Author's response to reviews

Title: Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study

Authors:

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Version: 3 Date: 26 January 2010

Author's response to reviews: see over
Dear Editor-in-Chief

Please find enclosed our revised manuscript entitled: *Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study*

The authors are Jørø Hjelmesæth (corresponding author), Jo Røislien, Njord Nordstrand, Dag Hofsø, Helle Hager and Anders Hartmann.

The manuscript has been revised according to the reviewers’ comments and suggestions, and it has also been proofread by an English native speaker.

Please see our point-by-point replies to the reviewers’ concerns below.

We hope that the revised manuscript is acceptable for publication in *BMC Endocrine Disorders*.

Sincerely,

Jørø Hjelmesæth
Reviewer's report
Title: Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study
Version: 2 Date: 2 December 2009
Reviewer: Carlos Lorenzo

Reviewer's report:
The aim of this article was to explore whether serum creatinine is a predictor of prevalent T2DM among morbidly obese Caucasian women and men with normal renal function. The authors concluded that low serum creatinine is an independent predictor of prevalent type 2 diabetes in Caucasian morbidly obese patients.
The relation of GFR as diabetes is interesting particularly early events in diabetic nephropathy. However, I have several comments regarding methodological issues in this paper.

1.- The conclusion of the paper may be true. It is already known that increased GFR and microalbuminuria are early manifestations of diabetic nephropathy. The decline of GFR tends to occur later in the disease process after the onset of macroalbuminuria. In this study, however, these events may be more difficult to demonstrate because of the characteristics of the participants. All participants are morbidly obese. In severe obesity, GFR levels tend to exceed those of lean individuals by 50% (Chagnac et al. Am J Physiol Renal Physiol 2000;278:F817). Therefore, hyperfiltration may be common in these morbidly obese subjects even in the absence of diabetes.

Reply: We agree with the reviewer that glomerular hyperfiltration may be common among morbidly obese subjects even in the absence of diabetes. In view of this, the topic of hyperfiltration has been elaborated upon in the introduction of the revised manuscript: "Notably, obesity may be considered a state of relative hyperfiltration, with several pieces of evidence indicating that absolute GFR is higher in severely obese subjects than in their lean counterparts (Griffin KA 2008, Chagnac et al 2000)". In addition, the categorisation of patients according to either the presence or absence of glomerular hyperfiltration has been removed from the revised manuscript, as has the suggested definition of hyperfiltration.

2.- Page 6, para 2, line 1: T2DM was diagnosed in patients who had a prior history of T2DM or a fasting serum glucose level ≥ 7.0 mmol/l.
The definition of diabetes may not adequate for this cohort of morbidly obese subjects. 2-h glucose concentration may be abnormal in a significant number of participants with undiagnosed diabetes. Another alternative is to use hemoglobin A1c as the parameter for the diagnosis of diabetes.

Reply: There is universal agreement upon the diagnostic criteria (ADA/WHO) of the cut-off level of fasting glucose ≥ 7.0 mmol/l in diabetes mellitus. The criteria are valid for all patient cohorts, including the morbidly obese. On the other hand, we acknowledge that a small number of patients with type 2 diabetes may have gone undiagnosed and were subsequently misplaced as absent. However, this is not a major problem in the present study since fasting glucose has been shown to identify the great majority of morbidly obese patients with unknown diabetes. (Hofsø D, Jenssen T, Hager H, Raïslien J, Hjelmésæth J. Fasting plasma glucose in the screening for type 2 diabetes in morbidly obese subjects. Obes Surg 2009 Dec 1. [Epub ahead of print]). This limitation has been addressed in the discussion section of the revised manuscript.

3.- Page 6, para 2, line 2: Homeostasis Model Assessment Insulin Resistance (HOMA- IR) was calculated as ([fasting serum glucose (mmol/l) * fasting serum insulin (pmol/l)] /135)
HOMA IR may not be an adequate parameter of insulin resistance in diabetic
individuals.

Reply: We acknowledge that the validity of HOMA-IR in the assessment of insulin resistance in subjects with type 2 diabetes has been disputed. However, Matthews et al. have demonstrated (in a small number of type 2 diabetic subjects treated with dieting alone) that HOMA IR highly correlated with the insulin resistance index assessed by the euglycemic-hyperinsulinemic clamp (clamp IR) (Matthews DR et al. Homeostasis model assessment: insulin resistance and b cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419, 1985). In addition, log (HOMA-IR) has been shown useful when evaluating the insulin resistance of glucose intolerant individuals, those with mild to moderate diabetes, and those with other insulin-resistant conditions (Muniyappa R, et al 2008). In our population of morbidly obese subjects the majority of patients had only mild to moderate diabetes. Approximately 66% of the female patients and 53% of the male patients were treated with either lifestyle intervention or metformin only, whilst only 13% of the female diabetic subjects and 18% of the male diabetic subjects received insulin.

