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Glutathione levels in patients with erectile dysfunction, with or without diabetes mellitus

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Summary

The reduced form of glutathione (GSH) is the most important cell antioxidant and is also an essential cofactor for nitric oxide (NO) synthase that synthesizes NO from L-arginine. Reduced levels of GSH, due both to a hyperglycaemia-induced increase of free radical production and to a decrease of NADPH levels [like in diabetes mellitus (DM)], can hamper the endothelial cell functions. This condition may play an important role in the aetiology of some clinical signs, like erectile dysfunction (ED). The aim of this study was to test the hypothesis that GSH concentration is reduced in patients with ED and type 2 diabetes mellitus. We studied 111 male patients with ED: 64 with diabetes (ED/DM) and 47 without diabetes (ED/wDM); 20 patients with diabetes but without ED (DM) and 26 male normal subjects as a control group (C). The GSH red blood cell concentration was significantly lower in ED than in C (X ± SD; 1782.12 ± 518.02 vs. 2269.20 ± 231.56 μmol/L, $p < 0.001$). In particular, GSH was significantly reduced in ED/DM vs. ED/wDM (1670.74 ± 437.68 vs. 1930.63 ± 581.01 μmol/L, $p < 0.01$). In DM, GSH was significantly lower than in C and significantly higher than in ED/DM (2084.20 ± 118.14 vs. 2269.20 ± 231.56 and vs. 1670.74 ± 437.68 μmol/L, $p < 0.002$ and $p < 0.001$ respectively). GSH showed a negative correlation with fasting glucose concentrations ($r = -0.34$, $p < 0.01$) and with the duration of DM ($r = -0.25$, $p < 0.05$). A GSH depletion can lead to a reduction of NO synthesis, thus impairing vasodilation in the corpora cavernosa.

Keywords: erectile dysfunction, glutathione, nitric oxide, type 2 diabetes mellitus

Introduction

Upon sexual stimulation, nerve impulses cause the release of cholinergic and non-cholinergic non-adrenergic neurotransmitters that mediate erectile function by relaxing the smooth muscle of the corpora cavernosa (Lue, 2000; Ziegler, 2002). The principal neural mediator of erection is nitric oxide (NO), a potent vasodilator and inhibitor of platelet adhesion and aggregation. Reduced levels of NO may contribute to vascular alteration facilitating platelet aggregation and decreasing endothelium-dependent vasodilation. NO is produced by NO synthase from L-arginine. Glutathione (GSH) is an essential cofactor for NO synthase (Snyder, 1992; Ghigo *et al.*, 1993).

Diabetes mellitus (DM) has been associated with erectile dysfunction (ED). The generation of reactive oxygen species

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(oxidative stress) and the reduction of antioxidant defence in diabetes may play an important role in the aetiology of this complication (Maas *et al.*, 2002; Maritim *et al.*, 2003). Hyperglycaemia may increase the generation of free radicals through glucose oxidation, lipid peroxidation, polyol pathway activation, prostanoid synthesis, non-enzymatic protein glycation, and subsequent oxidative degradation of glycated proteins. Furthermore, exposure of endothelial cells to high glucose leads to augmented production of superoxide anion, which may quench NO (Giugliano *et al.*, 1996). The antioxidant defence mechanisms involve both enzymatic and non-enzymatic strategies. Common antioxidants include vitamins A, C, E, GSH and the enzymes superoxide dismutase, catalase, glutathione peroxidase and reductase. GSH is an electron donor and is able to reduce and to scavenge free radicals; in the reduced form GSH is the most important cell antioxidant (Kidd, 1997). Reduced levels of GSH, due both to a hyperglycaemia-induced increase of free radical production and to a decrease of NADPH levels (like in DM), can impair the endothelial cell functions.

Aim of this study was to test the hypothesis that erythrocyte reduced GSH concentration is impaired in patients with ED and type 2 diabetes mellitus.

Materials and methods

Subjects

We studied 111 men with ED: 64 type 2 diabetics (ED/DM) and 47 non-diabetic patients (ED/wDM) (mean age ± SD: 52.28 ± 9.95 vs. 55.20 ± 5.70 years); 20 type 2 diabetics (DM) without sexual dysfunction (51.45 ± 2.60 years) and 26 healthy men without ED or hypertension or DM and normal BMI, who were evaluated as a control group (C) (52.10 ± 7.10 years).

