**Universidade Federal do Rio Grande do Sul**

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# The L-carnitine role

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**Gilian Batista Balbueno Guerreiro**

**Orientadora: Prof. Drª Carmen Regla Vargas**

**Co-orientadora: Caroline Paula Mescka**

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# Urinary biomarkers of oxidative damage in Maple Syrup Urine Disease: the L-carnitine role

***Gilian Guerreiro[1], Caroline Paula Mescka[3], Angela Sitta[2], Bruna Donida[4], Desirèe Marchetti[2], Tatiane Hammerschmidt[1], Jéssica Faverzani[1], Daniella de Moura Coelho[2], Moacir Wajner[2,3], Carmen Regla Vargas[1,2,3,4]***

*1 Faculdade de Farmácia, UFRGS, Av. Ipiranga 2752, 90610-000, Porto Alegre, RS, Brazil*

*2 Serviço de Genética Médica, HCPA, UFRGS, Rua Ramiro Barcelos, 2350, 90035- 903, Porto Alegre, RS, Brazil.*

*3 Programa de Pós-Graduação em CB:Bioquímica, UFRGS, Rua Ramiro Barcelos, 2600, 90035 000, Porto Alegre, RS, Brazil.*

*4 Programa de Pós-Graduação em Ciências Farmacêuticas, UFRGS, Av. Ipiranga, 2752, 90610-000, Porto Alegre, RS, Brazil.*

**Corresponding author:**

C.R.Vargas / G. Guerreiro. Serviço de Genética Médica, HCPA, Rua Ramiro Barcelos, 2350 CEP 90035-903, Porto Alegre, RS, Brasil. Tel: +55 51 33598011; Fax: +55 51 33598010; E-mail crvargas@hcpa.ufrgs.br (C.R. Vargas), gilian_guerreiro@hotmail.com (G. Guerreiro)

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**Abstract**

Maple Syrup Urine Disease (MSUD) is a disorder of branched-chain amino acids (BCAA). The defect in the branched-chain α-keto acid dehydrogenase complex activity lead to an accumulation of these compounds and their corresponding α-keto-acids and α-hydroxy-acids. Studies have shown that oxidative stress may be involved in neuropathology of MSUD. L-carnitine (L-car) have an important role as antioxidant through reducing and scavenging free radicals formation and by enhancing the activity of antioxidant enzymes. Our study evaluated the oxidative stress parameters, di-tyrosine, isoprostanes and antioxidant capacity, in urine of MSUD patients on protein-restricted diet supplemented or not with L-car capsules at a dose of 50 mg kg-1 day-1. We also determined the BCAAs, α-keto acids and α-hydroxy levels in urine and quantified the free L-car levels. Firstly, we found a deficiency of carnitine in patients before the L-car supplementation. Significant increases of di-tyrosine and isoprostanes, as well as reduced antioxidant capacity were observed in urine of MSUD patients before the treatment with L-car. The L-car supplementation was able to reduce the di-tyrosine and isoprostanes levels, as well as to increase the antioxidant capacity. We also quantified the BCAAs and metabolites in urine from these patients, detecting a significantly increase of leucine, isoleucine, α-ketoisocaproic acid, α-hydroxyisocaproate and α-hydroxy-B-methylvalerate after 2 months of treatment, compared to control group. In conclusion, our results suggest that L-car may have additional beneficial effects in the treatment of MSUD, not only by preventing oxidative damage, but also by increasing the excretion of MSUD metabolites, reducing the toxic effects caused by their accumulation.

**Keywords:** maple syrup urine disease - L-carnitine - oxidative stress – antioxidant

## 1. Introduction

Maple Syrup Urine Disease (MSUD) is a metabolic disease caused by a severe deficiency of the branched-chain α-keto acid dehydrogenase complex (BCKAD) activity. The blockage of this pathway leads to the accumulation in tissues and body fluids of branched-chain amino acids (BCAA) leucine (Leu), isoleucine (Ile) and valine (Val) and their respective α-keto-acids, α-ketoisocaproic acid (KIC), α-keto-β-methylvaleric acid (KMV) and α-ketoisovaleric acid (KIV), as well as the corresponding α-hydroxy acids, the α-hydroxyisocaproate (HIC), α-hydroxy-β-methylvalerate (HMV) and the α-hydroxyisovalerate (HIV), respectively (Treacy et al 1992; Chuang and Shih 2001; Harris et al 2004).

