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Simulated Lymphatic Fluid for In-Vitro Assessment in Pharmaceutical Development

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ABSTRACT

In addition to removing excess extracellular fluid and mobilizing immune cells throughout the body, lymphatic fluid provides a means for drug transport. Lymphatic drug delivery can impart higher efficacy and bioavailability, especially following oral administration. Currently, there is no standardized composition for simulated lymphatic fluid. Standardization of a simulated lymphatic fluid media would be an important and novel contribution to fill the current void in this emerging area of drug and formulation development. This study aims to review and analyze the composition and flow rate of prepared simulated lymphatic fluid with comparison to commercially available artificial fluid and biological fluid. The prepared simulated fluids were closer in makeup to biological fluid than the commercial artificial fluid, which suggests potential benefits for developing and optimizing lymphotropic formulations for in vitro studies.

KEYWORDS: Lymphatic fluid, simulated lymphatic fluid, lymphotropic drug development, dissolution

BACKGROUND

Lymph or lymphatic fluid is a biological fluid derived from the interstitial fluid of parenchymal cells throughout the body. The hydrostatic pressure within the capillary beds results in molecule ultrafiltration from arterioles, which drives some blood components along with proteins into the interstitial space (*1*). Approximately 90% of the 20–30 liters of the plasma that leaks into the interstitium returns to blood through the capillary venous end, and the remaining fluid is drained back along with other molecules from the extracellular space to the circulation by the lymphatic system in the form of lymph (*2*, *3*). Lymph also carries the invading pathogens and immune cells into lymph nodes where proper immune responses can be mounted (*2*, *4*). Thus, lymph serves in regulating and modulating immune response, thereby affecting some important immunological processes like immune tolerance in the body. Furthermore, lymph has two other main functions – maintaining fluid homeostasis and delivering some nutrients and fat absorption products from the intestine to the general circulation (*5*).

Lymph Flow

From the extracellular space, lymph first enters blind-ended lymphatic capillaries, termed the "initial lymphatics." Next, it drains into the lymphatic collecting vessels, then passes through at least one but usually several lymph nodes distributed throughout the body. Collecting vessels merge into larger trunks that empty into lymphatic ducts. Finally, the ducts return the lymph back into the venous circulation at the junction of the jugular and subclavian veins (Fig. 1), completing the circuit of fluid transport (*6*, *7*).

Dietary lipids and highly lipophilic drugs are usually packaged into lipoprotein vesicles (chylomicrons) in the cytoplasm of the enterocytes before being up taken by the lymphatic capillaries (*8*). Triglycerides of ingested lipids are usually hydrolyzed by lipases into monoglycerides and fatty acids prior to reaching the duodenum. When hydrolysis products enter the enterocytes, long chain fatty acids (C ≥ 12) and monoglycerides get re-esterified in the endoplasmic reticulum and are assembled into chylomicrons, which then migrate to the Golgi apparatus before being exocytosized into intestinal lymphatics (*9*, *10*).

Lymph flow rate differs among species, within species, and within subjects (*11*, *12*). Ultimately, it is a function of the blood flow through an organ and the proportion of the blood that leaks from the capillaries into the lymphatics

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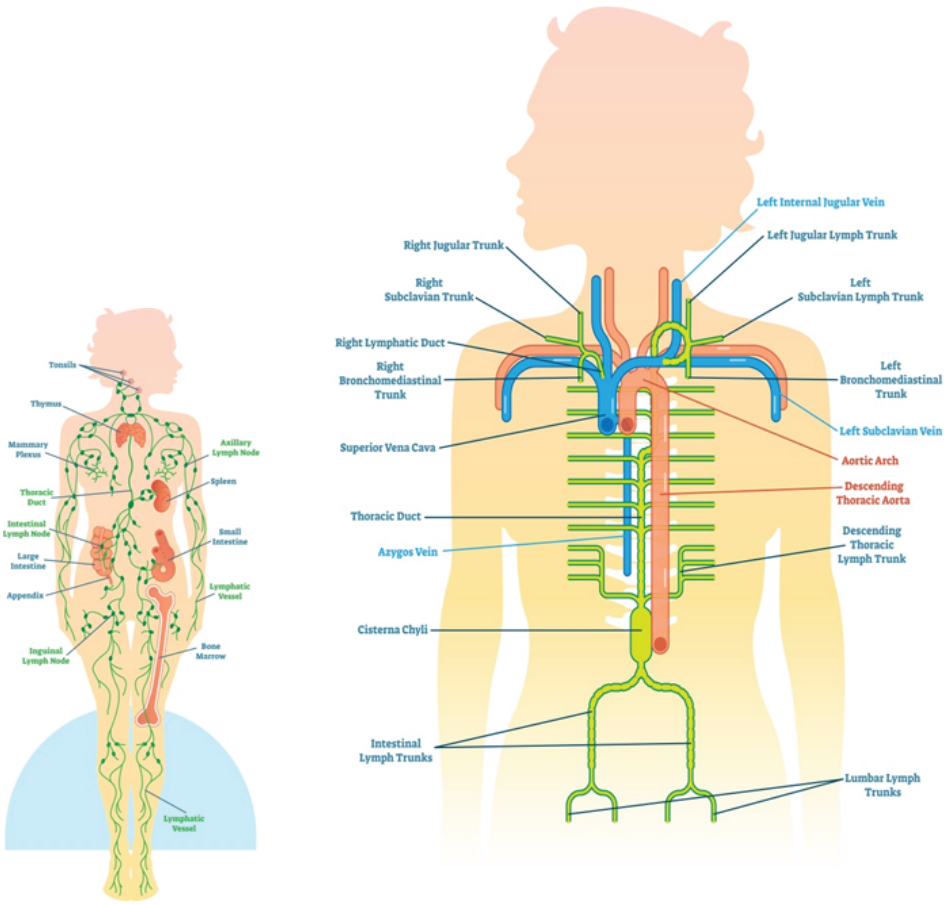

