Short Communication

# L-Carnitine supplementation decreases DNA damage in treated MSUD patients

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## ARTICLE INFO

## ABSTRACT

Maple syrup urine disease (MSUD) is an inherited disorder caused by severe deficient activity of the branched-chain α-keto acid dehydrogenase complex involved in the degradation pathway of branched-chain amino acids (BCAAs) and their α-ketoacid derivatives. MSUD patients generally present ketoacidosis, poor feeding, ataxia, coma, psychomotor delay, mental retardation and brain abnormalites. Treatment consists of dietary restriction of the BCAA (low protein intake) supplemented by a BCAA-free amino acid mixture. Although the mechanisms of brain damage in MSUD are poorly known, previous studies have shown that oxidative stress may be involved in the neuropathology of this disorder. In this regard, it was recently reported that MSUD patients have deficiency of L-carnitine (L-car), a compound with antioxidant properties that is used as adjuvant therapy in various inborn errors of metabolism. In this work, we investigated DNA damage determined by the alkaline comet assay in peripheral whole blood leukocytes of MSUD patients submitted to a BCAA-restricted diet supplemented or not with L-car. We observed a significant increase of DNA damage index (DI) in leukocytes from MSUD patients under BCAA-restricted diet as compared to controls and that L-car supplementation significantly decreased DNA DI levels. It was also found a positive correlation between DI and MDA content, a marker of lipid peroxidation, and an inverse correlation between DI and L-car levels. Taken together, our present results suggest a role for reactive species and the involvement of oxidative stress in DNA damage in this disorder. Since L-car reduced DNA damage, it is presumed that dietary supplementation of this compound may serve as an adjuvant therapeutic strategy for MSUD patients in addition to other therapies.

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## 1. Introduction

Maple syrup urine disease (MSUD; branched-chain ketoaciduria) is an inherited metabolic disorder caused by

mutations leading to deficient activity of in the mitochondrial branched-chain α-keto acid dehydrogenase (BCKDH) enzyme complex (E.C.1.2.4.4). This complex catalyses the irreversible oxidative decarboxylation of the branched-chain α-keto acids (BCKA) α-ketoisocaproate (KIC), α-keto-β-methylvalerate and α-ketoisovalerate, being also involved in the metabolism of the branched-chain amino acids (BCAA) leucine (Leu), isoleucine (Ile), and valine (Val). The metabolic blockage at this step results in the accumulation of BCKA and BCAA in tissues and body fluids of the affected individuals [1–3].

Patients affected by classical MSUD usually present poor feeding, ketoacidosis, apnea, hypoglycemia, convulsions, coma, ataxia, failure to thrive, psychomotor delay and mental retardation.

Central nervous system images reveal spongy degeneration of the white matter, reflecting hypomyelination/demyelination, edema and cerebral atrophy [1,4].

Although severe neurological damage occurs in most MSUD patients, the mechanisms involved in the neurotoxicity of this disease are still poorly understood. However, Leu and KIC have been considered the main neurotoxic metabolites in MSUD, since increasing concentrations of these metabolites are associated with the appearance and worsening of neurological symptoms [1,5].

Clinical management of MSUD comprises a lifelong strict and carefully adjusted semi-synthetic diet with restricted amounts of protein supplemented by vitamins, minerals and a mixture of essential amino acids [6]. Although patients improve significantly by the implementations of this therapy, protein restricted diet may lead to important nutritional deficiency and compromise of the antioxidant defense system [7,8].

L-Carnitine (L-car), a small quaternary amine highly polar and water soluble molecule obtained from dietary supply [9,10] has been used in the treatment of various organic acidurias [11,12]. Furthermore, L-car deficiency has been previously reported in treated MSUD patients with protein-restricted diet supplemented by a semisynthetic formula of essential amino acids without BCAAs [13]. Recent studies have observed that L-car has antioxidant properties and may protect cells from toxic reactive oxygen species in some metabolic disorders [14–17]. In this context, it was found that L-car administration prevents lipoperoxidation, protein damage and alterations on catalase and glutathione peroxidase activities in rat cerebral cortex in a chemically-induced acute model of MSUD [18]. Likewise, patients under BCAA-restricted diet plus L-car supplementation present a marked reduction of malondialdehyde content (lipid peroxidation) in relation to controls [13]. In vitro studies performed with human peripheral leukocytes have shown that L-car prevents DNA damage induced by the metabolites accumulating in MSUD [19]. However, to the best of our knowledge there are no reports in literature investigating DNA damage in MSUD patients and the role of antioxidant therapy in this process.

