Dynamic fluid shifts induced by fetal bypass

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Objective: Fluid shifts have been suggested to occur with fetal bypass. The degree or mechanisms behind these volume changes (or location) have not been defined. We characterized the preceding and correlated the findings to plasma vasopressin concentrations, the critical peptide of osmoregulation.

Methods: Seventeen ovine fetuses (105–111 days' gestation) were started on bypass and followed 2 hours after bypass. Hemodynamics and volume replacements needed to maintain minimum reservoir volume during bypass and normal physiologic parameters after bypass were recorded. Serial blood samples were collected to assess gas exchange and vasopressin levels. Changes in total tissue water content were measured for several organs and the placenta. Plasma volume, fluid shifts, and osmolarity were calculated.

Results: Hematocrit values decreased by 15 minutes of bypass to 28% from 33% and then increased to 34% by 120 minutes after bypass, corresponding to a decreased fetal plasma volume of 79 to 72 mL/kg by 120 minutes after bypass. The majority of volume shifts (approximately 100 mL/kg) occurred during bypass, but additional volume replacements were required after bypass to maintain normal hemodynamics, resulting in overall losses of 0.8 mL $\cdot$ kg^{-1} $\cdot$ min^{-1}. Losses were not accounted for by placental or organ edema. Vasopressin levels increased dramatically with bypass (39–51.5 pg/mL) and were strongly predicted by increased fetal plasma volumes ($R^2 = 0.90$), whereas osmolarity was not significantly associated with plasma volumes.

Conclusion: Fetal bypass leads to significant fluid shifts that correlate strongly with increasing vasopressin levels (but not changes in osmolarity). The placenta is not the primary site of volume loss. Rehydration of the fetus is necessary after bypass.

Each year, several hundred thousand babies around the world are born with heart defects. Most of these defects are effectively corrected through surgical intervention after birth; however, some continue to be associated with significant morbidity and mortality. There is increasing evidence that some of these defects might benefit from fetal interventions, including perhaps fetal cardiac surgery.[1-3] Previous studies have demonstrated the feasibility of fetal cardiac bypass.

Experimental studies of fetal cardiac surgery consistently observe placental dysfunction after bypass.[4-9] The mechanism or mechanisms for this placental dysfunction remain unknown,[7,10,11] although inflammation, an inappropriate fetal stress response, and activation of various biochemical pathways, among others, have all been implicated.[9,10,12-14] Some have also argued that fetal bypass leads to secondary changes in the placenta, such as increased edema through increased compliance and capillary leak, which would exacerbate placental function further. The latter seems reasonable in view of several reports of increased volume requirement for the fetus supported by extracorporeal circulation.[14-16] Such fluid losses are not surprising considering their reported occurrence in other settings, such as neonatal cardiac surgery,[17-19] in which the inflammatory insults of bypass are observed.

The magnitude and severity of such fluid losses with fetal bypass, however, have not been characterized, nor is there any information to quantify where these losses occur (and whether the placenta is the primary site of fluid loss). The mechanisms leading to fluid losses are not clear either. We have previously shown that profound changes in vasopressin release take place with the onset and progression of fetal bypass.[14] Vasopressin is a critical component of osmoregulation and therefore potentially capable of inducing significant fluid shifts. Uniquely, vasopressin receptors (but not vasopressin itself) are expressed at high levels within the placenta.[20]

With the preceding in mind, we set out to characterize the magnitude of changes (and their potential location) in fetal plasma volume and third-space fluid losses that occur with fetal bypass. Furthermore, we looked at any potential correlations of the preceding with changes in fetal plasma vasopressin levels. We hypothesized that postbypass rehydration of the fetus would be necessary for adequate fluid regulation, especially in the setting of associated placental dysfunction. Defining the role and contribution of each of these components has significant implications for clinical translation of fetal cardiac surgery.

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Abbreviations and Acronyms
PVR = placental vascular resistance
TTW = total tissue water

MATERIALS AND METHODS

Surgical Procedure

Twenty-six singleton and twin pregnant ewes from 104 to 114 days of gestation (average weight, 1.5 kg) were studied (term, approximately 148 days). Seventeen fetuses underwent 30 minutes of extracorporeal circulatory support and were followed for 2 hours after bypass. Of these, 7 fetuses were followed without any intervention, whereas 10 fetuses received treatment, as defined by volume resuscitation in the postbypass period as needed to maintain stable fetal hemodynamics. These bypass groups were compared with 9 equally instrumented control animals that did not undergo extracorporeal circulatory support. Surgical preparation and fetal bypass were performed as previously described by our group.[5,9,14,21]

Briefly, ewes are fasted for 24 hours before sedation with ketamine and diazepam, endotracheal intubation, and anesthesia maintenance with 2% isoflurane and oxygen. Ewes receive 0.3 mg of buprenorphine (Buprenex) administered intramuscularly and penicillin G. Catheters are placed in the maternal femoral artery and vein for measurement of blood gases and delivery of intravenous fluids, respectively. After midline laparotomy and minor hysterotomy, additional catheters are placed in the fetal femoral artery and vein for blood gas measurements, blood sampling, and pressure monitoring and for fluid resuscitation. Through the same hysterotomy, an umbilical flow probe (4–6 mm; Transonic Systems, Ithaca, NY) is placed to measure placental blood flow. Placental vascular resistance (PVR) is calculated as previously described.[22]

