Assessment of the redox status in patients with metabolic syndrome and type 2 diabetes reveals great variations

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Abstract. The aim of the present study was to examine the effectiveness of a new redox status marker, the static oxidation reduction potential (sORP), for assessing oxidative stress in 75 patients with metabolic syndrome (MetS) and type 2 diabetes (T2D). A total of 35 normal subjects were used as the controls. Moreover, conventional markers of oxidative stress were assessed, such as thiobarbituric acid reactive substances (TBARS), protein carbonyls, the total antioxidant capacity (TAC), glutathione (GSH) levels and catalase (CAT) activity in erythrocytes. The results revealed that sORP was significantly higher (by 13.4%) in the patients with MetS and T2D compared to the controls, indicating an increase in oxidative stress. This finding was also supported by the significantly lower levels (by 27.7%) of GSH and the higher levels (by 23.3%) of CAT activity in the patients with MetS and T2D compared to the controls. Moreover, our results indicated a great variation in oxidative stress markers between the different patients with MetS and T2D, particularly as regards the GSH levels. Thus, the patients with MetS and T2D were divided into 2 subgroups, one with low GSH levels (n=31; GSH <3 \( \mu \)mol/g Hb) and another with high GSH levels (n=35; GSH >4 \( \mu \)mol/g Hb). The comparison of the markers between the 2 subgroups indicated that in the low GSH group, the GSH levels were significantly lower (by 51.7 and 52.9%) than those in the high GSH group and the controls, respectively. Furthermore, sORP in the low GSH group was significantly higher (by 8.1%) compared to the high GSH group, suggesting its sensitivity for assessing oxidative stress in patients with MetS and T2D. Moreover, this variation in oxidative stress levels between the different patients with T2D suggests that the assessment of the redox status may be important in prediabetic conditions, since there is evidence indicating that differences in the redox status in pre-diabetes may result in different outcomes.

Introduction

Free radicals are products of normal metabolism, including reactive oxygen species (ROS) such as superoxide anion radical (O2•-), hydroxyl radical (OH•) and peroxyl radical (RO•2), and reactive nitrogen species (RNS), such as nitric oxide and the peroxynitrite radical (ONOO•) (1). Free radicals participate in several cellular functions, such as the regulation of signaling pathways and gene expression, and apoptosis (1,2). Endogenous sources of free radicals include the mitochondrial respiratory chain, inflammation, peroxisomes and cytochrome P450 (3). In addition, there are exogenous sources of ROS and RNS generation, such as smoking, air pollution, ultraviolet light and ionizing radiation (4). Free radicals are highly reactive species and can react with biological macromolecules (e.g., DNA, proteins and lipids), causing damage to these molecules (1). Living organisms have defense systems against free radicals, including antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and...
paraoxonase 1 (PON1), as well as non-enzymatic antioxidant compounds, such as glutathione (GSH), vitamins C and E, uric acid and ubiquinone (1). However, the overproduction of free radicals may lead to an imbalance in which the amount of ROS/RNS exceeds the antioxidant capacity, leading to oxidative stress associated with several pathophysiological conditions and diseases (1.5).

One of the pathophysiological conditions associated with oxidative stress is metabolic syndrome (MetS) (6). MetS is defined as a cluster of cardiovascular and type 2 diabetes (T2D) risk factors (7). MetS is diagnosed when a patient has at least three of the following risk factors: hyperglycemia, high blood pressure, high triglyceride levels, low high-density lipoprotein (HDL) cholesterol levels and obesity (7). There is evidence supporting the hypothesis that increased levels of oxidative stress may play an important role in MetS-related manifestations, including atherosclerosis and hypertension (8,9). Furthermore, oxidative stress is related with adiposity and insulin resistance in patients with MetS, suggesting that it is a crucial factor in the evolution of this pathological condition and not just a consequence (6,10,11).

As is already known, MetS may lead to the development of T2D, one of the most common metabolic disorders worldwide (12). T2D is characterized by hyperglycemia (i.e., high blood glucose levels) which occurs due to insulin resistance, that is, the cellular failure to respond normally to the insulin hormone (12). A number of studies have demonstrated that oxidative stress is associated with T2D, and particularly with its complications (12,13). In particular, some symptoms of T2D, such as hyperglycemia, insulin resistance and dyslipidemia induce oxidative stress through different mechanisms, such as increased advanced glycation end products (AGEs), inflammation, increased polyol pathway flux, increased hexosamine pathway flux and increased mitochondrial superoxide production (12-15). The increased levels of oxidative stress occurring in patients with T2D in turn aggravate some of the associated complications, particularly those involving the cardiovascular and neural system (12,14,16). Although the role of oxidative stress in diabetic complications has been established, its role as an etiological factor has not yet been fully elucidated (12,17).

