Thiol/Disulphide Homeostasis in Autosomal Dominant Polycystic Kidney Disease: A Single Center, Cross-Sectional Study

Otozomal Dominant Polikistik Böbrek Hastalığında Tiyol/Disülfid Homeostazı: Tek Merkezli, Kesitsel Bir Çalışma

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ABSTRACT

OBJECTIVE: The aim of this study was to determine a novel oxidative stress marker, plasma thiol-disulphide homoeostasis, in patients with autosomal dominant polycystic kidney disease (APKD).

MATERIAL and METHODS: Thirty-four patients with autosomal dominant polycystic kidney disease and 30 healthy volunteers were included in the study. The thiol-disulfide homeostasis assay was performed. Total antioxidant capacity was evaluated via the FRAP assay.

RESULTS: The median age was 46 (13) years in the patient group and 41 (13) years in the control group. In the APKD disease group, serum total and native thiol levels were statistically significantly lower ($p = 0.002$, $p = 0.002$, respectively). Serum native thiol levels were negatively correlated with age (r:-0.620 p: 0,000), systolic blood pressure (r: -0,697 p: 0,000), and diastolic blood pressure (r: -0,643 p: 0,000). A weak positive correlation was found between the disulfide/native thiol ratio and creatinine (r: 0.564 $p < 0.001$), and a negative correlation was found between the disulfide/native thiol ratio and glomerular filtration rate (r: -0.372 p: 0.030). There was a significant positive correlation between the native thiol level and GFR (r: 0.699, $p < 0.001$) level.

CONCLUSION: Our findings suggest that thiol/disulfide homeostasis may be associated with autosomal dominant polycystic kidney disease progression.

KEY WORDS: Oxidative stress, Autosomal dominant polycystic kidney disease, Thiol-disulphide, Creatinine

ÖZ

AMAÇ: Bu çalışmanın amacı, otozomal dominant polikistik böbrek (ODPK) hastalığı olanlarda yeni bir oksidatif stres markeri olan plazma tiyol-disülfid homeostazisini belirlemektir.

GEREÇ ve YÖNTEMLER: Otozomal dominant polikistik böbrek hastalığı olan 34 hasta ve 30 sağlıklı gönüllü çalışmaya dahil edildi. Tiyol-disülfid homeostazı ölçüldü. Total antioksidan kapasite FRAP testi ile değerlendirildi.

BULGULAR: Hastaların medyan yaşı 46 (13) yıl, kontrol grubunda 41 (13) yıldır. ODPK hastalarında serum total ve nativ tiyol düzeyleri istatistiksel olarak anlamlı derecede düşüktü (sırasıyla p=0,002, p=0,002). Serum nativ tiyol düzeyleri yaş (r:-0,620 p:0,000), sistolik kan basıncı (r:-0,697 p:0,000) ve diyastolik kan basıncıyla (r:-0,643 p:0,000) negatif korelasyon gösterdi. Disülfid / nativ tiyol oranı ile kreatinin arasında (r:0,564 $p < 0,001$) zayıf pozitif korelasyon bulundu, disülfid / nativ tiyol oranı ve glomerüler filtrasyon hızı arasında negatif bir korelasyon bulundu (r:-0,372, p:0,030. Nativ tiyol düzeyi ile GFR arasında pozitif korelasyon bulundu (r:0,699, $p < 0,001$).

SONUÇ: Bulgularımız tiyol/disülfid homeostazının otozomal dominant polikistik böbrek hastalığının ilerlemesi ile ilişkili olabileceğini düşündürmektedir.

ANAHTAR SÖZCÜKLER: Oksidatif stres, Otozomal dominant polikistik böbrek hastalığı, Tiyol-disülfid, Kreatinin

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INTRODUCTION

Autosomal dominant polycystic kidney (ADPK) disease is one of the most common hereditary disorders and is characterized by progressive cyst formation and the enlargement of the kidney and other organs (e.g., liver, pancreas, and spleen) (1). Ultimately, gradually growing cysts lead to kidney failure by the fifth of sixth decade of life (2).

Two genes, *PKD1* and *PKD2* (Polycystic Kidney Disease 1 and 2) have been identified as responsible for ADPK disease. According to the double-hit hypothesis, an initial germline mutation in one of these genes' alleles and a somatic second hit that cause the loss of the normal allele may lead to ADPK disease (3).

