

THORACIC DUCT FUNCTION IN FETAL, NEWBORN, AND ADULT SHEEP

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ABSTRACT

We measured thoracic duct lymph flow rate versus outflow pressure in 7 chronically catheterized adult sheep and in 6 newborn lambs and compared our results to data previously obtained from 10 fetal sheep. In fetal sheep the thoracic duct lymph flow rate was 34.5 ± 17.2 ml/hr or 11.7 ± 6.0 ml/kg/hr. Fetal thoracic duct lymph flow deviated from baseline between 8 and 12 torr outflow pressure and lymph stopped at 18 ± 2.5 torr. In newborn lambs the thoracic duct lymph flow rate was 49.5 ± 22.0 ml/hr or 7.4 ± 2.5 ml/kg/hr. The range of outflow pressures over which newborn lymph flow deviated from baseline was between 15 and 18 torr and lymph flow stopped at 26.2 ± 6.4 torr. Adult sheep thoracic duct lymph flow rate was 130 ± 74 ml/hr or 2.3 ± 1.3 ml/kg/hr. Adult lymph flow deviated from baseline between 25 and 35 torr and stopped at an outflow pressure of 41.7 ± 6.7 torr. The ability of the thoracic duct to return lymph against an outflow pressure improves with maturation. However, lymph flow rate corrected for body weight is greatest in immature animals. The higher corrected lymph flow rate in conjunction with the decreased ability to pump against an outflow pressure may help account for immature animals predisposition for edema.

Immature animals are particularly prone to develop edema. One mechanism by which edema occurs is when the thoracic duct is

unable to return lymph to the systemic circulation at the junction of the thoracic duct and the confluence of veins. For lymph to enter into the venous circulation, thoracic duct pressure must exceed systemic venous pressure. Studies in fetal sheep have shown that the outflow pressure at which lymph flow begins to diminish is only slightly greater than normal physiologic venous pressure (1,2). Whereas thoracic duct lymph flow rates have been measured in adult sheep, flow rates against outflow pressures have not been characterized, and no studies to our knowledge have been performed in newborn animals. We hypothesized that the ability of the thoracic duct to return lymph to the venous circulation against an outflow improved with maturation. To test this hypothesis, we measured thoracic duct lymph flow as a function of outflow pressure in chronically catheterized newborn lambs and adult sheep and compared the results to our previous fetal sheep data (1).

MATERIAL AND METHODS

We previously reported lymph flow measurements in 10 fetal sheep (1). For our present study, we compare the measurements in those 10 fetal sheep with newly obtained measurements of lymph flow in 6 newborn lambs and 7 nonpregnant, adult ewes (1).

Surgical Preparation

We have previously described the

surgical preparation of our fetal sheep in detail (1). In essence, we inserted a chronic, indwelling catheter into the thoracic duct in the left cervical area, such that the cervical and brachiocephalic branches continue to drain into the jugular vein. We also chronically placed catheters in the carotid artery, the superior vena cava, and into the amniotic cavity. By connecting the lymph catheter and the superior vena cava catheter, we created a fistula, which allowed lymph to return to the circulation during the 3 day postoperative, pre-experimental period.

For the six newborn lambs and the 7 nonpregnant adult sheep, we used isoflurane general anesthesia. We incised the skin over the left jugular vein and extended it to the thoracic inlet. By blunt dissection we located the thoracic duct as it joined the confluence of veins. Then, as in the fetus, we inserted a catheter 1 cm into the duct in a location that allowed the cervical and brachiocephalic lymphatic branches to continue to empty into the jugular vein.

The lymph catheters were made of .050 inch, internal diameter, Tygon® (Fisher Scientific, Pittsburgh, PA). They were 120 to 140 cm in length. To minimize the problem of clotting, all catheters were heparin impregnated (TDMAC Processing, Polysciences, Inc., Warrington, PA).

Next, we dissected free the carotid artery and jugular vein and inserted Tygon® catheters into them. We positioned the 8 F, heparin impregnated jugular venous catheter so that its tip rested in the superior vena cava. After all the catheters were sutured in place and the cervical incision closed in layers, we shortened the thoracic duct and jugular venous catheters to minimize resistance to flow. As in the fetal sheep, we then created a fistula to allow return of lymph to the circulation by inserting the thoracic duct catheter into the venous catheter. We stored all catheters in a plastic pouch sutured to the neck.

