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Effect of Lipids on Absorption of Carvedilol in Dogs: Is Coadministration of Lipids as Efficient as a Lipid-Based Formulation?

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Abstract

Lipid-based formulations (LBFs) is a formulation strategy for enabling oral delivery of poorly water-soluble drugs. However, current use of this strategy is limited to a few percent of the marketed products. Reasons for that are linked to the complexity of LBFs, chemical instability of pre-dissolved drug and a limited understanding of the influence of LBF intestinal digestion on drug absorption. The aim of this study was to explore intestinal drug solubilization from a long-chain LBF, and evaluate whether coadministration of LBF is as efficient as a lipid-based drug formulation containing the pre-dissolved model drug carvedilol. Thus, solubility studies of this weak base were performed in simulated intestinal fluid (SIF) and aspirated dog intestinal fluid (DIF). DIF was collected from duodenal stomas after dosing of water and two levels (1 g and 2 g) of LBF. Similarly, the *in vitro* SIF solubility studies were conducted prior to, and after addition of, undigested or digested LBF. The DIF fluid was further characterized for lipid digestion products (free fatty acid) and bile salt. Subsequently, carvedilol was orally administered to dogs in a lipid-based drug formulation and coadministered with LBF, and drug plasma exposure was assessed. In addition to these studies, *in vitro* drug absorption from the different formulation approaches were evaluated in a lipolysis-permeation device, and the obtained data was used to evaluate the *in vitro in vivo* correlation. The results showed elevated concentrations of free fatty acid and bile salt in the DIF when 2 g of LBF was administered, compared to only water. As expected, the SIF and DIF solubility data revealed that carvedilol solubilization increased by the presence of lipids and lipid digestion products. Moreover, coadministration of LBF and drug demonstrated equal plasma exposure to the lipid-based drug formulation. Furthermore, evaluation of *in vitro* absorption resulted in the same rank order for the LBFs as in the *in vivo* dog study. In conclusion, this study demonstrated increased intestinal solubilization from a small amount of LBF, caused by lipid digestion products and bile secretion. The outcomes also support the use of coadministration of LBF as a potential dosing regimen in cases where it is beneficial to have the drug in solid form, e.g. due to chemical instability in the lipid vehicle. Finally, the *in vitro* lipolysis-permeation used herein established IVIVC for carvedilol in the presence of LBFs.

Graphical Abstract

Keywords

Intestinal digestion, *in vivo* dog study, lipid-based formulation, coadministration, absorption, *in vitro* *in vivo* correlation (IVIVC)

Introduction

Oral intake is the desirable route of administration for many drugs. However, drug bioavailability from an oral formulation is dependent on favorable biopharmaceutical properties, including adequate gastrointestinal (GI) drug solubility and permeability. A diverse range of drug delivery systems have emerged to enable oral administration of the large number of drug candidates with poor oral bioavailability. [1, 2] One such strategy is to deliver the drug in a lipid-based formulation (LBF). For highly lipophilic and poorly water-soluble drugs (PWSD) the major advantages associated with LBFs are the avoidance of rate-limiting dissolution and increased intestinal solubility when the lipid excipients mix with the GI fluids; overall it is a viable strategy to overcome both dissolution rate-limited and solubility limited absorption of lipophilic compounds. Yet, this delivery strategy is still not widely used, and only a few percent of the market share of orally delivered products are LBFs. [3] Likely reasons are the complex nature of LBFs, reduced chemical stability of pre-dissolved drug in the LBF, and poor predictions of *in vivo* performance. [4, 5]

The ability of LBFs to increase oral bioavailability for PWSD, compared to standard formulations, has been shown in several studies. [6-9] LBFs may hold further potential for weak bases through reduced

1 risk of intestinal precipitation and prevention of erratic food-effects. [5] Because of the inherent lower
2 equilibrium solubility of weak bases at higher pH, supersaturation is likely to occur during transit from
3 the stomach to the small intestine. Supersaturated solutions are always at risk of precipitation, a
4 process that may lead to erratic and reduced drug absorption. The weak base cinnarizine has been
5 extensively studied in LBFs both *in vitro* and *in vivo*. [10-13] One finding from these studies is that
6 food effects can be minimized by LBFs, both through formulation in an LBF [10], and with
7 coadministration of lipids. [12] The reason for the LBF-mediated increased oral bioavailability and
8 reduced food effect of poorly soluble weak bases is probably multifaceted. Besides improved intestinal
9 drug solubilization when the formulation excipients mix with the GI fluids, the formulation excipients
10 may trigger endogenous secretion of bile. The presence of bile is suggested to aid solubilization of
11 LBF digestion products, which results in generation of lipid colloidal phases that maintain drug
12 solubilization in the small intestine. This bile effect has been shown to be more pronounced for basic
13 compounds compared to neutral and acidic ones. [14] Moreover, another potential solubility-
14 enhancing effect generated by LBFs is the presence of free fatty acids (FFAs), that are digestion
15 products from di-, and triglycerides commonly included in these formulations. In our recent *in vitro*
16 lipolysis study, which included a number of weak bases, the equilibrium solubility of weak bases was
17 significantly boosted when the formulation lipids were digested into FFAs. [15] The observed
18 solubility increase is a result of an attraction between the negatively charged FFAs and the positively
19 charged weak bases. [16, 17] However, to date the *in vivo* effect of FFA digestion products is
20 unknown, and complete understanding of the reason for increased oral bioavailability of weak bases
21 from administration of low dose LBF is lacking.

22
23 Thus, the aim of the current study was to examine the intestinal drug solubilization effect, and the
24 resulting effect on absorption, of a weak base from a low dose of LBF. The animal model used was
25 dogs with permanent stoma, where sampling can be performed from both duodenum and plasma. The
26 drug solubilization effect was explored in simulated intestinal fluid (SIF) and aspirated dog intestinal
27 fluid (DIF), prior to and after oral administration of low dose placebo-LBF, followed by analysis of
28 the fluid composition (digestion products and bile). Thereafter, oral administration of carvedilol, a

weak base of low solubility and with pK_a of 8.2, was studied for absorption using i) carvedilol pre-dissolved in an LBF as compared to ii) solid drug coadministered with LBF. These results were combined with *in vitro* lipolysis-permeation studies for assessment of the *in vitro in vivo* correlation (IVIVC). Through this broad *in vitro in vivo* approach we anticipated to elucidate the effect of *in vivo* processing of lipids versus the bile effect on the resulting solubilization, as well as examine the possibility to make use of a solid tablet coadministered with lipids rather than a lipid-based drug formulation.