Thus, the aim of the present study was to evaluate DNA damage in leukocytes from MSUD patients and the role of dietary L-car dietary supplementation on this damage. The relationship between DNA damage, MDA levels and Leu concentrations in plasma from MSUD patients was also studied.

## 2. Material and methods

### 2.1. Patients and controls

Six MSUD patients (classic form) admitted at the Hospital de Clinicas de Porto Alegre, aged 5–12 years old (mean age $8.28 \pm 2.87$ years) were included in this study. The main clinical symptoms and laboratorial signals at diagnosis were seizures, convulsions, hypotonia, hypoglycemia, poor feeding, ketoacidosis and psychomotor delay. The length of treatment ranged from 15 days to 9.83 years and consisted of a natural protein restricted diet with low BCAA, supplemented with a semi-synthetic formula of essential amino acids, vitamins and minerals and not containing L-car (MSUD 2-Milupa). These patients were also supplemented for 2 months with dietary L-car ($50 \, mg \, kg^{-1} \, day^{-1}$) not exceeding $1.5 \, g \, day^{-1}$. The control group was composed by six age-matched healthy individuals (mean age $6.0 \pm 3.12$ years). The comet assay, as well as malondialdehyde (MDA), Leu and free L-car concentrations were determined in blood of MSUD patients before (Group A) and after 1 month (Group B) or 2 months (Group C) of L-car supplementation and compared to control group. Blood Leu levels, expressed in µM, were measured by HPLC according to Joseph and Marsden [20] with slight modifications [21]. MDA was measured according to

Karatepe [22] and expressed as µM of MDA. Free L-car levels were determined in blood spots by liquid chromatography electrospray tandem mass spectrometry (LC/MS/MS), using the multiple reaction monitoring (MRM) mode [23] and the results were reported as µM. The present study was approved by the Ethical Committee of Hospital de Clinicas de Porto Alegre, RS, Brazil. Parents from all patients included in the present study gave informed consent. Data are represented as mean $\pm$ SD for the various measurements. For the statistical analysis, comparison between means was analyzed by repeated measures of ANOVA followed by the Tukey's multiple range test when the $F$ value was significant ($p < 0.05$). Correlations were carried out using the Pearson correlation coefficient.

### 2.2. Single-cell gel electrophoresis (comet assay)

The alkaline comet assay was performed as described by Singh et al. [24] in accordance with the general guidelines [25]. Isolated human leukocytes were suspended in agarose and spread onto a glass microscope slide pre-coated with agarose; the agarose was allowed to set at $4 \, °C$ for 5 min. The slides were incubated in ice-cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM TRIS, 1% Triton X-100 and 10% DMSO, pH 10) to remove the cellular proteins, leaving the DNA as histone-free nucleoids. After lysis, the slides were placed on a horizontal electrophoresis unit and covered with fresh electrophoresis solution (300 mM NaOH and 1 mM EDTA, pH > 13) for 20 min at $4 \, °C$ to allow DNA unwinding and the expression of alkali-labile sites. Electrophoresis was performed for 20 min (25 V; 300 mA; 0.9 V/cm). The slides were then neutralized, washed in bi-distilled water, and stained using a silver staining protocol [26]. After drying at room temperature overnight, the gels were analyzed using an optical microscope. One hundred cells (50 cells from each of the two replicate slides) were selected and analyzed. The cells were visually scored and received scores from 0 (no migration) to 4 (maximal migration) according to the tail intensity. Therefore, the damage index (DI) of the cells ranged from 0 (all cells with no migration) to 400 (all cells with maximal migration). All slides were analyzed under blind conditions by two different individuals and the average scores were used for the calculations.

