Response to Editorial Office:

1. Test your figures.
We tested all figures.

2. Copyright forms: No scanned copyright forms are acceptable. Each author must complete and digitally sign a copyright form and then the corresponding author should upload when the revised version will be uploaded.
All authors digitally signed the copyright forms.

Response to Reviewer Comments:

Reviewer #1:
This is a most interesting article concerning the impact of a non-specific beta-blocker on the hemodynamics of burned children. I have a few questions.
The authors thank Reviewer #1 for the feedback and important comments.

1. The regression plots have no data points on them. It would be desirable to overlay the data points on the plots so the reader may judge any bias involved in the plots. The authors state that each subject had at least one pH measurement performed over a 28 day period. This could reflect one point measured on day one followed by 20 points measured on day 28.
This is an interesting question. We included 862 daily median values of pH from the Propranolol group and 699 daily median values of pH from the control group in our linear regression analysis. Initially, we had a look at the graphs which showed all the raw data (each daily median) in the background. However, the graphs looked too busy especially when we added, for example as in this case, 1,561 data points onto the graph. It is also important to consider, as we stated in our methods, that we used daily median values for all of our study endpoints. Also our mixed multiple model was adjusted for potentially prognostic covariates such as age at burn, burn size, and days post burn, while blocking on subject. Further details of the statistical methods used were stated as follows in the Data analysis paragraph of the Methods section: *"For each outcome (CI, CW, EVLWI, MAP, arterial pH, PaO2, PvO2, RPP, SVRI, %HR, %SV, lactate levels, and Denver 2 score, as daily median values), mixed multiple linear regression was used to model the relation between the outcome and treatment group (control versus propranolol) while adjusting for effects due to potentially prognostic covariates such as age at burn, burn size, and days post burn, while blocking on subject. We log-transformed EVLWI, CW, PaO2, and PvO2 to improve approximations of normality prior to analysis, with results inverted for presentation. These models were also compared by likelihood-ratio tests with models that included an interaction between treatment and time; however, inclusion of the interaction term failed to improve the models. The analysis focused on the 28 days following burn injury, and all figures show the regression model estimate over time post burn with 95% shaded confidence intervals. Regression estimates are based upon holding constant the control treatment group and median values of other covariates. A 95% level of confidence was assumed."*

2. Are the authors convinced that beta blockade is indeed of value in this population? The reduction in cardiac output and heart rate may make the clinician more comfortable but is it really of value in the pediatric population. The mortality rates in both control and beta blockade groups were identical, the systemic pH values were not statistically different but consistently lower in the beta blockade group and the Denver 2 scores were

statistically the same but consistently higher in the blockade group. This is of some concern with this therapeutic approach. Do the authors have any comment on this? Also, what did the deaths result from? If a patient develops severe systemic sepsis and an increased metabolic demand is placed upon individual organ systems such as increased lactate production requiring increased lactate clearance, do the authors advocate continued beta-blockade use?
We agree, a reduction of heart rate and MAP makes us as clinicians more comfortable especially when children present with age predicted heart rate values of 160% or more. Our study showed that propranolol significantly reduced cardiac work as well as cardiac oxygen consumption; it further reduces cardiac index by not affecting peripheral perfusion. However, these observations using the PiCCO system are limited to the first 4 weeks post-burn, because our pediatric patients usually do not have arterial lines beyond that time-point. A recent review performed by Flores et al. showed that long-term benefits of propranolol administration in severely burned are a decreasing resting energy expenditure and decreased trunk fat, an increase in lean body mass and a decrease in insulin resistance (Flores O, Stockton K, Roberts JA, Muller MJ, Paratz JD. The efficacy and safety of adrenergic blockade after burn injury: A systematic review and meta-analysis. J Trauma Acute Care Surg. 2016 Jan;80(1):146-55.)

Our results showed that there was no significant difference regarding Denver 2 score (p=0.52) or pH (p=0.10) between both groups. The graphs might suggest that the propranolol group has had both, a higher Denver 2 score (Figure 3c) as well as a higher pH (Figure 3d), however, in both graphs, the confidence intervals are overlapping and there is thus no evidence of a statistical difference.