Figure 1. Lymphatic system through the body (left). The vessels part of this system starts with the initial lymphatic capillaries that are interlaced with arterioles and venules, then drain into lymphatic collectors and trunks before they empty into the right lymphatic and thoracic ducts, which in turn joins the venous circulation at the junction between the jugular and subclavian veins on both body sides (right). Illustrations used under a standard license from depositphotos. ©VectorMine (Eduards Kantāns).

(*12*). Pre-nodal (afferent) lymph flow collected from fasted-state rat mesentery is 15 µL/h, and post-nodal (efferent) flow of the mesenteric duct is approximately 1.3 mL/h (*13*). The rat lymph flow represents 1.1% of plasma flow (*12*). In humans, the afferent lymph flow is estimated to be twice that of the efferent, i.e., 8 and 4 L/day, respectively (*14*). This is attributed to the reabsorption that occurs at the lymph nodes through special blood vessels across. Lymph components can cross into either direction, thus changing the post-nodal lymph rate and composition (*3*, *15*, *16*).

Lymph Composition

Reported studies on lymph composition date back to the late 1920s. In 1932, Heim tested thoracic and cervical lymph of dogs for protein, non-protein nitrogen, urea, uric acid, creatinine, sugar, amino acids, chlorides, calcium, and phosphorus (*17*). After comparing his results with previously reported data, Heim concluded that the chemical composition of lymph overlaps with that of the plasma. The exception was for protein and its related substances (phosphorous and calcium), which were higher in plasma than in lymph (*17*). In a study that investigated rat ovarian lymph, the total concentration of protein was estimated to be 53% of the plasma (*12*). Yet, similar studies in other species have reported higher percentages of total protein concentration in lymph compared to plasma, with the highest level recorded in sheep as 90% (*18*, *19*).

Similarly in humans, the lymph composition was first believed to coincide with that of the plasma as the former was considered a filtrate of the latter. Challenges associated with cannulating lymphatic vessels with collecting only little amounts of lymph for analysis and the low sensitivity and resolution of the analytical instruments were barriers to a thorough analysis of lymph (*3*, *17*). Nevertheless, lymph biology has progressed over the years and these hurdles have been resolved. It is now known that pre-nodal lymph has a similar make up of salts, plasma proteins, sugars, and lymphocytes as interstitial

fluid. However, the post-nodal lymphatic fluid is more concentrated, having a higher count of lymphocytes and a two-fold higher concentration of the plasma protein (*2*, *15*, *20*).

Proteomic analysis revealed that the proteins essential to osmotic pressure maintenance, namely albumin, α1, α2, β globulins, and fibrinogen, in addition to the coagulation factors are higher in plasma than in lymph (*21–24*). Nevertheless, the lymph is richer in extracellular matrix proteins and intracellular proteins resulting from cellular metabolism, i.e., those released from apoptosis and lipoproteins (*25–29*). To date, more than 2000 proteins have been identified through protein mapping of lymph derived from various species including human (*30*). Mapping was mostly done on peripheral subcutaneous and mesenteric pre-nodal lymph; therefore, the full lymph profile is not yet completed (*30*).

The cellular component averages 12,000 ± 5200 cells/µL in rat mesenteric lymph (*13*). In human peripheral lymph, there are 162 cells per mm^3, with lymphocytes accounting for up to 96% of these cells (*31*). Low concentration of these cells does not appear to affect the bulk properties of the lymph (*2*).