## 3. Results

Data (represented as mean $\pm$ SD) from MSUD patients are divided into Group A (without L-car supplementation), Group B (1 month of L-car therapy) and Group C (2 months of L-car supplementation). Leu levels were measured in plasma from controls and MSUD patients before and after treatment. We found the following values expressed as µM: controls = $130.8 \pm 27.2$, Group A = $178.2 \pm 58.6$, Group B = $264.6 \pm 136.7$, Group C = $278.0 \pm 105.6$. There were no significant differences between the groups.

On the other hand, patients from Group A had significantly decreased levels of L-car ($20.9 \pm 6.6 \, µM$) as compared to the controls ($43.8 \pm 8.9 \, µM$) that normalized after L-car supplementation [Group B ($48.1 \pm 9.1 \, µM$) and Group C ($54.8 \pm 12.6 \, µM$)] [$F(3,21) = 6.253$, $p < 0.05$]. Moreover, MDA levels were significantly elevated in Group A ($0.087 \pm 0.06 \, µM$) as compared to the control group ($0.029 \pm 0.003 \, µM$), indicating lipid peroxidation, that was reversed after 2 months (Group C) ($0.036 \pm 0.003 \, µM$) of L-car therapy [$F(3,21) = 19.541$, $p < 0.05$]. With regard to DNA damage, we observed that only Group A had damage classes three and four (the highest damage class) before L-car supplementation and already in the first month of L-car administration this high damage was not more verified, being observed just zero, one and two damage classes (data not shown).

Fig. 1 shows a significantly higher DNA migration (damage class index = DI) in Groups A (DI = $72.3 \pm 4.5$), B (DI = $36.2 \pm 5$) and
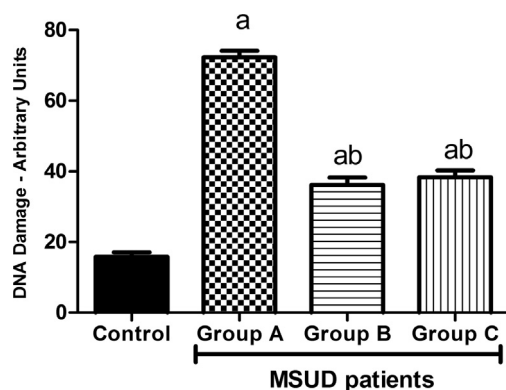
**Fig. 1.** DNA damage index (DI) of peripheral blood leukocytes from MSUD patients and controls. Group A: patients before supplementation with L-car. Group B: patients after 1 month of L-car supplementation. Group C: patients after 2 months of L-car supplementation. Data represent the mean ± SD (controls: $n = 6$; Group A: $n = 6$; Group B: $n = 6$; Group C: $n = 5$). (a) $p < 0.05$, compared to controls. (b) $p < 0.05$, compared to Group A (ANOVA, followed by the Tukey multiple range test).

C (DI = 38.3 ± 4.8) of MSUD patients, when compared to the control group (DI = 15.8 ± 3.1). Besides, L-car supplemented by 1 and 2 months was able to reduce DNA damage relatively to patients not treated with L-car [$F(3,21) = 168$, $p < 0.05$]. Moreover, a significant inverse correlation was observed between DNA damage and free plasma L-car levels ($r = -0.82$; $p < 0.05$) in treated MSUD patients (Fig. 2A).
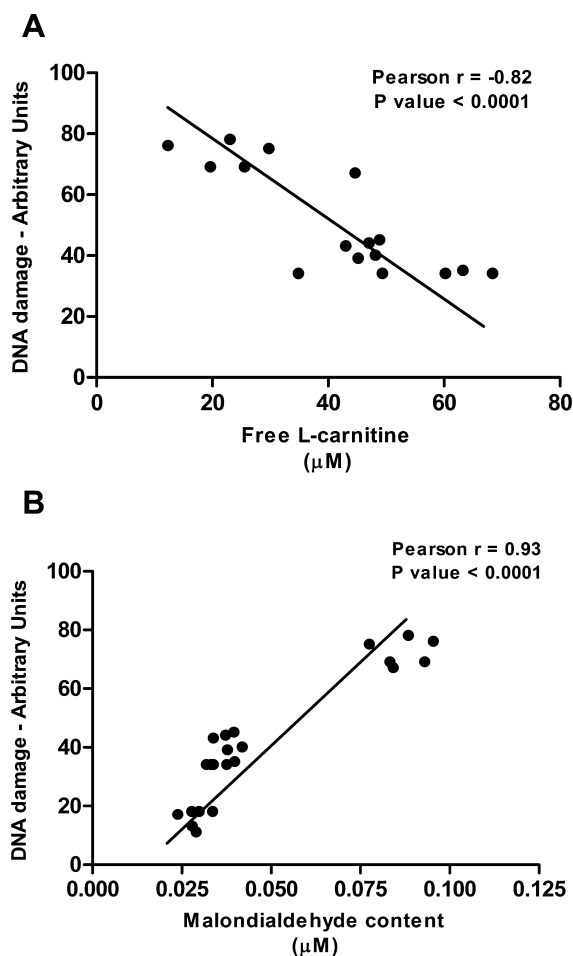