The inclusion criteria were: (i) age below 70 years; (ii) married or living a stable relationship for 6 months or more.

The exclusion criteria were: (i) concurrent endocrine disorders other than diabetes, hypertension and/or dyslipidaemia, or surgical procedures likely to impair sexual function; (ii) intake of beta blockers, diuretics or other drugs which have been shown to have deleterious effects on erectile function (Foresta *et al.*, 2004); (iii) alcoholism or other substance abuse; (iv) phosphodiesterase type 5 inhibitors intake and/or intracavernous prostaglandin E1 administration at study admission.

All subjects gave their written informed consent to participate in the study.

Procedures

The patients underwent clinical and diagnostic evaluations depending on their specific disorder (Krane *et al.*, 1989; NIH Consensus Conference, 1993; Chun & Carson, 2001): physiological, pathological, pharmacological history and in particular sexual and ED history were collected. Penile colour Doppler ultrasonography was performed in selected patients (Foresta *et al.*, 2004). Prolactin, TSH, total and free testosterone, HbA1c and fasting glucose and GSH concentrations were evaluated in all 157 subjects.

HbA1c was measured by high pressure liquid chromatography Autoanalyzer (Auto A1c-HA-8121; Menarini, Florence, Italy); the normal range was 3.9–5.1%; the coefficient of variation was 1.4%. Plasma glucose was measured by a glucose oxidase method (Beckman, Palo Alto, USA). The normal values of fasting plasma glucose are 70–110 mg/dL.

Testosterone levels were evaluated by RIA (DIRIA-TESTOK Diasorin, I); sensitivity was 0.05 ng/mL; intra- and inter-assay CVs were 7.2 and 12.2%, respectively; normal values are 3–10 ng/mL.

For dyslipidaemia we considered the American Association of Clinical Endocrinologists lipid guidelines (AACE Lipid Guidelines Committee. The American Association of Clinical Endocrinologists, 2000). Hypertension is defined as systolic blood pressure of 140 mmHg or greater, diastolic blood pressure of 90 mmHg or greater, or taking antihypertensive medication (The sixth report of the Joint National Committee, 1997). A patient was considered a smoker if he had smoked >1 cigarette/day for at least 1 year (Fedele *et al.*, 1998).

The method used for the determination of blood GSH concentration was described by Beutler *et al.* (1963) and is based upon the development of a relatively stable yellow colour when 5,5′-dithiobis-(2-nitrobenzoic acid, DTNB) is added to sulphydril compounds; 67 μL of whole blood were added to 333 μL of distilled water. One millilitre of precipitating solution (glacial metaphosphoric acid, EDTA, sodium chloride and distilled water) was mixed with the hemolysate. The mixture was centrifuged for about 5 min and 0.5 mL of surnatant was added to 0.75 mL of $Na_2HPO_42H_2O$. 0.25 mL of the DTNB solution was added and the optical density was measured at 412 nm. GSH was obtained commercially from Sigma and on the basis of a standard curve concentrations were expressed in μmol/L of blood; we divided these values by haematocrit and multiplied it by 100, to obtain the results in μmol/L of red blood cell. Normal values are between 2037 and 2500 μmol/L. Our laboratory normal range was calculated in 26 male blood donors attending the S. Giovanni Battista Hospital of Torino.

The presence and severity of ED were investigated by an abridged 5-item version of the 15-item International Index of Erectile Function (IIEF-5, normal values >21) (Rosen *et al.*, 1999).

Psycho-sexual diagnosis was made by psychological-sexual interview, including a semistructured questionnaire developed by the authors of this study, reported in the Appendix. Aim of this questionnaire is to obtain the patient's personality features and to contribute to the diagnosis of the sexual disease in order to choose among a: medical, sexological, psychological or psychiatric therapeutic approach (McCullough & Fine, 1999).

Statistical analysis

The results were expressed as mean ± standard deviation (SD) or in percentage terms and the statistical analysis was performed using the Student's *t*-test, the χ^2-test and Pearson linear correlation test; *p*-values <0.05 were considered significant. All test data, figures and tables are expressed as mean ± SD.

