PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Arterial Flow Patterns in Healthy Transitioning Near-Term Neonates
AUTHORS	Stritzke, Amelie; Murthy, Prashanth; Kaur, Sharandeep; Kuret, Verena; Liang, Zhiying; Howell, Sarah; Tyberg, John

VERSION 1 – REVIEW

REVIEWER	Reviewer name: Zach Vesoulis Institution and Country: Washington University School of Medicine, USA Competing interests: none
REVIEW RETURNED	20-Jun-2018

GENERAL COMMENTS	In this very interesting article, Dr. Stritzke et al. describe normative patterns of vascular resistance in three organs (brain, kidney, intestines) in a group of term infants. While the paper has limited generalizability (serial Doppler ultrasound measure are unlikely to be deployed clinically, no described relationship with outcomes) it is well written and demonstrates some important mechanistic concepts. However, without any correlation to clinical outcomes, the reported data does not add much value other than reference ranges. In its current form, there are several factors in the study design which should be addressed in a revision, primarily better definition of inclusion criteria and addressing the confounding factor of feeding. A summary of comments, divided by section, follows below. Abstract -no issues Introduction -In the review of factors which contribute to transitional hemodynamics, oxygen is curiously missing from the list. There is much discussion of changes in shunting patterns, without mention of the primary factor which drives pulmonary vasodilation. This is a significant omission and should be addressed. Methods -The inclusion/exclusion criteria need to be more detailed. We infants with evidence of distress on CTG included? What about infants with acidosis? Was there a cord pH cutoff for inclusion? -What was the rationale for including infants with and without delayed cord clamping. One of the theories of delayed cord clamping as a mechanism for improving neonatal health is that it “stabilizes” the cardiovascular system. Is it reasonable then to have half of the cohort receiving this hemodynamically active intervention? What was the reason for lack of DCC in the other infants?

	-The definitions of various indices should be included in a table, difficult to read as text -The most significant issue with the study design is the lack of attention paid to the timing of feeding. NIRS-based studies have shown significant changes in splanchnic in response to feeding. I would suggest that the authors review the chart and divide the measurements in to pre and post prandial. -The use of the Kruskal Wallis test is inappropriate. While KW does measure differences between more than one group, this is a repeated measures design, thus a statistical test which can account for this should be used (e.g. mixed model regression, Friedmans's). -Have the authors accounted for repeated statistical testing in the selection of significant p values? Results -"The SMA showed the highest blood flow pattern" What does this mean, what pattern? Discussion -Missing from the Discussion is speculation on the relationship between LVO and the blood flow regulation in each organ system. The authors show that the infants move from a high LVO, high resistance environment to a lower LVO, lower resistance environment, but what is the driving factor? -The authors should consider expanding their group of infants and focus on the relationship between these variables and meaningful clinical outcomes. One obvious outcome would be urine output, does the fall in RI/PI correlate with the expected diuresis during the second 48 hours of life?

REVIEWER	Reviewer name: Liesbeth Thewissen Institution and Country: University Hospitals Leuven, Department of Neonatology,Herestraat 49, 3000 Leuven, Belgium Competing interests: None
REVIEW RETURNED	02-Jul-2018

GENERAL COMMENTS	The current study is a prospective observational cohort study conducted in 2017. The authors describe in detail ultrasound derived serial hemodynamic measurements of the anterior cerebral artery, renal artery and superior mesenteric artery during the transitional phase in (near-) term neonates. The novelty of the study is the evolution of the Doppler-derived indices PI and RI, adding information regarding pattern evolution over time in the three organs. Furthermore, the objective was set to test these PI/RI patterns as a measure of vascular resistance, using Conductance. Comments to the authors. Overall, the paper contains consistent information and is clearly written. The evolution of flow patterns in the transitional phase between the three different organ systems in healthy (near) term infants is important information to improve understanding of pathological adaptation mechanisms. Interesting information about PI/RI among the different vascular beds is obtained. The exact meaning and measurement of conductance indicating the reciprocal of resistance is less clearly described.

	1)One of the objectives is to test PI and RI patterns as a measure of vascular resistance. This in not reflected in the primary or secondary outcome. The introduction of G, conductance, occurs very short in the introduction and more clarity about measurements of G in the clinical setting is needed. Furthermore, measurement of G is not clear in the method section (1/PI to calculate G). More clarity about figure 3 D-E-F is needed (pooled data?) Introduction: 2) Page 6 line 1: in the neonate, an adverse antenatal flow pattern…: do the authors refer to fetal flow patterns with reversed end diastolic flow or to aberrant neonatal flow? Methods 3) Page 9 line 1: A=πr2 ? 4) Page 9 line 9: please explain REB Page 9 line 16, table 2- 3 5)Was a post hoc test performed to distinguish which time points are different from each other, if the KW or FE test came back statistically significant? Results 6)Page 10 line 15-16: these lines are in contradiction. 'No significant changes to velocity or organ flow over time for any organ' versus 'ACA was the only organ increasing both Vmax and blood flow over time. This last statement is also repeated in the conclusion. Discussion 7)Page 12 line 5: Our values at birth…: to which specific values do the authors refer? 8)Page 12 Line 19: Organ blood flow versus renal blood flow values obtained in ref 29. The renal blood flow values seems to differentiate from the values obtained in ref 29. Can the authors comment on this? Limitation: 9) Did the authors take into account abnormal PI/RI of the uterine artery with or without abnormal fetal flow patterns in hypoxic pregnancies? In these cases, the hemodynamic indices obtained during the transitional phase might be different from the normal population. 10)Was the inter-rater variability between both neonatologists checked? Conclusions: 11)Page 15 line 1: While the brain shows an increase of flow over time: please confirm this with the results.

REVIEWER	Reviewer name: Emmanouil Bagkeris Institution and Country: University College London Competing interests: No competing interests
REVIEW RETURNED	12-Jul-2018

GENERAL COMMENTS	Dear authors, this was a very interesting manuscript to review. Below are my recommendations for improving the context of your manuscript. 1. The abstract states that the children included in the analysis are near term (>36 weeks), however the median GA is 40. Please edit text accordingly. 2. Please reference the growth charts used to define growth status of the infants. 3. Mixed ANOVA should be considered for both table 2 and table 3. The measurements are not independent (they come from the same patient), hence there is an association of the values and this dependence cannot be ignored. The current analysis considers 21 children in each time point i.e. 63 independent measurements in total. However the 63 measurements are nested within 21 children. A mixed ANOVA, (mixed model/multilevel model) will take the inter-patient variability into account and the p-values will be corrected accordingly. Patient ID should be considered as a random effect and the time (Time 1, Time 2 and Time 3) as fixed effect. 4. What was the rational behind comparing PI between organs in figure 1? Are they supposed to be the same or follow the same pattern? It would be interesting to provide as supplementary material the change over time of PI separately for brain, gut and kidney.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

In this very interesting article, Dr. Stritzke et al. describe normative patterns of vascular resistance in three organs (brain, kidney, intestines) in a group of term infants. While the paper has limited generalizability (serial Doppler ultrasound measure are unlikely to be deployed clinically, no described relationship with outcomes) it is well written and demonstrates some important mechanistic concepts. However, without any correlation to clinical outcomes, the reported data does not add much value other than reference ranges.

Response: We thank the reviewer for this positive review. We do see the point regarding limited generalizability and agree that likely serial Doppler ultrasound measures are not going to be deployed clinically in the future. However, our rationale was not to introduce serial Dopplers to clinical care, but rather to highlight the physiologic “normal” hemodynamic and vascular adaptation in the healthy neonate. To emphasize this point and to highlight the value of this data of our “normal” cohort, we added a sentence at the end of Rationale page 7, line 7-10: “Values for pathologic adaptations such as in premature, sick, or such with antenatal adverse vascular patterns may be compared in the future, highlighting vascular response mechanisms, which may help delineate beneficial clinical interventions.”