The kidney has a very active oxidative metabolism, especially due to the transport mechanism, which results in the production of reactive oxygen radicals (4). A large number of reactive oxygen radicals are produced in the kidney, and antioxidants are important in protecting kidney function (5). Increased renal oxidative stress can cause DNA damage and second hits, thus playing an important role in the progression of ADPK disease and cystogenesis (6-8). Meanwhile, oxidative stress in the kidney may lead to increased cell proliferation, extracellular cell matrix synthesis, inflammatory cell infiltration, and apoptosis (9,10). All of these are among the general pathological features seen in cystic kidneys.

Thiols are organic compounds that contain a functional carbon-bonded sulfhydryl group, or -SH. Plasma protein thiol groups act as buffers against oxidation, and they are the primary targets of reactive oxygen species (11). The oxidation of the thiol groups of proteins by reactive oxygen species leads to reversible disulfide bonds, which are the earliest signs of protein oxidation (12).

Decreased plasma protein thiols and increased S-thiolated proteins have previously been shown in end-stage renal disease patients (13-15).

The aim of this study was to investigate plasma thiol-disulphide homoeostasis in patients with autosomal dominant polycystic kidney disease using a new method (16) designed for use on automated chemistry analyzers.

MATERIALS and METHODS

Our study was performed at the Saglik Bilimleri University Bursa Yüksek İhtisas Education and Research Hospitals' Nephrology Department between November 2016 and February 2017. Ethic approval was obtained from the regional Ethics Committee on 2.11.2016 (2011-KAEK-25 2016/19-06). All experiments were carried out in accordance with the tenets of the Declaration of Helsinki (2013 Brazil version).

Thirty-four patients with APKD and 30 healthy volunteers were included in the study. All the participants signed an informed consent form. ADPK disease patients had been followed at the Nephrology Department of our hospital. The exclusion criteria were being younger than 20 and older than 65 years; having an active infection, acute renal failure, or type 1 and type 2 diabetes mellitus; being pregnant or lactating; being a smoker; having coronary artery disease, malignancy, liver cirrhosis, or inflammatory diseases; and using lipid-lowering drugs, antioxidants, or vitamin supplements.

After systemic examinations of all volunteers participating in the study, their body weight, height, waist circumference, and blood pressure were measured. Body mass index (BMI) was calculated based on the participants' weight in kilograms and height in meters.

Five ml of venous whole blood were taken after 8 hours of fasting. After waiting 20 minutes, these were centrifuged for 10 minutes at 1500 rpm, and the separated serum samples, were stored at -80° C. Glucose, uric acid, blood urea nitrogen (BUN), creatinine, total cholesterol, triglyceride, total protein, albumin, and high density lipoprotein cholesterol were measured using commercial kits on an Olympus AU 2700 instrument in our hospital's biochemistry laboratory. EDTA blood samples were analysed in an automated haematology analysis system, specifically a Coulter LH-750 haematology analyser (Beckman Coulter Inc., Fullerton, CA) CRP was measured in the serum via immunonephelometry (BN ProSpec; Siemens Healthcare Diagnostics Marburg, Germany). Kidney function assessed by serum creatinine through estimated glomerular filtration rate [MDRD formulae=175 x $(S_{Cr})^{-1.154}$ x$(age)_{-0.203}$ x 0.742 (if female) x1.212 (if Black)] (17). A plasma thiol-disulfide hemostasis assay was performed according to the protocol of Erel and Neselioğlu (2014) (16). In our study, the total antioxidant capacity of the sera was measured via the ferric reducing power of plasma (FRAP) method (Benzie et al., 1996), which was adapted to the microelisa plates (18).

Statistics

The IBM Statistical Package for Social Science software program (SPSS for Windows, Version 21.0, USA) was used to perform all statistical analyses. The normality of the continuous variables was analyzed with the Kolmogorov-Smirnov test and Shapiro-Wilks test. Data are expressed as means ± standard deviations (SDs) when normally distributed or with medians otherwise (Interquartile range; IQR). The Kruskal-Wallis and Mann-Whitney U-tests were used to compare the differences between the three groups. In the assessment of correlations, a Spearman test was used for data with nonparametric distributions. A p-value less than 0.05 was considered statistically significant with a 95% confidence interval.

