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EFFECT OF ALPHA-LIPOIC ACID SUPPLEMENTATION ON OXIDATIVE STRESS MARKERS AND ANTIOXIDATIVE DEFENSE IN PATIENTS WITH SCHIZOPHRENIA

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SUMMARY

***Background**: The purpose of this study was to examine the effects of alpha-lipoic acid (LA) supplementation on oxidative stress markers in patients with schizophrenia.*

***Subjects and methods**: Eighteen (18) medicated patients with schizophrenia and 38 healthy controls received daily supplements of LA (500 mg/day) for three months. At baseline, 45th and 90th days of supplementation, venous blood collected for analysis of oxidative stress markers [superoxide anion ($O_2^{\bullet-}$), thiobarbituric acid-reactive substances (TBARS) and advanced oxidation protein products (AOPP)] and antioxidative defense markers [superoxide dismutase (SOD), total sulfhydryl groups (-SH) and total antioxidant status (TAS)].*

***Results**: Increased plasma TBARS, TAS, SH groups levels and SOD activity were found in schizophrenic patients compared to control group. LA supplementation significantly reduced TBARS, AOPP and improved TAS levels in healthy subjects, while there were no significant differences in patients group. SH groups increased after 45 days and decreased to baseline levels after 90 days of supplementation in the control group. SOD activity decreased significantly in patients group after 45 days and 90 days of supplementation. After initial rose SOD activity in control group, decreased to baseline levels found after 90 days.*

***Conclusion**: LA supplementation decreased lipid peroxidation and oxidative damage of proteins and improved non-enzymatic antioxidant capacity in healthy controls. No significant changes were observed on oxidative damage in patients with schizophrenia.*

***Key words:** alpha-lipoic acid - oxidative stress - antioxidative defence - schizophrenia*

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INTRODUCTION

Schizophrenia is a severe disabling mental disorder that affects approximately 1% of the population worldwide (Perala et al. 2007). It is characterized clinically by positive symptoms (psychosis, hallucinations), negative symptoms (social withdrawal, flat affect, anhedonia), and cognitive dysfunction (Balu & Coyle 2011).

There is increasing evidence that oxidative stress, defined as imbalance between the production of reactive oxygen species (ROS) and clearance of ROS by components of the antioxidant defence system exist in schizophrenia (Bitanihirwe & Woo 2011, Ciobica et al. 2011). The possible mechanisms of increased ROS generation in schizophrenia include: mitochondrial dysfunction, auto-oxidation dopamine, pro-oxidant actions some antipsychotics (Bošković et al. 2011). The life style of schizophrenic patients (heavy smoking, drinking, high caloric intake and low physical activity) also contribute to increased susceptibility to oxidative stress (Mahadik et al. 2001) that may lead to changes in lipid membranes and alterations to DNA and proteins. In order to prevent oxidative damage, the body contains a large number of enzymatic (superoxide dismutase (SOD), glutathione peroxidase (GpX) and catalase (CAT)) and non-enzymatic antioxidants (e.g., glutathione (GSH), vitamin C and E, albumin and bilirubin) that prevent formation and/or scavenge ROS. Impaired antioxidant enzyme activities and reduced levels of antioxidants have been reported in drug-naive, first episode and chronically medicated schizophrenic patients (Mahadik et al. 2006).

Compared with the general population, patients with schizophrenia have increased prevalence of obesity, type 2 diabetes mellitus, and cardiovascular disease. Weight gain and increased adiposity are associated with decreases in insulin sensitivity, leading to an increased risk of hyperglycaemia and hyperlipidemia (Newcomer 2004). Oxidatively-modified lipoproteins, especially low-density lipoproteins (LDL) are formed via ROS activity. These species have been implicated in atherogenic processes (Stocker & Keaney 2004).

Recent years, increasing interest exists in using non-enzymatic antioxidants as adjunctive or supplements to prevent oxidative damage and to improve some of the

psychopathology and treatment-related side effects of antipsychotic drugs (Reddy & Reddy 2011).