**Fig. 2.** (A) Correlation between DNA damage index (DI) and L-car levels in MSUD patients. (B) Correlation between DNA damage index (DI) and MDA levels in MSUD patients. Graphs show the Pearson correlation coefficient and probabilities.

Finally, significant positive correlation between MDA content and DNA damage were highly correlated ($r = 0.93$; $p < 0.05$) in these patients (Fig. 2B).

## 4. Discussion

Cellular DNA is constantly exposed to endogenous and exogenous deleterious conditions that may affect its integrity. Thus, accumulated evidence have demonstrated that an increment in the generation of reactive oxygen and nitrogen species is a major process provoking DNA lesions, including single- and double-strand breaks, DNA-proteins cross-links, inter/intra-strand cross-links, and sugar fragmentation products. These alterations may induce mutations, chromosomal aberrations, microsatellite instability, loss of heterozygosity and cytotoxicity [27]. The comet assay is a rapid, simple, sensitive and versatile tool for detecting DNA strand breaks and alkali-labile sites in individual cells [28,29]. This assay is widely accepted as a standard method to investigate DNA damage and repair in different cell types and mainly reflects the pro-oxidant/antioxidant effects of various compounds [25,30].

In this study, we investigated DNA damage in erythrocytes from MSUD patients and also whether L-car dietary administration could protect against this damage in the same groups of patients treated with protein restricted diet supplemented by L-car for a period of up to 2 months. We also correlated DNA damage with L-car concentrations, MDA values (oxidative lipid damage) and Leu levels (the major metabolite accumulated in this disease) in order to get a better insight on the possible causes of this damage.

We first observed that MSUD patients treated only with protein restriction diet without supplementation of L-car have significantly higher DI when compared with the control group, indicating DNA damage. Damage classes 3 and 4 were observed in patients before L-car treatment (data not shown). Furthermore, L-car supplementation (50 mg kg$^{-1}$ day$^{-1}$) for 1 and 2 months provoked a significant decrease of DI when compared to Group A, but did not restore DNA damage at the level of the control group (healthy individuals). It was also verified a decrease in DNA damage classes 3 and 4 and an increase in DNA damage classes 0, 1 and 2 in Groups B and C of MSUD patients, indicating a decrease in the severity of this damage after L-car supplementation (data not shown). A significant inverse correlation between free L-car levels and DI was also found, reinforcing the role of L-car as a protective factor against DNA damage.

As regards to the mechanisms underlying the protective effect of L-car, we observed a significant positive correlation between DNA DI and the content of MDA in MSUD patients, indicating that DNA damage may be associated with the lipid peroxidation observed, and that probably there is an oxidative DNA damage in these patients. More recently, studies showed that L-car treatment (50 mg kg$^{-1}$ day$^{-1}$) reduced plasma MDA concentrations in patients with MSUD under treatment with protein-restricted diet [13]. Therefore, the protective role of L-car could be attributed to its antioxidant functions scavenging free radicals and metal-chelating properties decreasing cytosolic iron, which has a vital role in free radical production [31,32]. L-Car has also been reported to decrease lipid peroxidation and protect tissues from damage by repairing oxidized membrane lipids and decreasing MDA levels, besides facilitating fatty acid transport and therefore lowering the availability of lipids for peroxidation [33]. Still, it is important to note that in our study L-car supplementation was held for a relatively short period of time, so that the evaluation of the use of this antioxidant in MSUD treatment for a longer period may be interesting in order to check for other benefits.

