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ORIGINAL ARTICLE

Oxidative stress improvement is associated with increased levels of taurine in CKD patients undergoing lipid-lowering therapy

Angelo Zinellu · Salvatore Sotgia · Giacomina Loriga · Luca Deiana · Andrea Ercole Satta · Ciriaco Carru

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Abstract Lipid-lowering therapy has been reported to reduce several oxidative stress (OS) markers in hypercholesterolemia. Since OS is frequently associated with renal dysfunction, we aimed to investigate the effect of hypolipidemic drugs on oxidative stress and plasma taurine (Tau), a sulfur amino acid with a marked antioxidant effect, in chronic kidney disease (CKD). We enrolled 30 CKD randomized to receive three different hypolipidemic regimens for 12 months: simvastatin alone (40 mg/day) or ezetimibe/simvastatin combined therapy (10/20 or 10/40 mg/day). Low molecular weight (LMW) thiols including homocysteine, cysteine, cysteinylglycine, glutathione, and glutamylcysteine in their reduced and total form and oxidative stress indices as malondialdehyde (MDA) and allantoin/uric acid (All/UA) ratio were also evaluated. Tau concentration significantly increased throughout the therapy. The rise of taurine was more striking for the group with the concomitant administration of ezetimibe/simvastatin 10/40 mg/day (+31.6% after 1 year of therapy). A significant decrease of both MDA and All/UA ratio was observed during therapy for all patients (−19% for both MDA and All/UA ratio) with a more pronounced effect in patients treated with ezetimibe/simvastatin 10/40 mg/day (−26% for MDA and −28% for All/UA ratio). Besides, an increase of thiols reduced forms was found (+20.7% of LMW thiols redox status) with a greater effect in subjects treated with ezetimibe/simvastatin 10/40 mg/day (+24.7%). Moreover, we demonstrated that oxidative stress improvement during therapy was correlated with increased taurine levels. We hypothesize that taurine may be responsible for the oxidative stress improvement observed during lipid-lowering treatment through the reduction of superoxide anion production at the respiratory chain activity level.

Keywords Chronic kidney disease · Taurine · LDL · Oxidative stress

A. Zinellu
Porto Conte Ricerche Srl, Tramariglio, Alghero, Sassari, Italy

A. Zinellu (✉) · S. Sotgia · L. Deiana · C. Carru (✉)
Department of Biomedical Sciences and Centre of Excellence for Biotechnology Development and Biodiversity Research, University of Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy
e-mail: angelozinellu@libero.it

C. Carru
e-mail: carru@uniss.it

G. Loriga · A. E. Satta
Department of Internal Medicine, Azienda Ospedaliera Universitaria, University of Sassari, Sassari, Italy

C. Carru
National Laboratory of the National Institute of Biostructures and Biosystems, Osilo, Italy

Introduction

Chronic kidney disease is a well-known risk factor for cardiovascular disease (CVD) (Manjunath et al. 2003) and it has been reported that the severity of cardiovascular complications increases with the decrease of glomerular filtration rate (GFR) (Go et al. 2004). CKD patients are frequently affected by diabetes, hypertension, and obesity which are known traditional CVD risk factors in the general population (Sarnak et al. 2003). Moreover, in proteinuric patients, dyslipidemia has a highly atherogenic profile, with increased levels of triglycerides, total and LDL cholesterol, and decreased high-density lipoprotein (HDL) cholesterol (Vaziri 2006). Several biomarkers of

oxidative stress have been also found at elevated concentration in patients with CKD. These include products of lipid oxidation (lipid peroxides, malondialdehyde, and thiobarbituric acid reactive substances) and oxidized LDL (Agarwal 2004; Diepeveen et al. 2004), advanced oxidation protein products (AOPP) (Witko-Sarsat et al. 1998), F2 isoprostanes (Ikizler et al. 2002), and 8-hydroxyl 2-deoxyguanosine (marker of oxidative DNA damage) (Puchades Montesa et al. 2009). The nature of the oxidative stress in chronic kidney disease still remains unclear. In general, impaired oxidative balance may result from a combination of increased ROS production and reduced clearance, as well as an ineffective antioxidant defense mechanism, though some authors did not find differences in total antioxidant capacity between CKD patients and healthy people. Since it has been amply reported that the treatment of dyslipidemia with statin decreases cardiovascular morbidity and mortality, the control of plasma lipid levels is one of the targets for the treatment of CKD (Sever et al. 2003). Experimental and clinical evidences show that statin, as well as improving lipid profile, may have specific renoprotective properties and, combined with the renin–angiotensin system (RAS) inhibitor therapy, may synergize their antiproteinuric effects (Zoja et al. 2010). Moreover, preliminary data suggest that the combination of statin with ezetimibe (EZE), a cholesterol absorption inhibitor, provides complementary effects on lipids over that achieved with statin monotherapy (Dembowski and Davidson 2009).