We administered penicillin and gentamicin via continuous infusion intraopera-

tively and gave Liquamycin® intramuscularly every 72 hours for infection prophylaxis. For postoperative analgesia, we administered buprenorphine. All animals recovered for at least 3 days following surgery before we began the experiment.

Experimental Methods

In the fetal sheep, we made 15 minute collections of lymph at a known catheter outflow height relative to amniotic fluid pressure. Corrections for amniotic fluid pressure were made manually. We made at least 3 lymph flow collections before altering catheter height. If consecutive lymph flow rates varied by more than 10% at each height, then we made more collections until the variability decreased.

We made similar lymph collections for the lambs and adult ewes. We made 5 or 10 minute lymph collections at known lymph catheter outflow heights referenced to the height of the olecranon. Three consecutive collections whose volume varied by not more than 10% were collected prior to increasing the catheter outflow height. Then we changed the catheter outflow height, allowed 5 to 10 minutes for equilibration, and repeated lymph collections.

For all animals we returned the volume of lymph collected to the venous circulation, ml/ml, over the ensuing study period. For the fetuses, we returned the actual lymph collected; for lambs and adults, we returned 0.9% saline. In addition, since the newborn lambs were removed from their mothers and unable to nurse, we gave the lambs 10 ml 5% Dextrose every hour for hydration. The adults had free access to food and water.

For the fetuses, lambs, and adult ewes, we measured the catheter pressure at which thoracic duct lymph flow stopped. We also recorded the maximum pressure generated by the thoracic duct during no flow using Statham P-23b pressure transducers. This was termed the stop-flow pressure.

During all lymph collections, we

measured vascular pressures using Statham P-23b pressure transducers and recorded the pressures on an eight-channel amplifier recorder (Gould, Inc. Instruments Division, Cleveland, OH). We determined the heart rate from the phasic aortic blood pressure tracing. We obtained blood samples and measured pH, arterial oxygen tension, and arterial carbon dioxide tension using a Corning 178 pH blood gas analyzer (Corning Medical and Scientific, Medfield, MA), total protein concentration, and the hematocrit at baseline and at completion of the experiment.

At autopsy, we removed the thoracic duct and jugular venous catheters, measured their lengths, and determined their total resistance to flow by infusing 5% albumin-saline at 1 ml/minute and measuring the pressure drop across the length of the catheters. We then corrected all outflow pressures for catheter resistance:

$$\text{Outflow pressure} = (\text{Lymph flow rate} \times \text{Catheter resistance}) + \text{Catheter outflow height}$$

Statistical Methods

We compared lymph flow rates as a function of outflow pressures and used a two-way analysis of variance to determine differences. We used a student Neuman-Keuls for determinations of significance. We defined break-point range as the catheter outflow pressures between which the lymph flow rate significantly decreased. We considered a p value of <0.05 as significant. For comparisons of lymph flow rates, break-point pressures, and stop-flow pressures between fetuses, lambs, and adult ewes we used an unpaired student's t -test and considered a $p < 0.05$ as significant.

RESULTS

The resistance to flow in each fetal lymphatic catheter measured 2.1 ± 0.7 torr/ml/min at a flow rate of 1 ml/min; lamb

catheter resistance measured 2.7 ± 0.8 torr/ml/min; adult sheep catheter resistance measured 2.2 ± 0.8 torr/ml/min (mean \pm S.D.). All data have been corrected for catheter resistance.

The fetal sheep were 129 ± 7 days at time of operation and weighed 3.06 ± 0.57 kg at autopsy. Fetal lymph flow rates remained relatively constant over the range of lymph catheter outflow pressures encountered in physiologic situations, 0-8 torr. Flow rate at baseline (0 torr) was 34.5 ± 17.2 ml/kg/hr. The break-point in lymph flow rate, determined by ANOVA, occurred between 8 and 12 torr. The stop-flow pressure obtained from the catheter height at which lymph flow stopped was 16.4 ± 3 torr and that obtained from flow against a transducer was 18.0 ± 2.5 torr (*Table 1*).