Fetal Bypass

Using methods we have previously described,[5,9,14,21] fetal cannulation was performed with a 10F to 12F Bio-Medicus (Eden Prairie, Minn) venous cannula in the jugular vein and a 6F to 8F Bio-Medicus arterial cannula in the carotid artery. Direct central cannulation is not used to avoid the risk of hemorrhage that can occur with central cannulation and that would secondarily complicate the experimental model and observations because of required transfusions. Hemodynamic values are continuously recorded with a PowerLab data acquisition system (ADInstruments, Colorado Springs, Colo). Bypass is conducted with a roller pump system, with normothermia, vacuum-assisted venous drainage, and a heat exchanger and with the placenta functioning as the sole oxygenator. We use blood prime for our circuit, which is derived from a donor adult ewe (not maternal).[5,9,14,21] Bypass is conducted for 30 minutes, with a target flow rate of 200 to 250 mL $\cdot$ kg^{-1} $\cdot$ min^{-1} based on our prior studies (beating heart and continues to eject),[5,9,14,21] and fetuses are followed for 2 hours after bypass. The venous reservoir (Capiox Baby-RX; Terumo, Ann Arbor, Mich) is filled with 125 mL of prime at baseline. Thereafter, Plasmalyte-A (Baxter Healthcare Corp, Deerfield, Ill) solution is added to the circuit to maintain a minimum level of 50 mL in the reservoir. The remainder of the circuit consists of 1/4-inch tubing on the venous side and 3/16-inch tubing on the arterial side, and the pump head is brought as close as possible to the field, analogous to a remote pump-head situation. Ewes and fetuses are killed after the termination of the experimental protocol for autopsy assessment of fetal morphometrics and confirmation of catheter positions. All procedures in our laboratory are performed in accordance with Institutional Animal Care and Use Committee–approved protocols, and our laboratory is an Association for Assessment and Accreditation of Laboratory Animal Care–approved facility.

Sampling Regimen

For the purposes of this study, fetal arterial blood was collected before and after neck cannulation, at 15 and 30 minutes of bypass, and at 30, 60, 90, and 120 minutes after bypass for determination of blood gases and hematocrit values with an i-STAT clinical analyzer (i-STAT Corp, Windsor, NJ) and for glucose and lactate values with a YSI 2300-STAT analyzer (YSI Corp, Yellow Springs, Ohio). Placental cotyledons from bypass animals were collected before bypass and 30 and 120 minutes after bypass, wrapped in foil, and snap-frozen into liquid nitrogen for subsequent total tissue water (TTW) weight analysis. Similarly, after death, fetal left and right ventricles, lungs, livers, and kidneys were rapidly collected from bypass and sham bypass control animals and frozen in liquid nitrogen for later TTW analysis.

Vasopressin Measurement

Using methods we published previously,[14] fetal blood samples for vasopressin immunoassay were collected into lithium heparin–coated tubes (4 mL each; Monovettes; Sarstedt, Newton, NC). These were immediately placed on ice and centrifuged, and the separated plasma was frozen at –20°C until assay. The commercially available vasopressin EIA assay kit (Assay Designs, Ann Arbor, Mich) had a sensitivity of 3.5 pg/mL, with intra-assay and interassay coefficients of variation of 5.9% to 10.6% and 6.0% to 8.5%, respectively. This assay is capable of recognizing sheep proteins, and all results were read with a Multiskan-EX microplate reader (Thermo EC, Waltham, Mass) and Ascent software (Thermo EC). All samples were assayed in duplicate, and mean values are reported.

Calculations of Fluid Shift and Osmolarity

Fetal blood volume (FBV) was estimated as 11% of measured fetal body weight.[23] Prior studies of fluid extravasation with bypass in neonatal piglets estimated blood volume by injecting a known volume of carbon monoxide into the closed-circuit rebreathing system,[17,24,25] but this method is not possible in the fetal setting. Fetal plasma volume (FPV) and fluid shifts (FS) were calculated according to equations 1 and 2below by using measured hematocrit (HCT) values:

$$FPV = FBV \times (1 - [HCT/100]) \quad (1)$$

$$FS = (\text{Re}servoir\ volume\ lost\ during\ by\ pass + \text{Re}placement\ volume + FOPV_{initial}) - FPV_{final} \quad (2)$$

Osmolarity (cOsm) was calculated from measured sodium (Na^+) and glucose values, as shown in equation 3.

$$cOsm = \left(2 \times \left[Na^+\right]\right) + ([Glucose]/18) \quad (3)$$

Calculation of TTW

Placental cotyledons (n = 4, bypass; n = 4, control) and fetal lungs, livers, kidneys, and left and right ventricles (n = 6, bypass; n = 4, control) were examined. By using a variation of prior methods,[17] each 0.5- to 1.5-g sample of tissue was cut in 3 parallel pieces, placed on preweighed foil, and put in a 70°C drying chamber. Samples were weighed repeatedly until a stable weight was reached, usually within 48 hours. TTW is presented as both gram/gram of dry weight and percentage of water weight. TTWs from heart, lung, kidney, and liver tissue from noninstrumented control twins were compared with those from animals exposed to bypass at 120 minutes after bypass (ie, single time point). In contrast, serial comparisons could be carried out for placental tissue (before and 30 and 120 minutes after), comparing control animals and animals exposed to bypass.

Statistical Analysis

Longitudinal data were analyzed by means of mixed-models repeated-measures analysis of variance with SAS Proc Genmod (SAS Institute, Inc, Cary, NC), testing for between-group and within-group differences

over the course of the experiment. Least significant difference and Student's paired and 2-group *t* post-hoc tests were used to examine mean differences between groups and changes between time points. R^2 values, measuring the amount of variation explained by a given model, were determined by using least-squares regression. We defined an R^2 value of 0.7 or greater to be indicative of a strong predictor. Values are presented as the mean ± standard deviation. Software packages SAS 9.13, SPSS 15.0 (SPSS, Inc, Chicago, Ill), and Excel 2003 (Microsoft, Redmond, Wash) were used for data analysis. *P* values for post-hoc analyses must be viewed critically by the reader because the analyses were not preplanned and have not been adjusted for multiple comparisons. The analyses were performed to help describe the differences in mean values seen over time and between groups. Any significance of these results must be interpreted within this framework.