In the revised manuscript HOMA IR has been replaced by log HOMA-IR: In HOMA IR was included in the regression analyses of the first draft. This has been implemented in the methods section, page 5: “The log (HOMA-IR) is presented both because it has a stronger linear correlation with glucose clamp estimates of insulin sensitivity and because it is useful for the evaluation of insulin resistance in glucose intolerant individuals, those with mild to moderate diabetes, and those with other insulin-resistant conditions (Muniyappa R, et al 2008).”

4.- Page 5, last sentence: We aimed to explore whether serum creatinine is a predictor of prevalent T2DM among morbidly obese Caucasian women and men with normal renal function. Some of these individuals do not have normal renal function. A significant proportion has microalbuminuria and even macroalbuminuria. Furthermore, eGFR >60 ml/min x 1.73 m2 is not synonymous of normal GFR.

Reply: We agree that some of our patients have renal damage and that eGFR >60 ml/min x 1.73 m2 is not synonymous with normal GFR; implying that patients with albuminuria do have chronic renal disease grade I-II according to the KDOQI Guidelines. All individuals with GFR <60 mL/min/1.73 m2 for ≥3 months are classified as having chronic kidney disease, irrespective of the presence or absence of kidney damage. The rationale for excluding these individuals is that reduction in kidney function to this level or lower represents the loss of half or more of the adult level of normal kidney function, which may be associated with a number of complications. The term “normal renal function” has therefore been removed from the revised manuscript.

In addition, a recent paper indicates that a modified Cockcroft-Gault formula (replacing body weight with lean body weight) provides an unbiased, precise, accurate and clinically practical estimate of 24-hour measured creatinine clearance in morbidly obese patients (ref Demirovic JA et al 2009). (Better than both MDRD4 (which underestimates GFR) and ordinary CG (which overestimates CrCl)). Accordingly, eGFR has been removed from the analysis, with the modified Cockcroft-Gault formula implemented to estimate creatinine clearance (see revised table 1). Lean body weight (LBW) was estimated with gender specific equations which have been validated in morbidly obese subjects (Janmahasatian et al 2005). LBW (male) = (9270*body weight) / (6680+216*BMI) and LBW (female) = (9270*body weight) / (8780+244*BMI)

5.- Page 6, para 2, line 5: Glomerular hyperfiltration was defined as present if eGFR was higher than the gender specific mean eGFR+1.96 SD of non-diabetic subjects [17]. This definition is inadequate for the diagnosis of hyperfiltration. Individuals in this study are morbidly obese and a significant proportion of them may have hyperfiltration. Subjects of reference should have the following characteristics:
absence of diabetes and dysliipidemia, young, normal weight and blood pressure, and normoalbuminuria

Reply: We agree with the reviewer; the categorisation of patients according to either the presence or absence of glomerular hyperfiltration has been removed from the revised manuscript, as has the suggested definition of hyperfiltration.

6.- Page 9, para 1, line 4: When compared to their non-diabetic counterparts the serum creatinine levels of diabetic patients were significantly lower. By contrast, eGFR did not differ significantly between groups.

GFR tends to decrease with age. Subjects with and without diabetes do not differ in terms of eGFR. However, those with diabetes are 8-9 years older than nondiabetic counterparts. Adjustment for age may result in statistical significant differences.

Reply: We agree with the reviewer that GFR tends to decrease with age. However, the eGFR (original version) and creatinine clearance (revised version) equations are inclusive of age; eGFR= 30849 * serum creatinine^{-1.154} * age^{-0.203} * 0.742 (if female). Creatinine clearance (ml/min) = (140-age)*LBW*serum creatine*1.23*0.85 (if female). Creatinine clearance based on the latter formula did not differ significantly between groups (revised table 1). In addition, age was adjusted for in all logistic regression models (table 2).

7.- A more standard way of analyzing the data is to use tertiles or quartiles of creatinine or eGFR. This approach prevents the possibility of introducing bias by selecting an artificial cut-point that is associated with statistical significance.