Alpha-lipoic acid (LA) is a sulfur-containing compound that is synthesized naturally in very small amount by humans. Endogenous LA is a covalently bound for proteins and has essential role in energy metabolism in mitochondria (Packer et al. 1995). Besides its metabolic function, there is evidence that orally supply LA has potent antioxidant activity. As relatively small molecule, LA readily absorbed from the diet and across cell membranes. In the cell, LA converts to its reduced form dihydrolipoic acid (DHLA), a compound that also possesses biological activities (Gorąca et al. 2011). LA and DHLA have been shown that quenching of ROS, regeneration endogenous antioxidants such as vitamins C and E, and GSH, chelation of metal ions and repair of oxidatively damaged proteins (Biewenga et al. 1997, Bast & Haenen 2003, Bilska & Włodek 2005). Although the precise mechanisms are not completely defined, there is also some evidence that LA excerts anti-obesity and lipid-lowering effects (Carrier & Rideout 2013).

Taking into account these facts, the aim of the present study was to determine whether LA supplementation (500 mg per day) for three months could influence on oxidative stress markers and lipid profile in patients with schizophrenia and healthy controls.

SUBJECTS AND METHODS

Study population

The required sample size to obtain the power of 1-β=0.80 at α=0.05, was calculated according to the data from our preliminary results (Vidovic et al. 2010). Approximately 15 subjects per group were found to be sufficient. Eighteen patients (10 females and 8 males) diagnosed with schizophrenia according to International Classification of Diseases (ICD-10) criteria (WHO 1992) were recruited from the outpatient unit of the Clinic for Psychiatry, Clinical Center of Serbia, Belgrade. All patients have been compliant to their pharmacotherapeutic protocols and were in stable remission for at least two months prior to the inclusion of the study. All patients received antipsychotic therapy with 83.33% of them receiving second generation antipsychotics. All schizophrenic patients were of the chronic type, with duration of illness for at least 5 years, age between 25 and 60 years.

Control group consisted of 38 healthy subjects, similar age and gender (26 females and 12 males; mean age, 41.1±10.6 years), recruited from the general population and academic community who had no personal or family history of a psychiatric disorder.

A complete medical history including physical examination and laboratory tests was obtained from all subjects. The following exclusion criteria were applied to both patients with schizophrenia and controls: history of substance abuse or dependence, severe head injury, seizure disorders, diabetes, cancer, renal or liver diseases, the use of hypolipidemic drugs, or taking of any supplements at least 30 days prior the study beginning. Both patients and control subjects had similar socioeconomic status and dietary patterns.

All subjects gave signed informed consent to participate in the study protocol that was conducted in accordance with the Helsinki declaration and was approved by the Ethical Committee of the Clinical Center of Serbia in Belgrade.

Each participants received one LA capsule (500 mg) a day for 90 days. They were instructed to take the supplements 30 min before meals and to maintain their normal eating habits throughout the study. The patients were advised to continue their antipsychotic drugs as usual. To monitor compliance, subjects were asked to return any unused capsules.

All anthropometric measurements and blood sampling were performed at the beginning, after 45 days and 90 days of the study.

Symptom severity of subjects with schizophrenia was measured by using the Clinical Global Impression (CGI) scale (Guy 1976).

Anthropometric parameters and blood pressure

Body weight and total body fat were measured with Tanita digital scale (Inner Scan Body Composition Monitor, BC 587, Tanita Corp, Japan) to the nearest 0.1 kg with the subject wearing light clothes and without shoes. Body height was measured without shoes using a standard height bar. Body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of height in meters (kg/m^2). Waist circumference was measured midpoint between the iliac crest and the lowest rib. Hip circumference is the measure of maximum circumference over the buttocks. Measurements of both waist and hip circumference were taken twice to the nearest 1.0 cm and the average value was recorded. Waist to hip ratio (WHR) is waist circumference in centimeters divided by hip circumference in centimeters. Systolic and diastolic blood pressures were obtained by measuring the average value of two consecutive measurements in the sitting position with 5 min in between measurements, obtained by standard mercury sphygmomanometer.

Sample collections

Fasting venous blood was collected into tube with serum separator gel (for serum) and EDTA-containing tubes (for plasma) at baseline, 45 day and 90 day. Serum and plasma were separated by centrifugation and multiple aliqouts of each sample were stored at -80 ºC until analysis.

Biochemical parameters

Fasting glucose, lipid status parameters [total cholesterol (t-C), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG)] and uric acid (UA) concentrations were measured by standard laboratory procedures (ILab 300+ analyzer, Instrumentation Laboratory, Milan, Italy).