RESULTS

A total of 64 subjects were enrolled, 34 of whom were APKD patients, and 30 of whom were healthy volunteers.

The demographic and laboratory parameters of the volunteers are summarized in Table I. The median age was 46 (13) years (mean=45±9) in the patient group and 41 (13) years (mean=42±7) in the control group. The mean time after the initial diagnosis in the patient group was 6.7 ± 3.8 years.

The systolic blood pressure, diastolic blood pressure, BUN, creatinine, and fasting glucose values were statistically significantly higher in the patient group than in the control group (p<0.01, p<0.01, p:0.028, p:0.007, p:0.027, respectively) (Table I). GFR, hemoglobin, and lymphocyte levels were statistically

Table I: Characteristics of the study population.

Characteristics	Healthy Controls	Patients	p
Number of patients, n	30	34	
Age (years)	41(13)	46(13)	0.067
Gender (M/F)	10/20	11/23	0.934
Time after the initial diagnosis (years)	-	6.7±3.8	-
BMI (kg/m^2)	27.5±5	28±5	0.392
Waist circumference (cm)	87.9±11.8	89.7±15.1	0.589
TA systolic (mm Hg)	120(30)	130(20)	<0.01
TA diastolic (mm Hg)	70(10)	80(16)	<0.01
BUN (mg/dL)	13.5(5.9)	14.8(14.2)	0.028
Creatinine (mg/dL)	0.8(0.2)	1.0(0.7)	0.007
GFR (mL/min/1.73 m^2)	84±13	65±29	0.002
Fasting glucose (mg/dL)	90±8	96±12	0.027
T Chol (mg/dL)	215±54	216±42	0.931
Trig (mg/dL)	117(91)	123(100)	0.253
HDL(mg/dL)	52±12	56±17	0.317
LDL(mg/dL)	138±43	132±37	0.544
Hb (g/dL)	14.0±1.3	12.6±1.8	0.001
CRP (mg/L)	1(1)	1(1)	0.719
WBC (cellsx10^9/L)	7.150(2.050)	6.900(2.000)	0.275
Neutrophile (cellsx10^9/L)	4.122±1.292	4.051±1.192	0.820
Lymphocyte (cellsx10^9/L)	2.406±0.773	2.028±0.586	0.030
Platelet (cells/mL)	258500±56252	244470±60703	0.343
MPV(fL)	8.9(1.3)	8.8(1.1)	0.788
T protein (g/dL)	7.4(0.7)	7.3(0.3)	0.323
Albumin (g/dL)	4.6(0.3)	4.5(0.5)	0.101
FRAP (μmol/L)	853(169)	920(238)	0.489
Total thiol (μmol/L)	458±45	404±82	0.002
Native thiol (μmol/L)	442±41	371±78	0.002
Disulphide (μmol/L)	17.9±6.6	16.5±6.7	0.410
Disulphide/native thiol (%)	4.0(2.1)	4.0(2.0)	0.946
Disulphide/total thiol (%)	3.8(1.7)	4.1(1.7)	0.946
Native thiol/total thiol ratio	92.2(3.5)	91.6(3.4)	0.946

Data are expressed as means ± standard deviations when normally distributed and as medians (interquartile range) otherwise. **M:** Males, **F:** Females, **BMI:** Body mass index. **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure. *p<0.05 was considered significant for statistical analyses.

significantly lower in the patient group than in the control group (p:0.002, p:0.001, p:0.03) (Table I).

In the APKD group, serum total thiol levels and serum native thiol levels were statistically significantly lower than in the control group (p=0.002, p=0.002, respectively) (Table I).

A correlation analysis performed within the patient group showed that serum native thiol levels were significantly negatively correlated with age (r:-0.620 p:0.000), BMI (r:-0.436 p:0.010), waist circumference (r:-0.421 p:0.013), systolic blood pressure (r:-0,697 p:0,000), and diastolic blood pressure (r:-0.643 p:0.000) (Table II). Statistically significant positive correlations between the disulfide/native thiol ratio and systolic blood pressure (r:0.374 p:0.029), diastolic blood pressure (r:0.431 p:0.011), and disease duration (r:0.462 p:0.006) were detected (Table II).