Reply: Although we acknowledge that several authors use either tertiles or quartiles to analyse their data, we do not agree that such categorisation of data is superior to our use of Generalized Additive Models (GAM). To the contrary, any categorisation of continuous data, including generating tertiles or quartiles, weakens the power of statistical analysis as the cut-points are clearly “arbitrary” and dependent on the usage of tertiles/quartiles/quintiles...That is why when expecting non-linearity in the relationship between creatinine and T2DM we also fitted Generalized Additive Models (GAM). GAM is a natural extension of Generalised Linear Models (GLM), e.g. logistic regression, and allows for all types of functional relationships between dependent and independent variables using splines. As creatinine appeared to be piecewise linear with respect to T2DM in our data, we also fitted piecewise linear logistic regression models for all of the increasingly complex models described below, i.e. estimating the existence and location of a possible breakpoint in the relationship between creatinine and T2DM. Accordingly, we conclude that the cut points derived from this model cannot be considered “arbitrary”.

Which journal?: Not appropriate for BMC Medicine: an article whose findings are important to those with closely related interests and more suited to BMC Endocrine Disorders
What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests
Reviewer's report
Title: Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study
Version: 2 Date: 2 December 2009
Reviewer: Matthias Blüher
Reviewer's report:
Hjelmesæth and coworkers investigated in a large cross-sectional study of morbidly obese patients with normal renal function whether creatinine serum concentrations are associated with type 2 diabetes. The authors found that low serum creatinine is independently associated with prevalent type 2 diabetes in morbidly obese patients.
There are some critical points, which need to be clarified:
Comments:
1) The term predictor of type 2 diabetes should be avoided since with the cross-sectional design only relationships or associations can be detected.

Reply: Although we agree that no causal relationship can be documented in a cross-sectional study like ours, the term predictor is commonly used to refer to an “independent variable” and does not necessarily imply causality. Several cross-sectional studies, including the present, use the term predictor; indeed, a Google search (limited to the last 12 months) for “predictor cross-sectional study” revealed 7,020,000 results. For a recent example read the following article from the BMJ (2009) which in a cross-sectional survey uses the term predictor (Kath Moser BMJ 2009;338:b2025; see abstract below). “For cervical screening, ethnicity was the most important predictor”.

2) In the conclusion the term "independent" should be defined, i.e. independent of age, gender etc.

Reply: Thanks for this suggestion; this has been implemented in both the abstract and conclusion of the revised manuscript: “Low serum creatinine is a predictor of prevalent type 2 diabetes in Caucasian morbidly obese patients independent of age, gender, family history of diabetes, anthropometric measures, hypertension, and current smoking.”

3) Serum creatinine might be influenced by several individual factors including drinking behaviour, season of the year, hydration status. The authors should discuss these potential biases. Could the authors exclude that lower creatinine represents something like a "dilution" effect, because patients with hyperglycemia drink more? Therefore creatinine clearance rather than serum creatinine should be tested to exclude some of the potential biases.

Reply: We acknowledge that serum creatinine may have been influenced by confounding factors. However, serum creatinine shows little intra-individual variation in persons with normal renal function (ref Toffaletti JG et al. Clinical Chimica Acta 2008). Nevertheless, creatinine clearance has also been included in the revised manuscript.

4) It is inappropriate in a study which aims to use serum creatinine as surrogate parameter for lean body (or muscle) mass not to measure lean body mass. Hume’s equation is not sufficient to estimate lean body mass in particular in morbidly obese patients.

Reply: We agree that Hume’s equations may not be sufficient to estimate lean body mass in morbidly obese patients. Accordingly, the revised manuscript uses gender specific equations recently validated in morbidly obese subjects to estimate lean body weight (Janmahasatian S et al 2005). LBW (male) = (9270*body weight) / (6680+216*BMI) and LBW (female) = (9270*body weight) / (8780+244*BMI)
5) Was insulin measured at the same time as creatinine, i.e. 2006? How were the samples stored?