Oxidative stress status parameters

Thiobarbituric acid reactive substances (TBARS) were assayed in plasma according to Girroti et al. (1991). This assay is based on the formation of a complex between thiobarbituric acid and malondialdehyde, an end products of lipid peroxidation, which absorb at 535 nm. Advanced oxidation protein products (AOPP), which indicate the level of oxidative stress mediated protein damage, were measured using a spectrophotometric method at 340 nm and expressed as chloramine-T equivalents (μmol/L) (Witko-Sarsat et al. 1996). A spectrophotometric nitroblue tetrazolium (NBT) reduction assay was used to determine the production superoxide anione in plasma (Auclair & Voisin 1985).

Plasma superoxide dismutase (SOD) activities were assayed according to Misra & Fridovich method (1972) in which inhibition epinephrine auto-oxidation by SOD in the examined sample is recorded as an increase in absorbance at 505 nm for 3 min. One unit of SOD activity is defined as the activity that inhibits the auto-oxidation of adrenalin by 50 %.

The concentration of sulphydryl groups (-SH) in plasma was determined using 0.2 mmol/L 5.50-dithiobis (2- nitrobenzoic acid) (DTNB) reported by Ellman (1952).

Total anti-oxidant status (TAS) were measured in plasma according to Erel's method (2004). This assay is based on the bleaching of the characteristic coluor of a stable 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS+) by antioxidants present in serum. The colour change was measured using an ILab 300+ autoanalyzer. The reaction is calibrated with Trolox (a water-soluble analogue of vitamin E, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the TAS value of the samples tested is expressed as μmol Trolox equivalent/L.

Statistical analysis

Data were analyzed using SPSS for Windows 11.5 (Chicago, IL, USA), MedCalc (version 11.4 Software, Belgium) and G*Power 3.0.8 software. The normality of the distribution of variables was determined by the Kolmogorov-Smirnov test. Data was expressed as: mean ± standard deviation for normally distributed variables, geometric mean or 95% confidence interval for log-normally distributed data and absolute frequencies for categorical variables. An independent t-test analysis was performed to ascertain whether significant differences existed between the anthropometric, biochemical and oxidative stress parameters of the subjects in the patients and control group at baseline. The effect of the supplementation was assessed by using mixed–design analysis of variance (ANOVA) with time as the within-subject factor and group (patients versus controls) as between-subject factors. *Post hoc* comparisons were carried out by Bonferroni test were appropriate. Analysis of categorical variables was carried out using the Chi-square test for contingency tables. All statistical tests were considered significant at the 0.05 probability level.

RESULTS

The baseline characteristics of the study groups are summarized in Table 1 and 2, respectively. Patients and controls were matched for age and gender. There were no significant difference in smoking habits between patients with schizophrenia and control subjects (Table 1).

Table 1. Demographic and clinical characteristics of the study groups

	Patients with schizophrenia (n=18)	Control group (n=38)	P[1]
Males/females	8/10	12/26	0.348
Age, years	39.7±8.4	41.1±10.6	0.629
Body mass index, kg/m^2	26.7±5.4	24.9±3.2	0.140
Body fat, %	31.0±8.9	30.8±7.4	0.883
Waist circumference, cm	98.6±15.7	88.1±10.5	0.006
Waist: Hip Ratio	0.88±0.10	0.82±0.08	0.018
Smokers, n (%)	10 (55.6 %)	15 (39.5 %)	0.258
Systolic pressure, mmHg	115±14.4	124±14.9	0.018
Diastolic pressure, mmHg	81.8±12.2	81.4±8.5	0.963
Duration of illness, years	11.4±5.4	-	-
Age of onset, years	28.0±10.4	-	-
CGI-severity score	3.56±0.70	-	-
Antipsychotic doses (CPE mg/day)	435±358	-	-

CGI, Clinical Global Impressions; CPZ, chlorpromazine; Data are expressed as mean ± SD;
[1] Continuous variables were compared by Student's *t* test and categorical variables by Chi-square test

Although, there were no significant difference in the baseline body fat percentage and BMI among the study groups, patients with schizophrenia had significantly higher waist circumference ($p<0.01$) and waist to hip ratio ($p<0.05$) than control subjects. A significantly higher systolic blood pressure ($p<0.05$) was evident in control group (Table 1).