RESULTS

Fetal Hemodynamics and Placental Gas Exchange

Fetal hemodynamics with the conduct of bypass are shown in Table 1. Fetal mean arterial pressure increased acutely and profoundly during bypass, returning to baseline levels after bypass and then decreasing further by 120 minutes after bypass. Individual umbilical blood flow responses to the onset of fetal bypass were variable but, with termination of bypass, always returned to prebypass levels and then showed a progressive decrease by 120 minutes. The variable response to immediate initiation of bypass (first 2 minutes) is also seen in changes in PVR. After termination of bypass, however, PVR uniformly returns to prebypass levels before increasing, with the increase coincident with deterioration of fetal gas exchange, as reported previously.[4-9] From a hemodynamic standpoint, the first sign of hemodynamic deterioration after bypass typically manifests as an increase in fetal heart rate followed by a persistent decrease that accompanies decreasing umbilical blood flows and increasing umbilical vascular resistance; of note, however, central venous pressure does not change significantly during this time for the treated animals (6–9 mm Hg, data not available for untreated or control groups), remaining at around the baseline and normal range for the fetus. Fetal hemodynamics do not change in the sham/control group.

As reported previously[4,8,9,12,14] and in this study, fetal bypass leads to significant fetal mixed acidosis, as evidenced by progressive increases in P_{CO_2} and plasma lactate concentrations during and after bypass (data not shown). Conversely, the fetal P_{O_2} concentration is stable until the postbypass period, when it begins to decrease, typifying the late hypoxia observed with fetal bypass (data not shown). All 17 fetuses were successfully weaned off bypass and survived at least 60 minutes of the postbypass period.

Hematocrit and Plasma Volume Changes During Fetal Bypass

In prior studies we had noted that initiation of bypass was often associated with increased need for addition of crystalloid volume to maintain venous reservoir volume, which continued into the postbypass period.[14] At the same time, often despite this added volume (and resulting in lower hematocrit values during bypass), we would see an increase in fetal hematocrit values in the postbypass period, which is suggestive of possible hemoconcentration. Figure 1, *A*, shows this change for hematocrit value in a group of 7 bypass fetuses compared with control values: for the 2-group repeated-measures analyses (bypass vs sham), *P* values were .06 for time, .16 for group, and .10 for the interaction. Post-hoc analysis revealed a decrease in hematocrit value with onset of bypass ($P = .038$), and subsequent paired *t* tests showed hematocrit values remained less than baseline values ($P < .040$) until 60 minutes after bypass, whereas sham values did not change. In this group of animals, volume resuscitation was carried out during bypass to maintain the minimum reservoir level of 50 mL (as in the treatment group described next), but no additional volume was given after bypass. The volume changes during bypass in these 7 animals correlated with changes in fetal plasma volume (Figure 1, *B*) from approximately 79 mL/kg fetal body weight to approximately 72 mL/kg fetal body weight or approximately 8% loss over a period of 120 minutes. The statistical profile of plasma volume changes were the same as for hematocrit.

Hematocrit and Plasma Volume Changes After Fetal Bypass

Hemodynamic stability of individual animals varies during the postbypass period; some animals remain stable for protracted periods, whereas others deteriorate despite being

TABLE 1. Fetal hemodynamics before, during, and after bypass and in-group analysis of variance with least significant difference post-hoc analysis

		Before bypass	On bypass	30 Min after bypass	120 Min after bypass	In-group ANOVA
FMAP (mm Hg)		37.5 ± 7.0	54.4 ± 11.1	41.4 ± 8.7	32.9 ± 7.1	***P*** **< .01**
	P value		**.01**	.288	.240	
HR (beats/min)		137 ± 19	192 ± 32	166 ± 20	162 ± 35	***P*** **< .01**
	P value		**.01**	**.010**	**.036**	
Umb Q (mL/min)		261 ± 106	390 ± 152	340 ± 123	213 ± 140	***P*** **= .016**
	P **value**		**.021**	.147	.411	
Umb VR (mm Hg · mL^{-1} · min^{-1})		0.165 ± 0.093	0.171 ± 0.102	0.142 ± 0.069	0.250 ± 0.194	*P* = .227
	P value		.889	.642	.114	

Significance at a *P* value of .05 or less is shown in bold. *ANOVA*, Analysis of variance; *FMAP*, fetal mean arterial pressure; HR, heart rate; *Umb Q*, umbilical blood flow; *Umb VR*, umbilical volume resuscitation.

