Response to Reviewer 2 Comments

Dear Reviewer :

Thank you very much for your comments and suggestions on our manuscript. We have revised our manuscript accordingly. The changes are incorporated into the manuscript with red color. The point-wise responses to the comments is detailed below in red color.

Best wishes!

1. Line 33-41: The authors should provide a clear definition of pathologic jaundice. I think that a truly condition of pathologic jaundice is not discussed in this article.

Response: Thank you for your advice. Earlier, our definition of pathologic jaundice was indeed a bit too one-sided. We have reviewed the literature again and refined its definition. As follows:

Line 39-42: However, when jaundice appears too early, increases too rapidly or too high (blood bilirubin >256.5 μmol/L) or lasts for an abnormally long time, or also progressively worsens or is combined with pathologic factors, it is referred to as "Pathological Jaundice"[8-11].

2. I suggest that the term "suboptimal intake-related hyperbilirubinemia" is to substitute "breastfeeding jaundice" in Figure 1, and in the main text as well.

Response: Thank you for your comment. It was a labeling error due to carelessness in checking the images. We have corrected it. As follows:

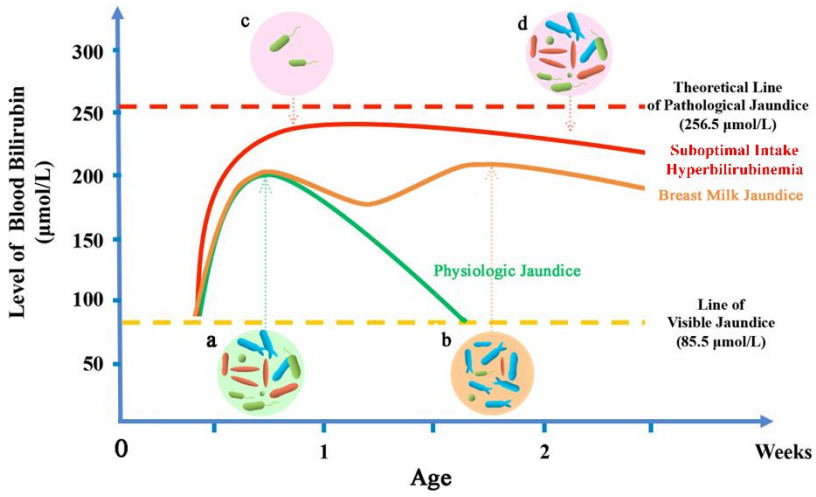


Figure 1. Blood bilirubin pattern of common physiological jaundice (green line), breast milk jaundice (orange line), and suboptimal intake hyperbilirubinemia (red line).

3. As shown in Figure 1, the bilirubin levels of the three kinds of jaundice all are lower than the theoretical line of pathological jaundice. Clinically, it is believed that physiologic jaundice and breast milk jaundice may not harm the baby. Therefore, the authors have to address the influence of neonatal jaundice on the neonatal health in the introduction.

Response: Thank you for your comment, it is indeed necessary to mention this key element. We have provided the following narrative:

Line 43-48: Pathologic jaundice, if not treated promptly and effectively, can progress further to acute bilirubin encephalopathy (acute phase manifestation of bilirubin toxicity occurring within 1 week of birth) or kernel jaundice (chronic, permanent clinical sequelae due to bilirubin toxicity). If the early treatment stage is missed and the condition further deteriorates, irreversible neurological damage will occur, seriously jeopardizing the health of the newborn [12].

4. Line 61-70: The authors have better provide appropriate references in the text.

Response: Thank you for your advice. Adding the appropriate references does improve the rigor of the article, and we have added them in the corresponding places. As follows:

Line 68-77: The relationship between the gut microbiota of neonates and jaundice has been proposed since the 1960s [23], but the previous view was incomplete and contradictory with many phenomena due to the limited knowledge of neonatal gut microbiota. Traditionally, it was proposed that bacterial strains of Escherichia coli and Clostridium species, which could produce β-glucuronidase and enhance enterohepatic circulation in the gut [24], could increase the blood bilirubin level, while Bifidobacterium and Lactobacillus as probiotics could help neonates in the fading of jaundice [25]. However, this hypothesis is contradictory to the fact breastfeeding neonates possess more Bifidobacterium in their gut but are more likely to be jaundiced. Thus the relationship between gut micro-biota and neonatal jaundice was doubted.