Material and Methods

Chemicals

Carvedilol, bile salt quantification assay kit (BIOVK209-100) and all analytical solvents were purchased from VWR International (Sweden). Orlistat, porcine pancreatin (8 x USP specifications activity), soybean oil, kolliphor EL, and free fatty acid quantification kit (MAK044) were obtained from Sigma-Aldrich (USA). Maisine 35-1 was a gift from Gattefossé (France). FaSSIF powder was bought from biorelevant.com (UK). Novozyme® 435 (immobilized lipase) was obtained from Strem chemicals (France). All culture media and supplements were purchased from Invitrogen AB (Sweden). Hard gelatin capsules were a kind gift from APL (Sweden).

Model Drug and Formulation

In this study the BCS class II drug carvedilol (Mw 406.5 g/mol, pK_a 8.2, logP 4.1, T_m 114.1 °C) [18] was selected as the weakly basic model compound. The composed LBF contained 32.5 % w/w soybean oil (long-chain triglyceride), 32.5 % w/w Maisine 35-1 (long-chain mixed mono-, di-, triglyceride), and 35. % w/w kolliphor EL (surfactant). To prepare the LBF, the excipients were preheated (soybean oil and kolliphor EL; 37 °C, Maisine 35-1; 70 °C (recommended by manufacturer)). Subsequently, the excipients were weighed into a vial in the above predefined fractions (% w/w), mixed and kept at 37 °C until further use. The maximum drug loading capacity (i.e. saturated drug concentration) in the LBF (37 °C) was determined according to a previously published protocol. [19] Prior to any experiment (~ 48 h), the formulation was loaded with carvedilol by weighing drug and LBF into a glass vial, vortexing the mix, and placing it in 37 °C until use.

Drug Solubility in Simulated Intestinal Fluids

In vitro lipolysis was performed similar to a previously described experiment. [20] A vessel containing digestion medium (37 °C) with a pH-stat (iUnitrode), coupled to a dosing unit was used (Metrohm 907 Titrando, Switzerland). The lipolysis medium consisted of a buffer (pH 6.5) containing 2 mM Tris-maleate, 1.4 mM CaCl₂·2H₂O, and 150 mM NaCl, supplemented with FaSSIF powder (3.0 mM sodium taurocholate and 0.75 mM lecithin). At the start of the experiment, lipolysis medium (54-60 ml, 37 °C) and LBF (0.75 g or 1.5 g) were added and allowed to mix for 10 min (450 rpm). During this dispersion phase, the pH was manually adjusted to 6.5 ± 0.05. Next, digestion was initiated by the addition of either pancreatic extract (6 ml) or immobilized lipase (750 mg, final concentration 125 PLU/ml), to reach a final volume of 60 mL. Pancreatic enzyme extract was prepared by mixing 1.6 g porcine pancreatin, 8 ml buffer, and 20 µl 5 M NaOH in a 15 ml tube, followed by centrifugation at 5 °C and 2,144 g for 15 min. The pancreatic enzyme was tested to have an activity of ~33 TBU/mg equal to ~ 6,600 TBU/ml extract, which resulted in complete digestion. [15] To maintain the pH at 6.5 in spite of release of ionized FFAs, 0.2 M NaOH was automatically titrated from the dosing unit during the digestion phase.

Solubility determinations prior to addition of LBF (lipolysis medium), after addition of LBF (dispersion medium), and after lipid digestion (digestion medium), were performed. Solubility in the dispersion and digestion mediums was determined by performing 'blank' lipolysis (no drug loaded into the LBF), with both pancreatic and immobilized enzyme, according to the procedure described above. The lipolysis medium was sampled (1 ml), at the end of the dispersion phase (10 min) and the digestion phase (30 min). To inhibit further lipolysis 5 µl/ml lipase inhibitor (0.5 M 4-bromophenyl boronic acid in methanol) was added, followed by centrifugation (37 °C, 21,000 g, 15 min) to separate the enzyme from the medium. Sampled dispersion and digestion medium, as well as plain lipolysis medium, was transferred to tubes with excess of crystalline carvedilol (4-5 mg), vortexed and placed on a shaker in an incubator (37 °C). After 1-2 h of incubation, pH was measured and adjusted to 6.5 if needed. After 24 h the samples were centrifuged (37 °C, 2 300 g, 10 min), and the supernatant sampled and quantified for drug with HPLC-UV. All samples were diluted 20-50 fold in acetonitrile

and a second time in mobile phase (3-5 fold). The solubility value was defined as the mean value of the 24 h triplicate samples.

***In vitro* Lipolysis with Absorption**

The *in vitro* lipolysis with absorption was carried out in a lipolysis-permeation setup at 37 °C, as described previously. [21] The device consists of two chambers separated by a Caco-2 cell monolayer, where the upper chamber is used to perform digestion studies and the lower chamber to determine drug permeation across the Caco-2 cells. The digestion experiment (upper chamber) was performed as described above with minor modifications. Formulation F1-F3 (Table 1) were digested with immobilized lipase, which has previously been shown to be compatible with the Caco-2 cell monolayer. [20] For formulation F4 (micronized drug only) the high presence of large undissolved drug particles disrupted the Caco-2 cell monolayers after 10-15 min, and thus the experiment could not be completed. The receiver chamber contained HBSS supplemented with 4% bovine serum albumin (235.4 ml, pH 7.4). Samples were withdrawn from both chambers after 10 min of dispersion and 5, 10, 20 and 30 min of digestion. Digestion samples were treated as described above, followed by dilution (5-20 fold) with acetonitrile, and a second centrifugation (20 °C, 21,000 g, 10 min). Prior to HPLC-UV analysis the samples were diluted (5-fold) in mobile phase. Samples withdrawn from the receiver chamber were diluted (1:8) with acetonitrile spiked with 100 nM warfarin as internal standard and quantified using UPLC-MS/MS. A stable pH in the digestion chamber (pH 6.5) was used as an *in situ* marker for membrane integrity. Loss of integrity will result in mixing of the digestion (pH 6.5) and receiver buffer (pH 7.4). Samples withdrawn 15 min prior to an increase in pH in the digestion chamber were discarded. [21]

Cell culture

For the permeation membrane, Caco-2 cells (American Type Culture Collection, VA, USA) were cultivated in an atmosphere of 90% air and 10% CO₂, as described previously. [22] Caco-2 cells (passage 95 to 105) were seeded on permeable, polycarbonate filter supports (0.45 µm pore size, 75-mm diameter; Transwell Costar, Sigma-Aldrich) at a density of 170,000 cells/cm² in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 1% minimum essential medium

nonessential amino acids, penicillin (100 U/mL), and streptomycin (100 µg/mL). Monolayers were used for experiments on days 21 to 24 after seeding.