Since oxidative stress is associated with MetS and T2D, its assessment in patients suffering from these disorders is useful for monitoring their progress and treatment, as well as for ameliorating the health-associated complications. Several biomarkers have been used for assessing oxidative stress levels in humans (18). However, the assessment of the redox status remains a time-consuming and impractical method in clinical settings, and thus there is a great need for developing new markers (19). In our previous studies, we measured a new marker, static oxidation reduction potential (sORP), in plasma using the RedoxSYS Diagnostic System for assessing oxidative stress induced by either physiological or pathophysiological conditions (20-23). sORP is the standard potential between a working electrode and a reference electrode with no driving current (or an extremely small current) which is proportional to the balance of reductants and oxidants and is what is classically termed ORP (i.e., a homeostatic parameter capturing the current balance of oxidants and reductants in a biological specimen). Low sORP values mean that the biological sample is in the normal range of oxidative stress, while higher than normal sORP values mean that the biological sample is in a higher state of oxidative stress.

The aim of the present study was to examine the effectiveness of sORP for assessing oxidative stress in patients having symptoms of both MetS and T2D. Moreover, conventional oxidative stress markers, such as thiobarbituric acid reactive substances (TBARS), GSH levels, CAT activity, protein carbonyl (CARB) levels and total antioxidant capacity (TAC) were measured in the blood of the patients in order to compare and correlate them with sORP.

Subjects and methods

Subjects. A total of 75 adult subjects manifesting both MetS and T2D, as well as 35 normal subjects participated in the present study. All experimental procedures were performed in accordance with the European Union Guidelines laid down in the 1964 Declaration of Helsinki and were approved by the Institutional Review Board of the University of Thessaly (Larissa, Greece).

Blood collection and handling. The participants visited the Standard Centre of Bioassays, ‘Hartografoi Hygeias’ in Athens (Greece) and blood samples were collected. Blood samples were drawn from a forearm vein of seated individuals and stored in ethylenediaminetetraacetic acid (EDTA; Becton-Dickinson, Franklin Lakes, NJ, USA) tubes for measuring the levels of TBARS, CARB and GSH, TAC, and CAT activity, and in heparin tubes for determining sORP. The samples were then centrifuged immediately at 1,370 x g for 10 min at 4°C and erythrocytes were divided from the plasma. The erythrocytes were lysed with distilled water (1:1 v/v), inverted and centrifuged at 4,020 x g for 15 min at 4°C, and the erythrocyte lysate was then collected for the measurement of CAT activity. A small amount of erythrocyte lysate (500 µl) was treated with 5% trichloroacetic acid (TCA; Sigma-Aldrich, Munich, Germany) (1:1 v/v), vortexed and centrifugated at 28,000 x g for 5 min at 4°C. The supernatants were then removed and the procedure was repeated in the same way. Subsequently, the clear supernatants were transferred to new Eppendorf tubes and were used for the determination of GSH levels. Plasma and erythrocyte lysates were stored at -80°C until further analysis.

Assessment of sORP using the Luoxis RedoxSYS diagnostic system. The sORP value was determined using the RedoxSYS diagnostic system (Luoxis Diagnostics, Inc., Englewood, CO, USA) as previously described (22). This marker exhibits the intergrated balance between oxidants and reductants in a specimen and is presented in mV. In this new and innovative method, 20 µl of plasma are applied to disposable sensors designed by Luoxis Diagnostics, Inc., which are then inserted into the RedoxSYS diagnostic system, and the sORP value is reported within 4 min.

Assessment of the levels of TBARS, GSH and CARB, TAC, and CAT activity. For the determination of the TBARS levels, the assay was based on the method described in the study by Keles et al (24). TBARS is a commonly and frequently
used method to determine lipid peroxidation. According to this method, 100 µl of plasma were mixed with 500 µl of 35% TCA (Merck KGaA, Darmstadt, Germany) and 500 µl of Tris-HCl (Sigma-Aldrich, St. Louis, MO, USA; 200 mM, pH 7.4) and incubated for 10 min at room temperature. One milliliter of 2 M sodium sulfate (Na₂SO₄) and 55 mM TBA solution were added and the samples were incubated at 95°C for 45 min. The samples were cooled on ice for 5 min and were vortexed following the addition of 1 ml of 70% TCA. The samples were centrifuged at 15,000 x g for 3 min and the absorbance of the supernatant was read at 530 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029; Hitachi). The GSH levels were measured based on the method previously described in the study by Patsoukis et al (25). In this assay, 50 µl of 20% TCA were added to 50 µl of plasma and this mixture was incubated in an ice bath for 15 min and centrifuged at 15,000 x g for 5 min at 4°C. The supernatant was discarded and 500 µl of 10 mM 2,4-dinitrophenylhydrazine (DNPH; Sigma-Aldrich, Munich, Germany) in 2.5 N HCl (pH 0.2) was added to the samples and the change in absorbance of the supernatant was read at 340 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029; Hitachi, Tokyo, Japan). The baseline absorbance was taken into account by running a blank along with all samples during the measurement. The calculation of the CARB concentration was based on the molar extinction coefficient of malondialdehyde.