Based on the clinical presentation and biochemical responses to thiamine administration, MSUD patients can be divided into five phenotypes: classic, intermediate, intermittent, thiamine-responsive and dihydrolipoyl dehydrogenase (E3) deficient (Chuang and Shih 2001). The main signs and symptoms presented by MSUD patients include psychomotor delay and mental retardation, coma, convulsions, poor feeding, apnea, ataxia, ketoacidosis, hypoglycemia, as well as generalized edema and hypomyelination/demyelination evidenced by magnetic resonance imaging studies of the central nervous system. The worldwide frequency of MSUD is approximately 1 in 185,000 newborns (Chuang and Shih 2001).

The mechanisms involved in the neurological symptoms presented by MSUD patients are still poorly understood. However, several studies have been demonstrated that Leu and/or KIC are the main neurotoxic metabolites in MSUD, and the high concentrations of these metabolites can be associated with the appearance of neurological symptoms (Snyderman et al 1964; Chuang and Shih 2001). The metabolites accumulated in this disease provoke convulsions (Coitinho et al 2001),

neuronal apoptosis (Jouvet et al 2000), impairment of neurotransmitter synthesis (Zielke et al 1997; Tavares et al 2000), myelin alteration (Treacy et al 1992) and affect energy metabolism in rat brain (Danner and Elsas 1989; Sgaravatti et al 2003).

The MSUD treatment consists of a low protein diet and a semi-synthetic formula poor in BCAA and supplemented by essential amino acids, vitamins and minerals (Snyderman et al 1964; Danner and Elsas 1989; Chuang and Shih 2001; Sitta et al 2014). However, natural protein restriction may lead to an increase of nutritional deficiencies and to a low total antioxidant status that contribute to oxidative stress (Barschak et al 2007, Barschak et al 2008; Mescka et al 2013; Sitta et al 2014). Oxidative stress is characterized as an imbalance between oxidants and antioxidants species in favor of oxidants, causing damage to many cellular constituents, such lipids, proteins and DNA (Finkel et al 2000; Halliwell and Gutteridge 2007).

L-carnitine (L-car) is a highly polar quatenary amine which plays important metabolic functions in the organism, like transport of long-chain fatty acids across the inner mitochondrial membrane for utilization in $\beta$-oxidation. Furthermore, this compound has demonstrated antioxidant activity by reducing and scavenging free radicals formation and by enhancing the activity of enzymes involved in the defense against reactive species (Derin et al 2004; Gulcin et al 2006). It was verified that MSUD patients have L-car deficiency, since this compound is obtained mainly from diet (Mescka et al 2013; Sitta et al 2014). Recent studies have demonstrated an increase of plasma antioxidant status in patients with inborn errors of metabolism supplemented with L-car (Ribas et al 2010; Mescka et al 2011; Sitta et al 2011; Mescka et al 2013; Ribas et al 2014). There are no studies in literature focusing urinary amino acids, α-keto acids, α-hydroxy acids and oxidative stress parameters in MSUD treated patients. It is

important to emphasize that BCAA metabolites are highly excreted in urine in this disease.

Thus, in this study, it was evaluated oxidative stress parameters and BCAA, α-keto acids and α-hydroxy acids concentrations in the urine of treated MSUD patients, supplemented or not with L-car. Our objective was to investigate a possible association between L-car, metabolites accumulation and oxidative stress in treated MSUD patients.

## 2. Material and methods

### 2.1 Patients, controls and biological samples

In this study it was studied seven patients (mean age, at collect moment, 8.28 ± 2.87 years) with late diagnosis and classical MSUD under protein restricted diet protocol from Medical Genetic Service of Hospital de Clínicas de Porto Alegre (HCPA), Brazil, which were diagnosed by elevated plasma BCAA levels. Whole blood on filter paper was used to evaluate the free L-car levels, plasma was used to amino acid quantification and urine samples were used to determine the parameters of oxidative stress and the concentrations of amino acids, α-keto acids and α-hydroxy acids.

Dietary treatment (median 0.95 year – range 15 days to 9.83 years) consisted of a protein-restricted diet supplemented with a semi-synthetic formula of essential amino acids (except leucine, isoleucine and valine), vitamins and minerals and not containing L-car (MSUD 2-Milupa®). The diet contained the following amounts of Leu (before 12 months of age: 40–80 mg kg−1 day−1; after 1 year of age: 275–535 mg day−1), Ile (before 12 months of age: 20–50 mg kg−1 day−1; after 1 year of age: 165–325 mg day−1) and Val (before 12 months of age: 20–60 mg kg−1 day−1; after 1 year of age: 190–375 mg day−1). In addition, for this study, MSUD patients were supplemented with L-car capsules, fractionated and mixed with the formula, at a dose of 50 mg kg−1

day−1, not exceeding 1.5 g day−1 for 2 months. Oxidative stress parameters, amino acids, α-keto acids, α-hydroxy acids and free L-car levels were analyzed in MSUD patients before (Group A) and after one (Group B) and 2 months (Group C) of L-car supplementation. The control group consisted of samples from six aged-matched healthy children (mean age 6.0 ± 3.12 years). The study was approved by the Ethics Committee of HCPA, RS, Brazil. All parents of the patients included in the present study gave informed consent.

