

Reviewer 1:

Comments

1. **Please include a short paragraph regarding the management options (if present) for equilibration of bilirubin levels in human body – any study with drugs or life style changes that has impact on bilirubin levels?**
 - Found one study <Am J Clin Nutr May 2008 vol. 87 no. 5 1141-1147> which showed a statistically significant increase in total bilirubin levels with low calorie diet but was not considered clinically significant. Therefore, the study was not included in the review.
 - In management options, I couldn't find any drugs that are specifically used to increase bilirubin levels, Drugs that are associated with an increase in bilirubin as a side effect include allopurinol, anabolic steroids, some antibiotics, antimalaria medications, azathioprine, chlorpropamide, cholinergics, codeine, diuretics, epinephrine, meperidine, methotrexate, methyl dopa, MAO inhibitors, morphine, nicotinic acid, birth control pills. However, in the expert commentary section in the 4th paragraph relationship between aspirin, statin and the bilirubin levels have been included.
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2. **Any animal study showing a direct evidence that bilirubin have had anti-oxidant effect and decrease atherosclerosis? Please include**
 - Couldn't find any animal studies to show all three in one study i.e. bilirubin's anti-oxidant effect and reduction in atherosclerosis. But bilirubin has anti-oxidant/anti-inflammatory effects (PMID: 17249379; 26232645: Rat study) and bilirubin levels are inversely associated with atherosclerosis (PMID: 23010146; 16534198; 12709588) -> which we've already shown in the "protective properties of bilirubin" section.

Reviewer 2:

1. **The title should be limited in CAD according to the manuscript. It is more appropriate as a mini review if published.**
 - Change title to **"Bilirubin in Coronary Artery Disease: cytotoxic or protective?"**
2. **The manuscript give more epidemiology description and ignore the comparisons within studies. Most importantly, the specific mechanism of protective or cytotoxic is only slightly mentioned, and this part should be extended.**
 - We have an entire section explaining mechanisms describing protective properties of bilirubin under "protective properties of bilirubin" and another section that describes "the evidence not supporting the protective role of bilirubin".
3. **The figure is unsharp.**
 - Better quality picture has been added.

Reviewer 3:

General Comments:

1. **"How likely is bilirubin to be directly associated with cardiovascular risks?"**
 - Addressed in the Expert Commentary section

- "First, it is possible that the protective effects seen with higher bilirubin levels are possibly mediated through heme oxygenase or by other substrates involved in the pathway of bilirubin production, namely, biliverdin and carbon monoxide. Although few studies have reported an inverse association between bilirubin and the risk of CAD, no such association was seen with UGT1A1 gene polymorphism and the risk of CAD. Thus, a conclusion can be safely inferred that if at all bilirubin is protective in CAD, it is likely that bilirubin production (by induction of heme oxygenase and accompanied by production of carbon monoxide) and not just its excretion indirectly confers the protective effect observed with CAD. This would reflect as bilirubin having a protective effect on CAD whereas, in reality it is only a mediator or a marker."
2. **"The protection from cardiovascular disease is most likely multifactorial. What are the main contributors beyond bilirubin?" As of yet, these things are commented upon throughout the manuscript, but trying to answer the main questions more directly would improve the paper.**
- Since the primary question in this review paper is "Whether elevated bilirubin has a protective effect on CAD", the discussion on other contributors beyond what is mentioned in the Expert Commentary section is beyond the scope of this article.
3. **Introduction, page 4 It is important to notice that the study by Vitek et al. showed that 1/50 patients with GS had IHD at baseline, and it is unclear how authors came to the conclusion that the prevalence was different in the two groups.**
- This is mentioned as one of the main results of the study by Vitek et al. (PMID: 11849670).
4. **The same study showed, that during three-year follow-up there was no difference in incident IHD in analyses adjusted for baseline factors. Using this as the main reference in support of lower risks of CVD with elevated bilirubin is questionable. Furthermore, the fact that no difference is observed when adjusting for baseline risk factors is interesting and in agreement with previous studies such as DOI: 10.1016/j.numecd.2013.12.009.**
- Under results section of this study, "Based on multivariable analysis of standard risk factors, the predicted 3-year incidence of IHD in patients with GS (Group A) was 3.1% for males and 0.5% for females. This was comparable to the predicted incidence of IHD in healthy comparable population (Group E; 3.2% for males, 1.1% for females). Poisson distribution probability test of observed and predicted 3-year incidence of IHD in male GS subjects (0 vs. 3.1%) revealed statistically significant difference between compared parameters ($P < 0.05$). Due to a limited number of patients, this test could not be used in females."
 - This statement shows that the study was not adequately powered; however, a trend of lower incidence of IHD with GS was observed (statistically significant within the males group).
5. **Bilirubin metabolism, page 5 The introduction of Crigler-Najjar and Dublin-Johnsson syndrome is interesting. The authors should consider relating this syndrome to cardiovascular risks specifically, if known, also including why the type 1 syndrome is lethal. Cardiovascular, hepatic, hematological causes or other?**
- Couldn't find anything about Crigler Najjar and changes in cardiovascular risk. However, the following statement has been included in the review "Patients with Crigler