5. Line 289: "According to our analysis, *Bifidobacterium* and *Lactobacillus* genera are neutral or only indirectly effective to neonatal jaundice." As being a new concept, the authors should provide more detailed information about their analysis.

Response: Thank you very much for your advice. The narrative here does seem a bit thin. On the one hand, we have added an appropriate analysis here. As follows:

Line 310-314: According to our analysis, *Bifidobacterium* and *Lactobacillus* genera are neutral or only indirectly effective to neonatal jaundice. Because, after the preceding discussion, for the most part, these strains do not have the bilirubin-reducing capacity and do not produce β-glucuronidase (and thus are unable to hydrolyze bound bilirubin directly)-they appear to be unrelated to bilirubin metabolism.

The phrase "after the preceding discussion" refers to chapter "3. The roles of different neonatal gut microbial taxa in the excretion of bilirubin".
On the other hand. *Bifidobacterium* is described in more detail in section "3.3. Three groups of gut microbiota with different roles". This is as follows:

Line 267-274: Among them, *Bifidobacterium* is more specific, although it has no direct effect on bilirubin excretion, it has been shown that the decrease in its abundance is positively correlated with the decrease in the expression of genes related to the galactose metabolism pathway, which reduces the upstream production of UDP-glucose, which leads to the lack of UDPGA and affects

the conversion of bilirubin [61]. *Bifidobacterium* promotes defecation, lowers intestinal pH, and inhibits β-glucuronidase activity [62, 63], thereby inhibiting bilirubin enterohepatic recycling and favoring bilirubin metabolism to some extent.

6. Line 302: “There are no studies on the relationship between breastfeeding times and the development of neonatal gut microbiota. We could conjecture that the limited nutrition input would delay the colonization and development of gut microbiota.” Is there any evidence to suggest the hypothesis?

Response: Thank you for your suggestion. We have added the appropriate evidence to support this hypothesis. As follows:

Line 326-332: There are no studies on the relationship between breastfeeding times and the development of neonatal gut microbiota. By virtue of the many common features of gastrointestinal physiology, microbiology, genetics, and diet, the pig is a valuable model for human nutritional research [80]. And it has been shown that early weaning causes changes in the intestinal corticotropin-releasing factor (CRF) system, which can lead to intestinal barrier disorders and intestinal flora imbalance in pigs [81]. We could conjecture that the limited nutrition input would delay the colonization and development of gut microbiota.

7. Line 334: “Two systematic reviews of randomized controlled trials of probiotic supplementation for prevention or treatment of jaundice in neonates were published in 2017.” Due to the controversial results, the author should provide more detailed information about the two reviews and try to explain why.

Response: Thanks to your suggestion, it does seem that the original manuscript did not recount the logical relationship between the two stories clearly. We have explained this section in more detail. As follows:

Line 362-373: Two systematic reviews of randomized controlled trials of probiotic supplementation for prevention or treatment of jaundice in neonates were published in 2017. One, by Chen and colleagues, showed that probiotics supplementation not only decreased the duration of phototherapy but also decreased the total blood bilirubin level [84], but this was mostly in the state of concomitant phototherapy. When probiotics were used as a single-strain preparation rather than in combination, i.e., without phototherapy, their therapeutic effect on jaundice was poor [85]. Secondly, the study by Deshmukh and colleagues showed that limited low-quality evidence indicated the reduction effect of the duration of phototherapy in jaundice neonates by probiotics treatment [86]. Thus there are not sufficient evidences to prove that *Bifidobacterium* and *Lactobacillus* probiotics strains were effective on reducing the level of blood bilirubin, which is coincident with our previous analysis of their ability to metabolize bilirubin.