2.2 Amino acids determination

The free amino acids in urine and plasma were determined by high-pressure liquid chromatography (HPLC) method according to Joseph and Marsden (Joseph and Marsden 1986). The quantification was performed by relating the chromatographic peak area of each amino acid to those obtained from a known standard solution and to the peak area of the internal standard (homocysteic acid) with known concentration. Results were expressed in μmol/L (plasma) and μmol/g creatinine (urine).

2.3 α-Keto acids and α-hydroxy acids determination

The α-keto acids KIC, KMV, KIV and the α-hydroxy acids HIC, HMV and HIV were determined in urine by gas chromatography-mass spectrometry (GC/MS) according to Sweetmann (Sweetmann et al 1995), using hexadecane and heptadecanoic acid as internal standards. The quantification was performed by relating the metabolites chromatographic peak area to those obtained from a known standard solution for each metabolite and to that of internal standard peak area. Results were expressed in μmol/L.

2.4 Free L-carnitine determination

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 (Chace et al 1997) and the results were reported in µmol/L.

## 2.5 15-F2t-isoprostane determination

15-F2t-isoprostane, a product of arachidonic acid metabolism and a biomarker of lipid peroxidation, was measure by a competitive enzyme-linked immunoassay (ELISA) (Oxford Biomed, EA 85), according the kit's instructions. First, the urine samples were mixed with dilution buffer. In this assay, the 15-F2t-isoprostane in the urine samples competes with the 15-F2t-isoprostane conjugated to horseradish peroxidase (HRP) for the binding to a specific antibody fixed on the microplate. The concentration of 15-F2t-isoprostane was determined by the intensity of color developed after the substrate was added (wavelength at 630 nm). Results were expressed as nanograms of isoprostanes per mg of urinary creatinine.

## 2.6 Di-tyrosine autofluorescence determination

Di-tyrosine (Di-tyr) content, used to determine in urine the levels of protein oxidation, was determined by autofluorescence, according Kirschbaum, 2002. For Di-tyr fluorescence determination, 50 µl of thawed urine was added to 950 µl of 6 mol/L urea in 20 mmol/L sodium phosphate buffer pH 7.4. After 30 minutes, the concentration was measured using a fluorometer (excitation 315 nm, emission 410 nm). Results were expressed as fluorescence units per mg urine creatinine (Kirschbaum et al 2002).

## 2.7 Antioxidant capacity determination

The urinary antioxidant capacity was determined using a chemical assay (Antioxidant Assay Kit, Cayman Chemical, 709001). This assay measures the capacity of antioxidants in the urine to inhibit the oxidation of 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) by metmyglobin. This reaction can be monitored by detecting the absorbance at 750 nm and the inhibition of the oxidation is proportional to antioxidants concentration. A standard curve using Trolox, a water-soluble tocopherol analogue, is used to calculate the capacity of the antioxidants in preventing the ABTS oxidation. Urinay antioxidant status was expressed as micromolar trolox equivalents.

### 2.8 Statistical analyses

Comparison between means was analyzed by Repeated Measures ANOVA followed by the Tukey multiple range test for parametric data and Friedman Test followed by Dunn's Multiple Comparison Test for nonparametric values when the F value was significant ($P < 0.05$). Correlations were carried out using the Pearson correlation coefficient.

## 3. Results

Figure 1 shows the measurement of free L-car in blood spots from MSUD patients, before and after the supplementation, and controls. The results demonstrated that MSUD patients had significantly reduced free L-car levels before the treatment (Group A) compared to control group. The supplementation with L-car was able to reverse this deficiency once it was observed a significant increase of L-car levels after 1 (Group B) and 2 (Group C) months of treatment [$F(3,19) = 6.253$, $P<0.05$].

We measured the levels of Leu in plasma from MSUD patients, before and after the treatment, and controls. We found the follow values, expressed in μmol/L: controls = 130.75 ± 27.16, Group A = 178.18 ±58.59, Group B = 264.57 ± 136.74, Group C = 278 ±105.55. Dates are represented as mean ± SD. There was no significant difference between groups.