Recently, an inverse relation between serum cholesterol levels and taurine concentration has been described (Murakami et al. 2010). Taurine is a sulfur-containing amino acid that is widely distributed in animal tissues and it is especially abundant in heart, liver, and kidney. It plays an important role in maintaining physiological homeostasis (Huxtable 1992) and also acts as an antioxidant agent. Taurine is involved in the catabolism of cholesterol. Cholesterol is converted into bile acids within the liver, which are then conjugated with either taurine or glycine before secretion into the bile. By this work, we aimed to evaluate the effect of hypolipidemic drugs on plasma taurine levels in proteinuric nephropathic patients. We enrolled 30 hypertensive patients in stable therapy with renin–angiotensin system (RAS) inhibitors, with low-density lipoprotein cholesterol superior to 100 mg/dl, treated with three different hypolipidemic regimens: simvastatin alone (40 mg/day) or EZE/simvastatin combined therapy (10/20 or 10/40 mg/day). Considering that taurine has important antioxidant effects, the evaluation of free plasma malondialdehyde (MDA) levels, the allantoine/uric acid ratio (All/UA), and the LMW thiols redox status was also performed to monitor the oxidative stress in patients during drug treatment.

Methods

Subjects

Thirty CKD patients were selected at the Istituto di Patologia Medica-Azienda Ospedaliero Universitaria with the following inclusion criteria: age >18; LDL cholesterol >100 mg/dl (without concomitant hypolipidemic drugs); presence of proteinuric chronic nephropathy defined as creatinine clearance >20 ml/min/1.73 m^2 combined to a urinary protein excretion rate >0.3 g/24 h, without evidence of urinary tract infection or overt heart failure (New York Heart Association class III or more). Patients were classified as CKD on stage 3 and 4 not receiving dialysis. Exclusion criteria were represented by: previous or concomitant treatment with steroids, anti-inflammatory and immunosuppressive agents, vitamin B6, B12, folate or statin; evidence or suspicion of renovascular disease, obstructive uropathy, type I diabetes mellitus, vasculitis. All patients were in stable treatment with RAS inhibitor therapy (ACE inhibition by benazepril plus angiotensin II antagonism by valsartan) for at least 6 months.

Enrolled patients were randomized to receive 40 mg/day simvastatin (group 1, $n = 10$) or EZE/simvastatin 10/20 mg/day (group 2, $n = 10$), or EZE/simvastatin 10/40 mg/day (group 3, $n = 10$). Patients were treated for 12 months and evaluated at baseline and at 4, 8 and 12 months of therapy. The control group included 30 subjects, recruited from accompanying relatives or friends of patients or from hospital personnel. Exclusion criteria for the control subjects were a history of diabetes, systemic hypertension, cardiovascular or cerebrovascular disease, renal failure, blood dyscrasias, tumors, retinal vascular disorders, age <18, and current medication with vitamin B6, B12, or folic acid. The control subjects were recruited concurrently during the patients' recruitment period. An informed consent was obtained from each patient and control, and the study was approved by our institution's ethics committee. The study complied with the principles of the Helsinki Declaration. This study has been registered with http://clinicaltrials.gov (NCT00861731).

Biochemical analysis

Taurine and plasma LMW thiols as homocysteine (Hcy), cysteine (Cys), cysteinylglycine (Cys-Gly), glutathione (GSH), and glutamylcysteine (Glu-Cys) were measured by capillary electrophoresis laser-induced detection, as previously reported (Zinellu et al. 2003, 2009; Carru et al. 2004). In particular, for reduced thiols, 200 μL of plasma sample was deproteinized by adding 200 μL of acetonitrile and centrifuged at 2,000×g for 5 min. Then, 100 μL sample was mixed with 100 μL of 100 mmol/L sodium

phosphate buffer, pH 12.5, and 15 μL of 0.8 mmol/L 5-IAF. After vortex-mixing, the samples were incubated for 15 min at room temperature. For total thiols, 200 μL of plasma sample was mixed with 20 μL of 10% v/v TBP in DMF for 10 min to reduce disulfide bounds. Plasma proteins were then precipitated by adding 200 μL of 6% SSA and removed by centrifugation (2000×*g* for 5 min). As much as 100 μL of 300 mmol/L sodium phosphate buffer, pH 12.5, and 25 μL of 4.1 mmol/L 5-IAF were added to 100 μL of supernatant. After vortex-mixing, the samples were incubated for 15 min at room temperature. Derivatized samples were diluted 100-fold in water and analyzed by capillary electrophoresis. The LMW thiol redox status was calculated by measuring the ratio of the sum of reduced (r) and total (t) forms of thiols as follows: (rCys-Gly + rCys + rHcy + rGSH + rGlu-Cys)/(tCys-Gly + tCys + tHcy + tGSH + tGlu-Cys).