Table 1. Comparison of the four test formulations used in the *in vitro* lipolysis-permeation study (F1-F3) and the *in vivo* dog study Part II (F1-F4). The ratio between amount of drug and LBF was kept the same for the *in vitro* and *in vivo* study.

Formulation	Description	<i>In vitro</i> Lipolysis-permeation		<i>In vivo</i> Part II	
		Carvedilol (mg)	LBF (g)	Carvedilol (mg)	LBF (g)
F1	Pre-dissolved carvedilol in LBF	37.5	1.50	25	1.0
F2	Carvedilol coadministered with LBF	37.5	1.50	25	1.0
F3	Carvedilol coadministered with LBF	37.5	0.75	25	0.5
F4	Micronized carvedilol	nd ^a	nd ^a	25	0.0

^anot determined (nd). The high presence of large undissolved drug particles disrupted the Caco-2 cell monolayers after 10-15 min, and thus the experiment could not be completed.

Summary of Dog Study Design (Part I and II)

The *in vivo* dog studies were performed at AstraZeneca, Mölndal, Sweden, and were approved by the Animal Ethics Committee of Gothenburg, approval number 34-2015. The study included three male Labrador dogs surgically equipped with a duodenal stoma,[23] age 3-6 years and weight 35-37 kg. Prior to the studies, the dogs were fasted overnight with free access to water. The *in vivo* study was divided into two different parts (I and II) (Figure 1). In Part I, the dogs were orally administered water, 1 and 2 g placebo LBF (i.e. no drug added) on three separate occasions. Intestinal fluid was sampled for analysis of digestion products (total concentration of FFA and bile salt (BS)) and determination of carvedilol solubility. In Part II, 25 mg carvedilol was orally administered in four different ways (F1-F4) (Table 1); pre-dissolved in 1 g LBF, coadministered with 1 and 0.5 g LBF, respectively, and as micronized powder, on four separate occasions. Plasma samples were withdrawn between 0-24 hours post administration (Figure 1 and Table 1). It was necessary to lower the LBF dose in Part II (1 and 0.5 g) compared to Part I (1 and 2 g), because of the maximum carvedilol dose (25 mg) that could be administered to the dogs. For the coadministration with LBF, a drug dose that was higher than the solubility in the LBF (37.5 mg/g) was targeted; by lowering the LBF dose, formulation F3 met this criterion. Further study details of the two parts are given in the below sections.

Figure 1. Summary of dog study design, divided into part I and II. Part I: sampling of intestinal fluid after oral administration of water and placebo LBF (time scale: minutes). Three samples from each subject (early, mid and late time point) were analyzed for total concentrations of free fatty acids (FFA) and bile salts (BS). Carvedilol solubility (37 °C) was determined and pH recorded in the collected fluids. Part II: on four separate days the dogs were orally administered F1-F4; 25 mg carvedilol pre-dissolved in 1 g LBF, coadministered with 1 and 0.5 g LBF, and as micronized powder, and drug plasma concentrations were determined (time scale: hours).

Sampling of Dog Intestinal Fluid (Part I)

Sampling of DIF was made on three separate days (experiment A, B, C) (Figure 1). The total volume of administered water was 75 ml on all occasions. Both LBF and water were administered via an orogastric tube. At the day of the DIF sampling, A: 75 ml water, B: 1 g of placebo LBF dispersed in 9 ml water, followed by 66 ml water, C: 2 g of placebo LBF dispersed in 8 ml water, followed by 67 ml water, were administered, respectively. The ratio between the LBF dose and the administered volume of water was adapted to match the ratio of LBF/lipolysis medium in the *in vitro* lipolysis studies (Table 2). DIF was sampled from duodenum stomas connected to plastic tubing for the duration of ≥ 1.5 h, and the time of collection and volume was noted. To inhibit further lipolysis of the formulation lipids, DIF was collected in vials standing on ice, and immediately treated with 1 μ l of 1 mM orlistat in ethanol. [24] All DIF samples were stored in -80 °C until further analysis. Three sample points (early, mid, late) from each subject were selected for analysis, and aliquots of the same sample were

used for all types of characterizations. DIF sampling from stomas is erratic and depend on the time point of gastric emptying in each subject, i.e. exact time points for sampling could not be predetermined. Thus, the samples were divided into three approximate time periods; early around 0-5 min, mid around 5-20 min, and late around 20-90 min after administration for comparability of the results.

Table 2. Comparison of the ratio between LBF dose and fluid volume used/administered during *in vitro* lipolysis studies and dog intestinal fluid (DIF) sampling (Part I).

	LBF "low" (g)	LBF "high" (g)	Fluid volume (ml)	Ratio "low" (LBF/Fluid)	Ratio "high" (LBF/Fluid)
<i>In vitro</i> lipolysis	0.75	1.5	60	0.013	0.025
<i>In vivo</i> DIF sampling (Part I)	1.0	2.0	75 ^a	0.013	0.027

^aEquals the administered volume of water, resting volume not taken into account.

Lipid Characterization of Dog Intestinal Fluid

The total concentrations of FFA (non-esterified fatty acids C8 and longer) and BS in the DIF samples were determined with commercially available enzymatic kits (see Chemicals section). The samples from all three subjects, including experiments A, B, and C, were analyzed individually in triplicates. Prior to analysis the samples were gently centrifuged (RT, 1800 g, 5 min) and diluted 10-100 fold in purified water. 96-well plates (3603, Corning®) was used for colorimetric detection at 570 nm (FFA), and 405 nm (BS) respectively, in a multi-mode plate-reader (Spark®, Tecan, Switzerland).

Drug Solubility in Dog Intestinal Fluid

Equilibrium solubility of carvedilol was determined in DIF samples from the three selected time points (early, mid, late) from all three subjects, including experiments A, B, and C (Figure 1). pH was measured (37 °C) at two time points; prior to addition of carvedilol and after ~ 20 h of incubation with drug. Aliquots of 300 µl DIF (preheated to 37 °C) was added to Eppendorf tubes with an excess of crystalline carvedilol (4-5 mg). The samples were gently mixed and placed on a shaking plate (200 rpm) in an incubator at 37 °C. After 24 h the samples were centrifuged (37 °C, 2 300 g, 10 min), followed by 10-fold dilution in acetonitrile. To remove precipitated proteins the samples were spun a second time (4 °C, 2 300 g, 10 min), and prior to HPLC-UV analysis the samples were diluted a second time in mobile phase (5-20 fold). The solubility value was defined as the mean value of the 24 h triplicate samples.