The baseline biochemical data of the study groups are shown in Table 2. A significantly higher glucose levels ($p<0.001$) and TG concentrations ($p<0.05$) were evident in the patients group. In contrast, their HDL-C was lower ($p<0.05$) compared to control subjects.

The changes in anthropometric characteristics and biochemical parameters following the supplementation with LA are shown in Table 2.

The mixed ANOVA analysis showed significant main effect of time on reduction BMI, and waist circumference ($p<0.05$, $p<0.01$ and $p<0.001$, respectively). A significant group x time interaction effect was observed between groups for changes in percentage body fat ($p<0.01$). *Post-hoc* comparison revealed significant decrease in body fat percentage in the control group after 45 and 90 days, when compared to baseline values ($p<0.01$ and $p<0.001$, respectively, Bonferroni test). Also, a significant interaction effect ($p<0.01$) was for SBP levels, which decreased siginificantly in the control group after 45 and 90 days ($p<0.001$ and $p<0.001$, respectively, Bonferroni test) compared with baseline, while no such changes were noticed in the patients group ($p>0.05$). There were also significant interaction group x time effect for glucose levels ($p<0.001$). After 45 days and 90 days of supplementation, we observed significantly lower glucose levels in the patients group ($p<0.05$ and $p<0.01$, respectively, Bonferroni test) but increased significantly in the control group ($p<0.05$ and $p<0.01$, respectively, Bonferroni test). No significant group x time interaction or main effect of time for t-C and TG levels, but there was a significant main effect of time for HDL-C levels ($p<0.05$). Its levels in control group significantly decreased by the end of the study compared to the 45 day ($p<0.05$, Bonferroni test). There were significant group x time interaction effect on LDL-C levels ($p<0.05$). LDL-C levels increased significantly in the control group by the end of the study compared to the 45 day ($p<0.05$ and $p<0.05$, respectively, Bonferroni test). Finally, there were significant group x time interaction for UA ($p<0.01$). In the control group, there were significant decrease of UA levels after 45 days ($p<0.001$, Bonferroni test), followed by an increase at the end of the study ($p<0.001$, Bonferroni test).

Table 2. Anthropometric and biochemical data of the study groups at baseline, after 45 days and after 90 days of supplementation with LA

	Group	Baseline	45 day	90 day	T	G x T
BMI, kg/m^2	Controls	24.9±3.2	24.8±3.3	24.4±3.5$^{**, \#\#}$	0.014	0.065
	Patients	26.7±5.4	26.6±5.5	26.6±5.4		
Fat, %	Controls	30.8±7.4	27.2±8.7**	25.8±8.2***	0.004	0.005
	Patients	31.0±8.9	32.5±11.3	30.5±9.8		
Waist, cm	Controls	88.1±10.5	85.7±9.9**	84.1±11.3***	<0.001	0.140
	Patients	98.6±15.7^{aa}	96.5±16.33^{aa}	96.9±16.4^{aa}		
WHR	Controls	0.82±0.08	0.81±0.07	0.82±0.07	0.106	0.675
	Patients	0.88±0.10^{a}	0.87±0.09^{a}	0.88±0.09^{a}		
SBP, mmHg	Controls	124±14.9	111±16.9***	111±15.4***	<0.001	0.002
	Patients	115±14.4^{a}	113±14.8	115±13.9		
DBP, mmHg	Controls	81.4±8.5	73.4±10.5^{a**}	76.2±9.2	<0.001	0.828
	Patients	81.8±12.2	74.1±7.1	78.5±7.7		
Glucose$^{\&}$, mmol/L	Controls	5.15 (4.99-5.31)	5.46 (5.29-5.64)*	5.54 (5.35-5.75)**	0.342	<0.001
	Patients	6.01 (5.40-6.67)^{aaa}	5.42 (5.17-5.69)*	5.29 (5.01-5.58)**		
t-C, mmol/L	Controls	6.09±1.28	5.95±1.27	6.36±1.29$^{\#}$	0.774	0.071
	Patients	6.36±1.38	6.39±1.78	6.18±1.54		
HDL-C, mmol/L	Controls	1.44±0.34	1.52±0.44	1.35±0.31$^{\#}$	0.030	0.646
	Patients	1.19±0.35^{a}	1.14±0.33^{a}	1.06±0.39^{a}		
LDL-C, mmol/L	Controls	4.13±0.99	4.00±0.99	4.49±1.02$^{\#}$	0.418	0.046
	Patients	3.99±0.86	4.16±1.55	3.96±1.30		
TG$^{\&}$, mmol/L	Controls	1.23 (1.09-1.39)	1.16 (0.94-1.44)	1.31 (1.13-1.52)	0.810	0.359
	Patients	1.91 (1.18-3.09)^{a}	2.05 (1.41-2.99)^{aa}	1.93 (1.36-2.74)^{a}		
Uric acid, µmol/L	Controls	334±145.6	211±94.2***	320±122.6$^{\#\#\#}$	<0.001	0.002
	Patients	333±181.7	315±200.1^{a}	309±120.4		

