Letter to the Editor

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Antioxidant capacity in patients with type 2 diabetes: a preliminary investigation on gender-specific differences in an Italian population

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To the Editor,

Men and women differences are important in epidemiology, pathophysiology and treatment of type 2 diabetes mellitus (DM2). On the other hand, international position statements on diabetes care do not provide specific recommendations for females, except in the case of pregnant women [1].

This is staggering if we think that women with DM2 carry 40% greater relative risk of coronary heart disease compared with men. The full explanation of the increased cardiovascular risk in diabetic women is far from being completely understood and many biological factors could be involved, like oxidative stress and antioxidant status.

Chronic inflammation, together with oxidative stress, is a pivotal factor in the development of DM2 and the progression of vascular complications [2]. Moreover, in DM2, a specific impairment in antioxidant capacity has been well described [3]. It is important to go in depth in the association between gender and oxidative stress because oxidative stress is crucial in many diseases that differently affect males and females, DM2 and atherosclerosis in particular. Recently, gender differences in antioxidant enzyme subunits' expression have been described, together with a different activity of these enzymes, between males and females [4]. Although these studies have been mainly performed on animal or *in vitro* models, it is now clear that estrogen may not be the only reason for the differences between males and females, and that a number of other mechanisms may play a role in this issue, especially for cardiovascular disease [4].

No study has investigated the gender differences in oxidative stress and antioxidant capacity of patient with DM2, in which cardiovascular disease, atherosclerosis and oxidative stress are strongly connected. We therefore aimed to assess gender-specific differences in markers of oxidative stress and antioxidant capacity, in a population of subjects with DM2 attending for routine care.

This study involved 56 consecutive patients with DM2 (36 males and 20 females) attending the Laboratory Unit of the University Hospital of Padua (Italy), between January and June 2012, for routine blood sampling. Forty-one healthy volunteers (24 males and 17 females) free of cardiovascular disease, none of whom referred history of significant illness, were also recruited in the same period as controls. DM2 patients without history of coronary heart disease, proliferative diabetic retinopathy, clinical diabetic neuropathy and chronic renal failure were recruited for this study. The information on medical history, treatments and dietary habits were recorded by a questionnaire completed by the patients. Patients or healthy subjects who reported to take any nutraceutical supplements in the previous 6 months were excluded from the study.

The study was approved by our Local Human Studies Committees and has been performed in accordance with the ethical standards of Declaration of Helsinki. Informed consent was obtained from all participants.

Plasma glucose and lipid profiles (total, high-density lipoprotein, low-density lipoprotein cholesterol and triglycerides) were measured using automated assays on Cobas 8000 (Roche Diagnostics, Mannheim Germany).

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Glucose was measured in plasma samples (sodium fluoride) using an enzymatic (hexokinase) assay [5].

HbA_{1c} was measured in whole blood samples with the high-performance liquid chromatography (HPLC) procedure (Adams HA-8180 Arkray, Kyoto, Japan). Inter-assay variation coefficient was 1.36%, whereas intra-assay was 1.03%, calculated using the National Glycohemoglobin Standardization Program values (%). This method for measuring HbA_{1c} was aligned with IFCC standardization [6].

Superoxide dismutase (SOD) was determined by Cayman Chemical Item no. 706002, using a colorimetric test that measures all three types of SOD (Cu/Zn, Mn and FeSOD), with an intra-assay coefficient of variation (CV) of 3.2% and interassay CV of 3.7%; this method was used either for catalase (CAT) determination (intra-assay CV of 3.8%, interassay CV of 9.9%). Concentrations of malondialdehyde (MDA) and glutathione (GSH) were detected by HPLC chromosystem procedure (intra- or interassay CV <3%/<9% and <6%/2.6%–10.8%, respectively) [7].

Vitamin E concentrations were measured by high-precision chromatography in a reverse-phase column using a diode array spectrophotometric detector, according to Laidman and Hall (intra- or interassay CV <9.5% and <13.6%) [8].

The sample size was determined by using the general formula suggested for pilot studies, when effect size of a quantitative variable is unknown [9]. Quantitative variables are expressed as mean ± SD or median and interquartile range, as appropriate. The normality of distribution of the considered variables was evaluated with Skewness and Kurtosis measures. Student's t-test, for paired or unpaired data and Wilcoxon singed rank test were applied, as appropriate. A Pearson's correlation analysis was attempted to evaluate the presence of any interrelationships between continuous variables.

Clinical features of patients with DM2 and healthy subjects are summarized in Supplementary Table 1. The questionnaire analysis did not show significant differences between males and females in relation to dietary habits and average amount of physical exercise.

Patients with DM2, considered as a whole, showed a reduction in plasma concentration of all markers of antioxidant capacity, namely, enzymes like SOD and GSH, and Vitamin cofactor E, in comparison with healthy subjects. Indeed, MDA level, and indicator of oxidative stress, was higher in patients with DM2.