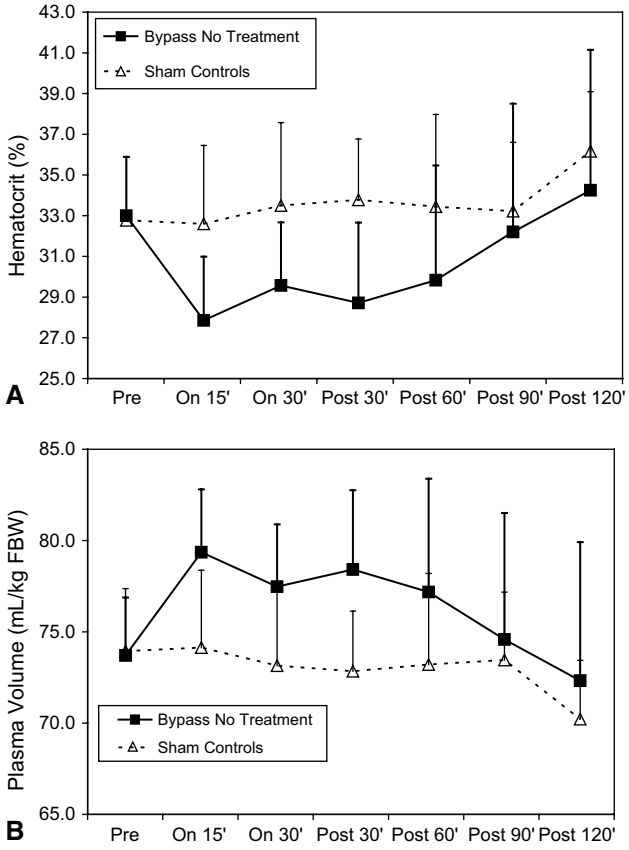

FIGURE 1. Fetal hematocrit levels decreased by 15 minutes of bypass (n = 7; $P < .05$, post-hoc analysis) and remained less than prebypass levels until 30 minutes after bypass ($P < .04$ vs before bypass, paired *t* test), followed by a sustained increase during the remaining postbypass period (A). The changes in hematocrit values observed with the bypass animals did not occur in the sham control animals (n = 9). Prime hematocrit values averaged 32% and were not an obvious cause of hemodilution. Calculated fetal plasma volumes during and after bypass ($P < .04$ vs before bypass) reveal the presence of a fluid shift in the postbypass period (B). *FBW*, Fetal body weight.

exposed to the identical experimental protocol. For this reason, we decided to further examine the use of postbypass volume resuscitation in a group of 10 fetuses in which volume resuscitation is carried out to improve or restore fetal hemodynamics. Figure 2 shows a representative case in which serial postbypass volume resuscitations in the form of 5 mL/kg boluses were administered to improve fetal hemodynamics, as would occur in the clinical setting. Volume given was in the form of remaining circuit blood. The protocol was continued until 120 minutes of observation, when the experiment was terminated. In Figure 2 each 10 mL of volume administered (dotted vertical markers no. 7-14) increased fetal mean arterial pressure and umbilical blood flow. As can be seen, fetal hemodynamics steadily decreased after cessation of this resuscitation protocol at the end of the 120-minute observation period (after marker no. 14).

Volume Additions During and After Bypass

To better assess the volume loss associated with fetal bypass, we quantified all volume required for maintenance of the minimum reservoir level during bypass and additional resuscitation in the postbypass period in the group of treated animals. Figure 3, *A*, shows that the largest amounts of fetal losses occur early and during extracorporeal support. The volume requirement, however, continues into the postbypass period (Figure 3, *A*). These fluid losses correspond to a dramatic change for the fetus, losing plasma volume or experiencing a fluid shift of approximately 0.8 mL · kg^{-1} · min^{-1} (Figure 3, *B*).

Total TWW

To find out where these fluid losses were occurring in the fetus, we measured total TWW from numerous sites in treated animals, as guided by prior literature.[17] TTW measurements in myocardium, lung, liver, or kidney samples demonstrated no differences between the sham and treated bypass groups (Figure 4, *A*). Although a few placental samples suggested mild increases in volume, overall comparison of serial placental samples from sham animals and animals undergoing bypass treatment before bypass and 30 and 120 minutes after bypass did not differ within groups or between the groups (Figure 4, *B*).

Vasopressin Levels Correlate to Fluid Shift

Vasopressin levels increased dramatically by 30 minutes of bypass, going from 39 pg/mL before bypass to 51.5 pg/mL, as we have shown previously.[14] Vasopressin levels were strongly predicted by increased fetal plasma volumes ($R^2 = 0.90$, Figure 5).

Osmolarity Changes with Fetal Bypass

Vasopressin release can be mediated by the fetal stress response, changes in osmolarity, or both. We therefore assessed the potential role of changes in fetal osmolarity in the observed vasopressin release, particularly in view of the substantial fluid losses. Plasma osmolarity was calculated from measured sodium and glucose levels throughout the protocol. Because of the potential confounding effects of excessive blood sampling, direct measurement of osmolarity (4 mL per sample) was not carried out in these studies, although we and others have previously shown that measured and calculated osmolarity are quite congruent.

Calculated fetal osmolarity remained increased after bypass in treated fetuses but decreased in nontreated fetuses ($P < .05$ at 30 minutes after bypass by using the paired *t* test, Figure 6). Osmolarity in sham animals did not change over time. Although osmolarity in volume-resuscitated animals went up during the postbypass period versus that seen in nonresuscitated animals, it was not significant, as determined by means of repeated-measures analysis, because of

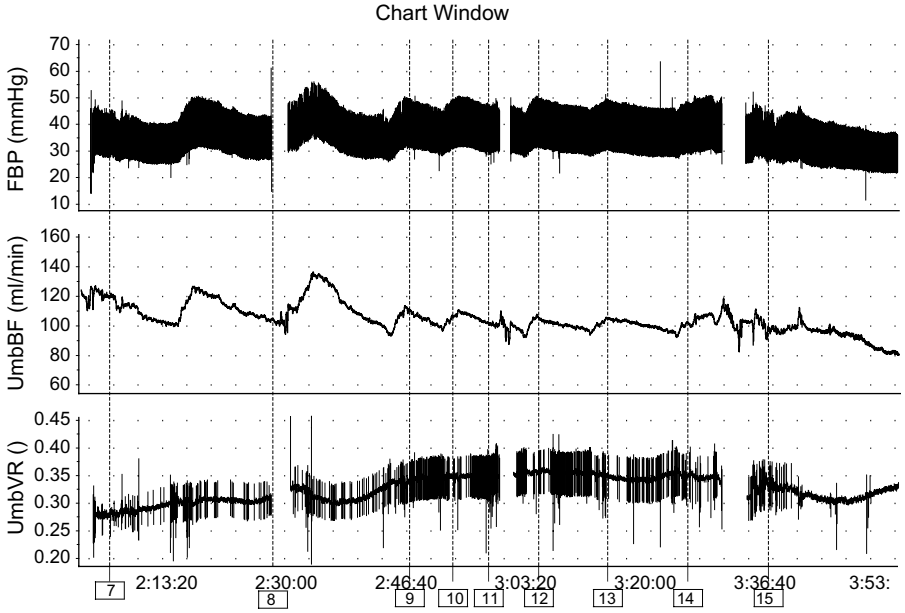

FIGURE 2. This representative tracing demonstrates the improvement in fetal hemodynamics (blood pressure *[FBP]* and umbilical blood flow *[UmbBF]*) with serial fetal transfusions (10 mL each) during the 2-hour postbypass period. Fluid resuscitation was applied each time hemodynamics began to decrease during the postbypass period *(vertical markers 7-14)* but not 2 hours after bypass. Note the steady deterioration in fetal hemodynamics by 2 hours after bypass *(marker 15)* and beyond when transfusions ceased. *UmbVR*, Umbilical volume resuscitation.

