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The Effect of Outflow Pressure upon Thoracic Duct Lymph Flow Rate in Fetal Sheep

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ABSTRACT. Edema develops when lymph does not return to the venous circulation at a rate equal to the rate of capillary filtration. Fetal sheep develop edema as well as an increased central venous pressure while undergoing atrial pacing at 320 beats per min. We hypothesized that the increased central venous pressure augmented the appearance of fetal edema by impairing the return of thoracic duct lymph to the venous circulation. To investigate this hypothesis, we studied the effect of outflow pressure upon thoracic duct lymph flow in 10 unanesthetized fetal sheep who had low resistance lymph catheters placed in the cervical thoracic duct near its junction with the left jugular vein. After the ewe and fetus recovered for 5 d, we altered the outflow pressure of the lymph catheter by adjusting its height with respect to amniotic fluid pressure and measured the resultant change in thoracic duct lymph flow rate. We found that lymph flow rate was constant over the range of outflow pressures (central venous pressures) normally encountered but decreased in a linear fashion at pressures greater than 0.68 kPa (5.1 torr). Lymph flow stopped at an outflow pressure of 2.40 kPa (18 torr). The data points are best fit by two lines obtained by a piecewise linear regression rather than a single line obtained from a linear regression. We conclude that fetal thoracic duct lymph flow is sensitive to elevations in outflow pressure. Lymph flow begins to diminish at outflow pressures corresponding to central venous pressures commonly encountered in pathologic conditions and may augment the appearance of fetal edema. (*Pediatr Res* 32: 585–588, 1992)

Abbreviations

I.D., inner diameter

Lymph returns to the systemic circulation at the junction of the thoracic duct and the confluence of veins, near the left thoracic inlet. For lymph to enter the venous circulation, thoracic duct pressure must exceed the systemic venous pressure at that junction. Interstitial fluid accumulates in the following situations: when fluid filtration from the systemic circulation is excessive to the extent that the lymphatic system is overwhelmed and does not have the ability to return all of the filtered fluid back to the circulation, when lymphatic function itself is impaired or has diminished pumping ability, or when there is a combination of the above. If the safety factor or buffering capacity of the interstitium is exceeded, then edema appears.

Previously, we and others have noted edema in fetal sheep undergoing continuous atrial pacing at 320 beats per min. We have also measured an increased central venous pressure coincident with the presence of edema (1–4). Brace (5) has reported a decrease in fetal thoracic duct lymph flow when outflow pressure exceeds 0 torr. However, in our paced fetal sheep and in our other published controls, the baseline venous pressures, or outflow pressures, were 3–4 ± 1 torr, yet these fetal sheep did not appear edematous nor did they have an increased total body water content as measured by the wet to dry method (1, 2).

We performed these experiments in chronic, unanesthetized fetal sheep to test the hypothesis that the ability of the thoracic duct to return lymph to the venous circulatory system is limited by a central venous pressure that is elevated above physiologic levels, thereby contributing to edema formation.

MATERIALS AND METHODS

Surgery. Our general fetal surgical techniques have been previously described in detail (1, 2). We operated on 10 singleton Western range ewes at 129 ± 7 d gestation (mean ± SD). For anesthesia, we injected 3 mL of 1% tetracaine hydrochloride into the epidural space and accompanied this with a continuous drip infusion of ketamine hydrochloride (1 g/L normal saline) at a rate sufficient to achieve sedation for the duration of the procedure. This combination also provided adequate anesthesia to the fetus in that we did not observe fetal movement during surgical procedures in most cases. If fetal movement occurred, we gave i.v. ketamine, 50 mg, directly to the fetus. For local anesthesia, we infiltrated 1% xylocaine into the skin and subcutaneous tissues of the fetus.

We then performed a midline laparotomy, palpated the fetal head, and withdrew it through a small uterine incision. Then we proceeded to catheterize the thoracic duct using the technique described by Brace (6).

Briefly, we infiltrated the area overlying the left external jugular vein near the thoracic inlet with 1% xylocaine and located the thoracic duct as it emptied into the confluence of veins. After ligating the duct, we inserted a large-bore, heparin-impregnated (TDMAC Processing; Polysciences, Inc., Warrington, PA) plastic catheter into it and sutured it in place.

We made the lymphatic catheters from a combination of materials: We made the segment introduced into the lymphatic from Vialon (Deseret Medical, Inc., Sandy, UT) that was 0.6 mm I.D. and 0.9 to 1.5 cm in length. We inserted this in turn into a piece of Tygon (Fisher Scientific, Pittsburgh, PA) that was 1.3 mm I.D. and 66.0 to 115 cm in length.