As shown in Table 1, among patients with DM2, female subjects differed by male counterparts only for fasting plasma glucose, whereas MDA concentration and markers of antioxidant capacity appeared not to be statistically different. Moreover, glycemic control as expressed by HbA_{1c} was overlapping between female and male.

No gender-specific differences emerged between male and female in healthy population, regarding markers of antioxidant capacity.

Pearson's analysis showed an inverse correlation between aging and concentrations of SOD and CAT in patients with DM2, whereas no significant association has been reported for other antioxidant markers, both in males and females (Supplementary Table 2). These correlations were closer in female group (Figure 1A). Otherwise, HbA_{1c} showed no significant correlations with any antioxidant markers in patients with DM2.

Also in healthy subjects, a close association between aging and concentrations of SOD and CAT was confirmed, even then more in the female subgroup (Figure 1B).

Biological, hormonal and sociobehavioral factors that distinguish males and females also influence their

Table 1: Differences in demographic and clinical markers between female and male subjects, considered in the two groups.

	Healthy		DM 2	
	Females (n = 17)	**Males (n = 24)**	**Females (n = 20)**	**Males (n = 36)**
Age, years	40.57 ± 20.90	44.96 ± 14.91	71.75 ± 12.47	68.62 ± 11.33
BMI, kg/m^2	23.43 ± 2.92	25.88 ± 4.02	28.92 ± 4.18	30.58 ± 6.16
Fasting glucose, mmol/L	4.33 ± 0.47	5.11 ± 0.90	7.77 ± 1.54	9.42 ± 3.05[a]
HbA_{1c}, %	5.13 ± 0.53	5.39 ± 0.44	7.30 ± 1.14	7.23 ± 1.27
HbA_{1c}, mmol/mol	33.0 ± 3.4	35.0 ± 2.9	56.0 ± 8.8	56.0 ± 9.8
MDA, μmol/L	0.18 ± 0.08	0.17 ± 0.08	0.26 ± 0.15	0.27 ± 0.21
GSH, μmol/L	87.14 ± 34.65	80.46 ± 30.89	69.45 ± 20.96	72.87 ± 30.92
SOD, U/mL	2.19 ± 0.56	2.23 ± 0.38	1.52 ± 0.28	1.56 ± 0.26
CAT, nmol/min/mL	24.84 ± 6.57	25.32 ± 4.40	13.66 ± 3.28	14.61 ± 3.16
Vitamin E, μmol/L	26.56 ± 4.47	30.96 ± 8.63	18.30 ± 9.37	18.83 ± 9.62

Data are presented as mean ± SD; Student's t-test for unpaired data. [a]p-value <0.01.

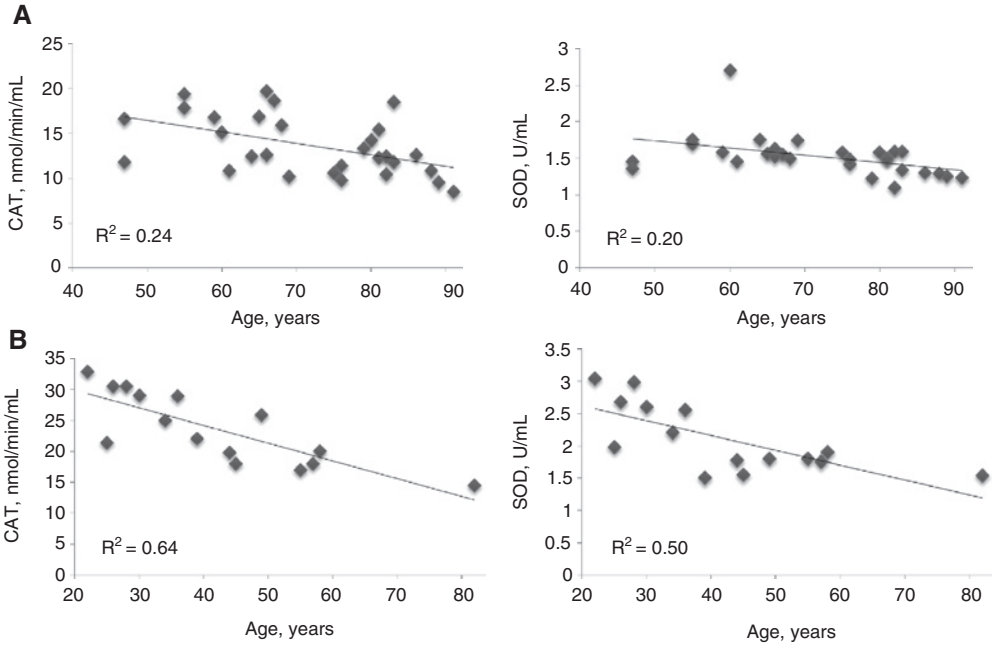

Figure 1: Scatter plots of correlation between aging and markers antioxidant capacity (SOD, CAT) in females.
(A) With DM2 and (B) healthy females. Aging was inversely associated with serum markers of antioxidation in both groups. R^2, is Pearson's coefficient. All correlations were statistically significant (p-value < 0.05).

different tendency to be enrolled in clinical trials. Probably because of poorly studied factors, such as the oxidative-antioxidant balance, different responses between genders to the same prevention strategies or pharmacological treatments are often observed. For this reason, descriptive studies that provide data on gender differences are very important.