Oral Administration of Drug Loaded LBFs (Part II)

On the day of the experiment, the dogs were pretreated with esomeprazole to increase pH in the stomach (~ 6). This was done by a 3 minute i.v infusion of 1 mg/kg esomeprazole (Nexium, AstraZeneca), 90 min prior to dosing of the test formulations. [25] The purpose of this pretreatment was to equalize pH in the stomach, avoid carvedilol from distributing out of the emulsion at low gastric pH, and to mimic the pH of the *in vitro* lipolysis. Approximately 90 min after the pretreatment the formulations were administered orally together with 75 ml of water (Figure 1 and Table 1). Carvedilol (25 mg) and LBF were administered in hard gelatin capsules as four different test formulations (F1-F4). F1: pre-dissolved carvedilol in 1 g LBF divided into three capsules, F2: one capsule of micronized carvedilol coadministered with 1 g LBF divided into three capsules, F3: one capsule of micronized carvedilol coadministered with 0.5 g LBF divided in two capsules, and F4: one capsule of micronized carvedilol. The dogs were fed four hours after administration of the test formulations. Each dog received each formulation in a cross-over design with at least 3-days wash-out period between dosing. Blood samples (1 ml) were collected from a leg or neck vein prior to administration and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours after administration in vacutainer K2-EDTA-containing tubes (BD, USA). The tubes were turned a few times to gently mix the blood with anticoagulant, followed by centrifugation at 4 °C, 3 000 g for 10 min. Thereafter the plasma was transferred into 2 ml tubes (Sarstedt AG & Co, Germany) and kept at -80 °C. Prior to UPLC-MS/MS analysis the plasma samples were diluted (1:3) with acetonitrile, spiked with warfarin (internal standard) at a concentration of 50 nM, and centrifuged at 4 °C, 2 300 g for 20 min.

HPLC-UV Analysis

Analysis was performed with an HPLC (Agilent Technologies 1290 Infinity) with a Zorbax Eclipse XDB-C18 column (4.6 x 100 mm) (Agilent Technologies, US). The mobile phase consisted of acetonitrile: sodium acetate buffer (pH 5) 50:50 (v/v). A linear gradient with a flow rate of 1 ml/min over 3 min up to 20:80 (v/v) was used, followed by 1 min linear decrease back to 50:50. The injection volume was 20 µL, absorbance monitored at 286 nm, and the retention time of carvedilol was 1.5 min.

UPLC-MS/MS analysis

The UPLC-MS/MS analysis was performed on a Waters Xevo TQ MS, electrospray ionization coupled to an Acquity UPLC system (Waters, Milford, MA), with a Waters BEH C18 2.1 × 50 mm (1.7µm) column. A 2 min gradient with a flow rate of 0.5 ml/min was used. Solvent A consisted of 5% acetonitrile and 0.1% formic acid in water, and solvent B consisted of 0.1% formic acid in acetonitrile. The LC run used a linear gradient from 0.5 to 1.2 min starting at 5% of solvent B and ending at 90% of B, followed by a hold for 0.4 min, and a linear gradient to return to the initial conditions from 1.7 min until the end of the run. The auto sampler tray temperature was 10 °C, injection volume 10 µl, and column temperature 60 °C. The mass spectrometer operated in positive electrospray mode for both carvedilol and warfarin (internal standard). The retention time of carvedilol and warfarin was 1.23 min and 1.37 min, respectively. The mass transitions followed were: 407.99, 99.73 m/z (cone voltage 40 V, collision energy 31 V) for carvedilol, and 309, 163 m/z (cone voltage 22, collision energy 14 V) for warfarin. Data acquisition and peak integration were performed with MassLynx software (Waters, Milford, MA).

Statistics and Pharmacokinetic Calculations

Data handling was carried out in Microsoft Excel, whereas visualization, statistical analysis and calculation of area under the curve (AUC) were performed in GraphPad Prism 7.0 (Graphpad Software Inc., USA). The data is expressed as mean values with standard deviation (n = 3). Solubility differences in SIF and DIF media, FFA and BS concentrations, as well as AUCs were assessed using a one- or two-way ANOVA, followed by a Tukey's multiple comparison analysis test, as appropriate. Results were deemed significant at p < 0.05. To calculate the AUCs of the *in vitro* lipolysis-permeation experiments (dissolved and permeated drug), and the drug plasma concentration-time profiles (Part II), the trapezoidal method was used. To assess differences between the test formulations, the relative bioavailability (F_{rel}), as compared to formulation F4 (micronized carvedilol without lipids present), was calculated based on the average AUC for each formulation from all three subjects (Equation 1).

$$F_{rel} = \frac{AUC_{formulation\ Fx}}{AUC_{formulation\ F4}} \quad \text{Eq 1.}$$

Results

Carvedilol Solubility in LBF and Simulated Intestinal Fluids

The loading capacity of carvedilol in the model long-chain LBF was 37.9 ± 2.69 mg/g (37 °C). The maximum solubility of carvedilol in various SIF media (lipolysis medium, dispersion and digestion medium, digested with immobilized lipase and pancreatic enzyme) is shown in Figure 2, with the corresponding mol drug per mol released free fatty acid presented in Figure S1. The lowest carvedilol solubility (0.15 ± 0.01 mg/ml) was observed in the lipolysis medium, in absence of any LBF. Upon addition of LBF, 0.75 and 1.50 g, the equilibrium solubility increased significantly in the dispersion medium (~10 fold), compared to the lipolysis medium solubility. As the lipids in the LBF were digested, carvedilol solubility was significantly boosted, with one exception; lower dose LBF (0.75 g) digested with the immobilized lipase. As the higher dose LBF (1.50 g) was digested with immobilized lipase the solubility (2.81 ± 0.12 mg/ml) was significantly higher than the corresponding dispersion medium solubility (1.35 ± 0.06 mg/ml). Additionally, as the pancreatic enzyme was used to digest the LBF (0.75 and 1.5 g), the solubility of carvedilol enhanced 3-fold compared to when the immobilized lipase was used. This reflects the higher digestion capacity of the pancreatic enzyme, compared to the immobilized lipase (Figure S2A and B), where a more extensive digestion leads to higher concentrations of FFAs, which increases carvedilol solubility. Interestingly, the solubility of carvedilol almost doubles with twice the amount of LBF, when digested with pancreatic lipase. This demonstrates the importance of FFAs for carvedilol solubility in the digestion medium. However, there is not a 1:1 relationship between carvedilol solubility and liberated FFAs (Figure S1). Although carvedilol solubility increases with the concentration of FFA, the amount of drug is 2-3 times higher than the amount of FFA *in vitro* (Figure S1).