subject loss late in the protocol. The changes in fluid shifts were not strongly related to changes seen in osmolarity ($R^2 = 0.16$).

DISCUSSION

This is the first report describing the dramatic fluid shifts that occur with conduct of fetal bypass. We also demonstrate that optimizing fetal hemodynamincs after bypass requires continued volume resuscitation of the fetus, which has significant implications for clinical translation of fetal cardiac surgery, particularly in the context of the current practice of open fetal surgery. Finally, our results are the first to indicate that bypass-induced fluid shifts are not associated with significant placental edema, an explanation provided in the past to account for ongoing placental dysfunction after bypass.

Large amounts of circuit volume are lost from the reservoir during fetal extracorporeal support in the absence of obvious hemorrhage. Furthermore, additional crystalloid volume is frequently necessary to maintain safe circuit operation during the 30 minutes of extracorporeal support, suggesting large amounts of fluid accumulation or extravasation occurring somewhere in the fetal–placental unit. Indeed, prior studies have demonstrated that the pregnant sheep has significant placental capacitance when driven by pressure and volume loading[15,26] and conversely by excluding the placenta during bypass,[4] suggesting it might accumulate intravascular fluid (increase capacitance) in the environment of bypass (Table 1). Similarly, increasing extracorporeal perfusion rates from 80 to 110 mL · kg^{-1} · min^{-1} in neonatal piglets (fetal sheep bypass is 200 mL · kg^{-1} · min^{-1}) increases fluid extravasation most prominently during bypass,[27] as seen in this study. This latter study also showed that increasing arterial pressures on bypass, however, does not result in increased fluid extravasation,[24] and conversely, lowering pressure does not reduce it.[25] We therefore suspect that the transient increase in fetal mean arterial pressure seen with fetal bypass (within 2 minutes and lasting approximately 5–7 minutes)[5,6,8] is unlikely to be responsible for the profound fluid extravasation we observed during bypass in this study.

The profound fluid shifts observed during bypass became less prominent during the postbypass period, although they were still persistent. Postbypass volume infusions increased or restored fetal hemodynamics, although often temporarily, suggesting that fluid was still actively shifting in the postbypass period. Prior studies by Brace and Gold[28] examining fetal hemorrhage and intravenous volume loading suggest that the fetus has 5 to 10 times the interstitial compliance and capillary filtration coefficient and about one half the vascular compliance of the adult ewe. Importantly, these prior authors showed that transplacental fluid movement was not considered to be a major factor in this dynamic. Thus the need to continually administer volume to some fetuses throughout the postbypass period might be a reflection of greater propensity of the fetus to rapidly move fluids across capillaries and interstitial space to regulate blood volume (ie, increased capillary leakage is normal in the fetus). It is also possible that the fetal endothelial layer is damaged or becomes

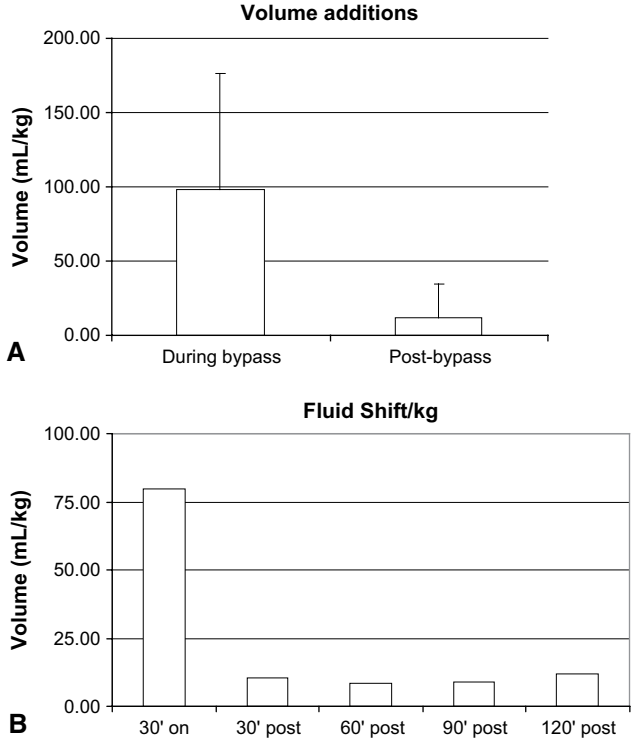

FIGURE 3. For animals in the treated group (n = 10), we tracked volume additions during bypass and recorded total transfusion amounts during the postbypass period. Sham animals received no exogenous fluids. Panel A shows that large amounts of circuit prime volume are lost during 30 minutes of fetal bypass and that substantial postbypass transfusion volume is also necessary to maintain fetal hemodynamics. Panel B shows that calculated fluid shift induced by bypass reveals a dramatic extravasation of plasma volume during bypass that continued in the postbypass period. Intravascular fluid loss averaged 0.8 mL · kg^{-1} · min^{-1}, suggesting the fetal circulation shifts more than 200 mL of plasma volume over the 150-minute experimental protocol.

