Author's response to reviews

Title: Metabolic correlates of subcutaneous and visceral abdominal fat measured by ultrasonography: a comparison with waist circumference

Authors:

Simona Bertoli (simona.bertoli@unimi.it)
Alessandro Leone (alessandro.leone1@unimi.it)
Laila Vignati (laila.vignati@unimi.it)
Angela Spadafranca (angela.spadafranca@unimi.it)
Giorgio Bedogni (giorgiobedogni@gmail.com)
Angelo Vanzulli (angelo.vanzulli@gmail.com)
Elena Rodeschini (elena.rodeschini@unimi.it)
Alberto Battezzati (alberto.battezzati@unimi.it)

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Author's response to reviews: see over
Reviewer 1: (Dr Jean Abed)

Discretionary Revisions:

What makes this article unique when we compare it to published manuscripts about the same subject?

This study demonstrates, in a large sample of Caucasian subjects, that US-determined VAT and SAT are both independently associated with MS. Moreover, to our knowledge, we are the first to show that VAT, being associated to all of the MS components in addition to hyperuricemia and altered liver enzymes, performs equally or better than WC except for high blood pressure and low HDL.

These data reinforce the existing evidence that the components of abdominal fat may play, at least in part, independent roles in the development of cardiometabolic risk factors.

We highlighted the novelty of this study in the conclusion section (Lines: 291-299)

“*In conclusion, US-determined VAT and SAT are both independently associated with MS. VAT is associated to all of the MS components in addition to hyperuricemia and altered liver enzymes, and performs equally or better than WC except for high blood pressure and low HDL. In contrast, SAT is independently associated only with high blood pressure and high ALT. These data are of clinical interest and suggest that the components of abdominal fat, as measured by US, may play at least in part independent roles in the development of cardiometabolic risk factors. Cohort studies are needed to test whether changes in US-measured VAT and SAT are associated with changes in the CMD risk profile.*"
Reviewer 2 (Dr Ian J Neeland)

Major Compulsory Revisions

Q1. This paper focuses on the outcomes of metabolic syndrome, uric acid, and liver markers. The authors do not provide sufficient justification as to why these 3 specific outcomes were chosen in comparison with other potential outcomes. Was this a post-hoc data derived choice as to what outcomes most closely tracked with VAT and SAT?

No, it was not a post-hoc data derived choice. Our outcomes (glucose, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, alanine transaminase, gamma-glutamyl-transferase and uric acid) were chosen before performing the analysis because we considered them the most relevant biomarkers associated to metabolic syndrome and NAFLD. We routinely measure such metabolic parameters in all subjects we see on the first visit. Never the less, we recognize that it could be interesting to study the association with some other important risk factors, such as insulin, hemoglobin glycated and inflammatory parameters.

In the light of this, we added the following sentences in the discussion as a limitation of the study (Lines: 277-283)

“Third, our outcomes (glucose, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, alanine transaminase, gamma-glutamyl-transferase and uric acid) were chosen because we considered them the most relevant biomarkers associated to metabolic syndrome and NAFLD. Never the less, we recognize that it could be interesting to study the association with some other important risk factors, such as total and LDL cholesterol, insulin, hemoglobin glycated and inflammatory parameters in particular stratifying for obesity degree”

Q2A. The main conclusions that a specific adiposity marker such as VAT or WC predicts the outcome better than other markers is no sufficiently justified either. The only comparison here uses BIC, which if the data are examined closely, are all in the same relative range.

BIC is to be interpreted in absolute rather than in relative terms (1). This is a strength of BIC as the absolute delta(BIC) is guaranteed to be the same across different BIC metrics. The delta(BIC) that we used in the present study is the one suggested by Raftery (2). The reason why we chose BIC as information criterion for this study is simply that it penalizes severely for the number of predictors. Therefore, BIC is guaranteed to choose the simplest model whenever possible. However, according to the request number Q2B of this Reviewer, we now report other measures of model fit and predictive power (see below).

(1) Long JS & Freese J. Regression models for categorical dependent variables using Stata. College Station, TX: Stata Press, 2014
(2) Raftery AE. Bayesian model selection in social research. Sociological methodology 1995;25:111-164

Q2B. Furthermore, these are modeled separately and the most informative method to compare their relative strengths for prediction of the outcome would be to
incorporate them all into the same model and examine the Chi Square or R-squared values for each independent variable.

We thank the reviewer for the suggestion to incorporate WC and US-measurements into the same model. However, our multivariable models were pre-specified and were aimed at evaluating the relative association of WC vs. VAT vs. SAT vs. VAT + SAT with the outcomes of interest. Sex and age were used as covariates because of their known association with the outcomes of interest. We believe that this pre-specified hierarchy of models is the one needed to answer the specific study question. Incorporating WC, VAT and SAT in the same model would not answer the question that led to this analysis in the first place. Another problem with the present study population is that WC and VAT are highly collinear. As detected by Spearman’s rho, the correlation of WC with VAT is in fact 0.80 ($p < 0.001$, $n = 2414$) and a formal analysis of collinearity using the Belsley-Kuh-Welsch criteria shows a condition number greater than 30, discouraging the use of WC and VAT in the same model. Incidentally, centering on means (or medians) did not solve the problem.