Results

The age of ED/DM and ED/wDM at the first andrological examination was not significantly different (52.28 ± 9.95 vs. 55.20 ± 5.70 years) and both groups presented the same duration of ED (4.02 ± 3.35 vs. 3.18 ± 1.88 years). The BMI was higher in ED/DM than in ED/wDM (29.16 ± 2.99 vs. 27.65 ± 3.35 kg/m^2, $p < 0.02$). The prevalence of arterial hypertension in ED was higher than in C (79 vs. 0%, $p < 0.001$) and in ED/DM was higher than in DM (78 vs. 50%, $p < 0.05$). No significant difference in lipid levels and smoking habits was recorded in the different groups. In ED/DM the IIEF-5 score was significantly lower than in ED/wDM (11.20 ± 5.50 vs. 13.80 ± 5.40, $p < 0.02$).

In ED/DM the duration of the disease (8.47 ± 3.39 years), the HbA1c (8.40 ± 1.36%) and fasting glucose concentrations (176.13 ± 41.30 mg/dL) were higher than in DM (4.05 ± 1.55 years, $p < 0.001$; 6.94 ± 1.16% $p < 0.001$; 150.60 ± 36.12 mg/dL, $p < 0.05$, respectively). These characteristics are outlined in Table 1.

The GSH concentration in ED was significantly lower than in C (1782.12 ± 518.02 vs. 2269.20 ± 231.56 μmol/L, $p < 0.001$). In particular, the GSH concentration in ED/DM was lower than in ED/wDM (1670.74 ± 437.68 vs. 1930.63 ± 581.01 μmol/L, $p < 0.01$). Moreover, the GSH concentration in DM was significantly lower than in C (2084.20 ± 118.14 vs. 2269.20 ± 231.56 μmol/L, $p < 0.002$). ED/DM showed a lower GSH concentration than DM (1670.74 ± 437.68 vs. 2084.20 ± 118.14 μmol/L, $p < 0.001$) (Fig. 1).

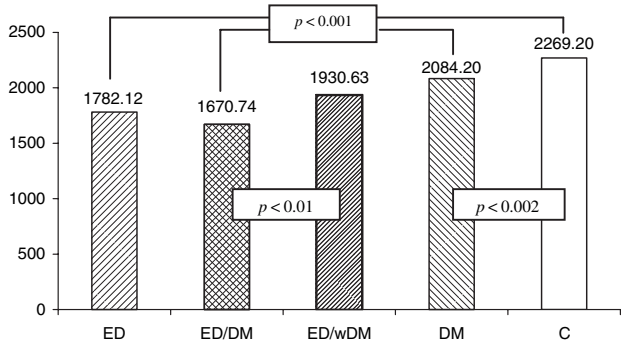

Figure 1. Red blood cell glutathione concentrations (μmol/L) in 111 men with erectile dysfunction (ED): 64 with diabetes mellitus (ED/DM) +47 without diabetes mellitus (ED/wDM); 20 diabetics (DM) and 26 normal subjects (C).

In all 84 diabetic patients, with and without ED, GSH showed a negative correlation with fasting glucose concentrations ($r = -0{,}34$, $p < 0.01$), and with the duration of DM ($r = -0.25$, $p < 0.05$).

Hormonal assessment excluded the presence of any endocrinological disease responsible for sexual dysfunction: prolactin, total and free testosterone and TSH were normal. No patients were taking medications that could affect the sexual function.

Discussion

The age of patients affected by ED with and without DM was not significantly different, nor was that of the control

Table 1. Clinical, biochemical and hormonal characteristics in: C, control group; DM, diabetes mellitus (diabetics without erectile dysfunction), ED: erectile dysfunction (all patients): with diabetes mellitus (ED/DM) and without diabetes mellitus (ED/wDM)