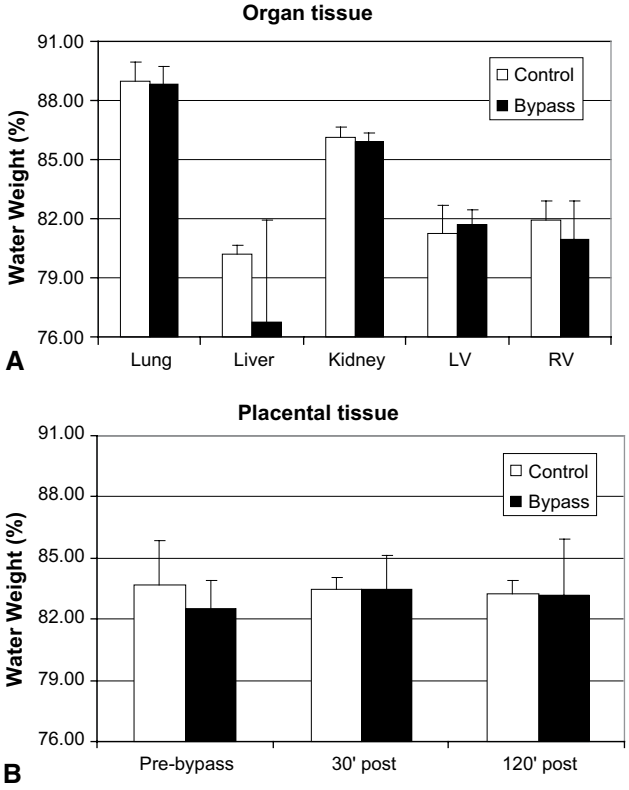

FIGURE 4. Panel A shows the percentage of total tissue water weight measurements in myocardium, lung, liver, or kidney samples and demonstrates no differences between the sham control animals (n = 4, *open columns*) and treated bypass groups (n = 6, *solid columns*) by using the nonparametric *t* test. Panel B shows that serial placental samples from sham control animals (n = 4) and treated bypass animals (n = 4) before bypass and 30 and 120 minutes after bypass did not differ within groups (paired *t* test) or between the groups by using the nonparametric Student *t* test. *LV*, Left ventricle; *RV*, right ventricle.

dysfunctional during the process of fetal bypass. We have recently demonstrated that circulating in vivo nitric oxide levels decrease in the postbypass period, whereas paradoxically cyclic guanosine monophosphate levels increase, suggesting that normal vasodilatory mechanisms of the placenta are disrupted with bypass, an indirect reflection of dysregulation of the fetal endothelial system.[9] Further studies examining endothelial function and integrity in placental and organ tissues are currently underway in our laboratory.

Our data also suggest that the fluid losses with fetal bypass are not prominent in the organs we studied. Importantly, and rather surprisingly, we did not see evidence of placental edema through our water weight determinations in serial placental samples collected before and after bypass, a protocol uniquely possible because of the sheep cotyledonary placenta. We had previously studied placental histology and architecture but had never found any profound evidence of severe edema (data not shown). These histologic studies, however, are not sensitive enough to detect more subtle degrees of fluid accumulation, as assessed by means of standard TWW analysis. Prior studies in neonatal piglets have demonstrated that organ edema occurs with normothermic bypass in the liver, gut, muscle, and skin[17] and that hypothermic or high-flow bypass increases edema formation and edema extends to more organs.[17,24] Our studies of TWW were confined to myocardium, lung, liver, and kidneys from treated animals undergoing normothermic bypass and sham animals. Our model is limited in the application of before-and-after sampling for certain tissues or organs. Its possible animals without treatment during bypass could show water weight changes or that changes are confined to organs we did not assess (eg, skin, gut, or muscle), but future studies are planned to address this. The placental samples, however, do allow for a before-and-after evaluation and as such do not show evidence of substantial fluid extravasations and corresponding weight gain in the placenta.

Similarly, we could not measure fetal urine output into the amniotic cavity (although unlikely in the setting of high

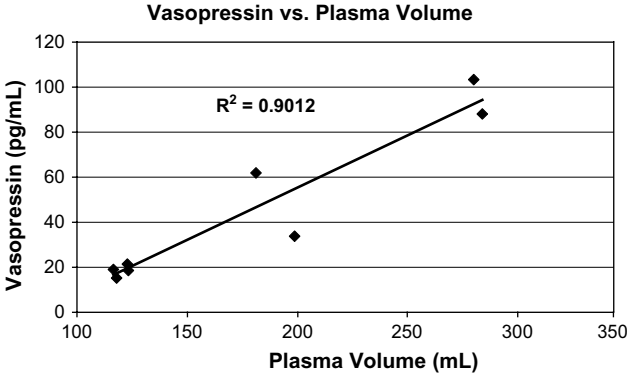

FIGURE 5. Vasopressin levels increased dramatically from baseline to before bypass and further throughout bypass. Increasing vasopressin levels were strongly related to increased fetal plasma volumes ($R^2 = 0.90$, n = 4 animals), suggesting a potential role for vasopressin-mediated osmoregulation.

vasopressin levels, see below) without significant additional surgical manipulation because a Foley catheter is not feasible in the fetus. Neither could we measure ''insensible losses'' with our experimental protocol, which could be significant, especially in view of the needed fetal chest exposure during open fetal surgery. Admittedly, our protocol of continued fetal observation in the postbypass period, during which the fetus is exposed, increases the likelihood of insensible losses that would be perhaps reduced in the clinical setting. Nevertheless, our study highlights that there is potential for significant fluid losses (0.8 mL · kg^{-1} · min^{-1}) during fetal manipulation and exposure to extracorporeal circulation that should be taken into account in potential conceptualization of clinical translation. Current practice of open fetal surgery does not allow for fluid resuscitation of the fetus, at least directly, after intervention. In a circumstance that placental transfer of fluids might be limited (as in fetal bypass with