We completed the statistical analysis including the R-squared values for each independent variable. Because all our outcomes are dichotomous (high triglycerides, low HDL, high blood pressure, high glucose, MS, high uric acid, high ALT and high GGT), we suppose that the “$R^2$” referred to by the Reviewer is actually a “pseudo $R^2$” (1). We have chosen to report McFadden pseudo-$R^2$ for the reasons stated here: http://statisticalhorizons.com/r2 logistic.

(1) Long JS & Freese J. Regression models for categorical dependent variables using Stata. College Station, TX: Stata Press, 2014

Q2C. Alternatively, standardized beta coefficients may be used to compare relative contribution to the outcome.

We would be pleased to use standardized regression coefficients if our models were apt for them (e.g. http://www.biomedcentral.com/1471-230X/6/33). Unfortunately, our models are not apt for them because they include a dichotomous predictor (sex). Such dichotomous predictor makes uninterpretable not only the standardized regression coefficient associated with it but also the standardized regression coefficients associated with the other predictors (http://www.statalist.org/forums/forum/general-stata-discussion/general/655772-standardized-regression-coefficients-with-categorical-independent-variables).

We did not include in the manuscript the above-described considerations for reasons of space, but if this reviewer or the editor should consider this advisable, we can insert a couple of paragraphs in the methods or in the discussion.

Q2D. Just using a 10 unit difference in BIC where the absolute values are in the 2000 range does not substantiate the authors conclusions in this reviewer’s viewpoint.

Agreed, we now take into account the combination of the three statistical parameters (BIC, PSEUDO-$R^2$, AUC-ROC). If $\Delta$BIC < 10, and PSEUDO-$R^2$ and AUC-ROC were not different, we defined the models similar associated to the outcome considered.
We reported the following sentence in the statistical section (Lines: 150-151)

“If ΔBIC < 10, and PSEUDO R² and AUC-ROC were not different, we defined the models similar associated to the outcome considered”

Q2E. Additional metric such as the C-statistic for MS and the Hosmer Lemeshow test for model calibration would add more robust information to support their conclusions.

According to the request of this Reviewer, we now give in Appendix 1 the areas under the ROC curve (AUC-ROC), which correspond to Harrell’s C statistic. We now report the results of the procedure for testing goodness of fit (GOF) presently suggested by Hosmer & Lemeshow (1).


Q3. The authors state that their study population is “at-risk” and different compared with other studied population, potentially explaining divergent results. However, the authors do not describe what “at-risk” means and how this is defined. One could argue that everyone is "at-risk" for cardiometabolic disease.

We agree with the Reviewer for the comment. In order to avoid potential misunderstandings, we have removed the term “at-risk”.

The new title is:
“Metabolic correlates of subcutaneous and visceral abdominal fat measured by ultrasonography: a comparison with waist circumference”

Q4. The differential relationships between adiposity markers and outcomes among the obese vs. non-obese are not sufficiently explored. A secondary analysis stratifying by this important variable would be of interest.

We thank the Reviewer, that this is very interesting question. Unfortunately, it is unlikely that it can be answered in the present population, at least using conventional regression techniques. The reason is the very high association between WC and BMI (Spearman's rho = 0.88, p < 0.001, n = 2414). More formally, there is a very high collinearity (condition number much greater than 30, Belsley-Kuh-Welsch test) between BMI and WC that discourages their use in the same models. Incidentally, centering on means (or medians) did not solve the problem. This is what we regularly find in samples selected at tertiary care centers such as ICANS. We suggest that this relationship should be evaluated in samples from the general population where collinearity is expected to be low (e.g. http://www.biomedcentral.com/1471-230X/6/33).

Therefore we added in the limitations of the study that an analysis stratifying by obesity could reveal further insights in suitable samples drawn from the general population.

The following sentence has been included in the limitation of the study (Lines: 280-283).

“Never the less, we recognize that it could be interesting to study the association with some other important risk factors, such as total and LDL cholesterol, insulin, hemoglobin glycated and inflammatory parameters in particular stratifying for obesity degree”
Q5A. What clinical relevance do these relationships have to determine the outcome of laboratory values and MS (which is based on laboratory values). Why would the clinician want to measure US based VAT or SAT to determine risk of having elevated uric acid or GGT when they could just as easily measure the actual laboratory values?

The Reviewer is absolutely right that this would be a silly enterprise. We used the term “predictor” mostly in its statistical meaning, that is the “X”(s) in Y = f(X_1, X_2, ... X_n) but because this generated confusion, we now use the term “association” instead of “prediction” throughout all the manuscript.