The concentration of CARB, an index of protein oxidation, was determined based on the method described in the study by Aebi (27). In particular, 4 µl of erythrocyte lysate (diluted 1:10) were added to 2,991 µl of 67 mM sodium potassium phosphate (pH 7.4) and 20 µl of erythrocyte lysate was treated with 5% TCA, mixed and then centrifuged at 15,000 x g for 3 min at 4°C. The supernatant was discarded and 1 ml of 10% TCA was added, vortexed and centrifuged at 15,000 x g for 5 min at 4°C. The supernatant was discarded and 1 ml of 10% TCA was added, vortexed and centrifuged at 15,000 x g for 5 min at 4°C. The supernatant was discarded and 1 ml of ethanol-ethyl acetate (1:1 v/v) was added, vortexed and centrifuged at 15,000 x g for 5 min at 4°C. This washing step was repeated twice. The supernatant was discarded and 1 ml of 5 M urea (pH 2.3) was added, vortexed and incubated at 37°C for 15 min. The samples were centrifuged at 15,000 x g for 3 min at 4°C and the absorbance was read at 375 nm. The calculation of the CARB concentration was based on the molar extinction coefficient of DNPH. Total plasma protein was assayed using the Bradford protein assay. The GSH levels were measured based on the method previously described in the study by Reddy et al (26). A total of 20 µl of erythrocyte lysate was treated with 5% TCA, mixed with 660 µl of 67 mM sodium potassium phosphate (pH 8) and 330 µl of 1 mM 5,5-dithiobis-2-nitrobenzoate (DTNB; Sigma-Aldrich, Munich, Germany). The samples were incubated in the dark at room temperature for 45 min and the absorbance was read at 412 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029; Hitachi). The GSH concentration was calculated relative to a calibration curve made using commercial standards.

The measurement of CAT activity was based on the method described by Aebi (27). In particular, 4 µl of erythrocyte lysate (diluted 1:10) were added to 2,991 µl of 67 mM sodium potassium phosphate (pH 7.4) and the samples were incubated at 37°C for 10 min. A total of 5 µl of 30% hydrogen peroxide (H₂O₂) was added to the samples and the change in absorbance was immediately read at 240 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029; Hitachi) for 130 sec. The determination of CAT activity was based on the molar extinction coefficient of H₂O₂.

Finally, the determination of TAC was based on the method described in the study by Janaszewska and Bartosz (28). In this assay, 20 µl of plasma were added to 480 µl of 10 mM sodium potassium phosphate (pH 7.4) and 500 µl of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The samples were then incubated in the dark for 30 min at room temperature and then centrifuged at 20,000 x g for 3 min. The absorbance was read at 520 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029; Hitachi). TAC was presented as mmol of DPPH reduced to 2,2-diphenyl-1-picrylhydrazine by receiving one hydrogen atom from the antioxidants of plasma.

Statistical analysis. For statistical analysis, data were analyzed by one-way ANOVA followed by Dunnett's test for multiple pairwise comparisons. The level of statistical significance was set at P<0.05. For all statistical analyses, SPSS software version 13.0 (SPSS, Inc., Chicago, IL, USA) was used. Data are presented as the means + standard error of the mean (SEM).

Results

The results revealed that the sORP values in plasma were significantly (P<0.05) higher by 13.4% in the patients with MetS and T2D compared to the controls, indicating an increase in oxidative stress (Fig. 1A). No statistically significant differences were observed in the CARB and TAC levels, and TAC in the plasma between the patients with MetS and T2D and the controls (Fig. 1B-D). The GSH levels in erythrocytes were significantly (P<0.05) lower by 27.7% in the patients with MetS and T2D compared to the controls (Fig. 2A). CAT activity in erythrocytes was significantly (P<0.05) higher by 23.3% in the patients with MetS and T2D compared to the controls (Fig. 2B).