In all 10 fetuses, before returning the head to the uterus, we infiltrated the skin overlying the left or right jugular vein and performed a cutdown to cannulate the jugular vein and carotid artery. For the jugular venous catheter, we used a 30- to 60-cm piece of 2.3 mm I.D. polyvinyl tubing into which we could insert the end of the lymph catheter. After closing this cervical incision, we attached a plastic catheter to the fetal skin for recording amniotic fluid pressure, returned the fetal head to the uterus, directed all plastic catheters out of the uterine opening, and then closed the uterine incision.

Next, we palpated a fetal hindlimb, withdrew it through a

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second small uterine incision, and performed a cutdown in the popliteal area to insert and advance polyvinyl catheters into the fetal abdominal aorta and inferior vena cava. After securing the catheters in place and returning the extremity to the uterus, we closed the uterine incision with a purse string suture.

We led all plastic catheters through the ewe's peritoneal cavity and tunneled them through the skin overlying the flank area, then secured them in place and stored them in a plastic envelope. We shortened the thoracic duct and jugular venous catheters as much as possible to minimize resistance to flow, then created a fistula by connecting the two catheters together, permitting continuous recirculation of thoracic duct lymph.

Immediately postoperatively and for the next 2 d, we administered analgesic agents (buprenorphine hydrochloride, Norwich Eaton, Norwich, NY) to the ewes. For prophylaxis against infection, we injected penicillin (600 000 units) and kanamycin (50 mg) into the amniotic cavity daily. We also treated the ewes with intramuscular penicillin and dihydrostreptomycin (Combiotic, Pfizer Inc., Agriculture Division, New York, NY; 3 mL) daily after surgery. All animals recovered for at least 5 d before we performed any experiments.

Experimental methods. We measured the thoracic duct lymph flow rate by collecting thoracic duct lymph sterilely into test tubes over 15-min periods at known lymph catheter outflow heights with respect to amniotic fluid pressure. In all instances, we manually subtracted amniotic fluid pressure from the pressure corresponding to the catheter height. Then we altered the outflow height of the catheter, waited at least 15 min for equilibration, and repeated the collection. When the catheter height was increased, lymph flow temporarily decreased but would equilibrate after 10 to 15 min; the opposite occurred when the catheter height was decreased. Before changing the catheter outflow height, we made at least three consecutive 15-min collections of thoracic duct lymph. If the lymph flow rate during a collection period varied by more than 10% from the lymph flow rates of previous collection periods, we made subsequent collections until the variability decreased.

Additionally, we measured the catheter outflow pressure at which thoracic duct lymph flow stopped and we recorded the maximum pressure generated by the thoracic duct during no flow using Statham P-23b pressure transducers. During each lymph collection, we measured vascular pressures and amniotic fluid pressure using Statham P-23b pressure transducers and recorded the pressures on an eight-channel amplifier recorder (Gould, Inc., Instruments Division, Cleveland, OH). Vascular pressures were referenced to amniotic fluid pressure by manually subtracting amniotic fluid pressure from each vascular pressure. We determined the heart rate from the phasic aortic blood pressure tracing. We obtained samples of fetal blood at hourly intervals 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 using the Bradford Protein Assay (Sigma Chemical Co., St. Louis, MO), and the hematocrit.

At the end of each 15-min collection, we returned the collected lymph sterilely to the fetal venous circulation over the ensuing 15-min period.

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/min and measuring the pressure drop across the length of the catheter.

Statistical methods. We used a two-way analysis of variance for statistical comparisons and a p value of < 0.05 as being indicative of significance. For estimating the break point in thoracic duct lymph flow as a function of lymph catheter outflow pressure, we fit the data points to a piecewise linear regression as described by Vieth (7).

This research protocol was reviewed and approved by the Baylor College of Medicine Animal Protocol Review Committee.

RESULTS

The resistance to flow in each lymphatic catheter-jugular venous catheter combination measured 0.27 ± 0.09 kPa/mL/min (2.1 ± 0.7 torr/mL/min) at a flow rate of 1 mL/min. We used this measurement to correct the catheter outflow pressures. All data presented have been corrected for catheter resistance.

Lymph flow rates for each individual fetus remained relatively constant over the range of lymph catheter outflow pressures encountered in physiologic situations (0–0.67 kPa; 0–5 torr), but lymph flow rates varied widely between individual fetuses. At a lymph catheter outflow pressure equivalent to 0 kPa (torr) relative to amniotic fluid pressure, the lymph flow rate varied from 16 to 68.8 mL/h. Our fetuses weighed 3.06 ± 0.57 kg (mean ± SD) at autopsy. Based on this weight, lymph flow averaged 11.49 ± 5.98 mL/kg/h (mean ± SD).