The MDA and All/UA ratio was determined by capillary electrophoresis UV detection as previously described (Zinellu et al. 2011a, b). Total cholesterol, HDL, and triglycerides were measured by enzymatic methods using commercial kits (Boehringer-Mannheim, Mannheim, Germany).

Statistical analysis

All the results are expressed as mean values (mean ± SD) or median values (median and range). The distribution of variables in the study group was assessed by the Kolmogorov–Simirnov test. The statistical differences among controls and patients were compared using unpaired Student's *t* test or Mann–Whitney rank sum test, as appropriate. The effect of the drug treatments was evaluated by one-way repeated measures ANOVA. The correlation analysis between variables was performed by Pearson's correlation. The calculations were performed using the Statgraphics plus 5.1 package for Windows (Rockville, MD, USA).

Results

Taurine biosynthesis is strictly linked to the metabolism of sulfur amino acids, involving the enzymatic oxidation and conversion of Cys derived from methionine (Fig. 1); therefore, the quantification of LMW thiols as Cys, Cys-Gly, Hcy, GSH and Glu-Cys was performed in the study.

The clinical characteristics of controls and all CKD patients, at baseline, are reported in Table 1. As previously reported (Zinellu et al. 2010), nephropathic patients showed elevated levels of plasma triglycerides, LDL, and plasma thiols in comparison to normal values in the healthy population. In particular, as expected, more than 50% of

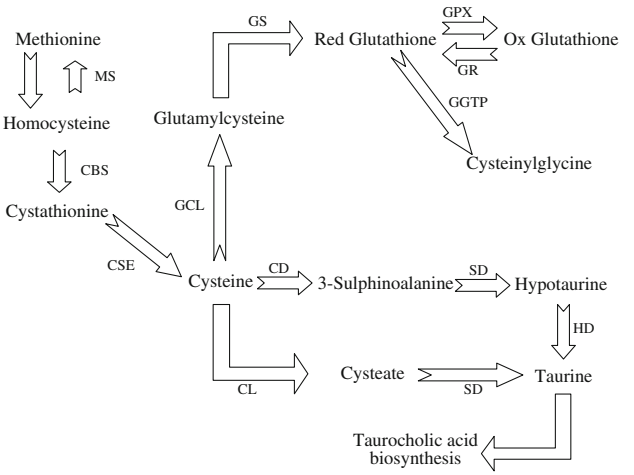

Fig. 1 Diagram of taurine metabolism. *CBS* cystathionine beta-synthase, *CD* cysteine dioxygenase, *CL* cysteine lyase, *CSE* cystathionine gamma-lyase, *GCL* glutamate cysteine ligase, *GGTP* gamma glutamyltranspeptidase, *GPX* glutathione peroxidase, *GR* glutathione reductase, *GS* glutathione synthetase, *HD* hypotaurine dehydrogenase, *MS* methionine synthase, *SD* sulfoalanine decarboxylase

CKD subjects were hyperhomocysteinemic (Hcy >15 μmol/L) versus 10% of the healthy population. All the oxidative stress indices evaluated were significantly altered in CKD (MDA: 218 ± 147 vs. 140 ± 81, $p = 0.013$; All/AU ratio: 1.5 ± 0.7% vs. 0.9 ± 0.1%, $p < 0.001$; LMW thiols redox status: 8.2 ± 2.1% vs. 10.3 ± 1.5%, $p < 0.001$). Baseline levels of both MDA and All/UA were inversely related to GFR ($r = -0.42$, $p = 0.02$ and $r = -0.44$, $p = 0.015$, respectively). Moreover, we found lower levels of plasma taurine in nephropathic subjects than in controls (51.1 ± 13.3 vs. 62.3 ± 16.1, $p < 0.01$). Taurine levels were inversely correlated with total cholesterol levels in controls ($r = -0.33$, $p < 0.05$) but not in patients.