In a previous study, we found that the induction of oxidative stress exhibited great variability between different individuals, since the outcome of an oxidant stimulus may be affected by several different factors (e.g., genetic, physiological and biochemical) (21,29). Based on this observation, the individual variability of the tested oxidative stress markers within the patients with MetS and T2D was examined (Fig. 3). Among these markers, the GSH marker exhibited the greatest variability, since there was a 6-fold difference between the lowest value and the highest value (Fig. 3A). GSH was also one of the three markers that exhibited a significant difference in its levels between the patients with MetS and T2D and the controls. In addition, GSH is considered one of the most important endogenous antioxidant molecules and a major contributor to the cellular redox status of living organisms (30). Thus, the patients with MetS and T2D were divided into 2 subgroups, the first one with low GSH levels (n=31; GSH <3 µmol/g Hb) and the second one with high GSH levels (n=35; GSH >4 µmol/g Hb). Nine patients had intermediate GSH values, that is, between 3.1 and 3.9 µmol/g Hb. Nine patients had intermediate GSH values, that is, between 3.1 and 3.9 µmol/g Hb, and thus they were not included in any of the 2 subgroups, so as to have a clear distinction of patients as regards the GSH levels. Between the average values of these 2 GSH groups, there was a statistically significant (P<0.05) difference (by 51.7%) in GSH levels in erythrocytes (Fig. 4A). Moreover, the GSH levels were significantly (P<0.05) lower (by 52.9%) in the low GSH group compared with the controls (Fig. 4A). In addition, in these 2 GSH groups, the differences between the other oxidative...
stress markers were also examined. There were no significant differences observed in CAT activity in erythrocytes between the 2 GSH groups (Fig. 4B). However, CAT activity was significantly (P<0.05) higher in the low and high GSH groups by 20.4 and 26.7%, respectively than in the controls (Fig. 4B). The sORP values in the plasma of the patients in the low GSH group were significantly (P<0.05) higher (by 8.1%) compared with those of the patients in the high GSH group (Fig. 5A). Moreover, the sORP values were significantly (P<0.05) higher in the patients in the low and high GSH groups (by 15.6 and 6.9%, respectively) compared with the controls (Fig. 5A). In addition, the CARB levels in plasma were significantly (P<0.05) higher (by 16.7%) in the low GSH group compared with the high GSH group (Fig. 5B). There were no significant differences observed in TBARS and TAC levels in plasma between the 2 GSH groups (Fig. 5C and D).

Figure 1. Markers of the redox status in the plasma of patients with metabolic syndrome (MetS) and type 2 diabetes (T2D) and normal controls. (A) Static oxidation reduction potential (sORP), (B) thiobarbituric acid reactive substances (TBARS), (C) protein carbonyl (CARB), (D) total antioxidant capacity (TAC). *P<0.05, significantly different compared to the controls.

Figure 2. Markers of the redox status in erythrocytes of patients with metabolic syndrome (MetS) and type 2 diabetes (T2D) group and normal controls. (A) Glutathione (GSH), (B) catalase (CAT). *P<0.05, significantly different compared to the controls.
Figure 3. Values of redox biomarkers of each patient with metabolic syndrome and type 2 diabetes. (A) Glutathione (GSH) (in erythrocytes), (B) thiobarbituric acid reactive substances (TBARS) (in plasma), (C) protein carbonyls (CARB) (in plasma), (D) catalase (CAT) (in erythrocytes), (E) static oxidation reduction potential (sORP) (in plasma), (F) total antioxidant capacity (TAC) (in plasma).

Figure 4. Markers of the redox status in erythrocytes of patients with metabolic syndrome (MetS) and type 2 diabetes (T2D) exhibiting low and high glutathione (GSH) levels, and the normal controls. (A) GSH, (B) catalase (CAT). *P<0.05, significantly different compared to the controls. #P<0.05, significantly different compared to the high GSH group.
Discussion

MetS is a cluster of medical conditions, including abdominal obesity and insulin resistance, plus any two of the following four factors: i) increased triglyceride levels, ii) decreased HDL cholesterol levels, iii) increased blood pressure, and iv) increased fasting blood glucose levels (7). The prevalence of MetS is approximately 22.9% in the US population and up to 36% of Europeans aged between 40-55 suffer from the disease (31,32). MetS is also a risk factor for developing T2D, another type of metabolic disorder, characterized basically by elevated blood glucose levels due to insulin resistance and affects approximately 380 million individuals worldwide (12). Both of these disorders are also associated with oxidative stress, a pathophysiological condition in which there is an overbalance of free radicals production against antioxidant mechanisms (12,13,33,34). Oxidative stress occurring in patients with MetS and T2D may further aggravate the associated complications, particularly those involving the cardiovascular system (14,16,35,36). Thus, the assessment of oxidative stress in patients with MetS and T2D is considered useful for monitoring their health status (37-39). In previous studies, we demonstrated that the determination of the sORP values in plasma, a new marker of oxidative stress, was effective for assessing the redox status in different physiological conditions and diseases (20-23). Thus, the aim of the present study was to examine the effectiveness of sORP for assessing oxidative stress in patients manifesting both MetS and T2D.