Figure 2. Carvedilol solubility in lipolysis medium (37 °C, pH 6.5), after addition of 0.75 and 1.50 g of placebo LBF (Dispersion), following digestion with immobilized lipase (Digestion IL) and pancreatic enzyme (Digestion PE). The presence of formulation components significantly increased carvedilol solubility in the dispersion medium compared to lipolysis medium. Generally, carvedilol solubility was boosted when the formulation lipids were digested into free fatty acids. However, digestion with PE gave 3-fold higher solubility levels than when the same amount of LBF was digested with IL. Solubility differences were statistically significant ($p < 0.05$) between all different media, except when noted as non-significant (ns).

In vitro Lipolysis with Absorption

In vitro lipolysis of formulation F1-F3 (Table 1) was performed in the lipolysis-permeation setup.

Concentrations of dissolved carvedilol in the aqueous phase and permeated carvedilol into the receiver chamber are shown in Figure 3A and B, respectively. The AUCs of the corresponding concentration time profiles were used to assess differences in carvedilol exposure between the three tested formulations (Figure 3C and D). The resulting carvedilol distribution between aqueous (dissolved drug) and pellet phase (F1; precipitated, F2-3; undissolved drug) are presented in Figure S3A-C.

Figure 3. Carvedilol concentration in aqueous phase and transfer across the monolayers during 30 min *in vitro* lipolysis-permeation of formulation F1, F2 and F3 with immobilized lipase. Formulation details, see Table 1. (A) Concentration of dissolved carvedilol in the aqueous phase. (B) Mass-transfer of carvedilol to the receiver chamber. (C) AUCs of carvedilol concentrations in the aqueous phase vs time profiles. (D) AUCs of carvedilol mass-transfer vs time profiles. Values are expressed as average values $\pm$ SD (n=3). *represent significant differences between AUCs: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

For formulation F1, a majority of carvedilol remained dissolved in the aqueous phase during the dispersion (0.65 mg/ml, ~ 100%). As the digestion proceeded, an increasing level of carvedilol precipitated, and at 30 min of digestion around 20% of the dose had precipitated (Figure 3A and S3A). For F2 and F3, the full dose of carvedilol did not dissolve during the lipolysis experiment, and hence, the concentrations of dissolved carvedilol in the aqueous phase were significantly lower compared to F1 (Figure 3A and C). It should be noted that for all three tested formulations, the concentrations of dissolved carvedilol are below the determined equilibrium solubility of carvedilol upon both dispersion and digestion (Figure 2), i.e. supersaturation was not occurring and the full dose should be possible to dissolve over time. For both F2 and F3 approximately 50% of the carvedilol dose was found in the aqueous phase during the dispersion phase (Figure S3B and C), although as the digestion progressed differences were observed between the two formulations. When F2 was digested the concentration of carvedilol in the aqueous phase kept a rather constant level between 0.30 mg/ml – 0.36 mg/ml (48% - 57%), while for F3 the aqueous concentration of carvedilol dropped gradually from 0.31 mg/ml (50%) down to 0.19 mg/ml (31%), at the 30 min sampling point. The difference in

concentration of dissolved drug between F2 and F3 after 30 min of digestion can be linked to amount of coadministered LBF, since carvedilol thrives in the presence of higher concentrations of lipid digestion products, FFAs (Figure 2).

Carvedilol transfer across the Caco-2 cell monolayers into the receiver chamber during dispersion and digestion of formulation F1-F3 is depicted in Figure 3B. The carvedilol flux during lipolysis of F1 and F2 was similar, whereas the carvedilol flux of F3 proved to be lower than the flux observed for F1. Looking at the AUCs (Figure 3D), the total mass-transfer of carvedilol from F3 was significantly lower compared to F1. Noteworthy is that while the AUC of the dissolved carvedilol in the aqueous phase is significantly lower for F2 compared to F1 this was not reflected in the mass-transfer of carvedilol for the two formulations. Hence, no significant difference was observed between the AUCs of the total mass-transfer for F1 and F2.

Lipid and pH Characterization of Dog Intestinal Fluid

Determined duodenal concentrations of FFA and BS at the three time points (early, mid, late) are shown in Figure 4A-B. Each time point shows the average molar concentration in DIF collected from three dogs. Elevated levels of FFA were observed after administration of 1 and 2 g of LBF, compared to only water. After administration of water the FFA concentrations ranged between 0.87 – 1.28 mM, whereas 1 g of LBF provided 3.89 – 6.58 mM FFA, and 2 g LBF resulted in 9.99 – 12.37 mM of FFA. Interestingly, the duodenal FFA concentrations after administration of 2 g LBF were significantly increased compared to after administration of water at all three time points. The higher LBF dose also provided higher FFA concentration at the mid and late time points compared to after administration of 1 g of LBF. The increase in FFA concentration after administration of placebo LBF primarily originates from digestion of the formulation glycerides. The BS concentrations followed a similar rank order as the FFA concentrations (Figure 4A-B), where the BS concentrations becomes elevated after administration of LBF, compared to water. However, a lag time was observed for the increase in BS concentration. At the early sample point (0-5 min post administration) the average BS concentrations for water and the two LBF doses were at comparable levels, in the range of 4.3 – 10.1 mM. At the

following time points the concentrations of BS were higher after administration of LBF, compared to water. No significant difference in BS concentrations between the two doses of LBF were observed. However, after administration of 2 g LBF the highest concentration of BS (22.7 ± 9.2 mM) was observed at the mid time point, while after administration of 1 g LBF the highest concentration was observed at the late time point (18.8 ± 8.9 mM). The DIF was also analyzed for pH (Figure 4C), and the pH at 37 °C of the duodenal fluids ranged between 1.8–7.3. However, no significant differences in pH was observed between the sample points or as LBF was administered (1 and 2 g) compared to water. An additional pH measurement was done after addition of carvedilol to perform the solubility study. On average, pH of the DIF increased 0.9 pH units by the addition of carvedilol (~20 h of incubation at 37 °C).

Figure 4. Total free fatty acid (FFA) and bile salt (BS) concentrations, pH, and carvedilol solubility in dog intestinal fluid (DIF), after administration of water, 1 and 2 g of placebo LBF at three different time points (early, mid, late), respectively. A schematic overview of the DIF sampling (part I) is given in Figure 1. In general, carvedilol solubility was elevated with higher levels of FFA and BS, while pH was not affected by administration of LBF. (A) Molar concentrations of FFA (B) Molar concentrations of BS. (C) pH of the sampled DIF. (D) Carvedilol solubility (mg/ml). *represents the significant difference towards water, and # represents the significant difference towards 1 g LBF. ****p < 0.0001; ***p < 0.001, ##/***p < 0.01, and #/***p < 0.05.