	C (26)	DM (20)	ED (DM + wDM) (111)	ED/DM (64)	ED/wDM (47)
Age (years)	52.10 ± 7.10	51.45 ± 2.60	53.96 ± 7.88	52.28 ± 9.95	55.20 ± 5.70
ED duration (years)			3.63 ± 2.79	4.02 ± 3.35	3.18 ± 1.88
BMI (Kg/m^2)	≤ 25	28.89 ± 2.91	28.81 ± 3.30	29.16 ± 2.99*	27.65 ± 3.35*
IIEF-5				11.20 ± 5.50†	13.80 ± 5.40†
DM duration (years)		4.05 ± 1.55‡		8.47 ± 3.39‡	
HbA1c (%)	4.05±0.64	6.94 ± 1.16‡	6.80 ± 0.52	8.40 ± 1.36‡	4.40 ± 0.52
Glucose (mg/dL)	87.23 ± 5.46	150.60 ± 36.12‡	143.13 ± 23.30	176.13 ± 41.30‡	89.15 ± 5.57
Testosterone (ng/mL)	5.88 ± 1.55	5.59 ± 1.94	5.50 ± 1.66	5.69 ± 1.58	5.24 ± 1.76
Dyslipidaemia (%)	0	55	55	61	47
Hypertension (%)	0	50§	79	78§	81
Smoking (%)	0	40	50	48	53

Data are expressed as X ± SD or percentage values.
Significant differences: *BMI in ED/DM versus ED/wDM: $p < 0.02$; †IIEF-5 score in ED/DM vs. ED/wDM: $p < 0.02$; ‡duration of the disease, HbA1c and fasting glucose concentration in ED/DM vs. DM: $p < 0.001$, $p < 0.001$ and $p < 0.05$ respectively; §prevalence of arterial hypertension in ED/DM vs. DM: $p < 0.05$.

group, making all groups comparable. No significant difference in the duration of sexual dysfunction at the time of first examination was found between diabetics and non-diabetics, indicating the difficulty that all patients experience in reporting this problem (Tiefer & Schuetz-Mueller, 1995; Dunsmuir & Holmes, 1996). In fact over 70% of cases of sexual dysfunction remains undiagnosed (Althof *et al.*, 1999). In patients with ED BMI is higher in diabetics than in non-diabetics (Bonadonna *et al.*, 1990). In the present study the prevalence of arterial hypertension was significantly higher in diabetics with ED than in those without sexual problems, as already described in many epidemiological studies (DeFronzo & Ferrannini, 1991; Hypertension in Diabetes Study, 1993; Feldman *et al.*, 1994; Johannes *et al.*, 2000). It is very difficult to separate DM from hypertension. These diseases can be associated to ED and are known to increase the risk of both microvascular and macrovascular complications. These conditions are also known to be associated with endothelial dysfunction; impaired NO formation may therefore be considered a key pathogenic mechanism in both endothelial and ED (Maas *et al.*, 2002). In diabetics without ED the prevalence of smokers was not significantly different from that in impotent diabetics. In accordance with previous studies, a longer duration of the disease, a poor metabolic control, as demonstrated by higher HbA1c percentage, and higher blood pressure values were present also in our diabetic patients with ED. In fact, these conditions are well known causes of ED in DM (McCulloch *et al.*, 1980; Klein *et al.*, 1996; Fedele *et al.*, 1998).

In this study, GSH in patients with ED is significantly reduced in comparison with the healthy control group. GSH is an essential cofactor for NO synthase and NO plays a pivotal role in the relaxation of human penile smooth muscle and penile erection (Angulo *et al.*, 2000). We hypothesize that low levels of this cofactor can contribute to ED by reducing NO production (Ghigo *et al.*, 1993). Recently, a new class of NO donors, such as S-nitrose-glutathione and S-nitrose-acetylcysteine-ethylester has been developed. Their use has been proposed in vascular disorders and conditions associated with malfunctions of endothelial cell NO production. S-nitrosothiols have been especially suggested to act as physiologic nitrinergic factors in the mammalian corpus cavernosum (Buyukafsar *et al.*, 1999) and to induce relaxation in the isolated human corpus cavernosum (Seidler *et al.*, 2002). S-nitrosothiol has been reported to induce vascular and penile trabecular smooth muscle relaxation even when a poor response to prostaglandin E1 is present (Angulo *et al.*, 2000).