The results revealed that the sORP values in plasma were significantly higher in the patients with MetS and T2D compared with the controls, suggesting the induction of oxidative stress in the patients affected by these two metabolic disorders. In our previous studies, we observed increased sORP values in patients with sepsis and in conditions of strenuous exercise-induced oxidative stress (21-23).

The significantly lower GSH levels in the erythrocytes of the patients with MetS and T2D compared with the controls also supported the induction of oxidative stress in the patients with MetS and T2D. Another study also reported decreased GSH levels in patients with MetS (40). Likewise, a decrease in GSH levels in human erythrocytes and serum has been demonstrated in other studies on patients with T2D (41-43). GSH is one of the most important antioxidant mechanisms in living organisms, and thus low GSH levels are associated with oxidative stress and the manifestation of various diseases (30,44,45). As regards the mechanisms through which T2D is associated with low GSH levels, it has been suggested that in hyperglycemia, glucose is used in the polyl pathway, resulting in a decrease in nico-

![Figure 5: Markers of the redox status in plasma of patients with metabolic syndrome (MetS) and type 2 diabetes (T2D) exhibiting low and high glutathione (GSH) levels, and the normal controls. (A) Static oxidation-reduction potential (sORP), (B) protein carbonyls (CARB), (C) thiobarbituric acid reactive substances (TBARS), (D) total antioxidant capacity. *P<0.05, significantly different compared to the controls. **P<0.05, significantly different compared to high GSH group.]
In this study, the patients with MetS and T2D exhibited a significant increase in CAT activity compared to the controls. CAT is the main regulator of hydrogen peroxide metabolism, which is associated with diabetes mechanisms, such as the expression of glucose receptor and insulin secretion (47). Other studies have demonstrated conflicting results, reporting either a decrease (47), increase (48) or no change (49) in CAT activity in hyperglycemic conditions. It has been proposed that an organism may increase CAT activity in some cells, such as erythrocytes in order to protect itself from free radical-induced cell damage in diabetic conditions, particularly in cells with low CAT activity, such as pancreatic beta cells (50). Thus, increase in CAT activity may also indicate the induction of oxidative stress in patients with MetS and T2D, as similarly shown by sORP and GSH markers.

However, in this study, no differences were observed in the TBARS and CARB levels (indicating lipid peroxidation and protein oxidation, respectively), in plasma between the patients with MetS and T2D and the controls. Although studies have demonstrated that T2D is accompanied by increased lipid peroxidation, the latter is not a prerequisite for MetS (42,51). On the contrary, it seems that for some unclear reason, lipid peroxidation may even be decreased in MetS (52). Thus, the co-occurrence of both MetS and T2D in the patients may explain the absence of increased TBARS levels in their plasma. Moreover, no increase was observed in the CARB levels in the patients with MetS and T2D compared with the controls, although protein oxidation is considered a characteristic of either MetS or T2D (51,53). This absence of increase in CARB levels may be explained by the fact that advanced oxidation protein products (AOPPs) instead of CARB have been shown to be the most appropriate marker for protein oxidation in MetS (51). AOPPs have also been reported to be increased in T2D (54). AOPPs are generated by the action of chloraminated oxidants (e.g., hypochlorous acid and chloramines) produced by myeloperoxidase in activated neutrophils during oxidative stress (55). CARB are produced on protein side chains (particularly of Pro, Arg, Lys and Thr) when they are oxidized (18).

Furthermore, TAC marker did not differ significantly between the patients with MetS and T2D and the controls. Since TAC is considered a marker of the total redox status, this finding was in contrast to the induction of oxidative stress indicated by other markers (actually, TAC would be expected to be reduced). However, this result may be explained when considering that TAC is based on the assessment of the reductant compounds, which along with the antioxidant enzymes constitute the antioxidant defense mechanisms. Although some antioxidants (e.g., GSH) are reduced in MetS and T2D disorders, some others such as uric acid have been reported to be increased (51). Uric acid accounting for approximately 60% of the antioxidant activity in human plasma is believed to be increased in MetS subjects as insulin may reduce uric acid elimination in the urine (51,56). Thus, although TAC may remain unchanged due to this parallel increase and decrease in different antioxidants in MetS and T2D conditions, oxidative stress occurs as oxidant compounds are increased more than the antioxidants. For this reason, and as we have suggested previously (21,23), the sORP marker may be a better marker than TAC for assessing the total redox status, since the former is based on the evaluation of the difference between oxidants and reductants while the latter only on the reductants (i.e., antioxidants).