The lambs were 6.2 ± 2.2 days old at time of study and weighed 6.7 ± 1.9 kg. Lymph flow rates for the lambs and the adult sheep decreased in a more linear manner than in the fetuses, without a distinct plateau, but we were able to determine by ANOVA a range of pressures over which flow became distinctly different. Lamb lymph flow rate at 0 torr outflow pressure was 49.5 ± 22.0 ml/hr or 7.4 ml/kg/hr. In the lambs the break-point occurred at a catheter outflow pressure between 15 and 18 torr and lymph flow stopped at an outflow pressure of 22.8 ± 8.4 torr by catheter height and at 26.2 ± 6.4 torr by transducer (*Table 1*).

The adult sheep were all greater than 1 year old and weighed 54.8 ± 8.1 kg at time of study. The lymph flow rate at 0 torr outflow pressure was 130 ± 74 ml/hr or 2.3 ± 1.3 ml/kg/hr. In adult sheep the break-point in lymph flow rate occurred between 25 and 35 torr and lymph flow stopped at 42.3 ± 6.4 torr by catheter height and 41.7 ± 6.7 by transducer (*Table 1*). *Fig. 1* graphically displays lymph flow rates as a function of outflow pressures for fetal sheep, lambs, and adult ewes.

Heart rate, vascular pressures, pH, arterial oxygen and carbon dioxide tensions, total protein concentration, and hematocrit

TABLE 1
Lymph Flow Rate, Break-Point Pressure, and Stop-Flow Pressure
in Fetal Sheep, Lambs, and Adult Sheep

	Lymph Flow Rate (baseline)		Break-Point Range (torr)	Stop-Flow (catheter height) (torr)	Stop-Flow (transducer) (torr)
	(ml/hr)	(ml/kg/hr)			
Fetuses (n=10)	34.5±17.2	11.7±6.0	8-12	16.4±3.0	18.0±2.5
Lambs (n=6)	49.5±22.0*	7.4±22.0*	15-18	22.8±8.4*	26.2±6.4*
Adults (n=7)	130±74*	2.3±1.3*	25-35	42.3±6.4*	41.7±6.7*

Mean±SD; *different from preceding value, p<0.05, unpaired t test

TABLE 2
Heart Rate, Aortic (P_{AO}) and Venous (P_{SVC}) Pressures
in Fetal Sheep, Lambs, and Adult Sheep Whose Lymph Flow Rate
was Measured as a Function of Outflow Pressure

	Heart Rate (beats/min)	P _{AO} (torr)	P _{SVC} (torr)	Hct (%)	Protein (g/dl)
Fetuses (n=10)					
baseline	173±9	45±3	4±1	32±3	3.4±0.3
final	172±19	44±3	3±1	32±3	3.5±0.3
Lambs (n=6)					
baseline	207±64	84±5	2±1	33±4	5.8±0.9
final	209±59	84±7	2±1	36±4	5.8±0.7
Adults (n=7)					
baseline	107±10	86±6	6±4	33±3	6.3±0.4
final	105±5	87±5	7±2	31±2	6.1±0.4