Erythrocyte GSH is also significantly decreased in type 2 diabetic patients without sexual dysfunction compared with controls as shown in other studies (Murakami *et al.*, 1989; Seghrouchni *et al.*, 2002). This reduction is strongly evident in diabetics with a vascular complication, like ED and hypertension. A negative correlation between GSH and markers of metabolic control of diabetes like HbA1c has been shown (Giugliano *et al.*, 1996), suggesting an important role for hyperglycaemia (Jain & McVie, 1994). A similar behaviour is described in platelets (Thomas *et al.*, 1986) and plasma (Samiec *et al.*, 1998) while increased GSH levels have been recently described in penile cavernosal tissue from patients with ED and DM (Tuncayengin *et al.*, 2003). Indeed in hyperglycaemic conditions, glucose is preferentially used in the polyol pathway (Greene *et al.*, 1987; Lee & Chung, 1999) that depletes NADPH necessary for GSH regeneration through the GSH-Red enzyme. Hyperglycaemia is therefore the indirect cause of GSH depletion. As GSH is an important antioxidant molecule, its depletion leads to the increase of oxidative stress (Seghrouchni *et al.*, 2002). Decreased GSH levels in DM may be also caused by a limited regeneration of oxidized glutathione (GSSG) due to the increased consumption of NADPH in the polyol pathway. GSSG is actively pumped out from the erythrocyte leading to a decreased level of GSH. GSH levels are higher in well controlled than in poorly controlled DM, suggesting a prominent role of glycaemic control on the antioxidant system in diabetics (Dincer *et al.*, 2002). Thus, complications in patients with type 2 diabetes have been linked to oxidative stress and impaired antioxidant defence (Zaltzberg *et al.*, 1999). Furthermore, our previous data demonstrated that the administration of GSH is able to improve platelet cNOS activity in type 2 diabetics (Martina *et al.*, 2001). There are also evidences that in experimental diabetes low doses of the antioxidant (α-lipoic acid and γ-linolenic acid) improve NO-mediated neurogenic and endothelium-dependent relaxation of corpus cavernosum (Keegan *et al.*, 2001) and that vitamin E combined with sildenafil enhances erectile function in rat with streptozotocin-induced diabetes (DeYoung *et al.*, 2003). Vitamin E has been shown to reduce oxidative stress and its potential benefit within diabetic population has been supported.

In conclusion, our data provide evidence that red blood cell GSH is lower in patients with ED and even lower when associated with poorly controlled diabetes. This work suggests that GSH administration is a possible complementary therapeutic approach to the treatment of male ED, especially in case of failure of other pharmacological therapies, such as phosphodiesterase type 5 inhibitors. These inhibitors are frequently used in the treatment of ED in diabetic patients, although the ED is dependent on psychological, vascular, neurological, and local penile aetiologies. For this reason, chemoprevention methods including NO precursors and free radical scavenger administration (Costabile, 2003) have been proposed.

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Appendix: Questionnaire for Psychosexual Interview

1. Sexual desire: Yes No
   Level of sexual desire: low/moderate/high
   Realistic: Yes (oriented towards: female/male/a specific person) No
   In case you resolve your sexual disease do you think your sexual desire would increase?
   Yes (oriented towards: female/male/a specific person) No
2. Erectile dysfunction: Yes No
   Lifelong/Acquired/Situational
   Able to penetration: Yes No
   Able to maintain erection: Yes No
3. Spontaneous nocturnal erections: Yes No
   Erections with sexual stimulation (pictures, movies, fantasy etc.): Yes No
4. Ejaculation: Yes No (retrograde/anejaculation)
   Premature: Yes (lifelong/acquired/situational) No
   Delayed: Yes (lifelong/acquired/situational) No
5. Other sexual disorders
6. Masturbation: Yes (individual with partner) No
   Ability to achieve/maintain valid erections: Yes No
   Frequency:
   Satisfaction: Yes No
7. Couple relationship:
   Frequency of sexual intercourse:
   Satisfaction: Yes No
   Contraception: Yes No
   Type of contraception:
   How do you perceive yourself? As a single/as a member of a couple

8. Quality of couple relationship:
9. Sexual intercourse outside the couple relationship
   Past: Yes No
   Present: Yes No
10. Homosexual orientation:
11. Educational level:
12. Social development:
13. Affective expression:
14. Mood:
15. Ability in managing real life situations:
16. Quality of sleep:
17. Anxiety: Yes (state/trait/performance anxiety) No
18. Depression: Yes No
19. Eating disorders
20. Hypochondriasis: Yes No
21. Psychiatric disorders: Yes No
    With admission to hospital (when, how, how long?)