In this study, the patients with MetS and T2D exhibited great variations in the values of oxidative stress markers, particularly those of GSH, and thus the patients were divided into 2 subgroups, one with low GSH (<3 µmol/g Hb) and the other with high GSH (>4 µmol/g Hb) levels. The statistical comparison of the average values of oxidative stress markers between the two subgroups indicated that the low GSH group had significantly higher sORP levels than the high GSH group, suggesting greater oxidative stress in the former group compared to the latter. This finding was also supported by the higher protein oxidation levels as shown by CARB in the low GSH group compared with the high GSH group. There were no significant differences observed in CAT, and in the CAT and TBARS levels between the two GSH groups. Since oxidative stress has been associated with the severity of complications in patients with either MetS or T2D (33-36), the observed variation of the induction of oxidative stress in such subjects emphasizes the need for assessing their redox status. Namely, higher oxidative stress levels in patients with MetS and T2D may be an alarming sign for applying appropriate interventions (e.g., antioxidant supplementation), so as to reduce the aggravation of complications (12,37). Among the two oxidative stress markers assessing total redox status (i.e., sORP and TAC), sORP seems to be a suitable marker for assessing oxidative stress levels in patients with MetS and T2D, since it was associated with lower GSH and higher CARB levels.

Moreover, the assessment of the redox status may be important in prediabetic conditions. According to a new theory suggested by Watson (57) and Sharoff et al (58), there may be a close association between T2D and the redox status. According to this theory, a main cause of diabetes is a reductive environment in the endoplasmic reticulum, impairing disulphide bond formation needed to stabilize the 3D conformation of physiologically active proteins (57). Namely, an oxidative environment seems to be required for the proper folding and the normal function of proteins. Major evidence supporting this theory is that the membranous sacs of the endoplasmic reticulum of insulin-resistant rodents contain higher amount of unfolded polypeptides and many fewer S-S bonds than normal endoplasmic reticulum (59,60). Moreover, it has been demonstrated that supplementation with antioxidant decreased the ability of exercise to make cells more sensitive to insulin (61). In addition, subjects carrying mutations impairing the synthesis of antioxidant molecules manifested increased insulin sensitivity (62). Based on this theory, our findings showing that oxidative stress levels varied greatly among MetS and T2D subjects emphasize the need for the assessment of redox status in prediabetic subjects, which may help to discern those with reductive redox status from those with oxidative one, and so to make the appropriate interventions. It has often been suggested without distinction the antioxidant supplementation in prediabetic subjects, although as explained above this may be harmful for those having a reductive redox status. In
future studies, we will investigate the association between the redox status and clinical signs of prediabetic subjects.

In conclusion, the present results suggest that sORP may be an effective marker for assessing oxidative stress in MetS and T2D patients, since it was higher in these subjects compared to control ones. Moreover, sORP was effective for discerning the oxidative stress levels among MetS and T2D patients, since it was associated with low GSH and high CARB levels. Thus, the use of such a marker may be useful for identifying eagerly high oxidative stress levels in MetS and T2D patients, and consequently reducing complications for identifying eagerly high oxidative stress levels in MetS compared to control ones. Moreover, sORP was effective in MetS and T2D patients, since it was higher in these subjects may be an effective marker for assessing oxidative stress in the redox status and clinical signs of prediabetic subjects. More over, sORP may be useful for discerning high from low oxidative stress levels in prediabetic subjects, which may also determine the type of intervention.

References

1. Halliwell B: The wanderings of a free radical. Free Radic Biol Med 46: 531-542, 2009.
2. Ghosh J and Myers CE: Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. Proc Natl Acad Sci USA 95: 13182-13187, 1998.
3. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J: Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39: 44-84, 2007.
4. Orient A, Donkó A, Szabó A, Leto TL and Geiszt M: Novel sources of reactive oxygen species in the human body. Nephrol Dial Transplant 22: 1281-1288, 2007.
5. Mylonas C and Kourtetas D: Lipid peroxidation and tissue damage. In Vivo 13: 295-309, 1999.
6. Ford ES, Mokdad AH, Giles WH and Brown DW: The metabolic syndrome defined using population-specific waist circumference thresholds. Diabetes Care 27: 1486-1493, 2004.
7. Mehran R, Dreyer KD, Fang J, Grines CL, Leon MB, Holmes DR Jr, et al: A randomized trial comparing clopidogrel with prasugrel in patients undergoing percutaneous coronary intervention. N Engl J Med 360: 869-880, 2009.
8. Anderson TJ, Adams CD, Antman EM, Atkins JN,融创在, et al: 2011 ACCF/AHA focused update of the guideline for the management of patients with unstable angina/non-ST-elevation myocardial infarction. J Am Coll Cardiol 57: 496-511, 2011.
9. OGINO K and Wang DH: Biomarkers of oxidative/nitrosative stress: An approach to disease prevention. Acta Med Okayama 61: 181-189, 2007.
10. Spanidis Y, Goutzourelas N, Bar-Or D, Ntontou AM, Bella E, Becker AT, Statiri A, Kafantaris I and Kouretas D: Application of a new oxidation-reduction potential assessment method in strenuous exercise-induced oxidative stress. Redox Rep 20: 154-162, 2015.
11. Stagos D, Goutzourelas N, Ntontou AM, Kafantaris I, Deli CK, Poulis A, Lamartas AZ, Bar-Or D and Kouretas D: Assessment of eccentric exercise-induced oxidative stress using oxidation-reduction potential markers. Oxid Med Cell Longev 2015: 204615, 2015.