Simulated Lymphatic Fluid

In the field of pharmaceutical sciences, lymph-targeted drug delivery can open a new era to medicines and vaccination (*5*). This approach has relevant therapeutic and pharmacokinetic benefits (*32*). The intestinal lymphatic transport, in particular, can demonstrate various advantages over portal blood absorption following oral administration of drugs (*33*). Intestinal lymphatic absorption can aid drugs by shunting away from first pass hepatic metabolism, thus imparting higher bioavailability. Thus, drugs with a high extraction ratio are the most ideal candidates to be delivered via lymphatic fluid. Additionally, intestinal lymphatic delivery can boost efficacy of chemotherapeutic and immunomodulatory agents, with the lymphatic system being primarily involved in cancer metastasis and immune modulation (*6*, *10*, *33*). Examples of lymphotropic drugs include carvedilol, halofantrine, praziquantel, docetaxel, valsartan, among others (*34–40*).

The importance of standardized physiologically relevant simulated fluids for use in biopharmaceutic and dissolution studies have been highlighted in multiple articles and reviews; however, there is no mention of standardized simulated lymphatic fluid in these publications (*41–43*). Standardization of a simulated clinically or physiologically relevant lymphatic fluid media would be an important and novel contribution to fill the current void in this emerging area of drug and formulation development.

Simulated lymphatic fluid can be composed of the components listed in Table 1 (*2*, *41*). The composition of salts in the proposed fluid is similar to that of the extracellular fluid (*41*). Protein composition is approximated to be less than 0.01 g/mL in pre-nodal lymph (*2*). As albumin is a main protein that affects drug binding and pharmacokinetics, it can be added into the simulated lymphatic fluid. It composes nearly 60% of the total protein in lymph and would be used in the concentration of less than 0.006 g/mL (*12*, *21*, *44*).

Table 1. Concentration of Various Constituents in Lymphatic Fluid (2, 41)

Component	General Simulated Lymphatic Fluid (mM)	Intestinal Simulated Lymphatic Fluid or Chyle (mM)
HCO^{3-}	4.2	4.2
K^+	5	5
Cl^-	148.8	148.8
Na^+	142	142
Ca^+	2.5	2.5
Mg^+	1.5	1.5
HPO_4^{-2}	1	1
SO_4^{-2}	0.5	0.5
Tris(hydroxymethyl) aminomethane	50	50
Hydrochloric acid	45	45
Proteins	< 0.01 g/mL	0.02–0.06 g/mL
Triglyceride fat globules (chylomicrons)	-	> 0.01 g/mL

Lymph derived from the intestine is termed "chyle." It has a rich protein content (0.02–0.06 g/mL) and a milky color, unlike the clear lymph from other parts of the body (*45*, *46*). The white color is attributed to the fact that this portion of lymph is rich in chylomicrons (*47*, *48*). Chylomicrons are mainly composed of triglycerides as well as phospholipids, proteins, and cholesterol (*49*).

Intralipid (Fresenius Kabi Canada Ltd, Toronto) 20% is a commercial product used for providing fats and calories to patients in need of total parenteral nutrition (*50*). The components of Intralipid are listed in Table 2. Interestingly, the components are comparable to those of endogenous chylomicrons, which are used as a main component to simulate intestinal lymphatic fluid (*50*, *51*). The size of globules lies within the range of the chylomicron's; furthermore, it contains the main components that constitute chylomicrons and chiefly affect their drug uptake. The acids present in soybean oil, from which Intralipid is prepared, resemble the typical

acids packaged into the chylomicrons (*52*, *53*). As a consequence, the simulated chyle would be similar to the previously described lymphatic fluid, with a slightly higher albumin level (0.012–0.036 g/mL). As a surrogate for the chylomicron component of lymphatic fluid, Intralipid has a triglyceride concentration of more than 0.01 g/mL (*2*, *50*).

Table 2. Composition of Intralipid and Endogenous Chylomicrons

	Chylomicrons	Intralipid
Size	75–1000 nm (1 µ)	0.5 µ
Components	Triglycerides (84%) Phospholipidis (7%) Protein (2%) Cholesterol (7%) Cholesterol esters (2%)	Soybean oil* (20 g) Egg phospholipids (1.2 g) Glycerol (2.2 g) q.s water for injection (100 mL)

**Acids in soybean oil include 52% linoleic and 22% oleic in addition palmitic (13%), linolenic (8%), stearic (4%), myristic (< 1%), and other acids (1%).*

MATERIALS AND METHODS

Preparation of Simulated Lymphatic Fluid

For pharmaceutical compounding and preparation of simulated lymphatic fluid, reagents in Table 3 are used. The method of preparation for both general and intestinal lymphatic fluid involves successive addition of the reagents in the specified amounts after each being fully dissolved in 700 mL water, before adjusting to the required pH with 1 M HCl and completing the volume to 1 L (*41*). If stored at 2–8 °C, simulated general lymphatic fluid is stable for more than 2 months, whereas simulated intestinal lymphatic fluid requires gentle shaking before use if stored under similar conditions and for the same period of time.

Analysis of Simulated Lymphatic Fluids

The lymphatic fluids (prepared and a commercially obtained product) were analyzed to compare their composition and properties with the reported data for biological lymph. Variables used for comparison of the fluids included pH, density, chemical content, and solubility.