We also added on page 10, line 17/18 that in hospital record follow-up to up to median 12 months it could be ascertained that there were no significant cardiopulmonary disease in either participant.

Introduction

-In the review of factors which contribute to transitional hemodynamics, oxygen is curiously missing from the list.

There is much discussion of changes in shunting patterns, without mention of the primary factor which drives pulmonary vasodilation. This is a significant omission and should be addressed.

Response: We agree completely with the reviewer and just assumed the oxygen's effect as the most important driver to all the changes described. Hence, we did add that oxygen is the driver on page 5 line 3-4 in adding: "…the rising oxygen tension with…the first breaths" decrease pulmonary vascular resistance…

Methods

-The inclusion/exclusion criteria need to be more detailed. Were infants with evidence of distress on CTG included? What about infants with acidosis? Was there a cord pH cutoff for inclusion?

Response: We thank the reviewer for these very relevant and thoughtful points. Care was taken in order to gather data on a random group of normal, healthy, near-term babies, as this study was conducted in a low-risk maternity hospital with a level II NICU only. In order to ascertain a healthy population as much as possible we did exclude maternal diabetes, maternal selective serotonin re-uptake inhibitors (SSRI), and clinical suspicion of persistent pulmonary hypertension of the neonate (PPHN) as stated under exclusion criteria. We did however, add under exclusion criteria page 8 line 3 that known antenatal uterine artery adverse Doppler changes were also an exclusion criterion, not mentioned before.

There was no cut-off a priori for cord pH or Apgar, as informed consent was taken from the parents before these were known, at least 6 hours before delivery.

The participants' wellbeing is evidenced by the median Apgar score of 9 (Q1-Q3 of 9-9) at 5 minutes (See Table 2). In order to address this question further, we also added the arterial cord pH and base excess to Table 2. Further details on the two babies who were admitted to the NICU was added to on page 10, lines 12-14: One was admitted for Transient Tachypnea of the Newborn (TTNB) with CPAP support for <24hours and culture-negative rule out sepsis, NICU stay was 48 hours. The second was admitted for mild Hypoxic-Ischemic encephalopathy (HIE), NICU stay 72 hours. The neurological follow-up at 3 months of this latter baby was normal.

-What was the rationale for including infants with and without delayed cord clamping. One of the theories of delayed cord clamping as a mechanism for improving neonatal health is that it "stabilizes" the cardiovascular system. Is it reasonable then to have half of the cohort receiving this hemodynamically active intervention? What was the reason for lack of DCC in the other infants?

Response: We thank the reviewer for this very relevant point. We agree that delayed cord clamping (DCC) is relevant in this context and report on the data for that reason in Table 1. Of the 21 included babies, 9 received >60 seconds of DCC, for 2 the data was not known (we added this information to Table 1), and 11 did not receive >60 seconds of DCC, for no apparent reason. Since there was no contraindication to DCC, we asked for the deliverers' practices at the hospital this data was obtained. The response was that "DCC is done at term if the family wishes". We do not think in consideration of the small numbers that it would make sense to divide the group in DCC and non-DCC receivers. Therefore, we agree that unfortunately the inconsistency regarding DCC in our cohort is a limitation we added under the appropriate section, page 15, lines 8-9.

-The definitions of various indices should be included in a table, difficult to read as text

Response: We thank the reviewer for this helpful hint and added Table 1 to the main document with the respective indices used and their derivation.

-The most significant issue with the study design is the lack of attention paid to the timing of feeding. NIRS-based studies have shown significant changes in splanchnic in response to feeding. I would suggest that the authors review the chart and divide the measurements in to pre and post prandial.

Response: We again thank the reviewer for this thoughtful comment. We agree with the fact that feeding may elicit a splanchnic perfusion response, however, more so in an established rhythm of substantial meals which is hardly expected in the first 24 hours of life. Due to the nature of small, frequent, and really impossible to ascertain amount of feeding -as it is mainly breastfeeding- in the first 24 hours of life, this data was impossible to gather. May we also please remind the reviewer of the small size of the stomach of a newborn infant <24hours? These babies are with their mothers and within the first 24 hours we do not expect more than 1-2 wet diapers because of the initiation of feeding and transitioning. We did list this limitation under the limitations' section. The other arteries' perfusion is hardly dependent on feeding status.

-The use of the Kruskal Wallis test is inappropriate. While KW does measure differences between more than one group, this is a repeated measures design, thus a statistical test which can account for this should be used (e.g. mixed model regression, Friedmans's).

-Have the authors accounted for repeated statistical testing in the selection of significant p values?

Response: Thank you for pointing this out. We have not accounted for repeated statistical testing in the selection of significant p values. We re-ran the analysis by applying mixed model ANOVA and updated the result in the manuscript.

Results

-"The SMA showed the highest blood flow pattern" What does this mean, what pattern?

Response: Table 4 shows the blood flow pattern of how many ml/kg/min were calculated per organ:

ACA Flow (ml/kg/min) 4.30 (5.30) 4.70 (3.70) 7.80 (7.70) 0.07

SMA Flow (ml/kg/min) 14.70 (11.90) 12.20 (13.90) 16.80 (16.10) 0.37

Renal Flow (ml/kg/min) 3.80 (5.70) 4.20 (3.70) 4.60 (5.80) 0.88

On page 11 lines 1-2 we clarified the sentence in changing the rounded up "15" to the actual '14.7ml/kg/min" and added "compared to brain with 4.3 and kidney with 3.8ml/kg/min." The blood flow pattern is further elucidated in Figure 3, C)

Discussion

-Missing from the Discussion is speculation on the relationship between LVO and the blood flow regulation in each organ system. The authors show that the infants move from a high LVO, high resistance environment to a lower LVO, lower resistance environment, but what is the driving factor?

Response: We thank the reviewer for the comment but respectfully disagree. We did not show the infants move from a high LVO, high resistance environment to a lower LVO, lower resistance environment: The LVO, as the RVO, did not change significantly over the observed time, they remained constant (See Table 3). This is confirmed by a very recent publication which we added to the reference list due to its very relevant nature (Ref 32): J Pediatr. 2018 May 23. Cardiopulmonary Adaptation During First Day of Life in Human Neonates. Jain A.

Our aim was to show differential organ Doppler derived indices and blood flow DESPITE the constant LVO which serves as arguments for an organ-level independent autoregulation.

The driving factor is largely unknown, we can only speculate it being organ-specific vascular tone regulating cytokines such as prostaglandines which are known to play a role in the vascular regulation of the kidney. We added a sentence as to clarify above issue and these speculations on page 14 lines 6-8: “Despite the constant central outputs, organ flows and indices differ distinctly, supporting independent vascular regulation of flow, arguably via paracrine and autocrine mechanisms.”

-The authors should consider expanding their group of infants and focus on the relationship between these variables and meaningful clinical outcomes. One obvious outcome would be urine output, does the fall in RI/PI correlate with the expected diuresis during the second 48 hours of life?

Response: We thank the reviewer for this very thoughtful comment and absolutely agree that this clinically relevant parameter is important. We are actually aiming at addressing this very good question in a subsequent trial. However, for term infants such as the ones in this present study, we do not think that the timing of 24 hours after birth is sufficient to see the change in the renal RI/PI 3nough to correlate (yet) with urinary output (see ref 28). We know that renal adaptation, that is development of mature renal perfusion pattern, takes much longer, likely days to weeks. See also page 13, line 1-2 and ref 5.