Q5B. Furthermore, this is only cross-sectional data and no statements can be made about predicting future outcomes or disease states.

Please see our reply to Q5A. We now recognize this as a study limitation (Lines: 273-277).

“Second, this is a cross-sectional study. There is a general need of a cohort study aimed at evaluating the association of VAT and SAT changes with CMD risk changes. A large cross-sectional study with carefully standardized measurements of VAT and SAT, such as the present one, may help to plan such a study.”

Q5C. The relevance of this study as it pertains to biological questions vs. clinical questions should be clarified.

Agreed, we clarify this issue in the conclusion section (Lines: 295-297)

“These data are of clinical interest and suggest that the components of abdominal fat, as measured by US, may play at least in part independent roles in the development of cardiometabolic risk factors”

Q6. In the Supplementary data, SAT is generally (with the exception of BP and MS) not independently associated with the outcomes when VAT is included in the model. The possible reasons for this finding was not discussed in the paper.

We believe that the association of SAT with MS is mostly due to its association with high blood pressure. Explaining why SAT was independently associated with high blood pressure in causal terms is daunting because of the cross-sectional nature of the study (see our reply to Q5B). We prefer to suspend the judgment until cohort studies are performed. We are currently following a cohort of patients with the aim of evaluating the relationship of VAT and SAT changes with CMD risk changes.

We rephrased the discussion about the association between SAT and blood pressure (Lines: 213-218).

“An association between VAT and hypertension has been reported by most [2, 6, 20, 25, 26] but not all [4, 27] studies. The existence of an association between SAT and hypertension is more controversial. We found a greater association with high blood pressure for VAT than for SAT. In agreement with previous studies [2, 6, 27], VAT and SAT were independently associated with high blood pressure. This is of limited practical
utility, however, because WC, which is simpler to measure, was associated with high blood pressure more strongly than was VAT.

Q7. In this reviewer’s opinion, Figure 1 does not contribute much important information. A more informative figure might be the iterative ROC curves for MS using known criteria with the addition or in comparison to adiposity markers.

Figure 1 was deleted. We now give the ROC-AUC values for each model in the Appendix.

Minor Essential Revisions:

Q8: Page 11, 1st paragraph: "In partially agreement" should be "in partial agreement with".

We have rewritten the paragraph.
Reviewer 3: (Dr Hanen Samouda)

Comments to the Authors
A very important issue, related to the accurate and simple measurement of visceral adiposity and its relationship with cardiometabolic risk factors, was raised by the Authors in this study. The question posed by the authors is well defined. The methods are appropriate and well described, and sufficient details are provided to replicate the work. Data sound and seems well controlled. Figures appear to be genuine. The manuscript adhere to the relevant standards for reporting and data deposition. Discussion is well balanced and adequately supported by the data. I have only some useful comments.

We thank the Reviewer for the comment

Minor Essential Revisions:
Q1 The abstract seems to do not highlight the principal aims of the study, which is, as cited by the Authors in the paper on pages 3-4, lines 55-63:
1. To evaluate if VAT, as assessed by ultrasonography, is a more accurate predictor of several cardiometabolic risk factors than WC in adults, and;
2. To evaluate if US-SAT, associated to US-VAT, may improve this prediction.

Agreed, we have rephrased the aims of the study in the abstract (Lines: 22-25).

“The aim of this study was to evaluate whether dissecting abdominal fat in VAT and SAT using US may detect stronger and more specific association with MS, MS components, hyperuricemia and altered liver enzymes compared to waist circumference.”

Q2 This seems to be also the case for the conclusion (page 14, lines 259-264) and the title, as well. Title, abstract and conclusion should be rephrased.

Agreed, we have also rephrased the title and the conclusion of our study.

Title: “Metabolic correlates of subcutaneous and visceral abdominal fat measured by ultrasonography: a comparison with waist circumference”

Conclusion: (Lines: 291-299)

“In conclusion, US-determined VAT and SAT are both independently associated with MS. VAT is associated to all of the MS components in addition to hyperuricemia and altered liver enzymes, and performs equally or better than WC except for high blood pressure and low HDL. In contrast, SAT is independently associated only with high blood pressure and high ALT. These data are of clinical interest and suggest that the components of abdominal fat, as measured by US, may play at least in part independent roles in the development of cardiometabolic risk factors. Cohort studies are needed to test whether changes in US-measured VAT and SAT are associated with changes in the CMD risk profile.”

Q3 In perspective (page 14, lines 265-266), the Authors underline the importance to conduct longitudinal studies in order to follow the evolution overtime of VAT, SAT
and their potential relationship with cardiometabolic abnormalities, which is a very good point. However, please link this perspective with the measurement of abdominal adipose tissue by ultrasonography

Agreed, we have rephrased the sentence (Lines: 297-299)

“Cohort studies are needed to test whether changes in US-measured VAT and SAT are associated with changes in the CMD risk profile”.