Figure 1 shows the mean lymph flows occurring over a range of catheter outflow pressures. The data points represent 66 measurements of lymph flow in fetal sheep. At least three measurements were made for each outflow pressure at which flow occurred. The values obtained at stop-flow pressure are omitted from the figure for clarity. By visual inspection, lymph flow appears to be stable up to an outflow pressure of about 0.67 kPa (5 torr) then begins to decrease. However, by two-way analysis of variance, the fall off in lymph flow does not reach statistical significance at the < 0.05 level until an outflow pressure of between 1.07 and 1.60 kPa (8 and 12 torr) is attained. The lines drawn in Figure 1 show the results of fitting the data points to a piecewise linear regression (7). The stop-flow pressure measurements have been eliminated in performing the linear regressions. Two lines are obtained that cross at coordinates of $x = 0.68$ kPa or 5.1 torr, $y = 33.3$ mL/h. Using an F test to test the hypothesis that "rectilinear regression is the optimal fit" yields a p value of 0.009.

We also measured the pressure generated by the thoracic duct in a no flow state, the stop-flow pressure, by connecting the lymphatic catheter directly to a pressure transducer. This measured stop-flow pressure averaged 2.40 ± 0.13 kPa (18 ± torr)

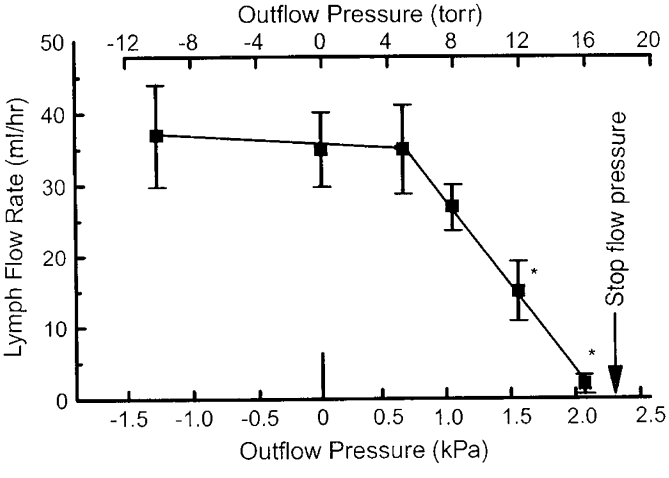

Fig. 1. The effect of lymph catheter outflow pressure on fetal thoracic duct lymph flow rate. The data points represent 66 measurements in 10 fetal sheep. At least three measurements were made for each fetus at each outflow pressure where flow occurred. Stop-flow pressure data points were excluded from the linear regression. Lymph flow rate is constant up to an outflow pressure of 0.68 kPa (5.1 torr), then decreases in a linear manner before ceasing at a measured outflow pressure of 2.40 ± 0.13 kPa (18 ± torr) or at an extrapolated stop-flow pressure of 2.19 ± 0.13 kPa (16.4 ± 1.0 torr). The lines represent the best fit of the data points obtained by applying a piecewise linear regression (7), as opposed to using a rectilinear regression and fitting the points to a single line. Data are expressed as mean ± SEM; *, different from previous lymph flow rate by two-way analysis of variance; $p < 0.05$.

Table 1. *Effect of lymph collection on heart rate, vascular pressures, pH, arterial blood gas tensions, hematocrit, and plasma protein concentration**

	HR	P_{aorta} (kPa)	P_{ivc} (kPa)	pH	PaO_2 (kPa)	$PaCO_2$ (kPa)	HCT	TP (g/L)
Baseline	173 ± 3	6.00 ± 0.13	0.53 ± 0.13	7.40 ± 0.01	3.07 ± 0.40	6.00 ± 0.13	0.32 ± 0.01	34 ± 1
Final	172 ± 6	5.87 ± 0.13	0.40 ± 0.13	7.39 ± 0.01	2.53 ± 0.13	6.13 ± 0.13	0.32 ± 0.01	35 ± 1

* Values are mean ± SEM. HR, heart rate; P_{aorta}, aortic pressure; P_{ivc}, inferior vena cava pressure; PaO_2, arterial O_2 tension; $PaCO_2$, arterial CO_2 tension; HCT, hematocrit; TP, plasma protein concentration. Conversion: 1 kPa = 7.5006 torr.

(mean ± SEM). The stop-flow pressure estimated by the linear regression is less, 2.19 ± 0.13 kPa (16.4 ± 1.0 torr) (mean ± SEM).

Heart rate, vascular pressures, pH, arterial oxygen tension, arterial carbon dioxide tension, total protein concentration, and hematocrit did not change significantly during alterations in lymph catheter outflow pressure (Table 1).

DISCUSSION

Previously, we produced hydrops fetalis in 28 fetal sheep by continuous atrial pacing at 320 beats per minute (1). These sheep had a baseline venous pressure of 0.53 ± 0.13 kPa (4 ± 1 torr). With atrial pacing, venous pressure increased to 1.07 ± 0.13 kPa (8 ± 1 torr). Our data suggests that, even if the fluid filtration rate remained the same with atrial pacing and did not increase, up to 144 mL of fluid could still accumulate in the fetal interstitium over 24 h solely on the basis of impaired drainage of thoracic duct lymph flow secondary to the increased central venous pressure. Any condition associated with an increased transvascular fluid filtration rate in conjunction with an increased central venous pressure could amplify edema formation.