In this population of term babies, logistically, it is much more difficult to follow them once they have left the hospital. Therefore we are planning to do investigations relating renal PI and RI with longer observations in urinary output, but in a more preterm and hence within-hospital population. For the purpose of this present study, however, we wanted to prove the concept that PI/RI does change, even within this short time frame of observation, characteristically, according to the organ.

Furthermore, as per Al-Dahhan J, Haycock GB, Chantler C, Stimmler L (1983) Sodium

homeostasis in term and preterm neonates. I. Renal aspects. Arch Dis Child

58:335–342 adaptation occurs with natriuresis in a characteristic manner after delivery. Therefore, we are assuming in this cohort of healthy babies, that the renal adaptation is somewhat similar for each of them. Nevertheless, we did mention the fact that we did not correlate the flows with the urinary output under limitations.

Reviewer: 2

Overall, the paper contains consistent information and is clearly written.

The evolution of flow patterns in the transitional phase between the three different organ systems in healthy (near) term infants is important information to improve understanding of pathological adaptation mechanisms. Interesting information about PI/RI among the different vascular beds is obtained. The exact meaning and measurement of conductance indicating the reciprocal of resistance is less clearly described.

Response: We thank the reviewer for these kind comments. See below regarding conductance.

1) One of the objectives is to test PI and RI patterns as a measure of vascular resistance. This in not reflected in the primary or secondary outcome.

Response: We have PI and RI as measure of vascular resistance as our first secondary outcome on page 9 line 1. We clarified in how we tested them in adding “… in testing 1/PI and 1/RI for their conductance.”

The introduction of G, conductance, occurs very short in the introduction and more clarity about measurements of G in the clinical setting is needed.

Response: Thank you for this comment. We did clarify why we think conductance is a superior measure than resistance on page 6, lines 16-20.

Furthermore, measurement of G is not clear in the method section (1/PI to calculate G).

Response: On page 9 line 14 we explained conductance further with “Conductance: (normalized) organ flow (G) / arterial pressure is VTI x CSA x HR /mean BP.

More clarity about figure 3 D-E-F is needed (pooled data?)

Response: On page 11 , lines 10-14 we explained Figure 3 D, E and F more clearly: “Because PI and RI have been taken as measures of resistance and because resistance is the reciprocal of conductance, we plotted 1/PI versus its respective conductance for ACA, SMA, and RA (Figure 3, D-F). Each slope was essentially zero and R2 ~ 0, indicating that the variance in organ conductance explained none of the variance in 1/PI. PI (and RI) are not measures of resistance.”

Introduction:

2) Page 6 line 1: in the neonate, an adverse antenatal flow pattern…: do the authors refer to fetal flow patterns with reversed end diastolic flow or to aberrant neonatal flow?

Response: Yes, we were. Thank you for pointing out that it is not clear. We did add some clarification page 6 line 2: “… such as absent, or reverse, end-diastolic uterine artery flow…”

Methods

3) Page 9 line 1: A=πr2 ?

Response: Thank you very much for catching this oversight. The area of a circle is calculated by

[π x (D/2)2]. We corrected that.

4) Page 9 line 9: please explain REB

Response: Research Ethics Board (REB) was added.

Page 9 line 16, table 2- 3

5)Was a post hoc test performed to distinguish which time points are different from each other, if the KW or FE test came back statistically significant?

Response: Yes, we were using Dunn’s test and Nemenyi test as the multiple comparison post hoc test if the KW test came back statistically significant. We are now using mixed model ANOVA instead of the KW test.

Results

6) Page 10 line 15-16: these lines are in contradiction. ‘No significant changes to velocity or organ flow over time for any organ’ versus ‘ACA was the only organ increasing both Vmax and blood flow over time. This last statement is also repeated in the conclusion.

Response: Thank you for pointing out that this statement needs clarification. Table 3 does confirm that there were no significant differences for neither Vmax nor blood flow over the three times. However, what we were trying to point out was that while the change was not significant over time, only for ACA was the direction of change for both Vmax and flow consistently going up, from 30.3 to 32 to 38cm/s and from 4.3 to 4.7 to 7.8ml/kg/min. In contrast, both kidney with Vmax 38.5 to 32.7 to 29.6cm/s, and splanchnic perfusion with Vmax 72.4 to 64 to 60cm/s were decreasing.

We changed the conclusion page 15, line 16 to "… is the only organ that shows a non-significant increase of flow over time…"

Discussion

7)Page 12 line 5: Our values at birth…: to which specific values do the authors refer?

Response: Page 12 line 20 we clarified: "Our closest values to birth taken within 1-2 hours of delivery…"

8) Page 12 Line 19: Organ blood flow versus renal blood flow values obtained in ref 29. The renal blood flow values seems to differentiate from the values obtained in ref 29. Can the authors comment on this?

Response: Thank you for pointing this out. We can only speculate that as renal maturation and adaptation after birth occurs, that blood flow will change with time. The reference 29 reports on values for both kidneys added together and the timing was from 7 hours to within the first week of life, therefore the adaptation may have been much more advanced than our very young cohort assessed within a few hours of birth. We did add page 13 line 14-15: "…, within one week of life, compared to our data within hours of birth when renal adaptation is likely still early."

Limitation:

9) Did the authors take into account abnormal PI/RI of the uterine artery with or without abnormal fetal flow patterns in hypoxic pregnancies? In these cases, the hemodynamic indices obtained during the transitional phase might be different from the normal population.

Response: We thank the reviewer for this very thoughtful comment and yes, indeed, we are fully aware of an adverse Doppler antenatal pattern having influence on postnatal adaptation patterns. In this first study we aimed to characterize normal adaption patterns, and antenatal adverse Doppler changes such as absent or reverse uterine Doppler flows if known, were excluded. In fact, only one single baby (Table 2) were SGA. We added this exclusion criterion to page 8, line 4. We aim to, in a second step, compare these normal adaptation patterns to premature, sick, and those with adverse antenatal Doppler patterns, as stated in the rationale page 7, page 9-12.

10) Was the inter-rater variability between both neonatologists checked?

Response: out of the 61 scans, 56 (93%) were done by one neonatologist (AS), 5 (7%) by the other (PM). The inter-rater variability was not checked, as the majority was done by one neonatologist. We added this information at page 10, line 10-11.

Conclusions:

11) Page 15 line 1: While the brain shows an increase of flow over time: please confirm this with the results.

Response: Thank you, we did change in response to comments page 15 line 19: "…while the brain is the only organ that shows a non-significant increase of flow…"

Reviewer: 3

Dear authors, this was a very interesting manuscript to review.

Response: Thank you very much for your kind comment!

The abstract states that the children included in the analysis are near term (>36 weeks), however the median GA is 40. Please edit text accordingly.

Response: This is correct. While the inclusion criteria included babies from 36 weeks and higher, there were only 2 babies both 36+6 weeks of gestation. The median therefore is 40 weeks with Q1-Q3 of 39-40.

2. Please reference the growth charts used to define growth status of the infants.

Response: Thank you, Fenton 2013 reference 21) added page 7 line 16.

3. Mixed ANOVA should be considered for both table 2 and table 3. The measurements are not independent (they come from the same patient), hence there is an association of the values and this dependence cannot be ignored. The current analysis considers 21 children in each time point i.e. 63 independent measurements in total. However the 63 measurements are nested within 21 children. A mixed ANOVA, (mixed model/multilevel model) will take the inter-patient variability into account and the p-values will be corrected accordingly. Patient ID should be considered as a random effect and the time (Time 1, Time 2 and Time 3) as fixed effect.