It is also of note that previous animal data have shown that accumulation of the major metabolite in MSUD induces DNA damage in the hippocampus and striatum of rats [34]. Furthermore, Mescka

et al. [19] evidenced that Leu and KIC induced DNA damage in peripheral whole blood leukocytes, which was prevented by ʟ-car. Taken together, it is presumed that DNA damage may be caused by the metabolites accumulating in this disorder.

On the other hand, an important function of ʟ-car, a small water-soluble molecule, is to act as a carrier for translocation of long-chain fatty acids from the cytosol into mitochondria for β-oxidation, therefore helping to keep energy supply in tissues that use fatty acids. Thus, although we cannot precise the causes of DNA damage prevention by ʟ-car, one of the possible mechanisms involved on DNA protection after ʟ-car administration in MSUD patients might be its ATP promotion action, which may induce protein phosphory-lation involved in the synthesis and processing of nucleic acid [35]. In this scenario, it was reported that ʟ-car may regulate DNA repair and also enhances the annealing effects by activation of DNA repair enzyme poly (ADP ribosyl) polymerase, a nuclear protein that is intimately linked with the occurrence of DNA strand breaks and also other related repair mechanisms [36]. Finally, we cannot rule out the possibility that ʟ-car may decrease DNA damage due to its property of binding to the toxic metabolites accumulated in MSUD helping their urinary excretion that were shown to cause directly or indirectly DNA damage [16,37].

To the best of our knowledge, this is the first report demon-strating that DNA damage occurs in MSUD patients possibly as a result of oxidative stress, although other causes cannot be excluded. In this context, reactive oxygen and nitrogen species can provoke DNA damage by direct chemical attack on DNA, as well as by indi-rect mechanisms, such as activation of $Ca^{2+}$-endonucleases and by interfering with enzymes of DNA replication and repair. Further-more, oxidative damage can also lead to mutations whose pattern depends on the conformation of the bases of the genes, as well as on the repair efficiency, the type of polymerase and the conforma-tion of the surrounding DNA affecting the accuracy of copying by polymerases [27].

In conclusion, ʟ-carnitine supplementation to MSUD patients may represent a new therapeutic approach and a possible adjuvant to the current treatment of this disease.

## Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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## References

[1] D.T. Chuang, V.E. Shih, Maple syrup urine disease (branched-chain ketoaciduria), in: C.R. Scriver, A.L. Beaudt, W.L. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York, 2001, pp. 1971–2005.

[2] E. Treacy, C.L. Clow, T.R. Reade, D. Chitayat, O.A. Mamer, C.R. Scriver, Maple syrup urine disease: interrelationship between branched-chainamino-, oxo- and hydroxyacids; implications for treatment; associations with CNS dysmyeli-nation, J. Inherit. Metab. Dis. 15 (1992) 121–135.

[3] K.A. Strauss, D.H. Morton, Branched-chain ketoacyl dehydrogenase deficiency: maple syrup disease, Curr. Treat. Option Neurol. 5 (2003) 329–341.

[4] A. Sitta, G.S. Ribas, C.P. Mescka, A.G. Barschak, M. Wajner, C.R. Vargas, Neuro-logical damage in MSUD: the role of oxidative stress, Cell. Mol. Neurobiol. 34 (2014) 157–165.

[5] B. Hoffmann, C. Helbling, P. Schadewaldt, U. Wendel, Impact of longitudi-nal plasma leucine levels on the intellectual outcome in patients with classic MSUD, Pediatr. Res. 59 (2006) 17–20.

[6] K.A. Strauss, B. Wardley, D. Robinson, C. Hendrickson, N.L. Rider, E.G. Puffen-berger, D. Shellmer, A.B. Moser, D.H. Morton, Classical maple syrup urine disease and brain development: principles of management and formula design, Mol. Genet. Metab. 99 (2010) 333–345.

[7] A.G. Barschak, A. Sitta, M. Deon, A.T. Barden, G.O. Schmitt, C.S. Dutra-Filho, M. Wajner, C.R. Vargas, Erythrocyte glutathione peroxidase activity and plasma selenium concentration are reduced in maple syrup urine disease patients during treatment, Int. J. Dev. Neurosci. 25 (2007) 335–338.

