Author’s response to reviews

Title: Sunflower oil and Mustard oil: Skin barrier function in newborns via a community-based, cluster randomized trial

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Author’s response to reviews:

Reviewer reports:

Raegan D. Hunt, M.D., Ph.D. (Reviewer 1): - This is a large randomized community based study comparing skin outcomes in both premature and term neonates undergoing traditional infant skin massage with two different plant-based oils: mustard oil and sunflower oil. The randomized groups seem comparable statistically. It was not possible to blind the subjects or the researchers as these oils are distinguishable. This limitation is well addressed. The increase in TEWL in both groups as infants aged over time seems surprising, but the manuscript correctly points out that the high relative humidity in the homes may have impacted this measurement. More standardized lower humidity settings are typically recommended for measurement of TEWL.

- The manuscript mentions that the infants were monitored for infection. In the Sunflower oil group, the pH lowered faster, suggesting more rapid formation of acid mantle. I did not see a specific comparison of skin infections observed between the two groups in the manuscript. Please consider adding clarification.

Response: Thank you for your suggestion. There was no difference in skin infections between oil groups, however this was not a main outcome of this substudy. The reason they were monitored
for skin infections is because they were part of the larger cohort included in the main study whose outcomes were morbidity and mortality. In line 209 we have added a clarifying sentence “There were no differences in skin infections by oil group.”

Orsolya Genzel (Reviewer 2): interesting study. it should be published, even if there was no difference between the oils. is mustard oil cheaper in Nepal than sunflower oil? this Information could be added if the authors wish to do so

Response: The cost of massage was about $0.10 per massage at the time of the study regardless of the type of oil.

Suzanne Pasmans (Reviewer 3): The authors aimed to quantify differences in skin barrier integrity and maturation between newborns randomly assigned to routine massage with mustard oil or sunflower seed oil over the first postnatal month, using skin condition (erythema, rash, dryness), skin surface pH, stratum corneum cohesion/protein concentration, and transepidermal water loss parameters.

We think this paper is novel and relevant for low resource settings. Furthermore, it is important to get insight in low-cost preventive strategies to improve morbidity and mortality due to skin infections. The impact of these data for Western countries could be improved by addition of a control group without oil exposure or a group with emollient therapy.

Methods section

- Please consider to delete information about the skin condition (erythema, rash, dryness) as this raises questions about the infection rate/mortality due to skin infections in both intervention groups. Otherwise, provide information about the infection rate in this manuscript.

Response: Thank you for your comment. Given the objectives of this study, we do not think it would be appropriate to delete the measures of erythema, rash and dryness. The reviewer rightly points out that these three features may be related to infection; however here we are focused on these features as possibly arising through irritation, inflammation and hyperproliferation as the skin barrier responds to insults. There was no difference in skin infections between oil groups, however this was not a main outcome of this substudy; we have now included a comment to this effect in the paper. The reason they were monitored for skin infections is because they were part of a larger cohort included in the main study whose outcomes were morbidity and mortality.

- Page 6, line 18-22: Please provide the name of the validated scoring scales for erythema, rash and dryness or add the scale to the supporting information. Could you explain the differences between scoring of rash and erythema?

Response: In response to the differences between erythema and rash, rash refers to discrete papules and/or pustules whereas erythema is increased visual redness, i.e., higher than a non-involved site on the body. There is no specific name for the scale but the following publications
listed below provide the basis and validation, etc. 1-4 The scale that was used is shown in the table below and included as Supplemental Table 1.

<table>
<thead>
<tr>
<th>Severity</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema Scale</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Faint or definite pink</td>
</tr>
<tr>
<td>2</td>
<td>Definite red</td>
</tr>
<tr>
<td>3</td>
<td>Very intense redness</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
</tr>
</tbody>
</table>

| Rash Scale | | |
| 0 | None | None |
| 1 | Papules | One |
| 2 | Pustules | 2-5 |
| 3 | Papules and pustules | &lt;10% |
| 4 | Clear fluid-filled vesicles | 10-50% |
| 5 | NA | &gt;50% |
| 6 | NA | Numerous and continuous/joining |

| Dryness Scale | | |
| 0 | None | None |
| 1 | Slight powderiness | &lt;10% |
| 2 | Early cracking | 10-50% |
| 3 | Moderate cracking & scales | &gt;50% |
| 4 | High cracking & lifting scales | NA |
| 5 | Bleeding cracks | NA |

- Page 6, statistical analysis: Did you perform a sample size calculation, if so please elaborate on this.

Response: As this study used a subset of infants enrolled in a larger study comparing morbidity and mortality, the sample size took many factors into account, including cost and logistics. By enrolling 500 infants in each group, we had the power to observe small differences in the means of the skin integrity measures between the two oils, with power dependent on the standard deviation.

Power to Observe a Difference in Means of Skin Integrity Measures (500 infants in each oil group)
Standard Deviation

Difference in means at each visit of infants massaged with sunflower oil versus mustard seed oil

<table>
<thead>
<tr>
<th></th>
<th>0.25</th>
<th>0.5</th>
<th>0.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>88</td>
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<td>100</td>
</tr>
<tr>
<td>1.5</td>
<td>56</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td>2.0</td>
<td>35</td>
<td>88</td>
<td>100</td>
</tr>
<tr>
<td>2.5</td>
<td>24</td>
<td>71</td>
<td>97</td>
</tr>
</tbody>
</table>

- Page 7, ethical approval: Please explain why no permission was needed from father and why only verbal (not written) informed consent was provided.

