymphatic transport is an alternative absorption pathway from the GIT, that have been shown to be significant contributor for the overall bioavailability of a number of highly lipophilic drugs, including fat soluble vitamins (23), halofantrine (24), probucol (25), ontazolast (26) and others.

We have previously demonstrated that 75% of the absorbed dose of vitamin $D_3$ is associated with lymphatic transport, and only 25% arrive to the systemic blood circulation via the portal vein (12). Co-administration of LCT, rather than shorter lipids, stimulates lipid turnover through the enterocyte, enhancing chylomicron synthesis and thereby increase the lymphatic transport pathway capacity. Caliph *et al*. have shown that while 70% of the bioavailability of halofantrine was contributed from lymphatic transport when the drug was dosed in LCT, following administration of the drug with MCT or SCT the majority of the drug was absorbed non-lymphatically, with significant decrease in total halofantrine bioavailability (27).

Hence, we hypothesized that when lymphatic transport is a primary mechanism of drug absorption from the enterocyte to the systemic circulation, the major role of a LCT component in the vehicle is to enhance this pathway and thereby to augment bioavailability, regardless of its impact on the digestion and dissolution properties provided by the formulation. Consequently, since the *in vitro* lipolysis experiment mimics only pre-enterocyte processes, the *in vitro* data for such molecules may fail to correlate with *in vivo* performance.

In order to confirm this hypothesis, we have utilized a unique investigational model developed in our lab that involves a pharmacological blockage of the chylomicron flow, and hence, inhibition of the lymphatic transport pathway, without affecting other absorption pathways (12).

The main mechanism of intestinal lymphatic drug absorption is via intra-cellular association of the drug with the lipidic core of the chylomicron (28). Following this association, the chylomicron is packaged in the golgi, secreted from the basolateral membrane of the enterocyte to the intracellular space, absorbed into the porous mesenteric lymph vessels and travel, with the lipophilic molecule in it, along the lymphatics until drainage into the systemic blood circulation. Cycloheximide is a protein synthesis inhibitor known to inhibit the secretion of chylomicrons from the enterocyte, apparently through anti-microtubular effects (18,19). We have previously showed that the absorption of vitamin $D_3$ in animals pretreated with cycloheximide is similar to the absorption of the vitamin in mesenteric lymph duct cannulated animals, with no adverse effects and no alteration of other absorption aspects (12). Hence, no lymphatic transport can occur in animals pretreated with cycloheximide whereas the non-lymphatic route is not altered. It can be seen that under conditions that do not allow lymphatic transport the MCT solution proved to be better than the LCT solution, and this outcome correlates with the results obtained by the *in vitro* lipolysis model (Fig 7).

Overall, this investigation highlights the effect of two different intra-enterocyte processes, pre systemic metabolism and lymphatic transport, on the ability of the *in vitro* lipolysis model to predict *in vivo* bioavailability. As the case of progesterone shows, significant pre systemic metabolism of the drug did not damage the ability of the *in vitro* lipolysis model to predict overall *in vivo* bioavailability. The data shows that in the case where there is no influence of the vehicle on the intra-enterocyte process and the investigated vehicle components do not affect the pre-systemic metabolism, the *in vitro* lipolysis model can serve as a predictive

tool. This is true as long as the metabolism is not saturated and maintains constant proportion between the available intracellular drug concentrations and the fraction metabolized (i.e., linear first order kinetics). On the other hand, in the case where the formulation components directly influence the intra-enterocyte processes, as affecting the lymphatic transport pathway, the *in vitro* lipolysis model is not suitable to predict the *in vivo* bioavailability. To have a rapid estimation of the lymphatic transport potential of a tested drug, a method suggested recently can be used (28).

The good IVIVC between the MCT and the SCT vitamin $D_3$ solutions evolves from the fact that these TG, unlike LCT, have no apparent influence on the lymphatic transport pathway, but do play a role in the absorption of the co-administered drug. In this case, the effect on the dissolution and solubilization properties becomes the factor governing the overall absorption process, and the increased aqueous phase drug amounts *in vitro* translated into augmented *in vivo* performance. This is adequately demonstrated by showing the good IVIVC when the lymphatic component was experimentally blocked.

Since lymphatic transport have been shown to contribute up to 70% of the total bioavailability of halofantrine (27), the lack of correlation between *in vitro* and *in vivo* halofantrine data (17) may be explained in the same manner as well.

## CONCLUSIONS

In conclusion, the results presented in this paper show that valuable information can be obtained from the *in vitro* lipolysis model, leading to the intelligent selection of suitable lipid components for enhanced oral bioavailability of BCS class 2 compounds.

The present study highlights the fact that in the case of significant component of lymphatic absorption of the drug, LCT enhances the overall bioavailability regardless of its pre-enterocyte effects. However, as shown by blocking the lymphatic pathway, there is also importance to include lipidic components that increase solubilization. Hence, the option of significant lymphatic transport of the drug must be taken into consideration, and the *in vitro* lipolysis data have to be interpreted adequately.