Response: Thank you for pointing this out. We have not accounted for repeated statistical testing in the selection of significant p values. We re-ran the analysis by applying mixed model ANOVA and updated the results and the tables in the manuscript.

4. What was the rationale behind comparing PI between organs in figure 1? Are they supposed to be the same or follow the same pattern? It would be interesting to provide as supplementary material the change over time of PI separately for brain, gut and kidney.

Response: Figure 1 shows the PI at time 1 for all three organs interrogated. They are not the same, the point is that PI differentiates between ACA and RA and between SMA and RA. We chose Figure 1 as at Time 1 there are the most significant differences between the organs: PI is significantly different between RA and ACA and between RA and SMA, indicated by the *. For Times 2 and 3, PI is significantly different only between RA and ACA, for RI the findings are exactly the same. Hence, we chose to show the most differential which is for Time 1 and not show the other times which are very similar. The data is available. We explain these findings on page 10 lines 17-18.

VERSION 2 – REVIEW

REVIEWER	Reviewer name: Zachary Andrew Vesoulis Institution and Country: Washington University School of Medicine, United States of America Competing interests:None
REVIEW RETURNED	15-Aug-2018

GENERAL COMMENTS	In this interesting pilot normative data, Stritzke et al provide a potentially valuable source of normative data on the transitional hemodynamic patterns of three different vascular beds— the brain, kidneys, and the GI tract. Although there is little direct translation of these results to practice at the bedside, this data is highly informative for other, more applied studies. The manuscript is generally well written and has a logical flow. There are several important limitations, particularly feeding practices and possibly the inclusion of infants who should have been excluded, which should be addressed in a more thorough manner. A complete review, organized by section, follows below.

	Abstract -the authors are encouraged to limit use of acronyms and abbreviations, where possible, to make the abstract as accessible as possible Introduction -Minor point- I assume when the authors speak of oxygen saturation measurement they are referring to the SpO2, not SaO2 (as this was a non-invasive study!) Methods -The authors state that they evaluated growth using the Fenton charts, but IUGR status is not listed as an exclusion criteria. As the authors explain in the introduction, placental insufficiency alters doppler flow patterns. -The authors use peripheral vessel diameter as a part of the metrics, but there is no discussion of peripheral vascular instability during transition. This can often be marked (as vividly shown by acrocyanosis or cutis marmorata) -Discussion of author contributions seems out of place in methods, is that the right spot for it? Results -The authors note, but do not comment, on the significant imbalance of imaging performed by one author. It would have been better for a single person to do all tests, or to have testing done by both to assess inter-rater reliability. -I question the inclusion of an infant with TTN and certainly an infant with HIE. Hypoxic-Ischemia causes significant instability to the autonomic nervous system, likely altering vascular auto regulation throughout the body. -I likewise question the validity of using hospital data to verify that the infants had “no significant cardiopulmonary disease.” What if the infants went to a different hospital? Or moved? Or died? -SD of brain and renal flow pattern not provided on page 11 line 4 Discussion -Rather than describe animal models for renal vascular regulation, why not discuss in context of renal dynamics of neonates. It is well known that infants have a relative oliguria for 24h followed by diuresis. Is this related to transitional vascular dynamics? -The authors appropriately point out the limited feeding data as a significant limitation. In fact, I would say this is the most significant limitation of the entire manuscript. In addition to variation in timing between feeds and imaging, the type of feeding is also important. Exclusively breastfed infants will take limited volumes in the first few days, likely inducing smaller amplitude changes in SMA flow. In contrast, formula fed infants may take greater volumes and have proportionally greater change in flow. The authors should provide more detailed feeding information, as they have it available. -The authors speculate about the upward trend in velocity and flow. Cerebral auto regulation is also heavily influenced by CO2 levels, volume status, etc. The current explanation is too simplistic. -It is interesting that all ductal flow was left to right, despite presence of TR jets (indicating elevated PVR in some infants) -The authors speculate that closure of PDA causes transient increase in PVR, leading to TR.

	Is it not also possible that the PDA had previously been serving as a “pop-off” for a stablely elevated PVR, and with closure this leads to greater back pressure on the AV valve? -Delayed cord clamping should be mentioned much sooner than the discussion. Why was it not performed in half of the cohort? -Another important, unanswered question is how stable are these measures. The authors attribute all changes with the passage of time and progression of transition, but what about measurement reliability? If one measures the MCA flow at 8am, how close of a measurement will he or she obtain at 10am? Figures -Figure 1 should show results for each vascular bed on each day -Figure 2 is difficult to interpret if one is unfamiliar with doppler envelope estimation.

REVIEWER	Reviewer name: Emmanouil Bagkeris Institution and Country: University College London, UCL UK Competing interests: No competing interest
REVIEW RETURNED	23-Aug-2018

GENERAL COMMENTS	Dear authors, thank you for taking my statistical comments into account. Please expand all abbreviations used at tables 2, 3 and 4 in footnotes under the tables.

REVIEWER	Reviewer name: Liesbeth Thewissen Institution and Country: University Hospitals Leuven, Department of Neonatology,Herestraat 49, 3000 Leuven, Belgium Competing interests: None
REVIEW RETURNED	27-Aug-2018

GENERAL COMMENTS	Overall, after the revision the paper reads very well and major shortcomings have been eliminated. However, some inconsistensies make it difficult to accept the paper in its current form. 1. Please make sure to refer to the correct tables in the text. 2. Blood pressure did not change significantly over time. Please adapt. 3. The referral to figure 3 C at page 11 line 4 is unclear. Figure 3 C measures a relative conductance. In the same section, the message of line 7-8 is unclear to me. Again in the same section, line 14-15, the authors state that the central outputs did not change significantly over time. However, in table 3, the p values comes back 0.01 for RVO (significant) and 0.05 for LVO (which makes it a trend). This also needs to be addressed in the discussion but will, to my opinion, not change the central message. 4. Page 13 line 8 and 13: Organ blood flow is mentioned in two different sentences with distinct values. Line 8: Is organ blood flow equal to SMA mesenteric artery blood flow in this sentence?

	Line 13: do the authors refer to renal artery blood flow in this sentence (organ blood flow of 21 ml/kg/min). 5. Conductance is defined as 1/pulsatility index in table 1. To my opinion, this is exactly the hypothesis tested by the authors. In the conclusion is stated: as 1/PI was independent of conductance,…. It would be more clear if the authors refer to the definition used to calculate conductance in table 1: VTIxCSAxHR/mean BP 6. Table 4/figure 2: renal PI/RI/Vmax/flow: please use consistent abbreviations throughout the text, figures and tables. In the text the authors refer to RA.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Abstract

The authors are encouraged to limit use of acronyms and abbreviations, where possible, to make the abstract as accessible as possible

Thank you for this suggestion. We reduced the use of abbreviations for the arteries and took out SMA, ACA and RA in the abstract, as well as Vmax and replaced them with superior mesenteric, anterior cerebral and renal arteries, as well as maximum velocity. We would argue to keep PI and RI for pulsatility and resistance indices as these are stand-alone commonly used Doppler indices that people may want to search the article for.

Introduction

I assume when the authors speak of oxygen saturation measurement they are referring to the SpO2, not SaO2 (as this was a non-invasive study!)

You are correct, and we apologize for that oversight. Measurement of oxygen saturation was always non-invasive; therefore we replaced all SaO2 with SpO2 designations.

Methods

The authors state that they evaluated growth using the Fenton charts, but IUGR status is not listed as an exclusion criteria. As the authors explain in the introduction, placental insufficiency alters Doppler flow patterns.