Response: Given the context of this setting, including low literacy, and reservation of signatures for signing of important documents (i.e. land title, etc) in these communities, and the fact that the underlying intervention was a modification of an existing cultural practice (i.e. massage of baby with natural vegetable oils), both Nepal and US Institutional Review Boards waived documentation of consent for this study. For this reason, the informed consent process did not include a signature.

Results section

- Page 8, line 38-39: The proportions of preterm infants in the MO (45.4%) and SSO (39.9%) groups do not correspond with the percentages of preterm (<37 weeks) patients in Table 1; 11.2+29.7=40.9% in the MO group and 11.4+28.3=39.7% in the SSO group. Please explain the differences.

Response: Thank you for your observation. The 45.4% and 39.9% were typos. This has been corrected on lines 203-204 to 40.9% and 39.7%

- Page 8, line 43-44: Please explain compliance with oil application and massage. Based on which data did you calculate the 100% compliance?

Response: At each visit we asked the mother/caregiver several questions about massage practices including whether the baby had been massaged with oil, how many times in the last 24 hours had the baby been massaged with oil, and what type of oil was used. If the baby was massaged with oil and they had used the correct oil for the group to which they were assigned, this was considered compliance.

Response: The sentence “Compliance was measured if the infant had been massaged with the correct oil for the intervention group they were assigned” in lines 205-207.

What were the massage frequencies after the first week, were they similar between the intervention groups (in the manuscript you only address the frequencies during the first week)?
Response: During the first week of life we had field staff visiting the households every day to monitor the massage practices (if they were massaging the infant, how many times per day). These massage monitoring visits only occurred during the first week of life and were not continued for the entire month. After the first week, we asked at all measurement visits (days 1, 3, 7, 14, and 28) whether the baby was massaged or not and with what type of oil, but we did not ask how many times they had massaged their infants since the last visit as we thought that, due to recall bias, the answer would not be reliable. Therefore we do not have frequency of massage after the first week.

Discussion

- What was the rationale behind the 28 days-intervention period in relation to skin barrier integrity and maturation? Do you expect changes between the groups after 1 month application of oil?

Response: Adaptive changes over the first month have been demonstrated in previous studies. Therefore, this time frame was selected to encompass the time of expected changes. After the first month of life, the skin barrier has adapted and matured and we would not expect further changes between the two groups.

- Page 11, line 48-51, "Regardless of... in severity": Is it possible that the worsening of skin condition is due to the oil and/or massage independent of the type of oil? You did not include a control group, so you cannot exclude that the worsening is due to the oil exposure.

Response: Thank you for your comment. On lines 288-291 we have included the sentence “In addition, as the worsening of skin condition during the first two weeks occurred in both oil groups and as we were unable to include a control group in this study for comparison, it is possible this could have been due to the use of oil or massage itself.”

- Page 13, line 16-17: Please consider to delete the sentence "Such information... is universal". In our opinion this is not a practical study (like the parent study) as you only quantify skin barrier integrity and maturation using skin surface pH, SC cohesion/protein concentration, and TEWL in this nested study. Including a control group without oil application would be of interest as this gives insight in the mode of action of the oils.

Response: Thank you for your comment. We agree that including a control group would be helpful to describe the mechanism for the oils. However due to the cultural practices in this area of Nepal of universal massage with vegetable (mustard seed) oil, the overall parent trial was focused on the question of whether or not substituting sunflower seed oil in place of the traditionally used mustard oil could lead to protective health benefits, without introducing substantive behavior change. We’ve made a change to this effect (see lines 316-319 of the manuscript)
Tables/figures
- Table 1: Please consider to delete the characteristic "has electricity" or otherwise explain the relevance.

Response: The variable “has electricity” has been removed.

- Table 2: Could you merge the scores per skin location into a general score of erythema, rash and dryness? Scores per skin location could be added to the supporting information.

Response: Thank you for your suggestion, however that is not something we would be able to do. The goal of these measurements and analyses was to determine what aspect of the skin barrier is being impacted. Erythema, rash and dryness indicate different types of skin barrier damage. Summing the scores would cause the reader to not know the type of damage, therefore score per skin location should stay disaggregated as is currently in the table.

- Figure 1: Do the "clusters" in figure 1 correspond to the "sectors" at page 5, line 14? Please use the same terms

The words sectors and clusters do correspond to the same thing. The word sectors was changed to clusters in rows 128-130 in the manuscript.

- Figure 1: The use of "n=" for both cluster numbers and patient numbers is confusing. Please adjust for this and change "eligible for mechanisms sub study" into "clusters eligible for..." and "mustard oil"/"sunflower oil" into "clusters assigned to mustard oil/sunflower oil".

Response: this has been corrected in Figure 1

- Figure 1: In the "selected" box "n=" is missing in both intervention groups.

Response: this has been corrected in Figure 1

- Figure 2: Please match the y-axis scales of mustard oil and sunflower oil (0 is not on the same height).

Response: this has been corrected in Figure 2

General

- Please use line numbers in following submission.

Response: Line numbers have been added.
References