After randomization, no significant differences of the clinical characteristics analyzed were found among the three groups except for All/UA levels (Table 1). As expected, a significant improvement in lipid profile was already observed for all groups after 4 months of therapy (Fig. 2). In particular, greater improvement in lipid profile was observed in group 3. A decrease of 40% in total cholesterol, 62% in LDL cholesterol, 21% in triglycerides, and an important reduction in LDL/HDL ratio (3.3 ± 1.6 at baseline vs. 1.1 ± 0.5 after 12 months, $p = 0.001$), with an 8% increase in HDL-c was observed in this group after 12 months of treatment. Drug treatment did not affect LMW plasma thiol levels also after categorization on the basis of the therapy type (data not shown), while taurine concentration significantly increased throughout drug treatment (Fig. 3a). The rise of taurine was more striking for the group with the concomitant administration of EZE/simvastatin 10/40 mg/day (+31.6% after 1 year of therapy) (Fig. 3b–d).

Table 1 Demographic and clinical characteristics of patients and controls

	Controls ($n = 30$) Mean ± SD or median (range)	CKD ($n = 30$) Mean ± SD or median (range)	Group 1 ($n = 10$) Simvastatin 40 mg/day Mean ± SD or median (range)	Group 2 ($n = 10$) Eze/simva 10/20 mg/day Mean ± SD or median (range)	Group 3 ($n = 10$) Eze/simva 10/40 mg/day Mean ± SD or median (range)
Sex [f/m] (% f)	11/19 (37%)	11/19 (37%)	2/8 (20%)	4/6 (40%)	5/5 (50%)
Age (years)	59 ± 10	60 ± 11	63 ± 11	58 ± 12	59 ± 9
Kidney profile					
Creatinine (mg/dL)	0.87 ± 0.24	1.75 ± 0.77***	1.92 ± 0.98	1.63 ± 0.62	1.70 ± 0.71
GFR (ml/min per 1.73 m^2)	–	55 ± 30	61 ± 48	52 ± 19	53 ± 8
Proteinuria (g/24 h)	–	0.99 ± 1.27	0.91 ± 0.63	0.81 ± 0.81	1.25 ± 2.00
Lipid profile					
Total cholesterol (mg/dL)	208 ± 42	239 ± 43**	232 ± 34	230 ± 41	254 ± 53
LDL-c (mg/dL)	131 ± 39	160 ± 37**	164 ± 34	156 ± 32	165 ± 47
HDL-c (mg/dL)	56 ± 18	49 ± 15	44 ± 8	47 ± 12	57 ± 19
LDL/HDL ratio	2.5 ± 1.1	3.5 ± 1.3**	3.75 ± 1.00	3.50 ± 1.12	3.26 ± 1.71
Triglycerides (mg/dL)	108 ± 54	143 ± 69*	141 ± 70	136 ± 62	151 ± 80
Total thiols					
tCysGly (μmol/L)	34.8 ± 6.9	35.6 ± 8.9	38.8 ± 9.1	34.4 ± 8.3	33.6 ± 9.2
tHcy (μmol/L)	10.7 ± 3.2	18.4 ± 11.2**	20.7 ± 15.0	18.5 ± 11.6	16.0 ± 5.9
tCys (μmol/L)	222 ± 49	299 ± 67***	296 ± 62	281 ± 51	319 ± 85
tGSH (μmol/L)	4.9 ± 1.5	6.5 ± 2.9**	6.43 ± 2.69	5.50 ± 1.66	7.45 ± 3.89
tGluCys (μmol/L)	3.2 ± 0.7	4.3 ± 1.1***	4.41 ± 1.06	3.94 ± 1.03	4.53 ± 1.23
tThiols (μmol/L)	275 ± 55	364 ± 75***	367 ± 63	344 ± 60	381 ± 99
Oxidative stress indices					
MDA (nmol/L)	140 ± 81	218 ± 147*	248 ± 95	174 ± 163	230 ± 163
All/UA ratio (%)	0.90 ± 0.10	1.5 ± 0.7***	1.7 ± 0.10	1.1 ± 0.4[a]	1.6 ± 0.6
LMW thiols redox status (%)	10.3 ± 1.5	8.2 ± 2.1***	8.6 ± 2.1	8.4 ± 1.9	7.7 ± 2.4
Blood pressure					
Systolic BP (mmHg)	126 ± 8	130 ± 9	131 ± 9	127 ± 11	132 ± 8
Diastolic BP (mmHg)	80 (70–90)	80 (60–95)	80 (70–85)	80 (60–95)	80 (70–90)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus controls; after randomization, baseline characteristics of the three sub-groups were quite similar except for [a] $p < 0.05$ versus group 1 and 3