Reply: Yes, insulin was measured at the same time as creatinine (between November 28, 2005 and September 16, 2008). More details on the laboratory tests are given in our paper published in "Cardiovascular Diabetology" (ref 14 in the revised manuscript): “Laboratory analyses” Blood samples were obtained after an overnight fast by venipuncture in vacutainer gel tubes, and serum was separated from cells within 2 hours. Analyses of serum glucose, creatinine, calcium, albumin, magnesium, CRP and blood lipids were performed using dry reagent slide technology on the Vitros 950 Analyzer until November 2006 and Vitros FS 5.1 thereafter (Ortho-Clinical Diagnostics, New York, USA). Intact PTH was assayed using an electrochemiluminescence immunoassay on the Elecsys 2010 (Roche Diagnostics GmbH). The coefficients of variation for magnesium and PTH were 2% and 6%, respectively. Glycosylated hemoglobin was analyzed by high performance liquid chromatography (HPLC) using Tosoh HLC-723 G7 (Tosoh Corporation, Tokyo, Japan). Serum calcium was adjusted for albumin; serum calcium = (serum calcium [measured] - 0.018 * [serum albumin – 42]). Sera for analysis of Insulin and 25(OH)D were stored at -20 °C and analysed within 1 week of blood sampling at the Hormone Laboratory, Aker University Hospital. Insulin and 25(OH)D (the sum of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3) were analyzed in serum by radioimmunoassay (Linco Research Inc, St. Charles, MO, and DiaSorin, Stillwater, MN). The interassay coefficients of variation for insulin and 25(OH)D were 8% and 14%, respectively. All other analyses were performed on the day of blood sampling at Department of Clinical Chemistry at Vestfold Hospital Trust”.

6) The authors measured CrP, but did not mention the results. CrP levels have been frequently shown to be associated with prevalent type 2 diabetes and may have served as “internal control”.

Reply: The proportion of patients with CRP ≥ 7 mg/l is given in table 1 and did not differ significantly between groups.

Which journal?: Not appropriate for BMC Medicine: an article whose findings are important to those with closely related interests and more suited to BMC Endocrine Disorders

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests.

Matthias Blüher
Research
Inequalities in reported use of breast and cervical screening in Great Britain: analysis of cross sectional survey data
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Abstract
Objective To investigate the relation between women’s reported use of breast and cervical screening and sociodemographic characteristics.
Design Cross sectional multipurpose survey.
Setting Private households, Great Britain.
Main outcome measures Ever had a mammogram, ever had a cervical smear, and, for each, timing of most recent screen.
Results 91% (95% confidence interval 90% to 92%) of women aged 40-74 years reported ever having had a cervical smear, and 93% (92% to 94%) of those aged 53-74 years reported ever having had a mammogram; 3% (2% to 4%) of women aged 53-74 years had never had either breast or cervical screening. Women were significantly more likely to have had a mammogram if they lived in households with cars (compared with no car: one car, odds ratio 1.67, 95% confidence interval 1.06 to 2.62; two or more cars, odds ratio 2.65, 1.34 to 5.26), and in owner occupied housing (compared with rented housing: own with mortgage, odds ratio 2.12, 1.12 to 4.00; own outright, odds ratio 2.19, 1.39 to 3.43), but no significant differences by ethnicity, education, occupation, or region were found. For cervical screening, ethnicity was the most important predictor; white British women were significantly more likely to have had a cervical smear than were women of other ethnicity (odds ratio 2.20, 1.41 to 3.42). Uptake of cervical screening was greater among more educated women but was not significantly associated with cars, housing tenure, or region.
Conclusions Most (84%) eligible women report having had both breast and cervical screening, but 3% report never having had either. Some inequalities exist in the reported use of screening, which differ by screening type; indicators of wealth were important for breast screening and ethnicity for cervical screening. The routine collection within general practice of additional sociodemographic information would aid monitoring of inequalities in screening coverage and inform policies to correct them.
Reviewer's report

Title: Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study

Version: 2 Date: 27 November 2009

Reviewer: Giorgio Bedogni

Reviewer's report:

Major Compulsory Revisions

General. Your declared aim is to predict the "prevalence" rate of diabetes. However, the prevalence of diabetes in your population is too high for using logistic regression. With such prevalence, the odds ratio is not a synonym of the prevalence ratio. If your aim is instead to study the odds of diabetes due to creatinine – not the prevalence – logistic regression is OK but you should not say that you are modeling "prevalence". Binomial regression or Poisson regression are needed in the latter case.

Reply: With all due respect, we believe this to be a misunderstanding on your part. We did not aim to predict the “prevalence” rate of diabetes nor did we use the term “prevalence” rate in our article. The term “prevalent” diabetes was used to underline the cross-sectional nature of the relationship. Abstract: “Low serum creatinine is a predictor of prevalent type 2 diabetes”, Introduction: “We aimed to explore whether serum creatinine is a predictor of prevalent T2DM”. Yes, our aim was to study the odds of diabetes due to creatinine – not the prevalence – logistic regression is therefore OK; as was described in the Methods statistical analysis: “Logistic regression with predefined explanatory variables was used to assess the odds of T2DM”.