Data are expressed as arithmetic mean and standard deviation; &- means and SD. ranges from log-normal values. T-time, G x T-group and time interaction effect. The difference in relation to baseline was significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***). The difference in relation to 45 day was significant at $p<0.05$ ($^{\#}$), $p<0.01$ ($^{\#\#}$) and $p<0.001$ ($^{\#\#\#}$). The difference significantly different between the groups at the same time point $p<0.05$ (^{a}), $p<0.01$ (^{aa}) and $p<0.001$ (^{aaa}).

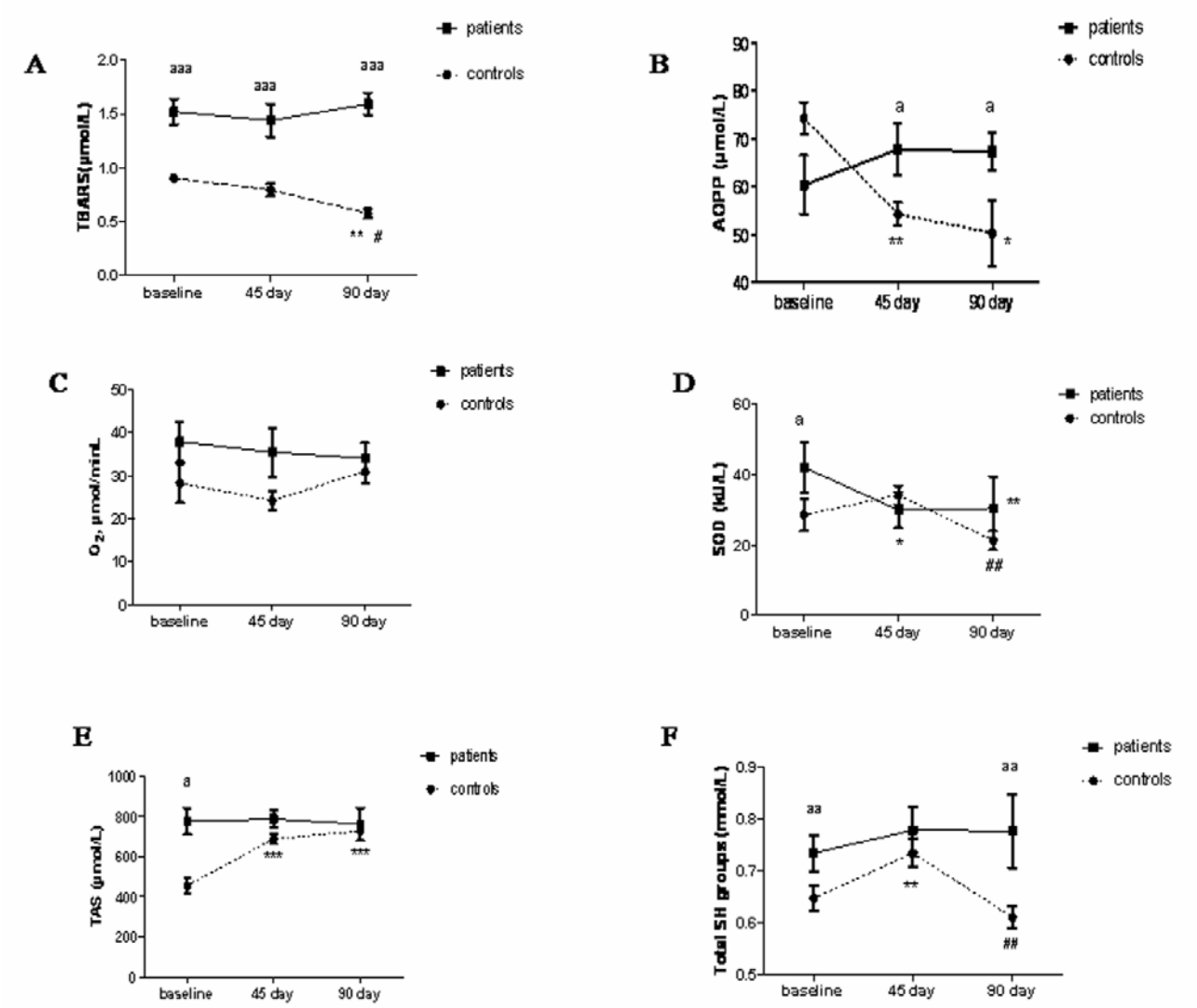

Figure 1. TBARS levels (A), AOPP levels (B), $O_2^{\bullet-}$ levels (C), SOD activities (D), TAS levels (E) and Total SH groups content (F), at baseline, after 45 days and after 90 days of supplementation with LA in the study groups

The baseline oxidative stress status parameters and changes following the supplementation are shown in Figure 1.

At the beginning of the study, we found significantly higher TBARS levels ($p<0.001$) in schizophrenic patients than in control subjects. The mixed ANOVA analysis showed a significant group x time interaction ($p<0.05$) on TBARS levels (Figure 1A). After 90 days of supplementation, we observed significantly lower TBARS levels in the control group compared to baseline values ($p<0.01$, Bonferroni test) and 45 day ($p<0.05$, Bonferroni test). There were also significant group x time interaction for AOPP levels ($p<0.01$), TAS levels ($p<0.05$) as well as marginally significant difference ($p=0.056$) for total –SH groups content. In the control group, there was a significant decrease of AOPP after 45 days ($p<0.01$, Bonferroni test), followed by an decrease at the end of the study ($p<0.05$, Bonferroni test) (Figure 1B). At the beginning of the study, we observed significant higher concentration of TAS, SOD activities and total –SH groups content in schizophrenic patients compared to the controls ($p<0.001$, $p<0.05$ and $p<0.01$, respectively). TAS levels in the control group significantly increased