12. Spanidis Y, Goutzourelas N, Stagos D, Kolyva AS, Gogos CA, Bar-Or D and Kouretas D: Assessment of oxidative stress in septic and obese patients using markers of oxidation-reduction potential. In Vivo 29: 595-600, 2015.
13. Spanidis Y, Goutzourelas N, Stagos D, Mespinis A, Priftis A, Bar-Or D, Spandidos DA, Tsatsakis AM, Leon Gans Kouretas D: Variations in oxidative stress markers in elite basketball players at the beginning and end of a season. Exp Ther Med 11: 147-153, 2016.
14. Keles MS, Tayri S, Sen N, Aksoy H and Akçay F: Effect of corticosteroid therapy on serum and CSF malondialdehyde and antioxidant proteins in multiple sclerosis. Can J Neurol Sci 28: 141-143, 2001.
15. Patsoukis N, Zervoudakis G, Panagopoulos NT, Georgiou CD, Angelatou F and Matsokis NA: Thiol redox state (TRS) and oxidative stress in the mouse hippocampus after pentylene-tetrazol-induced epileptic seizure. Neurosci Lett 357: 83-86, 2004.
16. Reddy YN, Murthy SV, Krishna DR and Prabhakar MC: Role of free radicals and antioxidants in tuberculosis patients. Indian J Tuberc 51: 213-218, 2004.
17. Aebi H: Catalase in vitro. Methods Enzymol 15: 121-126, 1984.
18. Januszewska A and Bartosz G: Assay of total antioxidant capacity: Comparison of four methods as applied to human blood plasma. Scand J Clin Lab Invest 62: 231-236, 2002.
19. Bloomer RJ and Fisher-Wellman KH: Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. Gend Med 5: 218-228, 2008.
20. Ristoff E and Larsson A: Oxidative stress in inborn errors of metabolism: lessons from glutathione deficiency. J Inherit Metab Dis 25: 223-226, 2002.
21. Wilson PWF and Grundy SM: The metabolic syndrome: practical guide to origins and treatment: Part I. Circulation 108: 1442-1443, 2003.
22. Balkau B, Charles MA, Drivsholm T, et al: European Group For The Study Of Insulin Resistance (EGER): Frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome. Diabetes Metab 28: 364-370, 2002.
23. Fukubayashi I, Hori M, Kato T, Yamasaki Y, Nakayama O, Iwataki M, Matsuoka M and Shimomura I: Elevated oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114: 1752-1761, 2004.
24. Roberts CK and Sindhu KK: Oxidant stress and metabolic syndrome. Life Sci 84: 705-712, 2009.
25. Cericola A and Motz E: Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 24: 816-823, 2004.
26. Armutcu F, Ataymen M, Aypaçan H and Gurel A: Oxidative stress markers, C-reactive protein and heat shock protein 70 levels in subjects with metabolic syndrome. Clin Chem Lab Med 46: 785-790, 2008.
27. Akbar S, Bellary S and Griffiths HR: Dietary antioxidant interventions in type 2 diabetes patients: A meta-analysis. Br Diabetics Vasc Dis 11: 62-68, 2011.
28. Neyestani TR, Shariat Zadeh N, Gharavi A, Kalayi A and Khajeh N: Physiological dose of lycopene suppressed oxidative stress and enhanced serum levels of immunoglobulin M in patients with type 2 diabetes mellitus: A possible role in the prevention of long-term complications. J Endocrinol Invest 30: 833-838, 2007.
29. Neyestani TR, Shariat-Zadeh N, Gharavi A, Kalayi A and Khajeh N: The opposite associations of lycopene and body fat mass with humoral immunity in type 2 diabetes mellitus: A possible role in atherogenesis. Iran J Allergy Asthma Immunol 7: 79-87, 2007.
40. Vávrová L, Kodydková J, Zeman M, Dušejovská M, Macášek J, Staňková B, Tvrzická E and Zák A: Altered activities of antioxidant enzymes in patients with metabolic syndrome. Obes Facts 6: 39-47, 2013.