The pH of the fluids was measured using a Fischer Scientific XL20 pH/conductivity meter.

The density of 80 mL-samples of each fluid were analyzed using a Mettler Toledo XPR/XSR-Ana density kit at 25 ± 0.2 °C. The specified amount was placed in the beaker, the sinker was completely immersed, and after ensuring no bubbles adhered to the immersed sinker, the draft shield was closed. After the balance reached stability, the obtained readings were recorded.

Medica's EasyRA analyzer was used to investigate the lymphatic fluids for chemical content, i.e., potassium (K^+), sodium (Na^+), calcium (Ca^{++}), magnesium (Mg^{++}), phosphorus (P^{2-}), iron (Fe^{3+}), and chloride (Cl^-) ions, as well as total carbon dioxide (CO_2), total protein, albumin, and triglyceride concentration. After calibration and a system check, cleaning the probe and the ISE (ion selective electrode), calibrated proper reagents were placed in sample holder with the blank (HPLC grade water) and 100 or 2000 µL samples of the simulated lymphatic fluids (or dilutions of the fluids). The required tests were processed and the acquired results were noted.

Solubility of a lymphotropic drug (rifampicin), a zwitterion with pKa 1.7 for the 4-hydroxy and 7.9 for the 3-piperazine nitrogen, was measured using the shake-flask method adopted from the literature in the prepared simulated lymphatic fluids and the commercially obtained artificial fluid.

Table 3. Reagents for Preparing Simulated Lymphatic Fluid

Reagent	CAS number	Amount for 1 L of Simulated Lymphatic Fluid
Sodium chloride	7647-14-5	8.035 g
Sodium bicarbonate	144-55-8	0.355 g
Potassium chloride	7447-40-7	0.225 g
Potassium phosphate dibasic	7758-11-4	0.231 g
Magnesium chloride hexahydrate	7791-18-6	0.311 g
1 M Hydrochloric acid	7647-01-0	39 mL
Calcium chloride dihydrate	10035-04-8	0.292 g
Sodium sulfate	7757-82-6	0.072 g
Tri(hydroxymethyl) aminomethane	7283-04-7	6.118 g
Protein (human serum albumin)	70024-90-7	40 g
Intralipids	68890-65-3	100 mL

RESULTS AND DISCUSSION

When compared with a commercial artificial lymphatic fluid (Biochemazone, batch no. BZ-0421A), the prepared simulated lymphatic fluids more accurately resembled the composition of biological lymphatic fluid (*2*, *52*). The data are presented in Table 4 for comparison.

The density values of both commercial and laboratory-prepared fluids were within the range reported for biological lymphatic fluid. However, the pH of the commercial fluid (6.98) did not lie within the physiological range reported for lymph collected from the thoracic duct (7.08–7.40) (*55*). Despite being close to the reported range, this pH could pose a hurdle to using commercial fluid for in-vitro drug studies in which the conditions are

usually set to mimic those in vivo.

The concentration of Na^+ in all fluids was less than the reported value in the biological fluid. The lowest concentration was recorded for in the artificial fluid, whereas the simulated lymphatic fluid values were closer to the reported physiological value. A similar result was determined with Ca^{++} concentration. The artificial lymphatic fluid had no Cl^- and very high concentration of K^+ and Mg^{++} whereas the values of both in the prepared simulated lymphatic fluids were within an acceptable range compared to biological fluid. All fluids contained a higher concentration of P^{2-} than the reported physiological value; the highest was the artificial fluid by almost 18-fold, and the simulated fluids were higher by approximately 2 and 3 fold for the simulated general and intestinal fluids, respectively. A negligible amount of iron was detected in the simulated intestinal lymphatic fluid, stemming from the reagents and the Intralipid used to prepare the fluid. The artificial fluid contained a high concentration of CO_2, unlike the others examined that had no CO_2.

All essential amino acids and serum proteins were present in the artificial lymphatic fluid with a total protein concentration of 0.004 g/mL. Albumin concentration was 0.002 g/mL, which coincides with the level of the albumin present in the general biological lymphatic fluid. The prepared simulated fluids only contained the albumin level known to be in the general and intestinal lymph (*2*). Usually, acidic drug binding in particular can be affected by albumin concentration, thus having an accurate representation of protein concentration in simulated lymphatic fluid is prudent. The presence of other protein moieties in the artificial commercial fluid, although at low concentrations, remains to be determined. The total protein in the simulated intestinal lymphatic fluid indicated that there are other proteins besides albumin. That additional protein was traced back to the soy protein in the soybean oil in Intralipid (Table 2).

Triglycerides were found in both the artificial and prepared simulated fluids in different amounts. The artificial fluid had 0.0009 g/mL triglycerides, whereas the simulated general fluid was developed deliberately to have none and the simulated intestinal fluid contained an amount within the stated range of the fat component found mainly in the intestinal lymph (> 1%) (*2*). The lipids used in the simulated intestinal fluid resemble those incorporated into the chylomicrons and transported through the intestinal lymph, as outlined earlier.