This is correct; antenatal Doppler changes would be able to alter postnatal Doppler adaptation. Presence of known antenatal Doppler changes was an exclusion criterion, and none of the included babies had them. The patient with SGA status was followed serially before birth, and did not have any of our exclusion criteria (maternal SSRI, maternal diabetes, known antenatal Doppler changes), he also was not exposed to maternal preeclampsia or hypertension. He did have a fetal unilateral dysplastic kidney which may explain his growth status, and measurements were done on the contralateral normal kidney.

We did not use SGA status as an exclusion criterion because we wanted an “all-comer” population of healthy, near-term neonates. We felt the criteria of known antenatal Doppler changes was more important as known to influence postnatal adaptation, than the growth status itself. Growth status and IUGR/SGA may be due to a variety of reasons not all of which could likely influence postnatal Doppler changes such as in this case. Therefore, we chose to include this baby wanting to not decrease the n further.

The authors use peripheral vessel diameter as a part of the metrics, but there is no discussion of peripheral vascular instability during transition. This can often be marked (as vividly shown by acrocyanosis or cutis marmorata)

That is an interesting point, however, it would be nearly impossible to observe the peripheral vascular stability or instability in these babies over an extended period of time. Might we remind the reviewers that all these babies were healthy normal, nursery babies cared for by their mothers on the postpartum ward, with the exception of the two babies briefly admitted to the NICU. None of the babies had any major peripheral vascular instability such as the ones mentioned, at the times of the measurement, but, of course, such incidents might have occurred between measurements, as in any other baby during the immediate postnatal adaptation period.

Discussion of author contributions seems out of place in methods, is that the right spot for it?

We have repositioned it immediately after the text, before the references. Thank you. Along with funding, competing interests, consent and ethics approval information.

Results

The authors note, but do not comment, on the significant imbalance of imaging performed by one author. It would have been better for a single person to do all tests, or to have testing done by both to assess inter-rater reliability.

We agree. It is mentioned in the Limitations section.

I question the inclusion of an infant with TTN and certainly an infant with HIE. Hypoxic-Ischemia causes significant instability to the autonomic nervous system, likely altering vascular auto regulation throughout the body.

Thank you for raising this point.

While we acknowledge the subtle physiological differences some clinical scenarios may come with, we had to draw a firm line for exclusion criteria such as antenatal Doppler changes or PPHN. The baby deemed “mild HIE” was admitted to the NICU after delivery for mild respiratory distress with a cord pH of 7.19, Base excess -10 and an Apgar of 7/8/9. He was not deemed as hypoxic ischemic encephalopathy (HIE) as his neurological exam remained completely normal, and therefore he was not treated with therapeutic hypothermia. After resolution of his respiratory distress which did not require respiratory support, he presented at age 38 hours with a seizure, and cranial MRI showed changes consistent with mild hypoxia and microemboli, the latter of which likely caused the seizures. He was discharged without anti-ictal medication.

Therefore, in review of the exclusion criteria, we decided to keep the baby in the study, as the initial hemodynamic evaluations were done on a baby with no indication that the baby had altered physiology.

I likewise question the validity of using hospital data to verify that the infants had “no significant cardiopulmonary disease.” What if the infants went to a different hospital? Or moved? Or died?

We again appreciate the question and, regarding validity, we do believe that following the hospital records usually does give a reliable view on what happened with these infants. The reason is that in Alberta we have a unified single health care system, Alberta Health Services, and all emergency and hospital contacts, medication application and potential deaths are accessible to the health care providers.

Granted, that does not cover any hospital or emergency visits outside of the province, the likelihood of which is small presumably. We added on page 10 line 5: “Hospital records of all infants up to a median of 12 months (range 8 to 18 months) in a unified health care system confirm…”

SD of brain and renal flow pattern not provided on page 11 line 4

Thanks for pointing out. The sentence is modified as follows:

The SMA showed the highest blood flow pattern and largest variation with mean 16.1 ml/kg/min (SD 9.7, Table 3), compared to brain with 5.8 ml/kg/min (SD 3.7) and kidney with 6.9 ml/kg/min (SD 9.4).

Discussion

Rather than describe animal models for renal vascular regulation, why not discuss in context of renal dynamics of neonates. It is well known that infants have a relative oliguria for 24h followed by diuresis. Is this related to transitional vascular dynamics?

We certainly agree with the reviewer that this is a fascinating topic and very likely that the adaptation in renal vascular physiology after birth has something to do with the oliguria after delivery. However there is very little known about the subject, as far as we can find, therefore we reverted to animal data to expand on this point.

The authors appropriately point out the limited feeding data as a significant limitation. In fact, I would say this is the most significant limitation of the entire manuscript. In addition to variation in timing between feeds and imaging, the type of feeding is also important. Exclusively breastfed infants will take limited volumes in the first few days, likely inducing smaller amplitude changes in SMA flow. In contrast, formula fed infants may take greater volumes and have proportionally greater change in flow. The authors should provide more detailed feeding information, as they have it available.

We again acknowledge this important shortcoming, but would have to say it is nearly impossible to account for frequent, inconsistent feeding the amount of which is equally difficult to ascertain. Similarly, breastfeeding and formula feeding may have different implications in terms of volume and the effect on gut perfusion, we agree. However, apart from the logistical difficulties controlling for this, we would like to point out that in the first 24 hours of life, neither breastmilk nor formula is usually taken in high quantities by the baby. Therefore, the effect of it, might, or might not, be that large. We do not have the feeding data available and did mention it in the limitations.

The authors speculate about the upward trend in velocity and flow. Cerebral auto regulation is also heavily influenced by CO2 levels, volume status, etc. The current explanation is too simplistic.

Thank you for pointing this out, we did add explanation to this section such as that we assume euvolemia, eucapnia and normal cardiac function in our cohort of healthy near term babies. We agree that cerebral autoregulation is intricately balanced and influenced by many factors, some of which were mentioned as well in the discussion page 12 line 5 (euvolemia and normal cardiac function is presumed). However, in this particular population we assume intact, mature autoregulation as these were healthy, mature babies who were in majority not admitted to the NICU.

It is interesting that all ductal flow was left to right, despite presence of TR jets (indicating elevated PVR in some infants). The authors speculate that closure of PDA causes transient increase in PVR, leading to TR. Is it not also possible that the PDA had previously been serving as a “pop-off” for a stablely elevated PVR, and with closure this leads to greater back pressure on the AV valve?

Thank you for this point and we do appreciate the intimate knowledge of physiology that speaks to it.

Yes, we agree that likely the presence of the TR jet may have been sign of a subtle increase (or lack of decrease) in right-sided pulmonary vascular resistance which the PDA may have served as pop-off for before.

Therefore, we found it remarkable and commented on having these two slightly differing populations, TR early (Time Point 1) with four of the five having a bidirectional moderate to large PDA, and one a left to right PDA. This group might indicate early transitional stages with the RV having to still cope with slightly increased right-sided resistance and therefore showing TR. This seemed distinct from the second, different group of babies which all 3 have late (Time Point 3) ONLY TR with all of them having their PDA closed, 2 of them had a PDA before, one never had the PDA open documented. These are the ones where the explanation about the pop-off valve that closed is entirely possible and, in fact, probable, however, due to small numbers these thoughts remain speculative.

Delayed cord clamping should be mentioned much sooner than the discussion. Why was it not performed in half of the cohort?

We thank the reviewers and agree. We added the discussion of delayed cord clamping into the introduction. Page 5 lines 15-17 we added: “A range of physiologic and pathologic parameters may effect neonatal transition, such as timing of the cord clamping, placental, maternal and neonatal disturbances such as maternal pre-eclampsia, chorioamnionitis, and prematurity.”