during the study ($p<0.001$), but not in the patients group (Figure 1E). After 45 days of supplementation we found a significant increase of total –SH groups in control group ($p<0.01$, Bonferroni test), followed by an decrease at the end of the study compared to 45 day ($p<0.05$, Bonferroni test) (Figure 1F). The significant group x time interaction effect ($p<0.05$), as well as significant main effect of time ($p<0.001$) were on SOD activities. *Post-hoc* analysis revealed significant decrease of SOD activities in the patient group, after 45 days and 90 days of supplementation when compared to baseline values ($p<0.001$ and $p<0.01$, respectively, Bonferroni test). SOD activities in the control group significantly decreased by the end of supplementation compared to 45 day ($p<0.01$, Bonferroni test) (Figure 1D). There were no significant changes in plasma levels $O_2^{\bullet-}$ in either group during the supplementation periods (Figure 1C).

DISCUSSION

In present study, we found a high prevalence of cardiometabolic risk factors in patients with schizophrenia (Table 1). Our finding of a higher prevalence of abdominal obesity, atherogenic dyslipidemia (low HDL and high TG) and hyperglicemia in schizophrenic patients compare to general population is consistent with other studies (De Hert et al. 2009). Weight gain is an established side effect of most antipsychotic drugs (Newcomer 2004). Previous studies showed that LA has antiobesity effects. Kim et al. (2008) have found that LA (1200 mg/day for 3 months) ameliorated antipsychotic-drug induced weight gain in patients with schizophrenia. Recently, the same author verified that LA can effectively decrease body weight and food intake in mice treated with olanzapine and reported that LA's antiobesity effect was related to supression of hypothalamic 5' AMP-activated protein kinase activity (Kim et al. 2014). Ratliff et al. (2013) also demonstrated significant weight loss in 12 non-diabetic schizophrenia patients consuming 1200 mg/α-LA over ten weeks.