Carvedilol Solubility in Dog Intestinal Fluid

Carvedilol solubility in DIF (37 °C) at the three selected time points (early, mid, late) is shown in Figure 4D, and mol drug per mol FFA in Figure S1. Each point shows average solubility in DIF collected from three dogs. Carvedilol solubility ranged from 0.21 mg/ml up to 4.6 mg/ml in the collected duodenal samples. When only water was administered the solubility of carvedilol in DIF was constant between 0.21-0.33 mg/ml at all three time points. Similarly, when 1 g LBF was administered a constant solubility range between 0.71 – 1.12 mg/ml was observed. Even if the overall solubility level was slightly higher in DIF after administration of 1 g LBF compared to only water, the difference was not statistically significant. However, after administration of 2 g LBF the carvedilol solubility increased significantly (mid time point), compared to after administration of only water and 1 g LBF. The solubility differences observed in the DIFs at the mid time point (Figure 4D) was compared to the levels of FFA (Figure 4A), BS (Figure 4B) and pH (Figure 4C) after administration of water, 1 g, and 2 g of LBF, respectively. From evaluating the profiles, pH of the duodenal fluid does not appear to be the major determining factor for carvedilol solubility. At the mid-time point carvedilol solubility is significantly different after administration of water and 2 g of LBF, and after administration 1 and 2 g LBF (Figure 4D), while the pH of the DIF at the mid-time point is similar for all three administrations (Figure 4C). However, comparing DIF solubility with the FFA and BS concentrations, the solubility follows the same rank order (water < 1 g LBF < 2 g LBF) as both FFA and BS concentrations (primarily mid-time point). In other words, these data indicate that carvedilol intestinal solubility is increasing with higher concentrations of FFA and BS already when a clinically relevant dose of LBF is administered.

Plasma Pharmacokinetics of Administered LBFs

Plasma concentrations of carvedilol were determined after oral administration of formulation F1-F4 to three Labrador dogs (Figure 1 and Table 1). The obtained plasma concentration time profiles are shown in Figure 5A-C, and Table 3 provides an overview of $C_{\max}$, $T_{\max}$, AUC, and relative bioavailability (F_{rel}) (Equation 1). The peak plasma concentrations of carvedilol occurred between 0.5 h up to 8 h after administration of the four test formulations. Formulation F3 showed double peaks in

the plasma concentration profiles for all three dogs. This trend was not observed for any of the other administered formulations, and possible reasons for the double peak phenomena is considered in the discussion.

Figure 5. Carvedilol plasma concentration time profiles (0-24 h) for formulation F1-F4 (Figure 1 and Table 1) orally administered to (A) Dog 1, (B) Dog 2, and (C) Dog 3. Note that the scales on the y-axes are different. The order of the carvedilol plasma exposure from the tested formulations follows the same trend for all three subjects. Pre-dissolved carvedilol in 1 g LBF (F1), and coadministered carvedilol with 1 g LBF (F2) gives similar plasma exposure, while it was considerably lower after coadministration of carvedilol with 0.5 g LBF (F3), and micronized carvedilol (F4).

From the plasma curves and the calculated AUCs it was observed that formulation F1 (pre-dissolved carvedilol in 1 g LBF) and F2 (coadministered carvedilol with 1 g LBF) provides similar plasma concentration profiles, while the plasma exposure from F3 (coadministered carvedilol with 0.5 g LBF) and F4 (micronized carvedilol) were considerably lower (Figure 5A-C and Table 3). Although the same trend of carvedilol plasma exposure from the four test formulations was observed among the three dogs, there were large interindividual variations in the carvedilol concentrations. In particular, the plasma concentrations ($C_{\max}$ and AUC) for Dog 3 were much higher than for the other two dogs. Thus, relative bioavailability was calculated to evaluate differences between formulations (Table 3). The relative bioavailability proved no difference in carvedilol plasma exposure between F1 and F2, i.e. pre-dissolved drug in LBF and drug coadministered with lipids demonstrated an equal level of carvedilol absorption. Additionally, the higher dose of LBF (1 g), both coadministered with micronized drug (F2), and with pre-dissolved drug (F1), provided significantly higher plasma exposure compared to the lower dose coadministered LBF (0.5 g, F3).

Table 3. Plasma pharmacokinetic parameters after oral administration of 25 mg carvedilol to three Labrador dogs in lipid formulation F1-F4 (Figure 1 and Table 1).

	Dog 1			Dog 2			Dog 3				
	C _{max} (ng/ml)	T _{max} (h)	AUC (ng/ml*h)	C _{max} (ng/ml)	T _{max} (h)	AUC (ng/ml*h)	C _{max} (ng/ml)	T _{max} (h)	AUC (ng/ml*h)	F _{rel} ^a (%)	±
F1	32.4	1.5	110.6	49.1	1.0	169.7	130.9	0.5	270.4	124 ^b	14
F2	33.9	3.0	128.8	57.1	1.0	196.1	142.4	1.0	283.2	139 ^c	13
F3	5.2	8.0	62.4	18.4	1.0	119.5	32.9	4.0	154.5	76	15
F4	15.1	2.0	102.4	18.2	0.5	130.0	55.1	1.0	202	-	-

^aRelative bioavailability compared to F4, see equation 1. ^bSignificantly different from F3 p < 0.01, ^cSignificantly different from F3 p < 0.001.

Evaluation of *In Vitro In Vivo* Correlation: Carvedilol Solubility and Absorption

When the *in vitro* lipolysis solubility (Figure 2) was compared to the *in vivo* DIF solubility of carvedilol (Figure 4D), the same tendency was observed; carvedilol solubility increases in the intestinal media as the LBF was added/administrated and digested. Determined carvedilol solubility in the lipolysis medium (0.15 ± 0.01 mg/ml) and average DIF solubility (all three time points: early, mid, late) after administration of only water (0.28 ± 0.20 mg/ml) showed to be comparable. Furthermore, when placebo LBF was administered and digested in the intestine, the solubility on average (all three time points: early, mid, late) increased to 1.05 ± 0.78 mg/ml for 1 g LBF (“low”), and 2.71 ± 1.93 mg/ml for 2 g LBF (“high”), respectively (Figure 6A). Surprisingly, the average carvedilol DIF solubility matches the *in vitro* lipolysis solubility better when the immobilized enzyme was used for the digestion compared to the standard pancreatic enzyme. Hence, the relatively low digestion capacity of the immobilized enzyme, as compared to the pancreatic extract (Figure S2A and B), more accurately predicted the *in vivo* DIF solubility of carvedilol in this study for both the “low” and “high” dose LBF (Table 2).