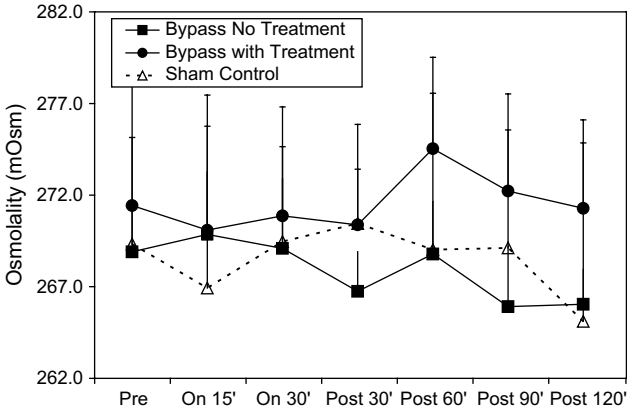

FIGURE 6. Plasma osmolarity was calculated from measured sodium and glucose levels throughout the protocol. Calculated osmolarity remained higher in treated bypass animals throughout the postbypass period versus that seen in nontreated bypass animals ($P < .05$ at 30 minutes after bypass by using the paired *t* test), likely reflecting the effect of fluid resuscitation.

associated placental dysfunction or in the setting of fetal hydrops, the likely scenario in which this technology would apply), strong consideration should be given to creating the means for continued fetal resuscitation and support in the postsurgical period, as in the form of intracardiac lines, which is common practice in pediatric cardiac surgery. In our laboratory we have shown that at least in the fetal lamb model, such an approach can be carried out safely, as in the neonatal setting.

We and others have previously noted that after bypass the fetus often needs and might benefit from significant volume resuscitation.[14-16] An alternative explanation for such volume changes could be changes in fetal fluid reabsorption at the renal tubules caused by the circulating vasopressin. Our results suggest that a dramatic increase in the potent vasoconstrictor vasopressin occurs during bypass that strongly correlates with the associated decreases in plasma volume. The changes in vasopressin expression, however, did not correlate with changes in osmolarity. Further evaluation of vasopressin receptor expression in the fetus and placenta along with studies by using vasopressin receptor antagonists are warranted to further explore the mechanism of fluid shift.

Vasopressin is believed to be the primary mediator of the fetal stress response and its ''brain-sparing effect.''[29] The brain-sparing effect is a well-described fetal adaptive mechanism that consists of preferential redistribution of fetal circulation to the brain, heart, and adrenals during stress.[8,30] This circulatory redistribution is certainly seen with experimental fetal cardiac surgery.[8,10] We have previously shown that significant release of vasopressin (along with other stress-response hormones) occurs with the conduct of experimental fetal cardiac surgery. These responses appear to reflect an overall stimulation of the inflammatory cascade associated with surgical intervention and extracorporeal circulation. Therefore the increase in vasopressin levels seen in our study is likely secondary (and not primary) in the observed fluid shifts. Certainly, the significant increases in vasopressin would be expected to reduce any fetal urine output, which would further accentuate the significance of our fluid losses into fetal tissues.

We also observed that maintenance of baseline osmolarity during the postbypass period was facilitated by means of volume resuscitation of the fetus because the sham and nonresuscitated animals both experienced significant decreases in osmolarity, primarily because of decreasing sodium levels. The effects of adult donor prime on fetal osmolarity must be considered because the calculated osmolarity of the prime is approximately 290 (sodium and glucose concentrations averaging 143 and 75, respectively) compared with prebypass fetal osmolarity that averages 270 mOsm. This is intriguing because both bypass groups experienced the same influence of increased prime osmolarity, yet the animals that were not volume resuscitated in the postbypass period displayed the same decreasing trend as the control

animals. Examining the osmolarity values on bypass reveals that no changes in osmolarity occur during bypass, indicating that prime osmolarity is not influential. This suggests that postbypass fluid resuscitation of the fetus with isotonic solutions, but not hypotonic solutions, would be preferable.

In summary, our results suggest that fluid shift in fetal cardiac surgery with bypass is substantial and begins early during bypass but is treatable with vigilant and appropriate fluid resuscitation during the postbypass period. As such, it is conceivable that some of the detrimental aspects of fetal bypass in prior studies might have been related to the management of the fetus before, during, and after bypass (and management of the fetal stress response) rather than directly to the effects of exposure to extracorporeal circulation. Miniaturization of extracorporeal circuit volume is a necessary engineering achievement likely to reduce inflammatory stimuli and should enhance successful fetal bypass, particularly if an oxygenator is used. However, current miniaturized systems are closed systems and severely limit flexibility during bypass. Our studies suggest that fluid resuscitation therapy could potentially improve outcomes and prevent the postbypass increase in PVR. Indeed, vigilant fluid resuscitation can ameliorate or stave off the peripheral vasoconstriction induced by hemoconcentration. Reduction of these symptoms might ameliorate fetal circulatory centralization and subsequent placental dysfunction and multiorgan failure.

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Discussion

Dr James S. Tweddell *(Milwaukee, Wis)*. Thank you, Dr Eghtesady. You and your colleagues are to be congratulated. That certainly is a complex animal model that you have chosen to tackle, and your studies are very interesting.

You have identified and attempted to characterize placental dysfunction and fluid loss in these fetuses, and you have shown that there are important fluid shifts. You have established that there is intravascular fluid loss of 0.8 $mL \cdot kg^{-1} \cdot min^{-1}$. This does not appear in any of the major organs, and it does not appear in the placenta either. You have concluded, correctly, I think, that placental edema cannot be implicated as a mechanism of placental dysfunction.

