Author's response to reviews

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

Authors:

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Author's response to reviews: see over
Thank you for permitting us to revise the manuscript: Bente Bryhni, Egil Arnesen, Trond G. Jenssen: “Associations of age with serum insulin, proinsulin and the proinsulin-to-insulin ratio: a cross-sectional study.”

Enclosed is a one revised version our manuscript with changes underlined.

The following changes have been made according to the comments by Referee 1:

We certainly agree that no conclusions on causality can be drawn from this cross-sectional observation study, as opposed to a longitudinal study.

Accordingly, we have reorganised the text.

Discussion, page 11, paragraph 2 has been moved to page 11, paragraph 1 and the following sentence has been added:

“Importantly, cross-sectional associations as in the present study, as opposed to longitudinal data, do not permit conclusions to be drawn about causality.”

Page 11, paragraph 3 has been moved to page 13, paragraph 1

Major comments

1. On page 9, paragraph 2 and page 10, paragraph 1, we have added the following sentences/clauses:

   “Adjustments by HbA1c did not change the associations with age. Also, exclusion of 38 men and 33 women with HbA1c at or above 6.5% (new criterion for the diagnosis of diabetes mellitus [13]) or 656 men and 726 women with HbA1c at or above 5.7% (the lower HbA1c level considered reasonable for identifying individuals with a high risk of future diabetes [13]) did not change the results.”

Page 12, paragraph 2 as been rewritten and we have added the following clauses:

   “Notably, a proportion of our subjects probably had unidentified diabetes or IGT, and the increase in proinsulin/insulin observed with age in this study could conceivably just reflect the increase in individuals with abnormal glucose tolerance.”
“However, a statement from the American Diabetes Association has recently affirmed that elevated HbA1c levels at or beyond 6.5% are sufficient to make a diagnosis of diabetes mellitus [13], and in addition, a HbA1c range of 5.7 to 6.4 is considered reasonable for identifying individuals with a high risk for future diabetes to whom the term prediabetes may be applied [13]. The omission of subjects with diabetes or prediabetes according to these definitions did not change the results.”

2. On page 14, paragraph 2 we have added the following sentence:

“Of note, however, since the half life of insulin and proinsulin are different, the kinetics of insulin differs from that of proinsulin in the postprandial state and direct comparisons of proinsulin/insulin ratios measured at different time points has not been validated.”

3. The percentiles of proinsulin/insulin have now been added to Figure 1 for men and women, as we consider that this variable logically belongs together with the percentiles of insulin and proinsulin. We agree that substitution of our Table 1 (age-adjusted means) by a new table with means according to strata of age would be helpful. However, we feel that the presentation of these data, in tables for men and women, would result in excessively sizeable tables.

The figure legend has been altered:

“**Figure 1** Medians (●) and 25 (▲) and 75 (▼) percentiles of serum insulin, proinsulin, and proinsulin/insulin by age. Non-fasting persons without self-reported diabetes mellitus. The Tromsø Study 1994-95”

The following changes have been made in the text:

Abstract, Page 2, paragraph 3:
“Proinsulin levels and proinsulin/insulin increased across age groups in both genders.”

Page 4, paragraph 3:

“In 6212 men and women who had random measurements of insulin and proinsulin in a population-based study, and who did not report diabetes mellitus, we describe the percentiles of insulin and proinsulin levels and the proinsulin-to-insulin insulin ratio (proinsulin/insulin) according to gender and age.”

Page 8, paragraph 2:

“We constructed plots for the medians and 25 and 75 percentiles of insulin and proinsulin concentrations, as well as proinsulin/insulin according to gender and age group and computed the trends across age for the medians by regression analysis weighted for the inverse of the squared SEM in each age group.”

Page 9, paragraph 2:
“However, serum proinsulin (p = 0.0002 for women and p = 0.0066 for men) and proinsulin/insulin (p < 0.0001 for women and p = 0.0002 for men) rose across age strata in both genders.”

Page 10, paragraph 2:

“However, proinsulin levels and proinsulin-to-insulin ratios increased across age groups in both men and women.”

4. Not only a decline in beta cell function but also a decline in insulin sensitivity contributes to the increase in incidence of diabetes with age. As we studied random insulin and glucose levels we could not calculate indices of insulin sensitivity such as HOMA-IR. However, we have adjusted the insulin and proinsulin levels and the proinsulin/insulin ratios by the waist circumferences and several other variables associated with insulin sensitivity, as stated in the text.

5. We agree that stratified analyses are helpful to control confounding. However, as there are many confounding factors in our study we believe that use of multiple regression analysis is preferable to stratified analyses to consider the independent associations between the dependent variables and age and other independent variables. We were also interested in the continuous associations between the dependent and independent variables. Accordingly, we prefer to retain the multiple regression models.

Minor comments

1. Adjustment for estimated GFR in our regression equations would be inappropriate as age, creatinine and body weight (in BMI) are already in the equations.

On page 14, paragraph 2 we have added the following sentence:

"However, as age, BMI, and serum creatinine were included in the regression equations and the analyses were performed separately for men and women, we did in effect adjust for the glomerular filtration rates [38].”

2. Tables 2-4 have been reorganised and merged into two tables (Tables 2 and 3), one for men and one for women. The table designations in text under Results have been altered accordingly.

3. The independent associations between heart rate and log_{10}(insulin), log_{10}(proinsulin), and log_{10}(proinsulin/insulin) are now stated under Results:

“Log_{10}(insulin) and log_{10}(proinsulin) were positively, but log_{10}(proinsulin/insulin) inversely related with heart rate in both genders (Tables 2 and 3).”

4. The independent associations between heart rate and insulin, proinsulin and proinsulin/insulin in both genders is now discussed in the text, page 15, paragraph 2:
“Resting heart rate can be perceived as an integrated marker of hemodynamic and autonomic nervous system states, and is an independent predictor of cardiovascular disease [43]. Elevated heart rate may reflect a shift in autonomic balance toward enhanced sympathetic tone [43] and is associated with higher insulin [44] and proinsulin concentrations [45], as also observed in the present study. A novel finding of our study is a negative association in both genders between heart rate and proinsulin/insulin. This result is consistent with a previous report of a positive association between heart rate and the acute insulin response, as measured by a frequently sampled intravenous glucose tolerance test [45].”

Other corrections:

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Page 4, paragraph 2:

“Although this finding could reflect beta cell failure in ageing, it might also be due to alterations in diet or gastric emptying, or even to an enhancement in insulin sensitivity in older age.”

Page 6, paragraph 1:

Among these, 863 women aged 50-54 years were excluded as they were all examined between 08 and 09 AM, and the time from the last meal had not been recorded, and 94 (aged 25-34 years) were excluded because the handling of their blood samples was inadequate. The final sample included 6212 persons, of whom 1116 had participated in a family intervention trial [11].