Moreover, the same rank order of the formulations was observed between the *in vitro* permeation (Figure 3B and D) and the *in vivo* relative bioavailability of carvedilol (Table 3), where $F3 < F1/F2$. The difference in carvedilol exposure for formulation F3, as compared to F1 and F2, is visualized in Figure 6B by comparing *in vitro* AUCs of carvedilol mass-transfer (Figure 3D) and *in vivo* AUCs of the relative bioavailability for each formulation (Table 3). That is, the *in vitro* mass-transfer of

carvedilol across Caco-2 monolayers during dispersion and digestion of LBFs showed to be related to the *in vivo* plasma exposure of carvedilol.

Figure 6. *In vitro in vivo* correlation (IVIVC) of carvedilol intestinal solubility and absorption. (A) Comparison of average carvedilol solubility (all three time points, see Figure 4D) in dog intestinal fluid (DIF) to solubility in digested lipolysis medium (immobilized lipase (IL) and pancreatic lipase (PE)), of the “low” and “high” level of LBF (see Table 2). (B) IVIVC of the relative bioavailability (F_{rel}) of carvedilol plasma exposure *in vivo* and *in vitro* mass-transfer across monolayers to the receiver chamber during dispersion and digestion. Values are expressed as average values $\pm$ SD ($n \geq 3$).

Discussion

Intestinal drug solubilization and absorption of a weak base after administration of a long-chain LBF was assessed in the present study. It was observed that clinically relevant doses of the explored LBF (2 g) triggered bile secretion in dogs (Figure 4B). Similar BS concentrations (~ 10 mM) have been observed when 2 g of long-chain LBF was administrated to humans. [26] However, in the previous study, intestinal drug solubility was not studied after the administration of lipids. We observed increased carvedilol solubility in the duodenal fluid after administration of 2 g LBF, as compared to only water (Figure 4D). The *in vitro* data also demonstrated increased carvedilol solubility upon addition of LBF, and then further improved solubility as the formulation lipids were digested (Figure 2). Thus, the *in vitro* data links the solubility increase to the presence of FFA. For the *in vivo* data, it is more complex since a low dose of LBF also proved to trigger bile secretion (Figure 4A-B). *In vitro* studies of lipid colloidal structures containing oleic acid suggest that the solubility decreases (cinnarizine) or is unaffected (halofantrine) for weak bases as the medium is diluted with bile. [14] Indeed, the effect of adding FFA has been shown to give a more pronounced solubility effect, compared to increased BS levels, for cinnarizine in simulated intestinal fluid. [27] In another *in vitro* study, the solubility of carvedilol in simulated intestinal fluid with extreme BS concentration (50 mM)

was ~ 0.7 mg/ml, and further increased in the presence of digested LBF to ~ 0.8 mg/ml. [28] Taken together, *in vitro* the presence of FFAs is the essential component for increased drug solubilisation of the weak base carvedilol, while *in vivo* the effect is mediated by both FFA and triggered BS secretion. Thus, the present study indicate that it is important to mimic bile secretion and make species adaptations to the *in vitro* setting for accurate *in vivo* predictions, this is further deliberated later in the discussion.

We showed that the approach of coadministering LBF with solid drug produced comparable plasma exposure to an LBF with pre-dissolved drug in the dog model used (Figure 5 and Table 3). Recently, a similar approach (referred to as the chasing principle) was evaluated for cinnarizine, danazol and halofantrine in a rat model. [29] In that study, the drug was administered either being pre-dissolved in the LBF, or as an aqueous suspension, together with the LBF on the side. The study showed comparable plasma exposure for the two formulation approaches. In another study, a lipid-based suspension of halofantrine was administered to beagle dogs. [30] The lipid-based suspension performed similar to the standard LBF with pre-dissolved drug. Compared to the two above mentioned approaches, the current approach with coadministration of solid drug in capsules provide a better alternative for drugs sensitive to oxidation, since this does not require predissolution in any type of vehicle (water or lipids). The tested principle also provides a possibility to use LBFs to deliver drugs that display both poor aqueous and lipid solubility. Typically, the use of LBFs is restricted by the amount of drug that can be dissolved in a clinically relevant dose, i.e. maximum 2 g, although the resulting intestinal solubilizing capacity might be greater. For example, in this study the average carvedilol solubility in DIF after administration of 2 g LBF was 2.71 mg/ml, the fluid volume given was 75 ml, and assuming the gastric resting volume for a larger breed of dog (> 20 kg) are similar to humans ~ 50 ml. [31] This would provide an intestinal solubilizing capacity of > 300 mg carvedilol, while the amount of drug possible to dissolve and deliver in 2 g of LBF is restricted to < 80 mg (loading capacity at 37 °C, 37.9 mg/g). This exemplifies why *in vivo* processing and the resulting intestinal solubility is important when designing lipid-based dosage forms; indeed, a much higher dose than that defined by the loading capacity of the LBF might be possible to deliver and dissolve in the GI tract.

The complex relationship between intestinal formulation processing and absorption of drug and lipid products complicates the establishment of robust IVIVC for LBFs. [5, 32] The standard *in vitro* lipolysis method (without an absorptive sink) [33, 34] as well as initial droplet size of the formulation [6, 7, 35] results in poor and inconsistent *in vivo* correlations. Thus, in the current study a newly developed lipolysis-permeation device, that enables simultaneous study of digestion and absorption was assessed for its potential to produce IVIVC. [21] Similarly to what was observed in the previous study, we found that the *in vitro* aqueous concentration of drug in the digestion chamber was not related to plasma exposure (Figure 3A and C, Table 3), whereas permeation of carvedilol across the absorptive membrane into the receiver chamber was in agreement with the plasma exposure in Labrador dogs (Figure 6B). These results highlights the importance of capturing lipid digestion simultaneously with drug absorption to obtain IVIVC.

In the *in vitro* lipolysis-permeation studies, the higher carvedilol aqueous concentration for F2 0.36 mg/ml (57% of the dose) compared to F3 0.19 mg/ml (31% of the dose) after 30 min of digestion (Figure 3A and C) can be related to the double LBF dose, and the resulting higher concentration of FFAs. [15] This apparent increase in the carvedilol aqueous concentration for F2 led to comparable flux to F1, while still not significantly different from F3. The reason could be that the actual free concentration of drug during digestion of F3 was not higher than during digestion of F2, assuming the colloidal partitioning was unaltered. [36] In the digestion medium a large proportion of a lipophilic drug molecule, such as carvedilol, is solubilized in colloidal structures formed by lipids and digestion products. Therefore the higher LBF dose mainly resulted in increased carvedilol solubilization, and hence, exemplified a solubility-permeability tradeoff with relatively low free fraction of carvedilol available for absorption. [37-39] However, *in vivo*, the higher LBF dose (F2) gave significantly higher plasma exposure compared to the lower dose (F3) (Figure 5 and Table 3). The dynamic changes *in vivo* in response to an increased amount of LBF are complex. In order to solubilize the entire carvedilol dose a sufficient amount of lipids, digestion and bile products needs to be present in the GI tract. Thus, a likely explanation for the hampered absorption *in vivo* from formulation F3 is that the

complete drug dose was not dissolved in the GI tract by the lower amount of LBF, and resulting lower concentration of FFAs and bile products. On the contrary, the higher dose of LBF resulted in an increased amount of lipids that were digested, which in turn led to solubilization of the entire drug dose and significantly higher plasma exposure. Additionally, in contrast to the *in vitro* situation, the LBF and bile products are exposed to continued dilution and absorption *in vivo*. This will reduce carvedilol solubilization along the GI tract, thus promoting supersaturation, and hence, stimulating absorption.