41. Hakki Kalkan I and Suher M: The relationship between the level of glutathione, impairment of glucose metabolism and complications of diabetes mellitus. Pak J Med Sci 29: 938-942, 2013.

42. Seghrouchni I, Drai J, Bannier E, Rivière J, Calmard P, Garcia I, Orgiazzi J and Revol A: Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. Clin Chim Acta 321: 89-96, 2002.

43. Ciuchi E, Odetti P and Prando R: Relationship between glutathione and sorbitol concentrations in erythrocytes from diabetic patients. Metabolism 45: 611-613, 1996.

44. Mazzetti AP, Fiorile MC, Primavera A and Lo Bello M: Glutathione transferases and neurodegenerative diseases. Neurochem Int 82: 10-18, 2015.

45. Pérez S, Pereda J, Sabater L and Sastre J: Redox signaling in acute pancreatitis. Redox Biol 5: 1-14, 2015.

46. Lee AY and Chung SS: Contributions of polyol pathway to oxidative stress in diabetic cataract. FASEB J 13: 23-30, 1999.

47. Góth L: Reactive oxygen species, hydrogen peroxide, catalase and diabetes mellitus. Redox Rep 11: 10-18, 2015.

48. Weidig P, McMaster D and Bayraktutan U: High glucose mediates pro-oxidant and antioxidant enzyme activities in coronary endothelial cells. Diabetes Obes Metab 6: 432-441, 2004.

49. Manea A, Constantinescu E, Popov D and Raicu M: Changes in oxidative balance in rat pericytes exposed to diabetic conditions. J Cell Mol Med 8: 117-126, 2004.

50. Tiedge M, Lortz S, Drinkgern J and Lenzen S: Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46: 1733-1742, 1997.

51. Venturini D, Simão ANC and Dichi I: Advanced oxidation protein products are more related to metabolic syndrome components than biomarkers of lipid peroxidation. Nutr Res 35: 759-765, 2015.

52. Sohet FM, Neyrinck AM, Dewulf EM, Bindels LB, Portois L, Malaisse WJ, Carpenter YA, Cani PD and Delzenne NM: Lipid peroxidation is not a prerequisite for the development of obesity and diabetes in high-fat-fed mice. Br J Nutr 102: 462-469, 2009.

53. Tabak O, Gelisgen R, Erman H, Erdene F, Muderrisoglu C, Aral H and Uzun H: Oxidative lipid, protein, and DNA damage as oxidative stress markers in vascular complications of diabetes mellitus. Clin Invest Med 34: Ei63-Ei71, 2011.

54. Cakatay U: Protein oxidation parameters in type 2 diabetic patients with good and poor glycaemic control. Diabetes Metab 31: 551-557, 2005.

55. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Junpers P and Descamps-Latscha B: Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 49: 1304-1313, 1996.

56. Muscelli E, Natali A, Bianchi S, Bigazzi R, Galvan AQ, Sironi AM, Fraschera S, Ciociaro D and Ferramini E: Effect of insulin on renal sodium and uric acid handling in essential hypertension. Am J Hypertens 9: 746-752, 1996.

57. Watson JD: Type 2 diabetes as a redox disease. Lancet 383: 841-843, 2014.

58. Sharoff CG, Hagobian TA, Malin SK, Chipkin SR, Yu H, Hirshman MF, Goodyear LJ and Braun B: Combining short-term metformin treatment and one bout of exercise does not increase insulin action in insulin-resistant individuals. Am J Physiol Endocrinol Metab 298: E815-E823, 2010.

59. Ron D and Harding HP: Protein-folding homeostasis in the endoplasmic reticulum and nutritional regulation. Cold Spring Harb Perspect Biol 4: 4, 2012.

60. Nardai G, Stadler K, Papp E, Korscma T, Jakus J and Csermely P: Diabetic changes in the redox status of the microsomal protein folding machinery. Biochem Biophys Res Commun 334: 787-795, 2005.

61. Ristow M, Zarse K, Oberbach A, Klötzing N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR and Blüher M: Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci USA 106: 8665-8670, 2009.

62. Schoenmakers E, Agostini M, Mitchell C, Schoenmakers N, Papp L, Rajanayagam O, Padidela R, Ceron-Gutierrez L, Döffinger R, Prevosto C, et al: Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. J Clin Invest 120: 4220-4235, 2010.