Statistically significant negative correlations were found between native thiol/total thiol and systolic blood pressure (r:-0.357 p:0.038), diastolic blood pressure (r:-0.412 p:0.015), and disease duration (r:-0.465 p:0.006) (Table II). There were significant negative correlations between serum native thiol level and BUN (r:-0.522 p:0.002) and creatinine (r:-0.579 p<0.001) levels. There was a significant positive correlation between native thiol level and GFR (r:0.699 p<0.001) level. A statistically significant but weak positive correlation was found between the disulfide/native thiol ratio and creatinine (r:0.564 p<0.001), and a statistically significant negative correlation was found between the disulfide/native thiol ratio and GFR (r:-0.372 p:0.030) (Table III). None of the thiol/disulfide hemostasis parameters were correlated with total antioxidant capacity, which was measured as the FRAP value.

DISCUSSION

Serum native thiol and total thiol levels were found to be significantly lower in the study group as compared to the control group. We suggest that this decrease is indicative of the inadequacy of antioxidant defense in ADPK patients.

Reduced thiol levels in ADPK disease can make the kidneys vulnerable to oxidative stress. Proteins achieve most of their antioxidant effect via its thiol groups (19,20). Additionally, the loss of the thiol group from proteins is the main molecular mechanism leading to structural and functional changes in proteins (18,19). In one study by Maser et al. (21), increased oxidative stress with reduced antioxidant enzyme production was observed in cystic kidney mice and rats. Torres et al. (22) found that a decrease in glutathione, the most abundant cellular thiol, aggravated polycystic kidney disease in mice and rats. Meanwhile, it has been demonstrated in a murine model of polycystic kidney disease that combination therapy that reduces interstitial inflammation and oxidative stress delays cyst growth (23).

Oxidative stress may result in thiol disulfide exchange reactions of free thiols towards disulfide bond formation. However, we could not find a difference between the patient and control groups in terms of serum disulfide, disulfide/total thiol, disulfide/native thiol, and native thiol/total thiol ratios.

Studies have shown that oxidative stress plays an important role in the progression of chronic renal damage (23,24). We found a statistically significant positive correlation between the duration of illness and the disulfide/native thiol and disulfide/total thiol ratios. Because there are no other studies investigating

Table II: Correlations between native thiol, total thiol, and disulphide and clinical parameters in the patient group (n = 34).

		Age	BMI	Waist circumference	SBP	DBP	Duration of disease
Native thiol	r	-0.620	-0.436	-0.421	-0.697	-0.643	-0.320
	p	0.000	0.010	0.013	0.000	0.000	0.065
Total thiol	r	-0.628	-0.486	-0.457	-0.677	-0.615	-0.256
	p	0.000	0.004	0.007	0.000	0.000	0.144
Disulfide	r	-0.189	-0.405	-0.317	-0.026	0.033	0.327
	p	0.285	0.017	0.067	0.886	0.852	0.059
Disulfide/Native thiol	r	0.182	-0.131	-0.052	0.374	0.431	0.462
	p	0.303	0.462	0.772	0.029	0.011	0.006
Disulfide/ Total thiol	r	0.173	-0.142	-0.066	0.357	0.412	0.465
	p	0.327	0.424	0.711	0.038	0.015	0.006
Native thiol/ Total thiol	r	-0.173	0.142	0.772	-0.357	-0.412	-0.465
	p	0.327	0.424	0. 066	0. 038	0. 015	0. 006

SBP: Systolic blood pressure, **DBP:** Diastolic blood pressure.

Table III: Correlations between native thiol, total thiol, and disulfide and biochemical parameters in patients (n = 34).