As reported in Table 2, a significant decrease of both MDA and All/UA ratio was observed during therapy for all patients (−19% for both MDA and All/UA ratio after 12 months), with a more pronounced effect in patients of group 3 (−26% for MDA and −28% for All/UA ratio after 12 months) and an increase of reduced forms of thiols was found (+20.7% of LMW thiols redox status after 12 months) with a greater effect in subjects of group 3 (+24.7% after 12 months). The mean values of taurine at baseline and 4, 8, and 12 months inversely correlated with the mean values of MDA and All/UA for all patients ($r = -0.978$, $p = 0.022$ and $r = -0.975$, $p = 0.025$, respectively) with a significant correlation in patients of group 3 ($r = -0.992$, $p = 0.008$ and $r = -0.956$, $p = 0.044$, respectively). The mean values of taurine were also positively related with LMW thiol redox status average for all patients ($r = 0.954$, $p = 0.046$), in particular in the group with the concomitant administration of EZE/simvastatin 10/40 mg/day ($r = 0.957$, $p = 0.043$).

Drug treatment did not significantly improve the kidney function as demonstrated by GFR mean value that was 55 ± 30 ml/min per 1.73 m^2 at baseline and 59 ± 40 ml/min per 1.73 m^2 after 1 year of treatment for all patients ($p = 0.18$ by ANOVA) and proteinuria mean value that was 0.99 ± 1.27 g/24 h at baseline and 0.85 ± 0.85 after 1 year of treatment for all patients ($p = 0.75$ by ANOVA).

Discussion

Oxidative stress is frequently associated with renal dysfunction and several markers, including plasma F2-isoprostanes, advanced oxidation proteins products, MDA and