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## REFERENCES

1. C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **46**:3–26 (2001).
2. C. W. Pouton. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and self-microemulsifying drug delivery systems. *Eur. J. Pharm Sci.* **11**:S93–S98 (2000).
3. A. J. Humberstone and W. N. Charman. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Adv. Drug Deliv. Rev.* **25**:103–128 (1997).
4. J. M. Dintaman and J. A. Silverman. Inhibition of P-glycoprotein by D-á-tocopheryl polyethylene glycol 1000 succinate (TPGS). *Pharm Res.* **16**:1550–1556 (1999).
5. G. L. Amidon, H. Lennernas, V. P. Shah, and J. R. Crison. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm Res.* **12**:413–420 (1995).
6. C. J. H. Porter and W. N. Charman. *In vitro* assessment of oral lipid based formulations. *Adv. Drug Deliv. Rev.* **50**:S127–S147 (2001).
7. J. O. Christensen, K. Schultz, B. Mollgaard, H. G. Kristensen, and A. Mullertz. Solubilisation of poorly water-soluble drugs during *in vitro* lipolysis of medium- and long-chain triacylglycerols. *Eur. J. Pharm Sci.* **23**:287–296 (2004).
8. G. A. Kossena, B. J. Boyd, J. H. Christopher, Porter, and W. N. Charman. Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs. *J. Pharm Sci.* **92**:634–648 (2003).
9. H. Ljusberg-Wahren, F. Seier Nielsen, M. Brogard, E. Troedsson, and A. Mullertz. Enzymatic characterization of lipid-based drug delivery systems. *Int. J. Pharm* **298**:328–332 (2005).
10. W. Nienstedt, A. Ojanotko, and H. Toivonen. Metabolism of progesterone, 17-hydroxyprogesterone and deoxycorticosterone by human small intestine *in vitro*. *J. Steroid Biochem.* **13**:1417–1420 (1980).
11. M. P. Harri, W. Nienstedt, and K. Hartiala. Steroid metabolism by the canine intestine. II. The metabolism of progesterone by the jejunal mucosa *in vitro*. *Scand J. Gastroenterol* **5**:415–419 (1970).
12. A. Dahan and A. Hoffman. Evaluation of a chylomicron flow blocking approach to investigate the intestinal lymphatic transport of lipophilic drugs. *Eur. J. Pharm Sci.* **24**:381–388 (2005).
13. L. Sek, C. J. Porter, A. M. Kaukonen, and W. N. Charman. Evaluation of the *in-vitro* digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. *J. Pharm Pharmacol* **54**:29–41 (2002).
14. L. Sek, C. J. H. Porter, and W. N. Charman. Characterisation and quantification of medium chain and long chain triglycerides and their *in vitro* digestion products, by HPTLC coupled with *in situ* densitometric analysis. *J. Pharml Biomed. Anal* **25**:651–661 (2001).
15. A. Hoffman and G. Levy. Kinetics of drug action in disease states. XXIX. Effect of experimental nephrotic syndrome on the pharmacodynamics of heptabarbital: implications of severe hypoalbuminemia. *J. Pharmacol Exp. Ther.* **249**:117–122 (1989).
16. D. B. Gomis, V. J. Escotet Arias, L. E. Fidalgo Alvarez, and M. D. Gutierrez Alvarez. Simultaneous determination of vitamins D3, E and K1 and retinyl palmitate in cattle plasma by liquid chromatography with a narrow-bore column. *J. Chromatogr B Biomed. Appl* **660**:49–55 (1994).
17. C. J. H. Porter, A. M. Kakunen, A. Taillardat-Bertschinger, B. J. Boyd, J. M. O'Connor, G. A. Edwards, and W. N. Charman. Use of *in vitro* lipid digestion data to explain the *in vivo* performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: studies with halofantrine. *J. Pharm Sci.* **93**:1110–1121 (2004).
18. A. Bernard and H. Carlier. Intestinal lymphatic absorption of labelled oleic acid in the normal rat and rat treated with actidione-cycloheximide or acetoxycycloheximide. *C R Seances Acad Sci. III* **292**:97–100 (1981).
19. S. M. Sabesin and K. J. Isselbacher. Protein synthesis inhibition: mechanism for the production of impaired fat absorption. *Science* **147**:1149–1151 (1965).
20. T. Gershanik and S. Benita. Positively charged self-emulsifying oil formulation for improving oral bioavailability of progesterone. *Pharm Dev. Technol* **1**:147–157 (1996).

21. K. J. MacGregor, J. K. Embleton, J. E. Lacy, E. A. Perry, L. J. Solomon, H. Seager, and C. W. Pouton. Influence of lipolysis on drug absorption from the gastro-intestinal tract. *Adv. Drug Deliv. Rev.* **25**:33–46 (1997).
22. I. Holmberg, L. Aksnes, T. Berlin, B. Lindback, J. Zemgals, and B. Lindeke. Absorption of a pharmacological dose of vitamin $D_3$ from two different lipid vehicles in man: comparison of peanut oil and a medium chain triglyceride. *Biopharm Drug Dispos* **11**:807–815 (1990).
23. A. Kuksis. Absorption of fat soluble vitamins. In A. Kuksis (ed)., *Fat absorption, Vol. 2*, CRC, Boca Raton, 1987, pp. 65–86.
24. S. M. Khoo, G. A. Edwards, C. J. H. Porter, and W. N. Charman. A conscious dog model for assessing the absorption, enterocyte-based metabolism, and intestinal lymphatic transport of halofantrine. *J. Pharm Sci.* **90**:1599–1607 (2001).
25. K. J. Palin and C. G. Wilson. The effect of different oils on the absorption of probucol in the rat. *J. Pharm Pharmacol* **36**:641–643 (1984).
26. D. J. Hauss, S. E. Fogal, J. V. Ficorilli, C. A. Price, T. Roy, A. A. Jayaraj, and J. J. Keirns. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor. *J. Pharm Sci.* **87**:164–169 (1998).
27. S. M. Caliph, W. N. Charman, and C. J. Porter. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. *J. Pharm Sci.* **89**: 1073–1084 (2000).
28. P. Gershkovich and A. Hoffman. Uptake of lipophilic drugs by plasma derived isolated chylomicrons: linear correlation with intestinal lymphatic bioavailability. *Eur. J. Pharm Sci.* **26**:394–404 (2005).