		BUN	Creatinine	GFR	FRAP
Native	r	-0.522	-0.579	0.699	-0.153
	p	0.002	<0.001	<0.001	0.387
Total	r	-0.483	0.527	0.681	-0.179
	p	0.004	0.001	<0.001	0.310
Disulphide	r	0.134	0.195	0.045	-0.197
	p	0.451	0.270	0.802	0.265
Disulfide/Native thiol	r	0.447	0.564	-0.372	-0.096
	p	0.08	<0.001	0.030	0.589
Disulfide/ Total thiol	r	0.446	0.553	-0.363	-0.102
	p	0.008	0.001	0.035	0.567
Native thiol/ Total thiol	r	-0.446	-0.550	0.363	0.102
	p	0.008	0.001	0.035	0.567

the dynamic thioldisulfide balance in polycystic kidney disease, given our results, disease duration and oxidative stress gradually increase together. Meantime, this result can also be related to the poor renal function of these patients and may not be typical of APKD. Severe impairment of thiol homeostasis in the plasma of terminal renal failure patients has been previously identified (25).

The correlations between the disulfide/native thiol ratio and systolic and diastolic blood pressures were statistically significant. We suggest that increased oxidative stress may be involved in the development of hypertension in APKD patients. Similarly, Ateş et al. (26) revealed that the disulfide/native thiol and disulfide/total thiol ratios were significantly higher in hypertensive patients.

In our study, there was a significant negative correlation between serum native thiol level and BUN and creatinine, as well as a significant positive correlation between native thiol level and GFR. There was a statistically significant negative correlation between the disulfide/native thiol ratio and creatinine and GFR. A statistically significant negative correlation was found between the disulfide/native thiol and disulfide/total thiol ratios and GFR. These results suggest that with impaired renal function, oxidative stress increases, and antioxidant defense weakens. Similar to our results, Ateş et al. (26) also found a correlation between GFR and serum thioldisulfide parameters.

In our study, TAC levels, indicating total antioxidant activity, were measured in the serum via the FRAP method. There was no significant difference between the two groups of FRAP measurement levels. Antioxidants such as uric acid (60%), ascorbic acid (15%), and vitamin E (5%) can be measured via the FRAP method. However, because thiols do not interact with ferric ions, the contribution of thiols to TAC cannot be determined via this method. Accordingly, no statistical correlation was found between serum thiol/disulfide parameters and FRAP values.

We can potentially explain the nonsignificant increase in FRAP levels as representing limited information about the eating habits affecting oxidative stress levels (27).

Our work involves certain limitations. First, the number of participants in both groups was low, which may have limited our statistical power. Second, this cross-sectional study was conducted at only one center. By increasing the number of participants from various populations, we might obtain clearer results. Despite the exclusion of participants who used vitamin supplements, nutritional habits that could cause oxidative stress were not standardized. Although non-diabetic volunteers were included in the study, fasting blood glucose, a factor affecting oxidative stress, varied between the two groups.

CONCLUSION

Our findings suggest that thiol/disulfide hemostasis may be associated with APKD disease progression.

REFERENCES

1. Tangri N, Hougen I, Alam A, Perrone R, McFarlane P, Pei Y: Total kidney volume as a biomarker of disease progression in autosomal dominant polycystic kidney disease. Can J Kidney Health Dis 2017;4:2054358117693355
2. Torres VE, Harris PC, Pirson Y: Autosomal dominant polycystic kidney disease. Lancet 2007;369:1287-1301