Mean±SD

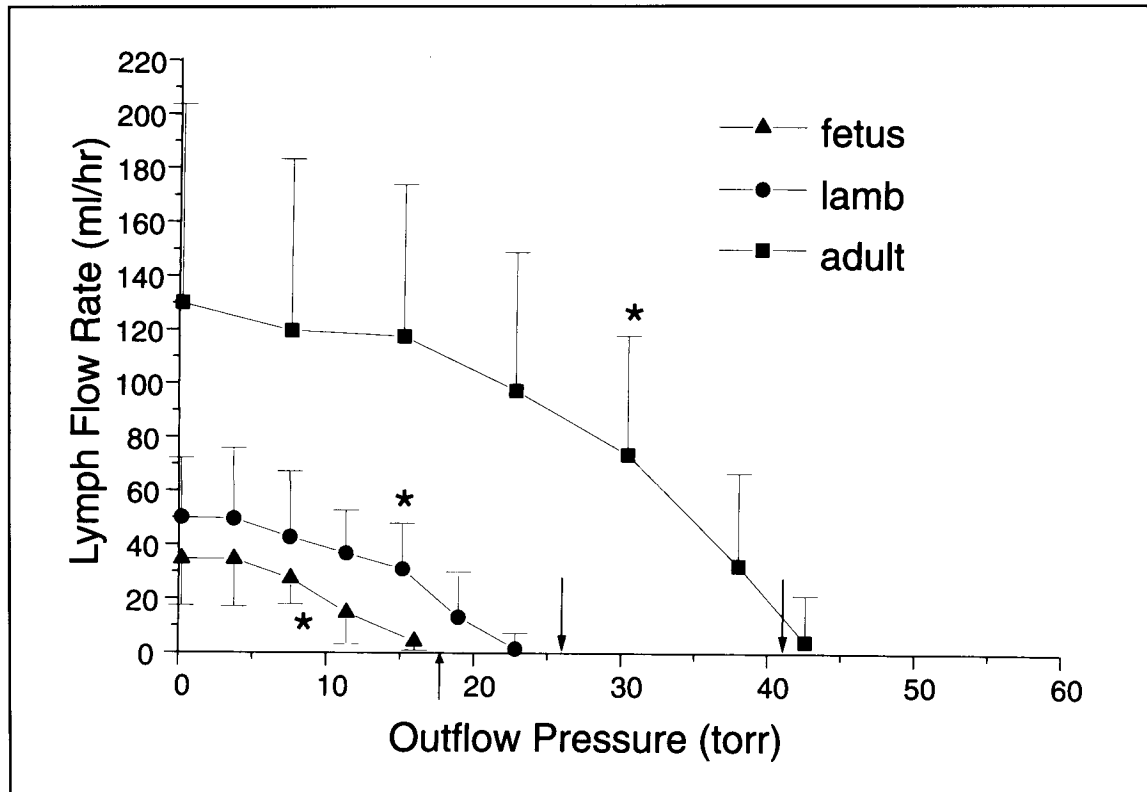


Fig. 1. Lymph flow rate as a function of outflow pressure in fetal, newborn, and adult sheep. * signifies beginning of break-point range. ↓ indicates stop-flow pressure obtained by pressure transducer.

did not change significantly during alterations in lymph catheter outflow pressure among animals in each group (Table 2).

DISCUSSION

The ability of thoracic duct lymph to flow against an outflow pressure improved with maturation. The stop-flow pressures at rest for each group of animals increased with maturation when determined by the outflow catheter height at which flow stopped or when measured by a transducer recording the pressure generated in a closed, no flow situation. In a similar way, the outflow pressure at which thoracic duct lymph flow deviated from baseline flow, the break-point, increased with increasing age and development. *In vivo*,

outflow pressure is central venous pressure. In fetal and newborn animals the central venous pressure could easily be elevated enough to account for a decrease in or even a complete stoppage of thoracic duct lymph flow, accounting for an increased propensity for edema.

Previously, from our fetal data, we were able to calculate a precise break-point in lymph flow by fitting the data to two straight lines obtained from a piecewise linear regression (3). Including lymph flow rates at outflow pressures of -5 and -10 torr made this possible, since these flow rates were essentially the same as the flow at 0 torr. We did not obtain lymph flow rates at negative outflow pressures in the lambs and adult sheep in this study and as a consequence, we

were not able to fit the data to a piecewise linear regression. ANOVA determines when lymph flow rate at one outflow pressure differs significantly from flow at another outflow pressure; thus, we can give only a break-point range rather than a precise break-point. Visual inspection of the lymph flow curves suggests that the break-point may actually be less than that obtained using ANOVA. This would suggest that edema might form more easily in response to an increased central venous pressure, impairing return of thoracic duct lymph flow.