General. The PDF I downloaded does not include references.

Reply: We are sorry to hear that the references were not included in the PDF; the references were included in the submitted final manuscript.

Abstract: this is a cross-sectional study, not a cohort study. Please, complete the phrase ending with “largely unchanged”.

Reply: We agree that this is a cross-sectional study. Accordingly, the term “cohort” has been removed from both the abstract and methods section of the revised paper. However, we do not understand the second comment about completing the phrase “largely unchanged”. In our view the following sentence is complete: “Adjustments for non-modifiable and modifiable risk factors left the piecewise effect for both women and men largely unchanged.”

METHODS: Was the MDRD equation validated in your VERY obese subjects? Age and gender being equal, most obese subjects have an expanded fat-free mass and one expects higher levels of creatinine compared to normal-weight subjects. This is a source of potential bias.

Reply: We acknowledge that the MDRD equation may not be the most appropriate for a population of morbidly obese subjects. In addition, a recent paper indicates that a modified Cockcroft-Gault formula (replacing body weight with lean bodyweight) provides an unbiased, precise and clinically practical estimate of 24-hour measured creatinine clearance in morbidly obese patients (Demirovic JA et al 2009). (Better than both MDRD4 (which underestimates GFR) and the ordinary CG (which overestimates CrCl)). Lean body weight (LBW) was estimated using gender specific equations which have been validated in morbidly obese subjects (Janmahasatian S et al 2005). LBW (male) = (9270*body weight) / (6680+216*BMI) and LBW (female) = (9270*body weight) / (8780+244*BMI). Accordingly, the modified Cockcroft-Gault formula has replaced the MDRD equation in the revised manuscript.

METHODS: Hume’s equation is likewise very limited to assess muscle mass in obese subjects. This is a source of potential bias.
Reply: We agree that Hume`s equations may not be the best formulas to estimate lean body mass in morbidly obese patients. Accordingly, we have removed the results based on Hume`s equations from the manuscript. In the revised manuscript lean body weight (LBW) is estimated with gender specific equations which have recently been validated in morbidly obese subjects (Janmahasatian et al 2005): LBW (male) = (9270*body weight) / (6680+216*BMI) and LBW (female) = (9270*body weight) / (8780+244*BMI)

RESULTS: The size of the association between creatinine and BMI is unimpressive. The fact that the p-value is significant is due to the large number of subjects. Please, report 95% confidence intervals for these associations.

Reply; We agree with the reviewer that "The size of the association between creatinine and BMI is unimpressive."; hence we did not stress that these results were particularly important. To the contrary, we wrote that; “Serum creatinine had a weak negative correlation with WC, WHR and BMI in women (all r=-0.08 and p=0.030-0.036) and with BMI in men (r=-0.11, p=0.043)”.

The Spearman correlation coefficient is a non-parametric method, and the calculation of corresponding confidence intervals is non-trivial. On the contrary, it is not even necessarily advised, and standard statistical software like SPSS does not include confidence intervals for the Spearman correlation coefficient. With the aid of stochastic simulation techniques they can be constructed in programming languages such as R and Matlab, but we suggest not going to such lengths for a rather minor aspect of the article.

RESULTS: Subgroup analysis was performed in 49% of the study subjects. Although the relationships were reported to be similar, I suggest to remove this analysis as it adds nothing to the main message and highly is debatable owing to the high missingness rate.

Reply: We acknowledge this suggestion. However, between November 28 2005 and September 16 2008 we included 1,017 consecutive patients; the subgroup analysis was performed in the last 495 consecutive patients (recruited between May 7th 2007 and September 16th 2008). Since physical activity might influence both creatinine and diabetes we continue to find it appropriate to briefly comment upon this finding in our manuscript.

DISCUSSION: Please, report 95% confidence intervals for the odds ratios.

Reply: Thanks for this suggestion; 95% confidence intervals for the odds ratios are reported in the revised manuscript.

Minor Essential Revisions
STATISTICAL ANALYSIS: log-transformed using natural logarithms may be more understandable than “ln-transformed”

Reply; Thanks for this suggestion; “ln” has been replaced with “log” in the revised manuscript.

STATISTICAL ANALYSIS: What is the rationale for including magnesium among predictors?


Which journal?: Not appropriate for BMC Medicine: an article of only archival
interest, but might be suited to BMC Endocrine Disorders

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**
I declare that I have no competing interests