Carbonelli et al. (2010) have evidenced that treatment with LA (800 mg/day for 4 months) induced significant weight loss and reductions of blood pressure in pre-obese and obese human subjects. On the contrary, some authors showed that 1800 mg/day of oral LA led to a modest weight loss in obese subjects without reductions in blood pressure and cholesterol concentrations (Koh et al. 2011). In the present study, supplemented 500 mg of LA daily significantly reduced BMI, body fat percentage, abdominal circumference and blood pressure in control group, while there were no significant changes in schizophrenia patients. Despite the fact that weight loss has a beneficial effect on lipid profile, we observed lower levels of HDL-C and higher t-C as well as LDL-C levels in control group at the end of the study compared to the 45 day of supplementation. The possible explanation may be in decrease lipoprotein lipase activity during active weight loss which has been reported in some studies (Cominacini et al. 1991, Dattilo & Kris-Etherton 1992).

Our results indicated that LA supplementation improved fasting glucose levels in patients with schizophrenia. This is in agreement with previously published data showing that LA improve glucose uptake by modulation redox-sensitive insulin-signaling pathways and is hellpful against insulin resistance (Jacob et al. 1999). However, we found increase of glucose levels in control group during the study. Despite, these changes were within referent ranges.

To our knowledge, this was the first investigation of the effect of LA supplementation on oxidative stress status in patients with schizophrenia. The dose of 500 mg/day LA was chosen on the basis of results of previous study which reported that LA is bioavailable and safe in moderate doses (Shay et al. 2009), and considering that high doses of antioxidants may be toxic, owing to prooxidative effects at high concentrations or their potential to react with beneficial concentrations of ROS normally present at physiological conditions that are required for optimal cellular functioning (Bouayed & Bohn 2010). Additionally, the LA/DHLA redox couple is recognized as one of the most powerful biological antioxidant systems (Gorąca et al. 2011). LA and it reduces form DHLA, met all criteria for an ideal antioxidant because they can quenching a broad range of ROS, chelating metals, interacting with and regenerating other antioxidants, have an amphiphilic character and they do not exhibit any serious side effects (Packer et al. 2001). The ability of LA to cross the blood-brain barrier and increases levels of endogenous antioxidants, especially GSH, who presents a major endogenous antioxidant in the brain, may have particular importance for schizophrenia (Packer et al. 1997, Seybolt 2010).

According to the results of previous studies, we expected increased susceptibility of patients with schizophrenia to oxidative stress compared with healthy subjects. Considering that the many inter-related mechanisms increase production of ROS and altered antioxidant defense in schizophrenic patients (Fendri et al. 2006), we want to test whether adjunctive supplementation of LA would modulate antioxidant defense and decrease oxidative damage in schizophrenic patients. In the present study we have confirmed an increase oxidative stress and impaired antioxidant defense in schizophrenia. Oxidative damage of lipids, measured as TBARS, has been frequently used marker of oxidative stress in schizophrenia. The result of our study is in agreement with previous studies have shown that lipid peroxidation is increased in schizophrenia (Zhang et al. 2010). In this study, for the first time we determined AOPP, as oxidative stress marker in schizophrenia. However, there was no significant difference for the baseline levels of AOPP in the study groups. Previous studies have shown strong antioxidant activity of LA against lipid peroxidation and oxidative damage proteins (Marangon et al. 1999, Zembron-Lacny et al. 2009). In the present study, we observed significantly decrease of TBARS levels in control group after 90 days of supplementation (Figure 1A). Changes in AOPP levels showed the similar pattern as for the TBARS levels during the study in control groups, while no significant changes were observed in the patients group (Figure 1B). These results suggested that LA supplementation protected the healthy subjects from oxidative damage, while it had no effect in the schizophrenic patients. The possible explanation may be that dose of 500 mg LA may be insufficient to prevent oxidative damage in the presence of higher level oxidative stress.

The antioxidative defense system includes enzymatic and non-enzymatic antioxidants. SOD is a key antioxidant enzyme responsible for the elimination of $O_2^{\bullet-}$ converting them into hydrogen peroxide and