Solubility of rifampicin (pKa 1.7 and 7.9) was 2.49 mg/mL in the artificial lymphatic fluid and 2.62 and 3.00 mg/mL in the prepared simulated general and intestinal lymphatic fluids, respectively (*56*). The drug solubility in the artificial and simulated general fluids was nearly the same. Yet, optimal results were encountered with the

Table 4. Comparison of Prepared Simulated Lymphatic Fluids, Commercial Artificial Fluid, and Biological Fluid

Property or Component	Commercial Artificial Lymphatic Fluid*	Simulated General Lymphatic Fluid	Simulated Intestinal Lymphatic Fluid	Biological Lymphatic Fluid (*2*, *52*)
pH	6.98	7.4	7.4	7.08–7.4
Density	1.007 g/mL	1.006 g/mL	1.005 g/mL	1.005–1.016 g/mL
Na^+	97.3 mM	135.7 mM	135.7 mM	142 mM
K^+	43.5 mM	5.23 mM	5.23 mM	5 mM
Cl^-	_	117.4 mM	117.4 mM	148.8 mM
Ca^+	0.91 mM	2.51 mM	2.51 mM	2.5 mM
Mg^+	3.65 mM	1.45 mM	1.45 mM	1.5 mM
P^{2-}	6.02 mM	1.06 mM	1.06 mM	1 mM (phosphate) 0.32 mM (phosphorus)
Fe^{3+}	-	-	0.007 mM	-
CO_2	48.6 mM	-	-	-
Albumin	0.002 g/mL	0.005 g/mL	0.021 g/mL	≤ 0.0054 g/mL for general lymphatic fluid 0.012–0.036 g/mL for intestinal lymphatic fluid
Total proteins	0.004 g/mL	0.005 g/mL	0.04 g/mL	< 0.01 g/mL for general lymphatic fluid 0.02–0.06 g/mL for intestinal lymphatic fluid
Triglycerides	0.0009 g/mL	-	0.03 g/mL	> 0.01 g/mL

**Artificial lymphatic fluid was purchased from Biochemazone, Waterloo, Canada (Batch no. BZ-0421A). Dash (-) indicates not detected.*

simulated intestinal fluid, which might be attributed to higher protein and triglyceride content in the simulated intestinal fluid compared to the other two fluids. This result demonstrates that the solubility of a compound can vary in simulated and artificial lymphatic fluids, which is essential when considering pharmaceutical, biochemical, or biological assays using these fluids. Solubility is also important for determining the dissolution profile of lymphotropic drugs and formulations.

CONCLUSIONS

Lymph-targeted drug delivery can open a new era for development of medicines and vaccines. Further study of lymphatic delivery of molecules is an important aspect of pharmaceutical research and development. Understanding and standardization of simulated lymphatic fluid and its relevance must be considered for optimal drug development research processes. This study reviewed and analyzed simulated general and intestinal lymphatic fluids that proved to be closer in makeup to biological fluid than a commercial artificial lymphatic fluid. These results are a step towards filling the current need for a standardized simulated lymphatic fluid in pharmaceutical investigations.

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CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this study.