Najjar Type 1 develop severe neurological impairment and carry a high early mortality unless they receive liver transplantation."

6. Prospective properties of bilirubin, page 5/6. The authors state that: "which in turn retards the peroxidation of lipids, hence, restricting the progression of atherosclerosis [10]." Reference 10 was an in vitro study which concluded that bilirubin may help reduce the risk of atherogenesis. The authors infer causal effects in vivo based on this study.

- Rephrased to : "Bilirubin sub-fractions (Bu and Bc) have demonstrated inhibition of low-density lipoproteins oxidation, which in turn retards the peroxidation of lipids, hence **could potentially** restrict the progression of atherosclerosis [10]."

7. Prospective properties of bilirubin, page 5/6. "Bilirubin protects" infer causality. Please rephrase.

- Rephrased to "Bilirubin **has been shown to be inversely associated with** increased arterial stiffness [12, 13]."

8. Genetic Polymorphisms of UGT1A1*28, page 13 The authors need to comment on potential genetic penetrance, because if the gene always leads to higher bilirubin levels, then the gene might very well be an intermediate variable which could explain why the gene is not associated with outcomes in multivariate analyses.

Rephrased to "Mendelian randomization is done to establish a causal relationship [50]. As mentioned above, a lack of significant association between the gene polymorphisms of UGT1A1 and risk for CAD goes in favor of bilirubin being a marker than a primary mediator for the cardioprotective effects observed with CAD. **Moreover, it also points out towards incomplete penetrance of the UGT1A1 gene.**" In the last paragraph of the paper.

9. Expert commentary, page 16. "Thus, a conclusion can be safely inferred that if at all bilirubin is protective in CAD, it might be possible that it is not just the bilirubin excretion but the production of bilirubin, which indirectly is by induction of heme oxygenase and is also accompanied by the production of carbon monoxide." This is confusing as the, by the authors, implied causal effects of bilirubin must be directly related to bilirubin to be causal. Implying that bilirubin may exert causal effects through other mechanisms (heme oxygenase, CO, etc.) is self-contradictory.

- Rephrased to "First, it is possible that the protective effects seen with higher bilirubin levels are possibly mediated through heme oxygenase or by other substrates involved in the pathway of bilirubin production, namely, biliverdin and carbon monoxide. **Although** few studies **have** reported **an** inverse association between bilirubin and the risk of CAD, no such association **was** seen **with** UGT1A1 gene polymorphism and the risk of CAD. Thus, a conclusion can be safely inferred that if at all bilirubin is protective in CAD, it is **likely that bilirubin production** (by induction of heme oxygenase and accompanied by production of carbon monoxide) **and** not just **its** excretion indirectly confers the protective effect observed with CAD. This would reflect as bilirubin having a protective effect on CAD whereas, in reality it is only a mediator or a marker."

10. Expert commentary, page 16. "And it is being reflected that bilirubin has protective effect on CAD whereas, actually it does not." Please rephrase.

- Rephrased as above

11. Expert comment, page 17. First paragraph. The hypothesis put forward is not

- Incomplete statement