[8] A.G. Barschak, A. Sitta, M. Deon, A.T. Barden, C.S. Dutra-Filho, M. Wajner, C.R. Vargas, Oxidative stress in plasma from maple syrup urine disease patients during treatment, Metab. Brain Dis. 23 (2008) 71–80.

[9] I. Gulcin, Antioxidant and antiradical activities of ʟ-carnitine, Life Sci. 78 (2006) 803–811.

[10] G.E. Bigford, G. Del Rossi, Supplemental substances derived from foods as adjunctive therapeutic agents for treatment of neurodegenerative diseases and disorders, Adv. Nutr. 5 (2014) 394–403.

[11] G.S. Ribas, V. Manfredini, J.F. de Mari, C.Y. Wayhs, C.S. Vanzin, G.B. Biancini, A. Sitta, M. Deon, M. Wajner, C.R. Vargas, Reduction of lipid and protein damage in patients with disorders of propionate metabolism under treatment: a possible protective role of ʟ-carnitine supplementation, Int. J. Dev. Neurosci. 28 (2010) 127–132.

[12] G.S. Ribas, G.B. Biancini, C. Mescka, C.Y. Wayhs, A. Sitta, M. Wajner, C.R. Vargas, Oxidative stress parameters in urine from patients with disorders of propio-nate metabolism: a beneficial effect of ʟ-carnitine supplementation, Cell. Mol. Neurobiol. 32 (2012) 77–82.

[13] C.P. Mescka, C.A. Wayhs, C.S. Vanzin, G.B. Biancini, G. Guerreiro, V. Manfredini, C. Souza, M. Wajner, C.S. Dutra-Filho, C.R. Vargas, Protein and lipid damage in maple syrup urine disease patients: ʟ-carnitine effect, Int. J. Dev. Neurosci. 31 (2013) 21–24.

[14] G.S. Ribas, A. Sitta, M. Wajner, C.R. Vargas, Oxidative stress in phenylketonuria: what is the evidence? Cell. Mol. Neurobiol. 31 (2011) 653–662.

[15] M. Dos Santos Mello, G.S. Ribas, C.A. Wayhs, T. Hammerschmidt, G.B. Guerreiro, J.L. Favenzani, A. Sitta, D. de Moura Coelho, M. Wajner, C.R. Vargas, Increased oxidative stress in patients with 3-hydroxy-3-methylglutaric aciduria, Mol. Cell. Biochem. 1–2 (2015) 149–155.

[16] G.S. Ribas, C.R. Vargas, M. Wajner, ʟ-Carnitine supplementation as a potencial antioxidant therapy for inherited neurometabolic diseases, Gene 533 (2014) 469–476.

[17] A. Sitta, C.S. Vanzin, G.B. Biancini, V. Manfredini, A.B. de Oliveira, C.A. Wayhs, G.O. Ribas, L. Giugliani, I.V. Schwartz, D. Bohrer, S.C. Garcia, M. Wajner, C.R. Vargas, Evidence that ʟ-carnitine and selenium supplementation reduces oxidative stress in phenylketonuric patients, Cell. Mol. Neurobiol. 31 (2011) 429–436.

[18] C. Mescka, T. Moraes, A. Rosa, P. Mazzola, B. Piccoli, C. Jacques, G. Dalazen, J. Coelho, M. Cortes, M. Terra, C. Regla Vargas, C.S. Dutra-Filho, In vivo neuro-protective effect of ʟ-carnitine against oxidative stress in maple syrup urine disease, Metab. Brain Dis. 26 (2011) 21–28.

[19] C.P. Mescka, C.A. Wayhs, G. Guerreiro, V. Manfredini, C.S. Dutra-Filho, C.R. Vargas, Prevention of DNA damage by ʟ-carnitine induced by metabolites accu-mulated in maple syrup urine disease in human peripheral leukocytes in vitro, Gene 548 (2014) 294–298.

[20] M.H. Joseph, C.A. Marsden, Amino acids and small peptides, in: C.F. Lim (Ed.), HPLC of Small Peptides, IRL Press, Oxford, 1986.