REFERENCES

1. Levick, J. R.; Michel, C. C. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc. Res*. **2010**, *87* (2), 198–210. DOI: 10.1093/cvr/cvq062.
2. Moore, J. E., Jr.; Bertram, C. D. Lymphatic system flows. *Annu. Rev. Fluid Mech*. **2018**, *50* (1), 459–482. DOI: 10.1146/annurev-fluid-122316-045259.
3. Hansen, K. C.; D'Alessandro, A.; Clement, C. C.; Santambrogio, L. Lymph formation, composition and circulation: a proteomics perspective. *Int. Immunol*. **2015**, *27* (5), 219–227. DOI: 10.1093/intimm/dxv012.
4. Santambrogio, L.; Ed. *Immunology of the Lymphatic System*. Springer; 2013. DOI: 10.1007/978-1-4614-3235-7.
5. Trevaskis, N. L.; Kaminskas, L. M.; Porter, C. J. H. From sewer to saviour - targeting the lymphatic system to promote drug exposure and activity. *Nat. Rev. Drug Discov*. **2015**, *14* (11), 781–803. DOI: 10.1038/nrd4608.
6. Yousef, M;, Silva, D.; Bou Chacra, N.; Davies, N.; Löbenberg, R. The lymphatic system: a sometimes-forgotten compartment in pharmaceutical sciences. *J. Pharm. Pharm. Sci*. **2021**, *24*, 533–547. DOI: 10.18433/jpps32222.
7. Tortora, G. J.; Derrickson, B. H. *Principles of Anatomy and Physiology*. Wiley; 2016.
8. Kim, H.; Kim, Y.; Lee, J. Liposomal formulations for enhanced lymphatic drug delivery. *Asian J. Pharm. Sci*. **2013**, *8* (2), 96–103. DOI: 10.1016/j.ajps.2013.07.012.
9. Yáñez, J. A.; Wang, S. W. J.; Knemeyer, I. W.; Wirth, M. A.; Alton, K. B. Intestinal lymphatic transport for drug delivery. *Adv. Drug Deliv. Rev*. **2011**, *63* (10-11), 923–942. DOI: 10.1016/j.addr.2011.05.019.
10. Cifarelli, V.; Eichmann, A. The intestinal lymphatic system: functions and metabolic implications. *Cell. Mol. Gastroenterol. Hepatol*. **2019**, *7* (3), 503–513. DOI: 10.1016/j.jcmgh.2018.12.002.
11. Trevaskis, N. L.; Lee, G.; Escott, A.; Phang, K. L.; Hong, J.; Cao, E.; Katneni, K.; Charman, S. A.; Han, S.; Charman, W. N.; et al. Intestinal lymph flow, and lipid and drug transport scale allometrically from pre-clinical species to humans. *Front. Physiol*. **2020**, *11*, 458. DOI: 10.3389/fphys.2020.00458.
12. Dharmarajan, A. M.; Bruce, N. W.; McArdle, H. J. Comparison of flow rates and composition of ovarian lymph and blood in the day-16 pregnant rat. *J. Reprod. Fertil*. **1986**, *77* (1), 169–176. DOI: 10.1530/jrf.0.0770169.
13. Dixon, J. B.; Greiner, S. T.; Gashev, A. A.; Cote, G. L.; Moore, J. E., Jr.; Zawieja, D. C. Lymph flow, shear stress, and lymphocyte velocity in rat mesenteric prenodal lymphatics. *Microcirculation* **2006**, *13* (7), 597–610. DOI: 10.1080/10739680600893909.
14. Renkin, E. M. Some consequences of capillary permeability to macromolecules: Starling's hypothesis reconsidered. *Am. J. Physiol*. **1986**, *250* (5 Pt 2), H706–H710. DOI: 10.1152/ajpheart.1986.250.5.H706.
15. Adair, T. H.; Guyton, A. C. Modification of lymph by lymph nodes. III. Effect of increased lymph hydrostatic pressure. *Am. J. Physiol*. **1985**, *249* (4 Pt 2), H777–H782. DOI: 10.1152/ajpheart.1985.249.4.H777.
16. Adair, T. H.; Guyton, A. C. Modification of lymph by lymph nodes. II. Effect of increased lymph node venous blood pressure. *Am. J. Physiol*. **1983**, *245* (4), H616–H622. DOI: 10.1152/ajpheart.1983.245.4.H616.
17. Heim, J. W. On the chemical composition of lymph from subcutaneous vessels. *Am. J. Physiol*. **1933**, *103* (3), 553–558. DOI: 10.1152/ajplegacy.1933.103.3.553.
18. Staples, L. D.; Fleet, I. R.; Heap, R. B. Anatomy of the utero-ovarian lymphatic network and the composition of afferent lymph in relation to the establishment of pregnancy in the sheep and goat. *J. Reprod. Fertil*. **1982**, *64* (2), 409–420. DOI: 10.1530/jrf.0.0640409.
19. Morris, B.; Sass, M. B. The formation of lymph in the ovary. *Proc. R. Soc. Lond. B Biol. Sci*. **1966**, *164* (997), 577–591. DOI: 10.1098/rspb.1966.0049.