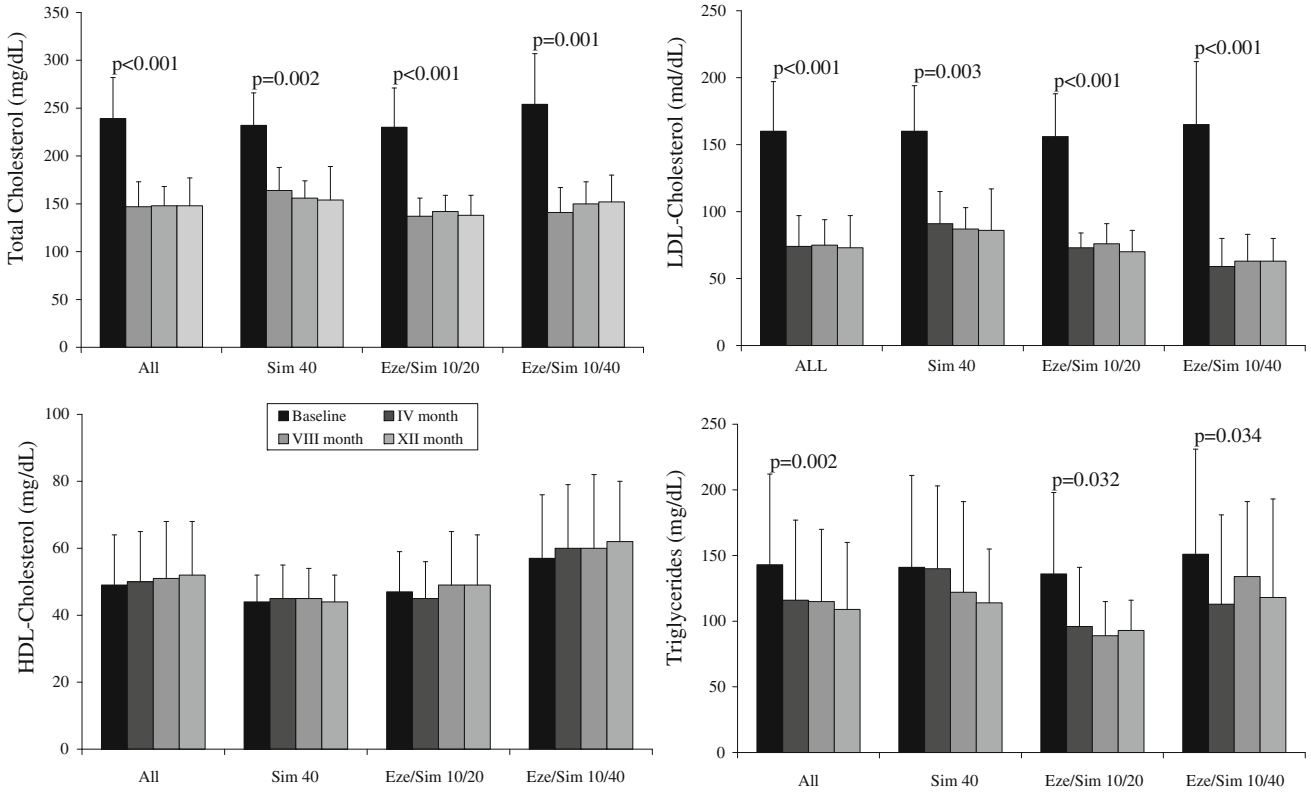

Fig. 2 Effect of drug treatment on plasma lipid parameters. *p* values refer to linear trend and has been evaluated by one-way repeated measures ANOVA

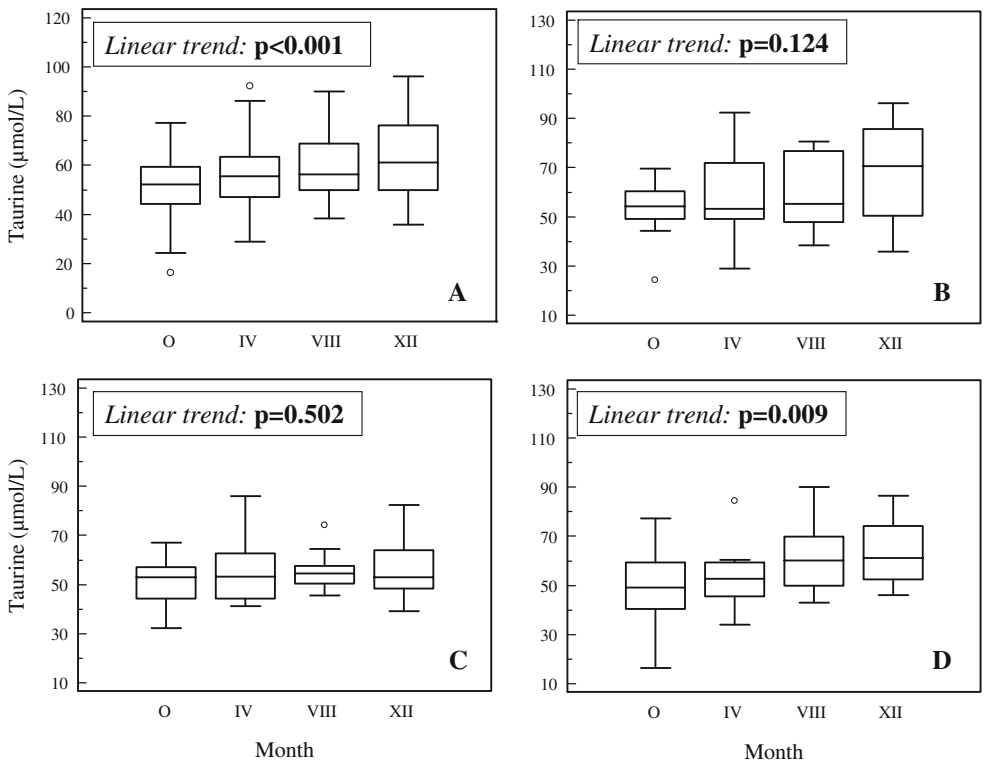

Fig. 3 Effect of drug treatment on taurine plasma levels in all patients (**a**) and after categorization for therapy type. **b** Group 1 ($n = 10$), **c** group 2 ($n = 10$), **d** group 3 ($n = 10$). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ versus baseline. *p* values were evaluated by one-way repeated measures ANOVA with Bonferroni correction. Analysis power for linear trend was 0.93 for **a**, and 0.75 for **d**