Figure 2 shows the evaluation of oxidative stress in urine from MSUD patients. The Figure 2A exhibits the di-tyrosine levels, a biomarker of oxidative damage to proteins. We verified that the Di-tyr levels before the supplementation with L-car (Group A) was markedly increased compared to control group, as well as after 1 month of treatment (Group B), and L-car therapy was able to reduce the di-tyr levels, reverting after 2 months of treatment (Group C). Group C was significantly different from groups A and B [$F(3,15) = 18.45$, $P<0.05$]. We also found a significant negative correlation between free L-car levels and Di-tyr levels ($r = -0.557$, $p<0.05$) (Figure 3).

Figure 2B shows the isoprostanes levels, an end product of arachidonic acid peroxidation. We found a significant increase in isoprostanes levels before the treatment (Group A) compared to the control group and the supplementation with L-car was able to reduce the lipid peroxidation in treated MSUD. Groups B and C show a significant decrease in the isoprostanes levels compared to the Group A [$F(3,16) = 4.184$, $P<0.05$].

Figure 2C shows the results of urinary antioxidant capacity in the groups. We found that before the treatment (Group A) and after one month (Group B) with L-car supplementation, the patients had a significant lower urinary antioxidant capacity compared to control group. However, the L-car supplementation after 2 months (Group C) was able to revert this process to control levels [$F(3,15) = 15.02$, $P<0.05$].

We also measured the urinary levels of branched-chain amino acids, α-keto acids and α-hydroxy acids in order to evaluate the effects of L-car on this compounds (Table

1). It was found an increase in the Leu and Ile levels in urine of patients only after 2 months (Group C) with L-car supplementation compared to control group [F (3,19) = 9.720, $P<0.05$) and F(3,20) = 9.24, $P<0.05$)], respectively. There was no significant difference between the groups for the amino acid Val.

The urinary concentrations of KMV, KIV and HIV were not altered by L-car supplementation. Increased levels of α-ketoisocaproic acid (KIC) and the α-hydroxy acids α-hydroxyisocaproate (HIC) and α-hydroxy-B-methylvalerate (HMV) were found, mainly after 2 months with L-car supplementation (Group C) compared to control group [F(3,18) = 1,018, $P<0.05$), F(3,15) = 20.33, $P<0.05$) and F(3,11) = 9.461, $P<0.05$)], respectively. Moreover, the levels of HIC were significant different between all the groups tested.

## 4. Discussion

MSUD was first described by Menkes and colleagues in 1954 and the dietary treatment was implemented ten years later by Snyderman (Snyderman et al 1964; Chuang and Shih 2001). The aim of the MSUD treatment is to keep the BCAA plasma concentrations in suitable levels for patients with this disease in order to minimize the brain damage found in MSUD patients without treatment or in metabolic crisis (Danner and Elsas 1989; Chuang and Shih 2001; Wendel et al 2006). As the treatment is based on a BCAA-restricted formula (without L-car), containing essential amino acids, vitamins and minerals, many studies have reported that MSUD patients have nutritional deficiency, including L-car (Mescka et al 2013), which can cause an antioxidant defenses impairment (Lombeck et al 1978; Borglund et al 1989; Barschak et al 2006; Barschak et al 2007; Barschak, et al 2008). In our study, we demonstrated that the levels of free L-car in MUSD patients before the treatment were significantly reduced and the

supplementation with L-car during 1 and 2 months was able to restore the normal levels. Our results corroborate with other studies, demonstrating that patients treated with protein restrict diet could have a deficiency of carnitine (Sitta el al 2011; Sitta et al 2014).

Several studies have shown that the accumulated metabolites in inborn errors of metabolism induce excessive free radical production and reduce the tissue antioxidant defenses (Colome et al 2000; Barschak et al 2008; Wajner et al 2004). In this context, *in vitro* and *in vivo* studies have been developed in MSUD (Bridi et al 2005; Bridi et al 2006; Barschak et al 2006; Barschak et al 2007; Barschak et al 2008; Mescka et al 2011; Mescka et al 2013), but the oxidative stress parameters in urine of these patients have not been studied. In other hand, these parameters in urine have been investigated in some metabolic diseases. It has been reported that patients with propionic and methylmalonic acidurias had high levels of urinary isoprostanes, that were reversed after the treatment with L-car. Furthermore, patients with these acidurias had a low urinary antioxidant capacity (Ribas et al 2012).