In this hospital, delayed cord clamping is not mandatory for term babies and is up to the delivering physician and the family to discuss and decide. For premature infants without contraindications, delayed cord clamping is highly recommended. Therefore, half of our cohort received delayed cord clamping, which is defined as >60 seconds to clamp the cord. In many of the deemed “non-delayed cord clamping” group, the cord was clamped between 30 and 60 seconds.

Another important, unanswered question is how stable are these measures. The authors attribute all changes with the passage of time and progression of transition, but what about measurement reliability? If one measures the MCA flow at 8am, how close of a measurement will he or she obtain at 10am?

That is an interesting point that is difficult to answer. Subtle cardiovascular changes are certainly prone to be influenced by a variety of factors. A baby that is crying due to hunger will have very different cardiovascular assessment than one that has just finished eating due to sympathomimetic activation. The SMA flow might be different before and after a feed, the renal artery flow before and after a void. There is little known about the intricate hemodynamics during this crucial neonatal physiological adaptive period. We aim to start shedding a light into what exactly happens and are planning to conduct further studies into populations with certain difficulties or pathologies. Due to logistical reasons, it is never possible to control for all these behavioral states for the measurements. However, the general direction of our flow measurements over time for each baby were going up, within physiological variability. This gives us confidence to be likely measuring genuine hemodynamic changes over time.

We would love to see other groups confirm (or challenge) our findings.

Figures

Figure 1 should show results for each vascular bed on each day

We thank the reviewer for this interest in the details of our hemodynamic data. However, the point of Figure one is really to take a snapshot of a typical difference in PI between the organ systems. The other time points were either very similar to slightly less of a difference. Therefore, due to space constraints with not unlimited numbers of figures, we decided to use this most interesting, as earliest, one as pars pro toto.

Figure 2 is difficult to interpret if one is unfamiliar with Doppler envelope estimation.

Thank you for this comment. We tried to make these Doppler images more accessible in supplying a longer explanation at the figure itself. They represent Doppler-measured Flow velocity curves in m/s over time. The figure 2 represents the visual information PI and RI are derived from with distinctly different shapes for each organ. This is why Figure 2 really visualizes the important findings. We added: “Flow velocity in m/s over time. Note that diastolic flow is greatest in the brain, less in the SMA and almost absent in the kidneys.”

Reviewer: 2

Please expand all abbreviations used at tables 2, 3 and 4 in footnotes under the tables.

Thanks for pointing this out, a good suggestion to make the data more accessible. All abbreviations used in tables 2, 3, and 4 were put in footnotes under the tables, expanded and explained.

Reviewer: 3

1. Please make sure to refer to the correct tables in the text.

Thank you, we did make those corrections.

2. Blood pressure did not change significantly over time. Please adapt.

Thank you, we did change this sentence in results to: “… BP remained stable over time…”

3. The referral to figure 3 C at page 11 line 4 is unclear. Figure 3 C measures a relative conductance.

Thanks for pointing out. The sentence is modified as follows:

… we plotted relative conductance for the three organs (Figure 3, C) and …

Furthermore, we corrected page 10 lines 19-21: “The SMA showed the highest blood flow pattern and largest variation with mean 16.1 ml/kg/min (SD 9.7, Table 3), compared to brain with 5.8 ml/kg/min (SD 3.7) and kidney with 6.9 ml/kg/min (SD 9.4).”

In the same section, the message of line 7-8 is unclear to me.

Thank you. Figure 3 D-F is trying to explore the relationship between the vascular conductance measured by each organ system and derived as I/PI (PI as potential measure of resistance) and then plotted over the organ blood flow. If the PI had indeed measured the resistance, a change in PI should change the organ flow, which we did not show. Indeed, we showed a slope of zero and R2 ~ 0, which indicated no correlation between the two measures, hence the grounds we reject PI as measure of resistance on. We attempted to clarify this in adding the sentence: “The claimed measure of conductance with 1/PI did not correlate with the organ flow.”

Again in the same section, line 14-15, the authors state that the central outputs did not change significantly over time. However, in table 3, the p values comes back 0.01 for RVO (significant) and 0.05 for LVO (which makes it a trend). This also needs to be addressed in the discussion but will, to my opinion, not change the central message.

This slight difference of LVO and RVO being significant in their increase over time came to be because of slightly different statistical tests used after the first revision. We revised both the results and the discussion section to reflect these findings which are consistent with literature.

4. Page 13 line 8 and 13: Organ blood flow is mentioned in two different sentences with distinct values.

Thank you for pointing out to us that “organ blood flow” is confusing if not clear which organ we were referring to. In that segment, the first “organ flow” was referring to splanchnic organs, which was added page 13 line 7. The second “organ flow” referred to the kidney which also was clarified page 13 line 13. Hence the two different values, for two different organ systems.

Line 8: Is organ blood flow equal to SMA mesenteric artery blood flow in this sentence?

Thank you, as above, organ flow was clarified.

Line 13: do the authors refer to renal artery blood flow in this sentence (organ blood flow of 21 ml/kg/min).

Thank you, as above, organ flow was clarified.

5. Conductance is defined as 1/pulsatility index in table 1. To my opinion, this is exactly the hypothesis tested by the authors. In the conclusion is stated: as 1/PI was independent of conductance,….

It would be more clear if the authors refer to the definition used to calculate conductance in table 1: VTIxCSAxHR/mean BP

Thank you. That is exactly correct, we are testing the hypothesis whether PI as claimed measure of resistance can uphold as 1/PI measure of conductance. We changed the definition of conductance in Table 1 accordingly to the suggested VTI x CSA x HR /BW /100mmHg = ml/min/kg BW/100mmHg, the one used in Figure 3 to test our hypothesis.

6. Table 4/figure 2: renal PI/RI/Vmax/flow: please use consistent abbreviations throughout the text, figures and tables. In the text the authors refer to RA.

Thank you, we did change Table 4 and Figure 2 to consistently use “RA” for Renal Artery.

VERSION 3 – REVIEW

REVIEWER	Reviewer name: Zachary Vesoulis Institution and Country: Washington University School of Medicine Competing interests: none
REVIEW RETURNED	27-Nov-2018

GENERAL COMMENTS	In this revised manuscript, Stritzke present their investigation into normative patterns of blood flow in transitioning term infants. Although the authors have addressed many of the previously identified issues, there are still several concerns which have gone unaddressed. Of particular concern is the lack of explanation for not excluding several infants and cursory explanation of the impact of feeding on the measured values. Finally, the authors note that their velocity measures match those of previous investigators, but that the PI/RI measures (the novel paper of the paper) are unrelated to conductance or resistance.

	The authors do not speculate on what these measures may correspond to or what clinical utility they might have. A complete review by section follows below. General -Minor typographical errors and incomplete sentences remain in manuscript Abstract -No concerns Introduction -The authors statement that the PDA closes between 12 and 24 hours is not accurate, and is undercut by the author's own data later in the paper. -It is also not accurate to say that all organ systems have "essentially equal" arterial and venous pressures. While the arterial pressures may be equal at the takeoff from a great vessel, the intraorgan pressures vary substantially by organ, and even by physiologic state (during exercise, post-prandial, etc). Likewise, venous pressure may be equal at the central level, but again depends on other physiologic factors. For example, during sepsis the venous pressure from the extremities is quite low due to leaky capillary beds Methods -The exclusion criteria are quite vague. What is a "factor known to prolong neonatal transitioning?" The authors later include an infant with TTN and another with HIE, both of which would seem to "prolong transitioning." -What about birth weight? The authors note that IUGR infants have altered cerebral blood flow during in utero studies, are they excluded in this study? -As previously noted, the authors should at least provide information about the pre/postprandial state or state that it was not available up front (rather than a single sentence at the end of the manuscript). The authors repeatedly note a wide variance in splanchnic blood flow, but only minimally acknowledge the existence of the factor most likely to drive this variance. -The authors use an oscillometric blood pressure measurement for their calculations. This method of blood pressure measurement is widely variable by device type and patient state and is generally not considered reliable. Did the authors take multiple measurements and average them? A single measurement? Was the patient crying or agitated during measurement? I recognized that this is a non-invasive study in healthy infants and invasive measurement is not possible, but this limitation should be prominently noted. Results -As previously noted, I find significant fault with the inclusion of the infant with TTN and HIE. Both of these states are associated with hypoxia, which which drive up PVR and slow down the typical peripheral vasodilation. The authors should provide an adequate justification for their inclusion (and explain how they do not meet the previously defined exclusion criteria). - The authors also include one SGA infant, although it is not clear if this is the same or different from the TTN or HIE infant. Per the author's own description, this infant should not be included in the study.