Five deaths were caused by sepsis, two from ARDS, one from intestinal perforation and one from brain death (herniation). As stated in the discussion, children in our studied cohort suffered from massive burns, with a burn size of an average 60% of the total body surface area. In pediatric burns, a 60% total body surface area burn is still a predictor for mortality (Taylor SL, Lawless M, Curri T, Sen S, Greenhalgh DG, Palmieri TL. Predicting Mortality from Burn Injuries: The need for age-group specific models. *Burns*. 40(6):1106–15, 2014.)
In case of sepsis, we did not stop the study medication and we were able to observe that there was no significant difference regarding mortality between both groups (p=1.00). However, to address if propranolol administration does have an impact on mortality, a larger group of pediatric burn patients is needed. In the current study, we only focused on patients who underwent hemodynamic monitoring with the PiCCO system (Figure 1).

Reviewer #2:
Well written manuscript by a group with expertise in propranolol in burned children.
We thank Reviewer #2 for the provided comments and for the positive feedback.

1. May be helpful to the readers not as familiar with hemodynamic monitoring if additional detail were added as to how EVLWI is calculated using PiCCO and how propranolol may effect EVLWI
The EVLWI was automatically derived from the PiCCO system during the transpulmonary thermodilution process. An education slideshow, provided by the manufacturer, shows that EVLW is calculated as ITTC (intrathoracic thermal volume) minus ITBV (intrathoracic blood volume).**(http://www.pulsion.com/fileadmin/pulsion_share/Education/ISICEM2006_EVLW_Belda_220306.pdf)** And EVLWI is the indexed (per body surface are in m2) EVLW.
As of today, there is no evidence that propranolol administration might have an impact on EVLWI. However, older case reports described that propranolol might have unopposed α effects

and thus may cause an elevation of afterload, leading to pulmonary edema. We added that information as follows to the Discussion:
"Case reports from the 1990s described that propranolol caused pulmonary edema by having unopposed effects on the α adrenergic receptor which caused an elevation of afterload (23,24). In our current study, there was no significant effect of propranolol on EVLWI, which can be used as a marker for pulmonary edema."

23. Pogson GW, Sharma J, Crouch TT. Pulmonary edema with low-dose propranolol in pheochromocytoma. South Med J. Jun;73(6):795–6, 1980.

24. Sloand EM, Thompson BT. Propranolol-induced pulmonary edema and shock in a patient with pheochromocytoma. Arch Intern Med. Jan;144(1):173–4, 1984.

2. With the advent of more sophisticated cardiac ultrasound in the ICU and less reliance on invasive and even noninvasive hemodynamic monitoring, can the authors comment on how the reported measurements are used to guide resuscitation and how this may compare to volume assessment using ultrasound. Also, why a PiCCO?
At our pediatric burn center, we use transthoracic echocardiography, transesophageal echocardiography, but the PiCCO system only in a certain patient population (Figure 1). To perform PiCCO measurements, patients must have an arterial line, and not all of our patients do receive one. We use the PiCCO since 2005 at our institution; the biggest benefit of the system is that it allows us to measure continuously hemodynamic parameters which can be of use in the critically ill.

Regarding the resuscitation; fluid requirements are estimated according to a formula based on total body surface area (BSA) and body surface area burned in square meters. Total fluid requirements for the first day are estimated as follows: 5000 mL/m2 BSA burned per 24 h + 2000 mL/m^2 BSA per 24 h. Urinary output is monitored hourly with target rates between 1.0 and 2.0 mL/kg/h. In addition, close hemodynamic observation is performed by using the PiCCO or other methods, but the primary endpoint is urinary output.
Each technique has its pros and cons; the aim of this study was to show if propranolol has an impact on hemodynamic measurements which have been derived by using the PiCCO system.