Table 2 Effect of drug treatment on oxidative stress

	Baseline	4 months	8 months	12 months	Linear trend p value (power)
MDA (nmol/L)					
All patients	218 ± 143	195 ± 129	183 ± 131	176 ± 123*	0.004 (0.97)
Sim 40	248 ± 95	224 ± 113	227 ± 136	220 ± 129	0.250
E/S 10/20	174 ± 163	156 ± 144	146 ± 140	135 ± 119	0.162
E/S 10/40	231 ± 163	206 ± 133	177 ± 114	172 ± 116	0.03 (0.82)
All/UA ratio (%)					
All patients	1.47 ± 0.72	1.33 ± 0.68	1.23 ± 0.56	1.19 ± 0.51	0.002 (0.98)
Sim 40	1.70 ± 0.97	1.66 ± 0.95	1.52 ± 0.75	1.53 ± 0.63	0.231
E/S 10/20	1.14 ± 0.35	1.03 ± 0.21	1.02 ± 0.23	0.92 ± 0.30	0.056
E/S 10/40	1.56 ± 0.64	1.29 ± 0.59***	1.16 ± 0.48*	1.13 ± 0.39	0.03 (0.91)
LMW thiol redox status (%)					
All patients	8.2 ± 2.1	8.4 ± 3.1	9.4 ± 2.7*	9.9 ± 2.7***	<0.0001 (0.96)
Sim 40	8.6 ± 2.1	8.1 ± 4.4	9.4 ± 2.7	10.1 ± 2.2*	0.021 (0.64)
E/S 10/20	8.4 ± 1.9	8.4 ± 2.7	9.7 ± 3.1	10.0 ± 4.1	0.077
E/S 10/40	7.7 ± 2.4	8.7 ± 2.0	9.1 ± 2.4	9.6 ± 1.4*	0.017 (0.68)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus baseline. ANOVA with Bonferroni correction

oxidized LDL, have been reported to be increased in nephropathic patients (Agarwal 2004; Diepeveen et al. 2004; Witko-Sarsat et al. 1998; Ikizler et al. 2002). It seems that ROS increases as renal function deteriorates, as reported from different studies in which an inverse relationship between oxidative stress indices and GFR was found (Go et al. 2004). In agreement with these observations, in the CKD patients we found increased levels of MDA and All/UA ratio and lower levels of reduced LMW thiols (decreased LMW thiol redox status) indicating a more marked oxidative stress, compared to controls. Even if the nature of oxidative stress in chronic kidney disease remains to be elucidated, some authors suggest that it might be a consequence of higher ROS production, since total antioxidant capacity has been found similar to that in healthy subjects (Cachofeiro et al. 2008). In addition to the oxidative stress that increases the risk for CVD, nephropathic patients are also characterized by a dyslipidemic profile with increased total and LDL cholesterol, triglyceride, and decreased HDL-c. The lowering of low-density lipoprotein cholesterol levels plays an important role in CVD prevention and represents the main target of hypolipidemic therapy. Statins are considered the most effective drug in terms of improving the serum lipid profile (Ong 2005). They reduce the cholesterol synthesis by inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase. The treatment with statins has been associated with improved outcomes either in the primary or the secondary prevention of CVD (Baigent et al. 2005). This benefit has been attributed not only to the lipid-lowering potency, but also to various pleiotropic anti-atherosclerotic properties of these drugs (namely anti-inflammatory, anti-oxidative, and anti-thrombotic) (Kostapanos et al. 2008). Nevertheless, not all patients tolerate high-dose statins and the incidence of abnormalities in liver function or myopathy may increase in a dose-dependent manner with this class of drugs (Conard et al. 2010); and some patients still do not meet any treatment goal. A statin therapy combined with a lipid-lowering therapy, which has a different mechanism of action, such as EZE, may provide complementary effects on lipids that surpass those of high-dose statins. EZE potently inhibits the intestinal absorption of cholesterol from dietary and biliary sources by blocking the Niemann–Pick C1-like 1 protein for cholesterol transport (Garcia-Calvo et al. 2005). Clinical studies showed that EZE, either as monotherapy or in combination with statins, decreased LDL cholesterol levels or beneficially modified serum lipid profile (Stein et al. 2008). We aimed to investigate the effect of lipid-lowering therapy on the levels of plasma taurine and oxidative stress indices in nephropathic proteinuric patients. Taurine is a sulfur amino acid with anti-atherosclerotic properties in part due to its antioxidant capacity. Reduced levels of taurine has been found in end-stage renal disease patients under hemodialysis treatment (Suliman et al. 2002, 2003); however, little information exists about its levels in CKD at stages 3 and 4. In these patients, we found lower taurine levels than in the controls (−18%). Considering that Tau is much more concentrated in the intracellular compartment (between 5 and 50 mmol/L), the difference in all body taurine levels may be also more substantial.