20. Ikomi, F.; Kawai, Y.; Ohhashi, T. Recent advance in lymph dynamic analysis in lymphatics and lymph nodes. *Ann. Vasc. Dis*. **2012**, *5* (3), 258–268. DOI: 10.3400/avd.ra.12.00046.
21. Rutili, G.; Arfors, K. E. Protein concentration in interstitial and lymphatic fluids from the subcutaneous tissue. *Acta Physiol. Scand*. **1977**, *99* (1), 1–8. DOI: 10.1111/j.1748-1716.1977.tb10345.x.
22. Wang, Y.; Lang, L.; Huang, P.; Wang, Z.; Jacobson, O.; Kiesewetter, D. O.; Ali, I. U.; Teng, G.; Niu, G.; Chen, X. In vivo albumin labeling and lymphatic imaging. *Proc. Natl. Acad. Sci. USA* **2015**, *112* (1), 208–213. DOI: 10.1073/pnas.1414821112.
23. Chi, J.; Xie, Q.; Jia, J.; Liu, X.; Sun, J.; Chen, J.; Yi, L. Prognostic value of albumin/globulin ratio in survival and lymph node metastasis in patients with cancer: a systematic review and meta-analysis. *J. Cancer* **2018**, *9* (13), 2341–2348. DOI: 10.7150/jca.24889.
24. Lucas, C. E.; Denis, R.; Ledgerwood, A. M.; Grabow, D. The effects of Hespan on serum and lymphatic albumin, globulin, and coagulant protein. *Ann. Surg*. **1988**, *207* (4), 416–420. DOI: 10.1097/00000658-198804000-00008.
25. Fang, J.-F.; Shih, L.-Y.; Yuan, K.-C.; Fang, K.-Y.; Hwang, T.-L.; Hsieh, S.-Y. Proteomic analysis of post-hemorrhagic shock mesenteric lymph. *Shock* **2010**, *34* (3), 291–298. DOI: 10.1097/SHK.0b013e3181ceef5e.
26. Dzieciatkowska, M.; Wohlauer, M. V.; Moore, E. E.; Damle, S.; Peltz, E.; Campsen, J.; Kelher, M.; Silliman, C.; Banerjee, A.; Hansen, K. C. Proteomic analysis of human mesenteric lymph. *Shock* **2011**, *35* (4), 331–338. DOI: 10.1097/SHK.0b013e318206f654.
27. Dzieciatkowska, M.; D'Alessandro, A.; Moore, E. E.; Wohlauer, M.; Banerjee, A.; Silliman, C. C.; Hansen, K. C. Lymph is not a plasma ultrafiltrate: a proteomic analysis of injured patients. *Shock* **2014**, *42* (6), 485–498. DOI: 10.1097/SHK.0000000000000249.
28. Meng, Z.; Veenstra, T. D., Proteomic analysis of serum, plasma, and lymph for the identification of biomarkers. *Proteomics–Clin. App*. **2007**, *1* (8), 747–757. DOI: 10.1002/prca.200700243.
29. Randolph, G. J.; Miller, N. E. Lymphatic transport of high-density lipoproteins and chylomicrons. *J. Clin. Invest*. **2014**, *124* (3), 929–935. DOI: 10.1172/JCI71610.
30. Santambrogio, L. The lymphatic fluid. *Int. Rev. Cell Mol. Biol*. **2018**, *337*, 111–133. DOI: 10.1016/bs.ircmb.2017.12.002.
31. Engeset, A.; Hager, B.; Nesheim, A.; Kolbenstvedt, A. Studies on human peripheral lymph. I. Sampling method. *Lymphology* **1973**, *6* (1), 1–5. DOI: 10.1007/978-1-4684-9030-5_36.
32. Bernier-Latmani, J.; Petrova, T. V. Intestinal lymphatic vasculature: structure, mechanisms and functions. *Nat. Rev. Gastroenterol. Hepatol*. **2017**, *14* (9), 510–526. DOI: 10.1038/nrgastro.2017.79.
33. Ahn, H.; Park, J.-H. Liposomal delivery systems for intestinal lymphatic drug transport. *Biomater. Res*. **2016**, *20* (1), 36. DOI: 10.1186/s40824-016-0083-1.
34. Sanjula, B.; Shah, F. M.; Javed, A.; Alka, A. Effect of poloxamer 188 on lymphatic uptake of carvedilol-loaded solid lipid nanoparticles for bioavailability enhancement. *J. Drug Target*. **2009**, *17* (3), 249–256. DOI: 10.1080/10611860902718672.
35. Holm, R.; Porter, C. J. H.; Edwards, G. A.; Müllertz, A.; Kristensen, H. G.; Charman, W. N. Examination of oral absorption and lymphatic transport of halofantrine in a triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (SMEDDS) containing structured triglycerides. *Eur. J. Pharm. Sci*. **2003**, *20* (1), 91–97. DOI: 10.1016/S0928-0987(03)00174-X.
36. Mishra, A.; Vuddanda, P. R.; Singh, S. Intestinal lymphatic delivery of praziquantel by solid lipid nanoparticles: formulation design, in vitro and in vivo studies. *J. Nanotechnol*. **2014**, *2014*, 1–12. DOI: 10.1155/2014/351693.
37. Attili-Qadri, S.; Karra, N.; Nemirovski, A.; Schwob, O.; Talmon, Y.; Nassar, T.; Benita, S. Oral delivery system prolongs blood circulation of docetaxel nanocapsules via lymphatic absorption. *Proc. Natl. Acad. Sci. USA* **2013**, *110* (43), 17498–17503. DOI: 10.1073/pnas.1313839110.
38. Beg, S.; Swain, S.; Singh, H. P.; Patra, ChN.; Rao, M. E. B. Development, optimization, and characterization of solid self-nanoemulsifying drug delivery systems of valsartan using porous carriers. *AAPS PharmSciTech* **2012**, *13* (4), 1416–1427. DOI: 10.1208/s12249-012-9865-5.
39. Chaturvedi, S.; Garg, A.; Verma, A. Nano lipid based carriers for lymphatic voyage of anti-cancer drugs: An insight into the in-vitro, ex-vivo, in-situ and in-vivo study models. *J. Drug Deliv. Sci. Technol*. **2020**, *59*, 101899. DOI: 10.1016/j.jddst.2020.101899.
40. Cote, B.; Rao, D.; Alany, R. G.; Kwon, G. S.; Alani, A. W. G. Lymphatic changes in cancer and drug delivery to the lymphatics in solid tumors. *Adv. Drug Deliv. Rev*. **2019**, *144*, 16–34. DOI: 10.1016/j.addr.2019.08.009.
41. Marques, M. R. C.; Loebenberg, R.; Almukainzi, M. Simulated biological fluids with possible application in dissolution testing. *Dissolut. Technol*. **2011**, *18* (3), 15–28. DOI: 10.14227/DT180311P15.
42. Galia, E.; Nicolaides, E.; Hörter, D.; Löbenberg, R.; Reppas, C.; Dressman, J. B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharm. Res*. **1998**, *15* (5), 698–705. DOI: 10.1023/A:1011910801212.
43. Amaral Silva, D.; Al-Gousous, J.; Davies, N. M.; Bou Chacra, N.; Webster, G. K.; Lipka, E.; Amidon, G.; Löbenberg, R. Simulated, biorelevant, clinically relevant or physiologically relevant dissolution media: The hidden role of bicarbonate buffer. *Eur. J. Pharm. Biopharm*. **2019**, *142*, 8–19. DOI: 10.1016/j.ejpb.2019.06.006.
44. Sleep, D. Albumin and its application in drug delivery. *Expert Opin. Drug Deliv*. **2015**, *12* (5), 793–812. DOI: 10.1517/17425247.2015.993313.
45. Hamill, J. M. Observations on human chyle. *J. Physiol*. **1906**, *35* (1-2), 151–162. DOI: 10.1113/jphysiol.1906.sp001187.
46. Natale, G.; Bocci, G.; Ribatti, D. Scholars and scientists in the history of the lymphatic system. *J. Anat*. **2017**, *231* (3), 417–429. DOI: 10.1111/joa.12644.
47. Bragdon, J. H. On the composition of chyle chylomicrons. *J.*