Our findings are similar in many respects to those of Brace (5). He also found lymph flow to be stable over a range of outflow pressures up to a certain break point, to decline in a linear manner at outflow pressures above that, and to cease at a stop-flow pressure. One difference in our findings is the site of the break point. Brace found the break point to be 0 kPa (0 torr); we measured the break point at 0.68 kPa (5.1 torr). Regardless, the major point that we wish to establish is that lymph flow rate is a function of the outflow pressure (central venous pressure) over the range of venous pressures often encountered. Possibly, there may be differences in lymph flow and lymphatic function among breeds of sheep that would explain the differences in our findings. Brace's sheep and our sheep were similar in gestational age. Our catheters are essentially the same as those of Brace and have a low resistance to flow, making catheter resistance an unlikely cause of the difference. Brace measured amniotic fluid pressure on-line and automatically subtracted it from venous pressure, whereas we manually subtracted averaged amniotic fluid pressures from averaged venous pressures. With on-line computer techniques, Brace was able to make measurements of lymph flow over 30 s. We, however, made at least three lymph collections over 15 min. If any collection varied by more than 10% from the others at each catheter outflow height, we made subsequent collections until the variability decreased. Changes in amniotic fluid pressure, usually secondary to movement of the ewe, accounted for the majority of the variation in lymph flow rate. These differences in methods of collecting lymph and in correcting for amniotic fluid pressure may account for our differing results.

We also found that the outflow pressure against which lymph could continue to flow was finite; that is, a certain outflow pressure exists at which lymph flow ceases. Although the stop-flow pressure that we measured, 2.40 ± 0.13 kPa (18 ± 1 torr), and that which we obtained from piecewise linear regression, 2.19 ± 0.13 kPa (16.4 ± 1.0 torr), were both greater than the value of 1.53 kPa (11.5 torr) reported by Brace, they are less than the stop-flow pressures of up to 100 mm Hg reported in the thoracic duct of adult sheep and humans (8, 9). This reduced stop-flow pressure indicates that in fetal sheep it is indeed possible that a venous pressure might be encountered that would totally inhibit lymph return to the circulatory system.

It is interesting that, although the stop-flow pressures obtained by Brace and us differ in absolute value from each other, both of us find that our measured and extrapolated values for stop-flow pressure are similar. (Our extrapolated value for stop-flow pressure is actually 1.6 torr less than our measured value.) This is different from the findings of Drake *et al.* (8) in the lung lymphatic vessels of adult, unanesthetized sheep. They found that the mean stop-flow pressure measured 40.7 ± 20.7 cm H_2O (mean ± SD) (4.12 ± 2.10 kPa, 30.9 ± 15.7 torr) but that the extrapolated pressure at which lymph flow should be zero was 24.7 ± 7.2 cm H_2O (mean ± SD) (2.50 ± 0.73 kPa, 18.8 ± 5.5 torr). When they raised outflow pressure above the value of extrapolated outflow pressure, they found that lymph flow deviated from a straight line, and, although lymph flow was slow, it was still greater than predicted by the extrapolated outflow pressure. They interpreted this higher than predicted flow as a result of the pumping ability of the lymphatic vessels. Although it may not be completely valid to compare lung lymphatic vessels with thoracic duct, it is suggestive, at least, that, because measured and extrapolated outflow pressures are nearly the same in both Brace's and our study, the thoracic duct of the fetus has less pumping ability than that of the adult. This would lend more support to our contention that the fetal lymphatic system is more sensitive to elevations in outflow pressure and more prone to the development of edema in situations where central venous pressure is only slightly elevated above normal.

In conclusion, our study shows that fetal thoracic duct lymph flow is limited by outflow pressure. The outflow pressure at which thoracic duct lymph flow begins to diminish corresponds to a central venous pressure that is only slightly above normal and that is encountered frequently in pathophysiologic situations. Lymph flow ceases at a measured stop-flow pressure that is within 0.23 kPa of the extrapolated stop-flow pressure obtained from a linear regression of lymph flow *versus* outflow pressure. This is in contrast to adult sheep studies, where the measured stop-flow pressure is greater than the extrapolated pressure and where both measured and extrapolated pressures are greater than those in the fetus. This may indicate that the pumping ability of the fetus is less than that of the adult. Although our results are quantitatively different in some aspects from those of Brace, our findings are qualitatively similar. The sensitivity of the fetal thoracic duct to increases in outflow pressure may play a major role in the development of fetal edema.

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