8. Line 343-351: Do the authors have any suggestion about probiotics strains for neonatal jaundice.

Response: Thank you for your question. Indeed, without the support of the available literature, we dare not make a judgment on this. However, we point out a direction of strain selection, i.e., to

consider strains in the favorable alteration of intestinal flora after phototherapy in conjunction with phototherapy. We make the following account of this:

Line 380-402: Phototherapy, the simplest treatment for neonatal jaundice, converts unconjugated bilirubin to a conjugated form, which can then be metabolized to urobilin analogs by the "traffic police" [87]. However, extended phototherapy is associated with potential dangers, including water and electrolyte imbalance, diarrhea, skin lesions, and bronze baby syndrome [88], and thus is often accompanied by adjuvants to shorten the duration of treatment, such as ursodeoxycholic acid [89]. In addition, there is much evidence that probiotics can be used in conjunction with phototherapy to treat neonatal jaundice. However, the mechanism of action of such combination therapy is not clear, and the effects of phototherapy and intestinal flora appear to be complementary. On the one hand, probiotic supplementation has been shown to improve recovery from neonatal jaundice and reduce the side effects of phototherapy by modulating bacterial colonization and enhancing immunity [90]. On the other hand, research has shown beneficial changes in the gut microbiota structure of neonates with jaundice after phototherapy [91], such as a significant decrease in the abundance of *Escherichia coli*, which is accompanied by a decrease in the activity of β-glucuronidase [92], thereby favoring the excretion of bilirubin. Based on this, the probiotic strain *Bifidobacterium animalis subsp. lactis* CP-9 obtained by Tsai et al. through screening has excellent anti-*E. coli* activity, which can synergistically improve the efficiency of phototherapy [93]. Similarly, *Saccharomyces boulardii* may promote treatment outcomes and prevent the recurrence of jaundice by modulating the gut microbiota of neonates in combination with phototherapy [94]. Perhaps synergistic treatment of neonatal jaundice can be further achieved by considering strains from the favorable alterations in gut flora after phototherapy when screening for new bilirubin-metabolizing strains, in conjunction with phototherapy.

9. The paper should undergo some copy-editing. For example, the use of capital and small letters needs to be revised.

Response: I'm very sorry, it was our oversight that led to errors that shouldn't have occurred. We checked the article to make changes including but not limited to the following:

Line 167: 3.1. *The Bacterial Taxa that Could Produce β-Glucuronidase and Hydrolyze Conjugated Bilirubin*
Line 223: 3.2. *Bacterial Taxa that Could Metabolize Bilirubin*
Line 248: 3.3. *Three Groups of Gut Microbiota with Different Roles*
Line 275: 4. *The Development of Neonatal Gut Microbiota and Jaundice*

10. The article would benefit from English language editing by a scientific editor.

Response: Thank you very much for your suggestions, and we have tried to improve the English quality of the article, which we hope will be helpful.

11. Minor suggestion: In Figure 3 and Figure 5, the words in white color were not comfortable to read.

Response: Thank you for your advice. It's a very detailed and helpful tip. We have changed the color of the text in the figures to make them easier to read. As follows:

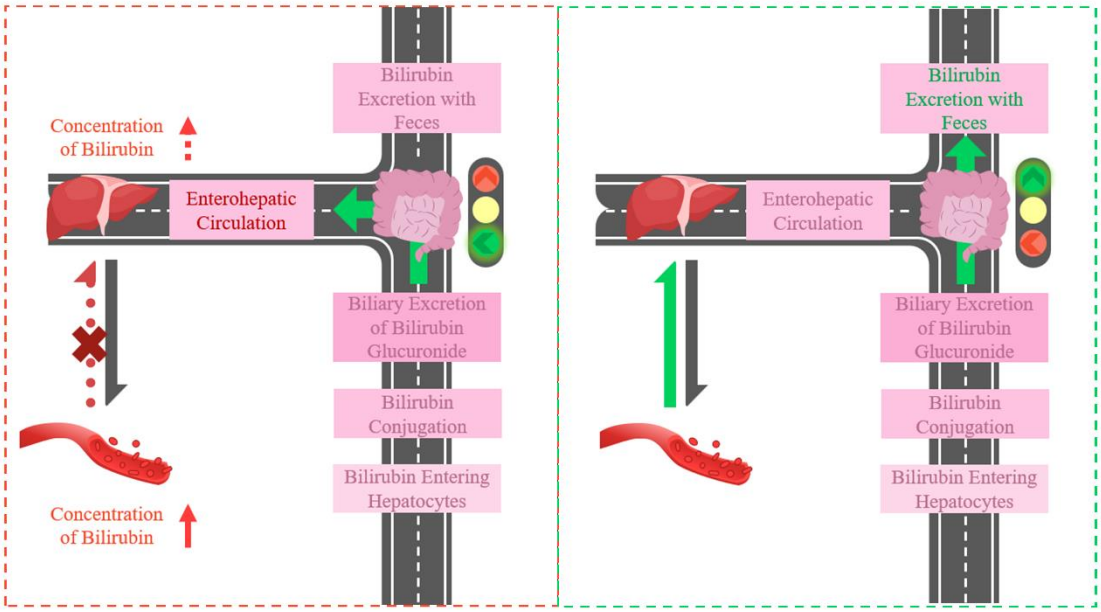


Figure 3. The Route of Bilirubin Excretion. The gut is the key junction.

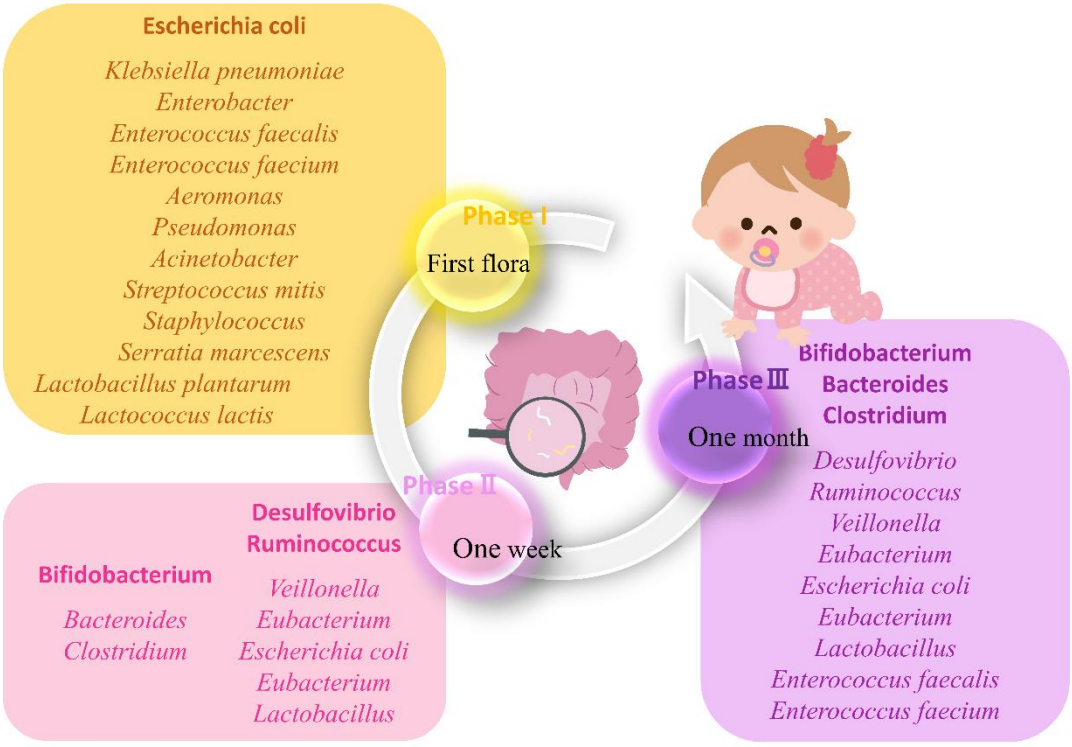


Figure 5. The Development of Neonatal Gut Microbiota.