As expected, the drug treatment significantly improved the lipid profile in all patients with better results for

subjects treated with EZE/simvastatin 10/40 mg day. An increase of Tau levels in all treated patients was found, but a greater efficacy was attained from combined therapy with higher simvastatin dose, by which a rise of 31.6% was reached after 1 year therapy, thus restoring the taurine levels of the healthy population (above 60 μmol/L). Interestingly, as previously reported (Milionis et al. 2003; Dierkes et al. 2004), the levels of Tau precursor as Cys and other thiols during drug treatment remained unchanged, suggesting that the taurine increase was not due to the rise of precursor levels. Since taurine is reabsorbed by the kidney, the recovery of kidney function by hypolipidemic therapy may explain the normalization of its levels. However in our population, we did not find significant improvement in GFR or proteinuria during the treatment, thus suggesting that the taurine increase was due to other factors than kidney function recovery. Since no evidence has been reported on the direct effect of hypolipemic drugs on Tau synthesis, the mechanisms for taurine increase during therapy with hypolipidemic drugs almost certainly involve the catabolism of cholesterol (Murakami et al. 2010). Cholesterol is converted to bile acids within the liver, which are then conjugated with either taurine or glycine before secretion into the bile. This process represents one of the major means for the ultimate excretion of sterols from the body. In accordance with this hypothesis, we found an inverse relationship between plasma total cholesterol and taurine levels in controls, as also already described in literature (Choi et al. 2006).

It has been reported that a hypercholesterolemic diet causes a significant decrease in the concentration of taurine in serum, liver, and heart compared to that found in the control group (Yokogoshi and Oda 2002) through the excessive employment of Tau in bile salts. Therefore, both statins (through the inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase) and EZE (by the reduction of the intestinal cholesterol absorption), by decreasing plasma cholesterol levels, allow reduction of the quantity of taurine excreted as conjugated cholesterol (Murakami et al. 2010), thus yielding an increase in the Tau plasma concentration.

It has been reported that both statin and EZE administration may reduce oxidative stress in diabetes and dyslipidemia (Kater et al. 2010; Kostapanos et al. 2011), but up to now there is little information about their anti-oxidant effects, alone or as combined therapy in CKD (Cachofeiro et al. 2008). To investigate the oxidative stress in our patient cohort, we measured plasma MDA, All/UA ratio, and LMW thiol redox state. As previously reported (Witko-Sarsat et al. 1998; Yilmaz et al. 2006; Dounousi et al. 2006), we found at baseline a significant inverse relationship between GFR and OS parameters. We also found a significant reduction of both MDA and All/UA ratio in all CKD patients during the therapy, with a great improvement in patients treated with combined therapy (EZE/simvastatin 10/40 mg day). Similarly we observed a significant increase in LMW thiol redox state that was more pronounced in group 3. The oxidative stress lowering allows the formation of more thiols in the reduced form, since the strength that pushes to form mixed disulfides is less intense. Moreover, we demonstrated that the OS improvement during the therapy was correlated with taurine levels suggesting that this sulfur amino acid may be responsible for the oxidative stress amelioration observed during lipid-lowering treatment. The relationship was mostly evident in patients treated with combined therapy (EZE/simvastatin 10/40 mg day). Although taurine is unable to directly scavenge the classic ROS, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, there are several studies suggesting that it is an effective inhibitor of ROS generation. By stabilizing the environment in the mitochondria, taurine prevents the leakage of the reactive compounds formed in the reactive mitochondrial environment and thus, indirectly, acts as an antioxidant (Hansen et al. 2006). In particular, some authors report the presence of taurine-conjugated tRNAs in the mitochondria (Schaffer et al. 2009; Jong et al. 2011). Because tRNA conjugation is required for normal translation of mitochondrial-encoded proteins, a taurine deficiency yields a drop in the production of these respiratory chain components. As a result, the flux through the electron transport chain decreases. The dysfunctional respiratory chain accumulates electron donors, which divert electrons from the respiratory chain to oxygen, forming superoxide anion in the process. The restoration of taurine levels increases the levels of conjugated tRNA, restores respiratory chain activity, and increases the synthesis of ATP at the expense of superoxide anion production (Schaffer et al. 2009; Jong et al. 2011). This well agrees with previous observations, which have suggested that OS in uremia may be due to higher ROS production rather than an impaired antioxidant defense (Cachofeiro et al. 2008).

In conclusion, our data indicate that the taurine levels increase in CKD patients undergoing lipid-lowering therapy, reaching the values of the healthy population. Better results are guaranteed by combined therapy with higher simvastatin doses. Moreover, a significant improvement in oxidative stress indices following treatment with hypolipidemic drugs has been found. This effect was previously observed without providing a rationale. We hypothesize that the decrease in serum cholesterol following drug administration allows to restore normal taurine levels which, in turn, yields oxidative stress lowering through the reduction of superoxide anion production at the respiratory chain activity level.

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