	Discussion -The authors describe their renal findings as suggestive of "incomplete renal adaption." Given the nature of the paper (transitional hemodynamics) I would have expected the authors to discuss the known normal trajectory in renal hemodynamic transition in all neonates (24 hours of relative oliguria followed by polyuria, limited GFR increasing with time). Doesn't the data support this already known physiologic phenomena? -Much of this manuscript is a replication of previously conducted studies. The most interesting/novel aspects (PI/RI) are not associated with conductance or resistance. What is the utility of report this data? What do the authors speculate is the clinical value of these indices? Is it possible that the study failed to show a relationship because of a technical issue (especially the use of oscillometric BP measurement)? -If the authors intend for this to be a paper purely providing descriptive norms, it is **essential** that they exclude the SGA, TTN, and HIE infant and provide significant detail about the prandial status of the infants at the time of measurement. -Likewise, is 3 points of data is really sufficient to judge the trajectory of the values? How can the authors state with certainty that the values are not varying around a mean? Tables -Table 2 would be better presented with measures of variance rather than range. -Table 5 is redundant. Either the description in the text or the table should be removed. Figures -Figure 2 is of limited value. The authors should either remove it or overlay measures to assist the reader in understanding how Doppler measurements are calculated

REVIEWER	Reviewer name: liesbeth thewissen Institution and Country: University Hospitals Leuven, Belgium Competing interests: None
REVIEW RETURNED	06-Dec-2018

GENERAL COMMENTS	I agree with the modifications in the manuscript.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 1 (Zachary Vesoulis, Seattle, Washington)

In this revised manuscript, Stritzke present their investigation into normative patterns of blood flow in transitioning term infants. Although the authors have addressed many of the previously identified issues, there are still several concerns which have gone unaddressed. Of particular concern is the lack of explanation for not excluding several infants and cursory explanation of the impact of feeding on the measured values. Finally, the authors note that their velocity measures match those of previous investigators, but that the PI/RI measures (the novel paper of the paper) are unrelated to conductance or resistance. The authors do not speculate on what these measures may correspond to or what clinical utility they might have. A complete review by section follows below.

We thank all the reviewers for their thorough review and making the manuscript better. We have addressed all the reviewers' issues point by point diligently in the previous 3 reviews. We are pleased to hear that reviewers saw the novelty and potential utility of our reporting PI and RIs in our small pilot cohort during hemodynamic transition, and recommended publication. We stated at the end of discussion that these PI/RIs are meant to be pilot values in normal adaptive patterns which are completely novel and have not been reported before.

We do speculate –and speculate is all it is at this point- on page 12 lines 14-15 that PI/RI despite being not related to organ conductance –in itself a very important point- may confer additional rheological information which we tried to showcase in Figure 2. It does not seem prudent, based on the presented data, to speculate further what kind of rheological information this might be, or their utility. The continued use of Doppler indices in obstetrics and neonatology, and their published relation to clinically relevant outcomes (refs 5, 9-12), clearly speaks to their clinical utility.

It is hardly feasible to add another time point or add parameters such as feeding time to a study that is done.

We are sorry Dr. Vesoulis thinks we did not explain thoroughly why we did not exclude the infants in question, and that he thinks our explanation of the impact of feeding was only cursory. We politely wish to point out:

Regarding the non-exclusion of the patient with seizure at 38 hours and the one with mild TTNB:

This baby did not qualify for the diagnosis of HIE at the time of the study. HIE is diagnosed with a clinical and laboratory component (Gluckman et al, Lancet 2005: Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: Multicentre randomized trial). This patient did not require any respiratory support and came to the NICU for observation of respiratory status.

Response to Reviewers from Sept 7, 2018, page 2/3:

While we acknowledge the subtle physiological differences some clinical scenarios may come with, we had to draw a firm line for exclusion criteria such as antenatal Doppler changes or PPHN. The baby deemed "mild HIE" was admitted to the NICU after delivery for mild respiratory distress with a cord pH of 7.19, Base excess -10 and an Apgar of 7/8/9. He was not deemed as hypoxic ischemic encephalopathy (HIE) as his neurological exam remained completely normal, and therefore he was not treated with therapeutic hypothermia. After resolution of his respiratory distress for which he never required respiratory support, he presented at age 38 hours with a seizure, and cranial MRI showed changes consistent with mild hypoxia and micro-emboli, the latter of which likely caused the seizures. He was discharged without anti-ictal medication. Therefore, in review of the exclusion criteria, we decided to keep the baby in the study, as the initial hemodynamic evaluations were done on a baby with no indication that the baby had altered physiology.

Response to Reviewers from Aug 09, 2018, page 3:

One was admitted for Transient Tachypnea of the Newborn (TTNB) with CPAP support for <24hours and culture-negative rule out sepsis, NICU stay was 48 hours.

Regarding the non-exclusion of the patient with SGA:

Response to Reviewers from Sept 7, 2018, page 1:

The authors state that they evaluated growth using the Fenton charts, but IUGR status is not listed as an exclusion criteria. As the authors explain in the introduction, placental insufficiency alters Doppler flow patterns.

This is correct; antenatal Doppler changes would be able to alter postnatal Doppler adaptation. Presence of known antenatal Doppler changes was an exclusion criterion, and none of the included babies had them. The patient with SGA status was followed serially before birth, and did not have any of our exclusion criteria (maternal SSRI, maternal diabetes, known antenatal Doppler changes), he also was not exposed to maternal preeclampsia or hypertension. He did have a fetal unilateral dysplastic kidney which may explain his growth status, and measurements were done on the contralateral normal kidney.

We did not use SGA status as an exclusion criterion because we wanted an “all-comer” population of healthy, near-term neonates. We felt the criteria of known antenatal Doppler changes was more important as known to influence postnatal adaptation, than the growth status itself. Growth status and IUGR/SGA may be due to a variety of reasons not all of which could likely influence postnatal Doppler changes such as in this case. Therefore, we chose to include this baby.

Regarding the feeding issue:

Response to Reviewers from Sept 7, 2018, page 3:

We again acknowledge this important shortcoming, but would have to say it is nearly impossible to account for frequent, inconsistent feeding the amount of which is equally difficult to ascertain. Similarly, breastfeeding and formula feeding may have different implications in terms of volume and the effect on gut perfusion, we agree. However, apart from the logistical difficulties, we would like to point out that in the first 24 hours of life, neither breastmilk nor formula is usually taken in high quantities by the baby. Therefore, the effect of it, might, or might not, be that large. We do not have the feeding data available and did mention it in the limitations.