[21] M. Wajner, D.M. Coelho, A.G. Barschak, P.R. Araujo, R.F. Pires, F.L. Lulhier, C.R. Vargas, Reduction of large neutral amino acid concentrations in plasma and CSF of patients with maple syrup urine disease during crises, J. Inherit. Metab. Dis 23 (2000) 505–512.

[22] M. Karatepe, Simultaneous determination of ascorbic acid and free malon-dialdehyde in human serum by HPLC-UV, LCGC N. Am. 22 (2004) 362–365.

[23] D.H. Chace, S.L. Hillman, J.L. Van Hove, E.W. Naylor, Rapid diagnosis of MCAD deficiency: quantitative analysis of octanoylcarnitine and other acylcarnitines in newborn blood spots by tandem mass spectrometry, Clin. Chem. 43 (1997) 2106–2113.

[24] N. Singh, M. McCoy, R. Tice, E.L. Schneider, A simple technique for quantification of low levels of DNA damage in individuals cells, Exp. Cell. Res. 175 (1988) 184–191.

[25] R.R. Tice, D. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, Y.F. Sasaki, Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35 (2000) 206–221.

[26] S. Nadin, L. Vargas-Roig, D. Ciocca, A silver staining method for single-cell gel assay, J. Histochem. Cytochem. 49 (2001) 1183–1186.

[27] B. Halliwell, M.C. Gutteridge, Free Radicals in Biology and Medicine, 4th ed., Oxford University Press Inc., New York, 2007.

[28] J.L. Ravanat, J. Cadet, T. Douki, Oxidatively generated DNA lesions as potential biomarkers of in vivo oxidative stress, Curr. Mol. Med. 12 (2012) 655–671.

[29] A.R. Collins, Measuring oxidative damage to DNA and its repair with the comet assay, Biochim. Biophys. Acta 1840 (2014) 794–800.

[30] E. Cemeli, A. Baumgartner, D. Anderson, Antioxidants and the comet assay, Mutat. Res. 681 (2009) 51–67.

[31] D. Haripriya, M. Devi, M. Kokilavani, P. Sangeetha, C. Panneerselvam, Age dependent alterations in mitochondrial enzymes in cortex, striatum and hip-pocampus of rat brain-potential role of ʟ-carnitine, Biogerontology 5 (2004) 355–364.

[32] T. Thangasamy, P. Jeyakumar, S. Sittadjody, A.G. Joyee, P. Chinnakannu, ʟ-Carnitine mediates protection against DNA damage in lymphocytes of aged rats, Biogerontology 10 (2009) 163–172.

[33] P.J. Rani, C. Panneerselvam, Effect of L-carnitine on brain lipid peroxidation and antioxidant enzymes in old rats, J. Gerontol. A: Biol. Sci. Med. Sci. 57 (2003) 134–137.

[34] G. Scaini, I.C. Jeremias, O.S. Morais, G.D. Borges, B.P. Munhoz, D.D. Leffa, V.M. Andrade, P.F. Schuck, G.C. Ferreira, E.L. Streck, DNA damage in an animal model of maple syrup urine disease, Mol. Genet. Metab. 106 (2012) 169–174.

[35] G. Famularo, C. De Simone, V. Trinchieri, L. Mosca, Carnitines and its congeners: a metabolic pathway to the regulation of immune response and inflammation, Ann. N. Y. Acad. Sci. 1033 (2004) 132–138.

[36] M.E. Boerighter, C. Franceschi, E. Arrigoni-Martelli, J.Y. Wei, J. Vijg, The effect of L-carnitine and acetyl L-carnitine on the disappearance of DNA single-strand breaks in human peripheral blood lymphocytes, Carcinogenesis 14 (1993) 2131–2316.

[37] G. Guerreiro, C.P. Mescka, A. Sitta, B. Donida, D. Marchetti, T. Hammerschmidt, J. Faverzani, D.M. Coelho, M. Wajner, C.S. Dutra-Filho, C.R. Vargas, Urinary biomarkers of oxidative damage in Maple syrup urine disease: the L-carnitine role, Int. J. Dev. Neurosci. 42 (2015) 10–14.