My first question is this: Where do you think the fluid is going?

Dr Eghtesady. We are now starting to look at some other organs, such as the skin, muscle, and gastrointestinal tract, specifically to see whether it is going there.

Dr Tweddell. Do you have anything you want to share with us today?

Dr Eghtesady. We have a couple of animals in which there is some suggestion that the gastrointestinal tract might be the culprit site, but I think N of 2 is too small to make any conclusions.

Dr Tweddell. You have also measured vasopressin levels throughout these studies, and others have implicated vasoconstrictors as part of the picture of placental dysfunction. In particular, 10 years ago, Frank Hanley demonstrated that products of cyclooxygenase might be important, possibly thromboxane or other prostanoids. If the production of these agents were blocked with indomethacin, then the rate of placental dysfunction would be reduced. One of the stimuli for vasopressin release is osmolarity, and you have shown quite nicely that it is probably not the case here. What is the stimulus for vasopressin release in these fetuses?

Dr Eghtesady. You are absolutely right. We became interested in this in part because of and building on the work of Dr Hanley and others, who have shown that fetal stress can be very important in terms of the evolution of placental dysfunction. Frankly, our discovery of vasopressin was somewhat serendipitous. What I mean by that is that we had previously looked at different stress response markers, including β-endorphin, cortisol, and vasopressin, and the impressive thing that we found was the increases in vasopressin with fetal surgical bypass. Normally, vasopressin levels are approximately 1 pg/mL in the fetal circulation, and with typical stress, those levels go up to approximately 10 pg/mL. In our protocol they increase to greater than 100 pg/mL. It just goes through the roof. We have also seen that when the level exceeds 90 pg/mL, those animals invariably do very poorly, whereas the animals that mount a response that is appropriate but not excessive do well.

The exact mechanism of release of vasopressin in the fetus is still not known. It is mediated by some effects from the pituitary gland, but interestingly enough, the placenta and the umbilical cord are not innervated. Therefore the mechanisms that actually regulate and perhaps go back and tell the fetus to release this potent hormone, which happens to be a powerful vasoconstrictor of placental vasculature, are not totally clear to us, and that is one of the things that we are looking into right now.

Dr Tweddell. Do you think it is a reduction in the atrial filling pressures with the initiation of bypass, something as simple as that?

Dr Eghtesady. It is possible. But what is interesting, actually, in the fetus is that we do not see a dramatic decrease in the filling pressures. One of the things that has been impressive to me is that we often talk about very little pulmonary blood flow in the fetus. Routinely we actually snare the pulmonary arteries because one of the things that can happen is that you can have increased pulmonary blood flow; although not on the percentage level of cardiac output, it is still not huge. However, one of the things that is often not noted in the literature that has been found to be very impressive is that there is substantial aortopulmonary collaterals—or whatever you want to call them—in terms of bronchial arteries that are equivalent or greater in their volume of return back to the heart through these other vessels. Therefore the heart does not completely empty out, as we normally see in the bypass situation. You actually have to put a vent separately into the left atrium, even though supposedly there is a patent foramen ovale.

Therefore I am not sure whether it is necessarily volume. Even with a vacuum, when we have totally sucked the heart, we still see the same vasopressin concentration. At this point, I do not have any thoughts on what is exactly the mechanism.

Dr Tweddell. From a mechanistic standpoint, the previous study suggested that indomethacin might be helpful in this. Have you considered using peripheral vasodilators, α-antagonists, or something of that nature? Is that in your future plans?

Dr Eghtesady. We have tried Regitine (phentolamine). We have not tried phenoxybenzamine. We have tried Nipride (nitroprusside), milrinone, and dobutamine, and we are planning to use some vasopressin antagonists that are now available.

Regitine does not work. For Nipride to work, the concentrations you have to use are unbelievable. Normally, as you know, in the clinical setting a half a microgram or maybe a microgram or 2. In a fetus, to see a response to Nipride in this setting, you have to go up to 10 to 15 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Milrinone has no effect whatsoever in the fetus.

Therefore of the things that we have tried thus far, we have not had any success in terms of regulating that. That is why we are interested in the vasopressin antagonist to see whether that works.

Dr Tweddell. Your study is one that could ultimately lead to a clinical translation. Would you speculate for us on that? Assuming you can get this to work, what would be some of the first lesions you might tackle?

Dr Eghtesady. One of the ones that we were focusing on in the laboratory is the intact atrial septum. I think when you see it in the operating room, it is very clear how thick that is, and when it is truly an intact septum, it is a challenge. When you look at the literature of what our cardiology colleagues have tried to do with stents and those things, it has not been very effective, certainly in the fetus. Even in the postnatal setting, often these kids that are coming out, and we have had this algorithm of being prepared to help these fetuses out, and they try to do a catheter-based approach to open up the septum. Invariably in the few cases that I have been involved in, it has not been successful, and we had to start them on bypass and resect the atrial septum. Therefore that is the model on which we have chosen to focus. It is a fairly simple operation. In some ways, to me, historically, the very first operations in congenital heart surgery dealt with the atrial septum. Therefore in a way we thought, ''Let's start with that.'' It is very simple and straightforward.

Dr Tweddell. That is terrific work, and you are to be congratulated for developing this model and getting this underway.

Dr Frank W. Sellke *(Boston, Mass)*. A lot is known about inflammatory stimuli in adult bypass, including the effects of neutrophils, complement, kinases, and so forth. Do you have any idea what is going on in the fetus during bypass and then later reperfusion?

Dr Eghtesady. A few studies have been done. We actually recently got results from a sort of a pseudoproteomics approach to try to look at this using a panel of antibodies from Kinexis. Clearly, a number of inflammatory markers are increased. We are currently digging our way through the data because there are so many changes. We have noticed a pretty dramatic change in some of the PKCs and we are trying to figure out what that means.