molecular oxygen. In the present study, plasma SOD activities in patients group before supplementation were found to be significantly higher in comparison to the control group. This elevation in SOD activity in patients with schizophrenia is consistent with other published reports (Dakhale et al. 2004, Kunz et al. 2008). We have also found the higher baseline level of $O_2^{\bullet-}$ in the patients group, but these increase was not statistically significant. These results indicate that increased activity of SOD may be compensatory mechanism in response to $O_2^{\bullet-}$ overproduction in chronic schizophrenic patients. In the present study, we observed significant decrease in SOD activity after supplementation with LA in both groups (Figure 1D). It is possible that LA supplementation results in decreased $O_2^{\bullet-}$ levels and SOD activity might be downregulated by a compensatory mechanism. Additionaly, a recent study has shown the relationship between the number of cardiometabolic risk factors and the degree of oxidative stress in metabolic syndrome patients (Yubero-Serrano et al. 2013). These authors suggested that subjects with more metabolic syndrome components have a higher degree of oxidative stress with compensatory increased plasma SOD and GPx activities. Thus, we could also assume that higher levels of plasma SOD activity in patients with schizophrenia observed in our study are possibly related to cardiometabolic risk factors. Therefore, the decrease in plasma SOD activities in schizophrenic patients could be explained as a possible consequence of decreased fasting glucose levels due to LA supplementation.

Non-enzymatic antioxidant defense was studied by estimating TAS, total SH groups and UA. The total antioxidant status (TAS) represents the sum of activities of all the antioxidants. There are several antioxidants, such as albumin, UA and ascorbic acid which account for >85 % of the total antioxidant capacity in human plasma (Yao et al. 1998). The results of this study indicated that LA supplementation had a beneficial effect on improvement TAS levels in control group. Consistent with the increased concentration of total SH group, we observed significant increase in TAS levels in control group after 45 day of supplementation in comparison with the baseline levels. From 45 to 90 days of supplementation, we observed significant decrease in SH groups to baseline levels in control group. These changes were accompanied by alterations in the level of UA during study.

Although previous in vitro studies have shown that LA and DHLA act as antioxidants directly, through radical quenching and metal chelation, there is evidence that in vivo LA may also act indirectly, through recycling of other antioxidants and/or induction of Phase II antioxidant defense mechanisms resulting in alteration of cellular redox status (Shay et al. 2009). There is evidence that DHLA has the capacity to directly regenerating ascorbic acid from dehydroascorbic acid and indirectly regenerating vitamin E (Scholich et al. 1989). There is evidence also that LA increases intracellular GSH (Busse et al. 1992) and coenzyme Q_{10} levels (Kagan et al. 1999). Considering that TAS reflect the sum of the activities of all antioxidants present in plasma, higher level TAS in the control group at the end of the study compare to baseline, although SH group significantly decreased from 45 day to 90 day, may be explain by increasing low molecular weight antioxidants. LA has been reported to increase GSH synthesis in the cell, by increasing the expression of gamma-glutamylcysteine ligase, the rate-limiting enzyme in glutathione synthesis and by increasing cellular uptake of cysteine, an amino acid required for GSH synthesis (Biewenga et al. 1997). We suggest that observed changes in total SH group contents in control group during study may results from exchange the thiols containing compounds between plasma and cells. On the other hand, it has been proposed that supplementation with LA could cause the removing of iron from the ferritin and stimulate the auto-oxidation of thiols. This reaction has been additionally enhanced by ascorbate with ensuing ROS production (Çakatay 2006). It is possible that observed increased UA after 45 day of the study, might be adaptive response in order prevents ROS generation and stabilize other plasma antioxidants such as thiols and ascorbic acid (Vina et al. 2000).

Study has some potential limitations to consider**.** The study included naturalistic sample of patients, using more than one therapeutic protocol. However, all patients received antipsychotic treatment, with the majority of them being medicated with atipsychotics with similar mechanisms of action. Although the study used relatively small sample size which could unable reaching definitive conclusions, the number of patients was sufficient to reach the recquired power of the study. Inclusion of another countrol group consisting of unsuplemented schizophrenia patients might provide further information on the role of supplementation on oxidative stress markers in schizophrenia. However, the study adressed limitation of previous cross-sectional studies lacking multiple measurements and the causal relationship between supplementation and oxidative stress in patients with schizophrenia over time.

CONCLUSIONS

The findings of the present study demonstrated compensatory mechanisms in terms of up-regulated antioxidant capacity and the increased levels of lipid peroxidation in schizophrenic patients. The supplementation with LA (500 mg/day for three months) improved glucose levels but not enough to prevented oxidative damage in schizophrenia. Further placebo-controlled and larger dose-ranging studies are needed to confirm the potential importance of supplementation with LA in patients with schizophrenia.

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