This study reported for the first time an assessment on gender-specific differences in markers of antioxidant capacity, both in patients with DM2 and healthy subjects. We did not observe any significant difference between males and females with regard to all these biochemical markers in this population.

On the other hand, we noted a very close relationship between reduced antioxidant capacity and aging in non-diabetic women. Interestingly, this is in agreement with the observations of an increased cardiovascular risk in young women not yet overtly diabetic [10].

This might lead one to suppose that reduction of markers of antioxidation, which have a pivotal role in offsetting the negative effect of oxidative stress on cardiovascular risk, is an early event and more pronounced in females. In addition, we observed that even in well-controlled patients with DM2, aging results in a severely impaired antioxidant capacity.

The lack of correlation between antioxidant markers and HbA_{1c}, already suggested by other authors [11], could in part depend on the fact that our DM2 patients were in good glycemic control and were free of major complications. Overall this suggests that, even in well-controlled DM2 patients, aging may determine an impaired antioxidant capacity in the long term, by means of other potential factors like advanced glycation end products (AGEs). Accumulation of these products, mainly influenced by diet and lifestyle, is closely related with aging, and it is linked with a number of chronic diseases, including cardiovascular disease [5].

Although our data do not suggest a gender-specific difference in the antioxidant capacity, we could not exclude that early impairment of antioxidant capacity may lead to an imbalance between oxidation and antioxidation that, along with other female-specific factors, could determine this increased risk.

Our study, as well as observational and "real-life", is vitiated by a low sample size and a cross-sectional design, which prevents drawing any cause-effect relationship. Because the recruitment of subjects was performed consecutively in a population of routine care, it was not possible to obtain an appropriate matching by age between healthy subjects and patients with DM2. On the other hand, when splitting the two groups by gender – which was the aim of our study – males and females were age comparable.

For these limitations, further prospective observational studies involving a larger number of subjects are needed.

Nonetheless, we considered several parameters of antioxidation and have for the first time assessed gender-specific differences in antioxidant capacity in patients with DM2.

Our observations suggest a role of aging in the impairment of antioxidant capacity in patients with DM2, both in males and females. Future research is needed to establish aging-related factors, such as AGEs, in order to suggest specific interventions aimed to improve antioxidant capacity in these patients.

Authors' contributions: NCC analyzed the data, performed statistical analysis and wrote the manuscript; CC collected and analyzed data; SB revised the manuscript; MP revised the manuscript; and AL analyzed data and revised the manuscript. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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References

1. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care 2012;35:1364–79.
2. Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA, et al. Inflammatory disease processes and interactions with nutrition. Br J Nutr 2009;101(Suppl 1):S1–45.
3. Chilelli NC, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: a "glycoxidation-centric" point of view. Nutr Metab Cardiovasc Dis 2013;23:913–9.
4. Kander MC, Cui Y, Liu Z. Gender difference in oxidative stress: a new look at the mechanisms for cardiovascular diseases. J Cell Mol Med 2017;21:1024–32.
5. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, et al. Tests of glycemia in diabetes. Diabetes Care 2004;27:1761–73.
6. Mosca A, Lapolla A, Gillery P. Glycemic control in the clinical management of diabetic patients. Clin Chem Lab Med 2013;51:753–66.
7. Hendricks T, Assmann RF. On the fluorimetric assay of circulating lipoperoxides. Clin Chim Acta 1998;174:263–70.
8. Laidman DL, Hall GS. Adsorption column chromatography of tocopherols. In: McCormick DB, Wright LD, editors. Methods in enzymology part C, vol 18. New York: Academic Press, 1971:349–56.
9. Viechtbauer W, Smits L, Kotz D, Budé L, Spigt M, Serroyen J, et al. A simple formula for the calculation of sample size in pilot studies. J Clin Epidemiol 2015;68:1375–9.
10. Legato MJ, Gelzer A, Goland R, Ebner SA, Rajan S, Villagra V, et al. Gender-specific care of the patient with diabetes: review and recommendations. Gend Med 2006;3:131–58.
11. Carmeli E, Coleman R, Berner YN. Activities of antioxidant scavenger enzymes (superoxide dismutase and glutathione peroxidase) in erythrocytes in adult women with and without type II diabetes. Exp Diabesity Res 2004;5:171–5.

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