Flow of lymph is due to inherent contractility of lymph vessels, compression and distention of lymph vessels by surrounding tissues and skeletal muscle contraction (movement), and the interstitial tissue fluid pressure, tending to "push" fluid into the lymphatics. Differences in each of these factors could account for differences in lymph flow rates, break-point pressures, and stop-flow pressures in fetal sheep, lambs, and adult sheep. Recently, we have found that there is a developmental maturation of thoracic duct and mesenteric lymph vessel smooth muscle in sheep (4). Fetal sheep have only a single, discontinuous layer of vascular smooth muscle cells lining their lymphatic vessels, whereas 4 month old lambs have 2-3 layers of vascular smooth muscle, and adult sheep have 5 layers of overlapping smooth muscle cells intermixed with collagen. If degree of smooth muscle development correlates with inherent lymphatic contractility, then the ability of lymph vessels to return lymph against an outflow pressure should improve with maturation. In a similar manner, the skeletal muscle of the more mature animals contain more and better organized contractile elements and relatively less water. Contractions and involuntary movement would likely more effectively compress lymphatic vessels in the more mature animals, propelling lymph along. Lastly, the interstitium of the fetus is more compliant than that in more mature animals (5). Any increase in interstitial fluid volume generates

less of an increase in interstitial fluid pressure and less of a force tending to drive interstitial fluid into terminal lymphatic vessels.

Paradoxically, when lymph flow rate is calculated per body weight, the lymph flow rate is higher in the fetus and decreases with maturation, despite the above reasons that more immature lymphatic vessels should be less suited to returning lymph to the circulation. The increased extracellular fluid volume of the fetus and lamb relative to the adult, the higher interstitial compliance of the fetus, and the increased fetal capillary filtration coefficient explain the need for a higher thoracic duct lymph flow rate in the fetus to maintain body water homeostasis. One explanation for this paradox and for the observation that edema occurs more readily in immature animals is that the lymphatic system of fetuses is operating near maximal capacity while more mature animals have a reserve ability to return fluid to the circulation should the need arise. In the fetus at baseline, a normal fluid filtration rate and a normal central venous pressure would result in a large volume of filtered interstitial fluid and a correspondingly large volume of lymph flow on a per body weight basis relative to more mature animals. However, edema would be absent if this equilibrium were maintained. A disturbance in the equilibrium by an increase in central venous pressure above 8-12 torr would impair return of lymph to the circulation and would result in edema. Lambs with their break-point range of 15-18 torr and adult sheep with their break-point range at 25-35 torr could tolerate pathological alterations in their central venous pressures up to this range before edema developed.

The adult group's absolute lymph flow rate was 2.6 times that of the lambs and 3.8 times that of the fetuses at 0 torr outflow pressure. Lymph flow varied among the animals in each group except near stop-flow pressure. Our fetal lymph flow rates are comparable with those of Brace (38.4 ml/hr, 15 ml/kg/hr) (6). Our adult thoracic duct lymph flow rates are less than those of

Valenzuela et al (4.6 ml/kg/hr) in chronically catheterized pregnant and nonpregnant ewes (7). The differences may be secondary to variations in intestinal lymph formation because of variability in intake, differences in lymph catheter resistance, and perhaps differences between breeds of sheep. Higher stop-flow pressures have been reported in adults of various species; however, these reports utilized lymphatic vessels other than the thoracic duct, thus making comparisons and any conclusions regarding maturational development difficult. McGeown et al reported lymph flow at an outflow pressure of 90 cm H₂O in hindlimb afferent popliteal lymphatic vessels of sheep (8). Pressures recorded from obstructed lymphatics in the legs of resting humans have reached values of over 100 mmHg (9). Drake et al noted that adult sheep lung lymph flow occurred until an outflow pressure of 30.9 torr was reached (10).

We do not know of another study examining lymph flow rates in lambs less than 1 week old. Since lamb cardiac output is almost twice that of the fetus (11), which should correlate with a higher interstitial fluid filtration rate and lymph flow rate, we expected the lamb lymph flow rates to be higher than we measured. The variations in lamb lymph flow probably relate to the time elapsed from the last nipping to the time of the study.

In conclusion, our study demonstrates that sheep thoracic duct lymph flow is limited by outflow pressure. The outflow pressure at which lymph flow begins to diminish and the outflow pressure at which lymph flow stops both increase with developmental maturation. Despite this, when standardized for body weight, lymph flow is greater in immature animals. This higher lymph flow in less mature sheep conforms with their increased body water content, and when considered in context with the limitations of their lymphatic vessels to function against an outflow pressure, offers one explanation for their propensity to develop edema.

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