Lab. Clin. Med. **1958**, *52* (4), 564–570. DOI: 10.1016/S0022-2275(20)39079-9.

48. Suy, R.; Thomis, S.; Fourneau, I. The discovery of the lymphatic system in the seventeenth century. Part II: the discovery of Chyle vessels. *Acta Chir. Belg*. **2016**, *116* (5), 329–335. DOI: 10.1080/00015458.2016.1195587.
49. Cooper, A. D. Hepatic uptake of chylomicron remnants. *J. Lipid Res*. **1997**, *38* (11), 2173–2192. DOI: 10.1016/S0022-2275(20)34932-4.
50. Dureau, P.; Charbit, B.; Nicolas, N.; Benhamou, D.; Mazoit, J.-X. Effect of Intralipid® on the dose of ropivacaine or levobupivacaine tolerated by volunteers: a clinical and pharmacokinetic study. *Anesthesiology* **2016**, *125* (3), 474–483. DOI: 10.1097/ALN.0000000000001230.
51. Férézou, J.; Gulik, A.; Domingo, N.; Milliat, F.; Dedieu, J.-C.; Dunel-Erb, S.; Chevalier, C.; Bach, A. C. Intralipid 10%: physicochemical characterization. *Nutrition* **2001**, *17* (11-12), 930–933. DOI: 10.1016/S0899-9007(01)00667-0.
52. Desmarchelier, C.; Borel, P.; Lairon, D.; Maraninchi, M.; Valéro, R. Effect of nutrient and micronutrient intake on chylomicron production and postprandial lipemia. *Nutrients* **2019,** *11* (6), 1299. DOI: 10.3390/nu11061299.
53. Oscarsson, J.; Hurt-Camejo, E. Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and their mechanisms of action on apolipoprotein B-containing lipoproteins in humans: a review. *Lipids Health Dis*. **2017**, *16* (1), 149. DOI: 10.1186/s12944-017-0541-3.
54. Souza, J. B.; Souza, J.; Castro, L. M. L.; Siqueira, M. F.; Savedra, R. M. L.; Silva-Barcellos, N. M. Evaluation of the losartan solubility in the biowaiver context by shake-flask method and intrinsic dissolution. *Pharm. Dev. Technol*. **2019**, *24* (3), 283–292. DOI: 10.1080/10837450.2018.1472610.
55. Bergofsky, E. H.; Jacobson, J. H., II; Fishman, A. P. The use of lymph for the measurement of gas tensions in interstitial fluid and tissues. *J. Clin. Invest*. **1962**, *41* (11), 1971–1980. DOI: 10.1172/JCI104655.
56. Gao, Y.; Zuo, J.; Bou-Chacra, N.; Pinto, T. d. J. A.; Clas, S.-D.; Walker, R. B.; Löbenberg, R. In vitro release kinetics of antituberculosis drugs from nanoparticles assessed using a modified dissolution apparatus. *BioMed Res. Int*. **2013**, *2013*, 136590. DOI: 10.1155/2013/136590.