Response to Reviewers from July 23, 2018, page ¾:

We agree with the fact that feeding may elicit a splanchnic perfusion response, however, more so in an established rhythm of substantial meals which is hardly expected in the first 24 hours of life. Due to the nature of small, frequent, and really impossible to ascertain amount of feeding -as it is mainly breastfeeding- in the first 24 hours of life, this data was impossible to gather. May we also please remind the reviewer of the small size of the stomach of a newborn infant <24hours? These babies are with their mothers and within the first 24 hours we do not expect more than 1-2 wet diapers because of the initiation of feeding and transitioning. We did list this limitation under the limitations’ section. The other arteries’ perfusion is hardly dependent on feeding status.

General

-Minor typographical errors and incomplete sentences remain in manuscript

We are sorry not to be able to identify any additional typographical errors and incomplete sentences that remain after 3 revisions.

Abstract

-No concerns

Introduction

-The authors statement that the PDA closes between 12 and 24 hours is not accurate, and is undercut by the author’s own data later in the paper.

We politely disagree: Most papers, one of the most recent ones (ref 34) quote a 12-14 hour closure window in term healthy neonates. McNamara et al (ref 34) quote in their cohort of 15 neonates with mean gestation 40 +/- 0.8 weeks “most (ductal flows) closing between 7 and 24 hours of age.”

Walther et al (ref 1) quotes: “by 24 hours all infants had a closed ductus arteriosus or nearly continuous left-to-right shunting.”

In our cohort we report 7/19 babies with small residual PDA shunt at 24 hours. These shunts are small, residual, and we assume in most published literature (as in ref 1) these are deemed “closed” as well, as they are not clinically nor hemodynamically relevant. Therefore we do not see a discrepancy between published literature and our data.

-It is also not accurate to say that all organ systems have “essentially equal” arterial and venous pressures. While the arterial pressures may be equal at the takeoff from a great vessel, the intraorgan pressures vary substantially by organ, and even by physiologic state (during exercise, post-prandial, etc). Likewise, venous pressure may be equal at the central level, but again depends on other physiologic factors. For example, during sepsis the venous pressure from the extremities is quite low due to leaky capillary beds

Thank you, this is correct. We stated that all the included babies were healthy, non-septic babies in a calm state. We agree insofar as that the actual point of our manuscript is to show the differential intra-organ pressures we believe is due to vascular autoregulation.

Methods

-The exclusion criteria are quite vague. What is a “factor known to prolong neonatal transitioning?” The authors later include an infant with TTN and another with HIE, both of which would seem to “prolong transitioning.”

We added reference 21 to back up our point that factors known to prolong neonatal transitioning were indeed SSRI treatment in the mother, maternal diabetes which is often associated with RDS, and antenatal Doppler changes, or clinical suspicion of PPHN. The list of predisposing factors in ref 21 for PPHN/delayed transitioning does indeed not include TTN or HIE.

-What about birth weight? The authors note that IUGR infants have altered cerebral blood flow during in utero studies, are they excluded in this study?

We excluded babies whose mothers had known uterine artery adverse Doppler changes.

-As previously noted, the authors should at least provide information about the pre/postprandial state or state that it was not available up front (rather than a single sentence at the end of the manuscript). The authors repeatedly note a wide variance in splanchnic blood flow, but only minimally acknowledge the existence of the factor most likely to drive this variance.

The wide variance in splanchnic blood flow was also seen in ref 3, a cohort in which measurements were controlled for feeding status, and aligns with NIRS findings (ref 29). We would like to point out that rather than “just than a single sentence at the end of the manuscript”, we mention on page 13: “Feeding is known to increase splanchnic flow in response in term babies.(3) The variability of feeding timing and amount may also explain some of the variation.”

We highlighted this limitation furthermore in the methods page 8 line 13. Furthermore, we added page 13 line 8 “in the first 24 hours of life” to highlight the fact that feeding in that time frame is indeed variable.

-The authors use an oscillometric blood pressure measurement for their calculations. This method of blood pressure measurement is widely variable by device type and patient state and is generally not considered reliable. Did the authors take multiple measurements and average them? A single measurement? Was the patient crying or agitated during measurement?

I recognized that this is a non-invasive study in healthy infants and invasive measurement is not possible, but this limitation should be prominently noted.

As in most non-invasive studies, the oscillometric method was the only feasible route to obtain blood pressure measurements. Methods, page 8: “via an oscillometric device with appropriately sized cuff (size 3 or 4) on the right arm, in a quiet physiological state immediately after the echocardiographic assessment, on an intermittent basis.”

Results

-As previously noted, I find significant fault with the inclusion of the infant with TTN and HIE. Both of these states are associated with hypoxia, which drive up PVR and slow down the typical peripheral vasodilation. The authors should provide an adequate justification for their inclusion (and explain how they do not meet the previously defined exclusion criteria).

- The authors also include one SGA infant, although it is not clear if this is the same or different from the TTN or HIE infant. Per the author’s own description, this infant should not be included in the study.

See above

Discussion

-The authors describe their renal findings as suggestive of “incomplete renal adaption.” Given the nature of the paper (transitional hemodynamics) I would have expected the authors to discuss the known normal trajectory in renal hemodynamic transition in all neonates (24 hours of relative oliguria followed by polyuria, limited GFR increasing with time). Doesn’t the data support this already known physiologic phenomena?

Response to Reviewers July 23, 2018, page 5

Furthermore, as per Al-Dahhan J, Haycock GB, Chantler C, Stimmler L (1983) Sodium

homeostasis in term and preterm neonates. I. Renal aspects. Arch Dis Child

58:335–342 adaptation occurs with natriuresis in a characteristic manner after delivery. Therefore, we are assuming in this cohort of healthy babies, that the renal adaptation is somewhat similar for each of them. Nevertheless, we did mention the fact that we did not correlate the flows with the urinary output under limitations.

-Much of this manuscript is a replication of previously conducted studies. The most interesting/novel aspects (PI/RI) are not associated with conductance or resistance. What is the utility of report this data? What do the authors speculate is the clinical value of these indices? Is it possible that the study failed to show a relationship because of a technical issue (especially the use of oscillometric BP measurement)?

See above. It is novel and “may be compared to pathologic adaptation in altered or immature physiology.” Page 15

-If the authors intend for this to be a paper purely providing descriptive norms, it is **essential** that they exclude the SGA, TTN, and HIE infant and provide significant detail about the prandial status of the infants at the time of measurement.

-Likewise, is 3 points of data is really sufficient to judge the trajectory of the values? How can the authors state with certainty that the values are not varying around a mean?

See above

Tables

-Table 2 would be better presented with measures of variance rather than range.

We appropriately showed the median for continuous variables and the frequency for categorical variables.

-Table 5 is redundant. Either the description in the text or the table should be removed.

We disagree. In the discussion page 14 we talk about the important features of the different closing patterns of PDA, namely that 4/21 babies had sustained bidirectional flow at Time 2. Table 5 in contrast gives detailed information about PDA flow patterns over time.

Figures

-Figure 2 is of limited value. The authors should either remove it or overlay measures to assist the reader in understanding how Doppler measurements are calculated

Figure 2 is one of the most important findings in our study, as it is complementary to the PI/RI indices which failed to show a relationship to conductance. PI/RI confer the numerical information of maximum/minimum flow velocity in relation to mean velocity. Visual representation of distinct organ-specific Doppler flow patterns is really portraying the important rheological information Dopplers may hold. We explained Figure 2 as flow velocity in meter per second over time. It is novel, it has not been reported before, and is indispensable.

Reviewer: 2

I agree with the modifications in the manuscript.

Ultimately, we respectfully request the editorial office to decide whether or not our explanation is sufficient to publish this dataset in its integrity.

We do ask politely for a timely decision to be able to submit this manuscript elsewhere should the decision be negative.