3. Reeders ST: Multilocus polycystic disease. Nat Genet 1992;1:235-237
4. Hansell P, Welch WJ, Blantz RC, Palm F: Determinants of kidney oxygen consumption and their relationship to tissue oxygen tension in diabetes and hypertension. Clin Exp Pharm Phys 2013;40:123-137
5. Zachara BA: Selenium and selenium-dependent antioxidants in chronic kidney disease. Adv Clin Chem 2015;68:131-151
6. Poulianiti KP, Kaltsatou A, Mitrou GI, Jamurtas AZ, Koutedakis Y, Maridaki M, Stefanidis I, Sakkas GK, Karatzaferi C: Systemic redox imbalance in chronic kidney disease: A systematic review. Oxid Med Cell Longev 2016;2016:8598253
7. Wu G, Somlo S: Molecular genetics and mechanism of autosomal dominant polycystic kidney disease. Mol Genet Metab 2000;69:1-15
8. Maser RL, Vassmer D, Magenheimer BS, Calvet JP: Oxidant stress and reduced antioxidant enzyme protection in polycystic kidney disease. J Am Soc Nephrol 2002;13:991-999
9. Lan X, Lederman R, Eng JM, Shoshtari SS, Saleem MA, Malhotra A, Singhal PC: Nicotine induces podocyte apoptosis through increasing oxidative stress. PLoS One 2016;11:e0167071
10. Iglesias-De La Cruz MC, Ruiz-Torres P, Alcamí J, Díez-Marqués L, Ortega-Velázquez R, Chen S, Rodríguez-Puyol M, Ziyadeh FN, Rodríguez-Puyol D: Hydrogen peroxide increases extracellular matrix mRNA through TGF-beta inhuman mesangial cells. Kidney Int 2001;59:87-95
11. Winther JR, Thorpe C: Quantification of thiols and disulfides. Biochim Biophys Acta 2014;1840:838-846
12. Nagy P: Kinetics and mechanisms of thiol-disulfide exchange covering direct substitution and thiol oxidation-mediated pathways. Antiox Redox Sign 2013;18:1623-1641
13. Üstündağ Budak Y, Kahvecioğlu S, Celik H, Alışık M, Erel O: Serum thiol/disulfide homeostasis in hemodialysis, peritoneal dialysis, and renal transplantation patients. Turk Neph Dial Transpl 2017;26:105-110
14. Colombo G, Reggiani F, Podestà MA, Garavaglia ML, Portinaro NM, Milzani A, Badalamenti S, Dalle-Donne I: Plasma protein thiolation index (PTI) as a biomarker of thiol-specific oxidative stress in haemodialyzed patients. Free Radic Biol Med 2015;89:443-451
15. Przemyslaw W, Piotr K, Grazyna C, Danuta KP, Malgorzata I, Barnedata M, Małgorzata S, Witold S: Total, free, and proteinbound thiols in plasma of peritoneal dialysis and predialysis patients. Int Urol Nephrol 2011;43:1201-1209
16. Erel O, Neselioglu S: A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-332
17. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F; Chronic Kidney Disease Epidemiology Collaboration: Expressing the modification of diet in renal disease study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007;53:766-772
18. Ustundag Y, Huysal K, Kahvecioglu S, Demirci H, Yavuz S, Sambel M, Unal D: Establishing reference values and evaluation of an in-house ferric reducing antioxidant power (FRAP) colorimetric assay in microplates. Eur Res J 2016;2:126-131
19. Erel O: A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277-285
20. Reischl E, Dafre AL, Franco JL, Wilhelm Filho D: Distribution, adaptation and physiological meaning of thiols from vertebrate hemoglobins. Comp Biochem Physiol Toxicol Pharmacol 2007;146:22-53
21. Maser RL, Calvet JP: Reduced expression of antioxidant and detoxicant enzyme genes in polycystic kidney disease (PKD). J Am Soc Nephrol 1996;7:1617
22. Torres VE, Bengal R, Litwiller R, Wilson D: Aggravation of polycystic kidney disease in Han: SPRD rats by buthionine sulfoximine. J Am Soc Nephrol 1997;8:1283-1291
23. Modlinger PS, Wilcox CS, Aslam S: Nitric oxide, oxidative stress, and progression of chronic renal failure. Semin Nephrol 2004;24:354-365
24. Modaresi A, Nafar M, Sahraei Z: Oxidative stress in chronic kidney disease. Iran J Kidney Dis 2015;9:165-179
25. Włodek PJ, Smolenski OB, Chwatko G, Iciek MB, Miłkowski A, Bald E, Włodek L: Disruption of thiol homeostasis in plasma of terminal renal failure patients. Clin Chim Acta 2006;366:137-145
26. Ateş İ, Ozkayar N, Altay M, Meriç Yılmaz F, Topçuoğlu C, Alışık M, Erel Ö, Dede F: Is disulphide/thiol ratio related to blood pressure in masked hypertension?. Clin Exp Hypertens 2016;29;38:150-154
27. Zamora-Ros R, Serafini M, Estruch R, Lamuela-Raventós RM, Martínez-González MA, Salas-Salvadó J, Fiol M, Lapetra J, Arós F, Covas MI, Andres-Lacueva C; PREDIMED Study Investigators: Mediterranean diet and non enzymatic antioxidant capacity in the PREDIMED study: Evidence for a mechanism of antioxidant tuning. Nutr Metab Cardiovasc Dis 2013;23:1167-1174