Reviewer's report

Title: Associations of age with serum insulin, proinsulin and the proinsulin-to-insulin ratio: a cross-sectional study

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Reviewer: Taro Maruyama

Reviewers report:

In this study, Bryhni et al. examined random insulin, proinsulin and proinsulin to insulin ratio (P/I) in 6212 persons without self-reported diabetes mellitus in the Tromso study 1994-95. As a result, proinsulin levels increased across age groups in both genders. After adjustment, greater age was associated with lower insulin and higher proinsulin and P/I. The authors concluded that these results point towards a loss of beta cell function inherent in the aging process.

Overall, this study is a cross-sectional observational study, not a longitudinal study, which cannot be drawn any “causal” association. The results should be interpreted carefully.

Major comments:

1) How many individuals in this study were diagnosed as diabetes defined by HbA1c 6.5% or more? In this study, unfortunately there was no information on glucose tolerance status (NGT/IGT/IFG/DM) other than self-reported diabetes. It has been shown that P/I ratio is increased in individuals with IGT or diabetes. Although the authors excluded the individuals with HbA1c 6.5% or more as definitive diabetes, it is quite likely that a proportion of individuals with IGT/IFG increases with age in a general population which was examined in this study. Thus the increase in P/I ratio with age observed in this study may just reflect the increase in individuals with abnormal glucose tolerance. One could think that the increase in P/I ratio may be a result, but not a cause, of increase in abnormal glucose tolerance with age.

2) Since the half life of insulin and proinsulin in blood are different, kinetics of insulin differs from that of proinsulin in postprandial state. Thus proinsulin to insulin ratio changes at each time point since last meal, which should not be simply adjusted by hours. Direct comparison among P/I ratios measured at different time points has not been validated.

3) There was no information on characteristics in each age group shown in Figure 1. The change in P/I ratio and glucose values should be added in Figure 1. The other information such as HbA1c and BMI should be shown as another Table since these values are important to assess beta cell function.

4) Not only decline in beta cell function but also decline in insulin sensitivity contributes to the increase in incidence of diabetes with age. However, there was
no information on insulin sensitivity such as HOMA-IR.

5) Overall, there are many confounding factors in this study which makes difficult to interpret the results. The authors should consider stratified analysis.

Minor comments:
1) Proinsulin levels were adjusted by creatinine. However, elder people tend to show lower creatinine levels compared to younger people. Thus estimated GFR (eGFR) should be used for adjustment.

2) Tables are redundant. Should be more organized.

3) Among many confounding factors, heart rate is significantly independently correlated with P/I in the both genders. The authors should discuss on this point.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.