Plasma concentration of carvedilol was determined in fasted Labrador dogs after oral administration of the four test formulations (Table 1). Formulation F3 resulted in double plasma peaks for all three subjects, while this was not observed for any other formulation. In a previous study, when cinnarizine-loaded LBFs were administrated to Labrador dogs, double peaks were also observed. [11] In that study the double peaks appeared arbitrarily, i.e. after administration of different types of LBFs in altering subjects, and was concluded not to be caused by the LBFs. However, in the present study, the double carvedilol peaks seem to be dependent on LBF dose, since it is occurring at the lower dose of LBF and not the higher. Known sources of multiple peaking are gastric emptying, multiple absorption sites in the intestine, enterohepatic recycling, and complexation. [40] The β -blockers acebutolol, nadolol and pafenolol have demonstrated erratic absorption after oral administration in rats and humans. For nadolol and pafenolol, the compromised absorption is related to association with unabsorbable BS micelles. [41, 42] Drug solubilization into BS micelles results in reduced absorption in the proximal part of the small intestine. Further down in distal ileum, where BS is reabsorbed, the free fraction of drug available for absorptions increases, and thus results in a second peak in the plasma concentration profile. [40] Supposing carvedilol is solubilized into BS micelles [43], and that both doses of LBF triggers a similar level of bile secretion, the observed plasma profile pattern for F3 may be caused by a larger fraction of drug molecules located in BS micelles unavailable for absorption, compared to F2, where more lipids are present to aid the dissolution process. Moreover, carvedilol solubilization into BS micelles might similarly explain the lower relative bioavailability of F3 compared to F4. However, from the data in the present study, it is not possible to conclude on the underlying mechanism for the

1 observed plasma profile pattern of carvedilol.

2
3 As mentioned earlier, this dog study confirmed the results of the human study by Porter *et al.* [26];
4 that a small clinically relevant dose of lipid increases intestinal BS concentrations. In light of this
5 finding, the relevance of 3 mM sodium taurocholate in the standard lipolysis medium, as introduced
6 by the LFCS consortium, can be discussed. [44] During lipolysis, BS facilitate lipase access at the
7 oil/water interface [45], and it has indeed been reported that the triglyceride lipolysis rate and extent,
8 as well as the fraction of ionized FFA increases with higher BS concentrations. [46-48] Elevated
9 concentrations of bile salts and phospholipids may also emulsify the lipid excipients, which will
10 influence the colloidal structures and their ability to aid drug solubilization. Moreover, even if the dog
11 BS concentration in this study is similar to what was previously found in humans after administration
12 of LBFs, differences in bile secretion exists between species. [31] Hence, instead of the standard 3
13 mM sodium taurocholate in the lipolysis medium, the concentration is suggested to be mimicking a
14 fed state, or alternatively, be supplemented with sodium taurocholate as the digestion phase starts, to
15 reach a level around 10-20 mM BS. This reflects the BS concentrations that was observed after dosing
16 of 1-2 g of LBF (Figure 4B). Additionally, it might be necessary to consider species differences in bile
17 secretion for *in vitro* evaluation of LBFs, to better reflect the *in vivo* conditions. However, it should be
18 noted that in the current lipolysis-permeation device with Caco-2 cell monolayers, a higher
19 concentration of sodium taurocholate may affect membrane integrity, [49] this is yet to be evaluated.

20
21 Additionally, in this study we observed that carvedilol intestinal solubility was in better agreement
22 with solubility in the digestion medium after use of immobilized lipase, compared to the standard
23 porcine pancreatic enzyme (Figure 6A). This finding was surprising, since the immobilized lipase has
24 reduced lipolytic rate compared to the pancreatic enzyme (Figure S2A and B). The reduced activity is
25 suggested to be due to that immobilized lipase contain a single enzyme (recombinant lipase B,
26 originating from *Candida Antarctica*), and also because of the immobilization onto polymeric beads
27 which hinders accessibility to the oil droplets. [20, 50] On the contrary, it has been shown that the
28 activity of the standard porcine pancreatic enzyme under optimized *in vitro* conditions is 1300-8000

fold higher than *in vivo*. [51] The higher porcine pancreatic enzyme activity *in vitro* compared to *in vivo* may partly explain the deviating solubility results, while yet another reason could be related to species differences in enzyme activity. The porcine pancreatic enzyme is used as the standard enzyme during *in vitro* lipolysis since it is available to purchase off the shelf, which for example, canine pancreatic enzyme is not. In other words, the porcine pancreatic enzyme may not be the most suitable enzyme for mimicking the *in vivo* conditions because of species differences and elevated enzymatic activity during *in vitro* lipolysis. Thus, as an alternative approach to the standard lipolysis setup and protocol [44], increased BS concentrations that can facilitate access to the oil/water interface for the immobilized lipase in the lipolysis-permeation device could be evaluated. This *in vitro* methodology has the potential to broaden the understanding of the LBF field, and improve IVIVC for this type of formulations.

Conclusion

This study demonstrated increased intestinal solubilization from a clinically relevant dose of LBF caused by lipid digestion products and bile secretion. The *in vitro* lipolysis-permeation used herein established IVIVC for carvedilol in the presence of LBFs when the fluxed amount over an absorption membrane was used for comparison. The detailed analysis of the *in vivo* sampled intestinal fluids indicates that both the digestion of the LBF as such and the triggered bile secretion play an important role in solubilization *in vivo*. Furthermore, the results also showed that coadministration of LBF with solid drug in capsule is as a potential dosing regimen for free weak bases. This is an attractive formulation strategy for compounds that are chemically not stable in a lipid vehicle. It would also be a more generic approach of producing LBF-like formulations, where any new solid dosage form can be converted to an LBF with minimal formulation efforts.

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- 3

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