3. The reported results are over a decade of time and it is unclear as to which patients received a PiCCO and which did not. How was it decided to place a PICCO? What was the primary endpoint of the study? Power analysis? How did resuscitation guidelines differ over the decade of the study?
These are important questions. We added the following section to the manuscript to clarify which patient received a PiCCO and how these decisions were made:
"At admission, central venous lines (inferior or superior vena cava) was placed in all patients. In addition, if indicated by the attending physician based on the severity of the burn, patients received an arterial line (femoral artery) for continuous arterial blood pressure monitoring. Beyond that, when indicated by the attending physician, a Pulsiocath 3- or 4-French thermistor-tipped catheter (Pulsion Medical Systems, Munich, Germany) was used to collect hemodynamic PiCCO measurements. The PiCCO catheter placement was in some cases limited due to its size, thus only patients aging 2 years and up underwent hemodynamic monitoring with the PiCCO system."

Regarding the study endpoint and power analysis, we would like to refer to following paragraph of the Methods section:

"We analyzed transpulmonary thermodilution measurements obtained using the PiCCO system (Pulse Index Continuous Cardiac Output, Pulsion Medical Systems, Munich, Germany) from severely burned children who were admitted to our institution between 2005 and 2015. This study has been approved by the Institutional Review Board at the University of Texas Medical Branch (Galveston, TX; protocol #15-0074). Inclusion criteria were defined as follows: age between 6 months and 18 years, thermal burns, received PiCCO measurements during the acute hospitalization, and received either propranolol (4 mg/kg/day), administered as part of a separately conducted prospective randomized controlled trial (protocol #04-157) or no acute study drug."

We have been using the PiCCO since 2005 at our institution and we did not perform a power analysis for the presented study because we studied a certain patient population (Figure 1, patients who received PiCCO catheters).
The primary endpoint of resuscitation of severely burned children is urinary output and this endpoint has not changed over the past 10 years. Both groups had patients over the same time-period and there is thus no expected bias regarding changes in any care protocol.

4. It would seem unlikely that propranolol could ever decrease perfusion to the extent that it would affect organ injury (Denver Score) in children. Is there any data in adults that such a relationship exists?
To the best of our knowledge, there is no data that shows that propranolol reduces cardiac index or peripheral perfusion in adult burn patients. We have to say that we give a high dose of propranolol (4mg/kg/day) to our pediatric burn patients to reduce heartrate and further decrease cardiogenic stress. Propranolol also improves long-term outcomes by increasing lean body mass, decreasing resting energy expenditure and reducing insulin resistance, as a recent review by Flore et al. described (Flores O, Stockton K, Roberts JA, Muller MJ, Paratz JD. The efficacy and safety of adrenergic blockade after burn injury: A systematic review and meta-analysis. J Trauma Acute Care Surg. 2016 Jan;80(1):146-55.)

Minor: under the competing interest section, add the year that the data was presented.
The research was presented in 2016; we added the year in the manuscript.

Reviewer #3:
The authors have a long track record studying severe burn injury in children. The current manuscript continues their work in this area. In particular, it provides additional data from a randomized prospective trial using propranolol versus control after severe burn injury. The authors measured hemodynamic variables using the PICCO system. They conclude that propranolol reduces cardiogenic stress without adversely affecting peripheral perfusion.
The authors thank Reviewer #3 for recognizing the volume of work to get to this point and for raising these important questions.

It is not clear to me exactly how the authors accumulated their data. They report data at 24 hours post admission and then up to 28 days. Their monitoring system captures data nearly continuously. Are the values expressed mean values for each day? Was there any degree of variability over each 24 hour period? The authors need to clarify this.
Thank for your comments and important concerns. We analyzed hemodynamic values from admission up to 28 days post-burn. The 24h post admission time-point was chosen because after that, patients were fully resuscitated but the study drug (propranolol) was given after this time-point. We created Table 2 to show that both groups started similar. To account for the

variability over each 24h period we used the daily median value as described in the methods. Furthermore, our mixed linear regression analysis was adjusted for age, burn size and days post burn. For further details we would like to refer to the Data analysis paragraph of the Methods section of the manuscript:
"For each outcome (CI, CW, EVLWI, MAP, arterial pH, PaO2, PvO2, RPP, SVRI, %HR, %SV, lactate levels, and Denver 2 score, as daily median values), mixed multiple linear regression was used to model the relation between the outcome and treatment group (control versus propranolol) while adjusting for effects due to potentially prognostic covariates such as age at burn, burn size, and days post burn, while blocking on subject. We log-transformed EVLWI, CW, PaO2, and PvO2 to improve approximations of normality prior to analysis, with results inverted for presentation. These models were also compared by likelihood-ratio tests with models that included an interaction between treatment and time; however, inclusion of the interaction term failed to improve the models. The analysis focused on the 28 days following burn injury, and all figures show the regression model estimate over time post burn with 95% shaded confidence intervals. Regression estimates are based upon holding constant the control treatment group and median values of other covariates. A 95% level of confidence was assumed."

It appears that the propranolol did its job. CI was reduced as was heart rate. Measures of peripheral perfusion however were the same. Peripheral oxygen consumption was not different. There was no difference in organ failure scores. Finally, there was no difference in degree of acidosis or serum lactate levels between the two groups. EVLWI was not changed by using propranolol. One could theorize that extra water in the lung would provide stress to the right side of the heart. Do the authors have any information on pulmonary vascular resistance?
This is well thought. Unfortunately, we did not have a look at the pulmonary vascular resistance index in our studied population because such a value cannot be measured by the PiCCO system. We agree that pulmonary edema, in this case an increase in EVLWI, might be reflected as an increased stress to the right heart. As mentioned in the manuscript, the EVLWI derived from the PiCCO can be very unspecific and variable in children, due to that we will validate the EWLI in our pediatric burn population as a future study of our group. The aim will be to compare chest x-rays to the actual EVLWI as an intrapatient comparison. Furthermore, we will also have a look at pulmonary edema, EVLWI and pulmonary vascular resistance, which we will be assessed using echocardiography.

Cardiovascular performance post injury or burn is designed to meet increases in oxygen demand produced by the insult. It would be helpful if the authors could provide some context for the reader. One might reasonably assume that a decrease in cardiac stress is advantageous, though I am not sure that is true in children. The end organ performance was not helped by using propranolol.
We agree that our studied showed that propranolol significantly reduced cardiac work as well as oxygen consumption; it further reduces cardiac index by not affecting peripheral perfusion. Indeed, end organ performance, as measured by the stroke volume, was not improved. However, these observations using the PiCCO system are limited to the first 4 weeks post-burn, because our pediatric patients usually do not have arterial lines beyond that time-point. A recent review performed by Flores et al. showed that long-term benefits of propranolol administration in severely burned are a decreasing resting energy expenditure and trunk fat, an increase in lean body mass and a decrease in insulin resistance (Flores O, Stockton K, Roberts JA, Muller MJ,

Paratz JD. The efficacy and safety of adrenergic blockade after burn injury: A systematic review and meta-analysis. J Trauma Acute Care Surg. 2016 Jan;80(1):146-55.)
Our current study aimed to evaluate the impact of propranolol on hemodynamic values up to 28 days post-burn. We showed that it had no adverse effect on peripheral perfusion, however, it reduced cardiogenic stress. The long-term benefits of propranolol in burns have herein been discussed and as mentioned by Flores et al. there is a need for further investigations regarding the impact of propranolol on outcomes following severe burns.

The authors' results are intriguing. It would be helpful if they could put them into some clinical recommendation for care of burned children. It would further help if they could theorize about potential applicability in other patient groups.
Thank you for this positive comment. We included your suggestions in the Discussion as follows:
"Cardiogenic stress, which could lead to cardiac failure during the acute stay, in adult burn patients is a common cause for death amongst adult burn patients. The long-lasting impact on the cardiovascular system in adults due to the with burns associated hyperdynamic response and increased length of stay have been recently described by Duke et al.(28). Propranolol could play an important role in the reduction of long-term cardiovascular morbidities following severe burns in adults. Prospective randomized controlled trials are currently ongoing to evaluate safety and efficacy of propranolol in adults with burns."

28. Duke JM, Randall SM, Fear MW, Boyd JH, Rea S, Wood FM. Understanding the long-term impacts of burn on the cardiovascular system. Burns J Int Soc Burn Inj. 2016 Mar;42(2):366–74.