In this study, we demonstrated that MSUD patients treated with protein restricted diet, but without L-car supplementation, had significantly increased Di-tyr levels in urine compared to control group and after 2 months of L-car supplementation, Di-tyr concentration were reduced to the normal levels. Furthermore, we found a negative correlation between urinary Di-tyr and blood free L-carnitine concentrations in MSUD patients, indicating that L-car treatment can be involved in the prevention of protein oxidative damage. Di-tyr is formed by the oxidation of adjacent protein tyrosine residues leading to the formation of a highly stable inter-phenolic bond that does undergo further metabolism (Kirschbaum et al 2002).

Protein oxidation by reactive species can lead enzymes, receptors and transport proteins to malfunction and, eventually, inducing alteration of cellular metabolism (Halliwell and Gutteridge 2007). Our results are in agreement with other studies. It has been seen that in plasma of MSUD patients, there was protein damage evidenced by increased of carbonyls content (Mescka et al 2013). Furthermore, studies in cortex of rats, evaluating carbonyls and sulfhydryl content as markers of protein damage, demonstrated that carbonyls content was significantly enhanced in cerebral cortex in MSUD group while sulfhydryl content was significantly reduced, indicating the occurrence of oxidized proteins, and the treatment with L-car prevented these effects, reducing to control levels this damage (Mescka et al 2011). It is known that for other inborn errors of metabolism, protein damage has already been verified since the patients with propionic and methylmalonic acidurias presented high levels of Di-tyr in urine and the treatment with L-car was able to reduce this damage to control levels (Ribas et al 2011).

We also demonstrated that treatment with L-car was able to reduce the isoprostanes levels progressively after 1 and 2 months of supplementation, indicating a decrease in the lipid oxidation in urine. Recently, studies showed that L-car proved to be effective in reducing the lipid oxidation, since malondialdehyde (Mescka et al 2013) and thiobarbituric acid-reactive substances (Barschak et al 2006; Barschak et al 2007) were markedly increased in MSUD patients and the therapy with L-car reversed oxidative damage to lipids in plasma of MSUD patients (Mescka et al 2013). Furthermore, L-car prevented lipid peroxidation in cerebral cortex of rats in a chemically-induced acute model of MSUD (Mescka et al 2011). L-car have been described in other studies as an antioxidant able to combat the lipid peroxidation (Abdul and Butterfield 2007; Miguel-Carrasco 2010)

Altered antioxidant capacity in plasma from MSUD patients was observed in other studies that demonstrated a decreased of the total antioxidant status, which represents the quantity of tissue antioxidants, and of the total antioxidant reactivity, which reflects the tissue capacity to react with free radicals (Barschak et al 2006; Barschak et al 2008). Our results are also in agreement, showing a reduction of urinary antioxidant capacity in patients before L-car supplementation. Possibly, these alterations can occur because of the BCAA restrict diet, normally used in the MSUD therapy, is poor in micronutrients necessary for the antioxidant status (Barschak et al 2007). Decreased urinary antioxidant capacity may reflect a reduction of antioxidants in the blood, such as uric acid and dietary antioxidants. Otherwise, it was verified in our study that after 2 months of L-car administration, this process was reversed to control levels, reinforcing an important antioxidant effect of L-car in MSUD patients.

In this study, we quantified the most accumulated metabolites in urine from MSUD patients. We found an increase, compared to control group, mainly after 2 months of treatment, of the amino acids Leu and Ile and of the metabolites KIC, HIC and HMV. Among these compounds, Leu and KIC are considered the main neurotoxic agents in MUSD (Chuang and Shih 2001). The appearance of these two compounds, as well as HIC and HMV, in significantly high concentrations after 2 months of L-car treatment suggested that supplementation with this coumpond may influence the urinary excretion of these metabolites, decreasing the concentration in the blood and tissues. It should be emphasized that because the toxic effects of the accumulating metabolites in MSUD patients, the beneficial effects of L-carnitine supplementation, such as correction of carnitine deficiency and restoration of intramitochondrial acyl-CoA/CoA ratios, may improve the metabolic status of these patients (Ribas et al. 2014). All these factors can

have contributed to lower levels of oxidative damage observed in MSUD patients during treatment with L-carnitine.

It is important to emphasize that Di-tyr and antioxidant capacity were reversed only 2 months after L-car treatment. Carnitine supplementation is frequently used in patients with organic acidemias, as for example, propionic, methylmalonic, isovaleric, 3-hydroxy-3-methylglutaric and glutaric type I acidemias and beneficial effects are well described in these patients (Hoffmann et al 1996; Bykov et al 2004; Kölker et al 2011; Ribas et al 2014). In these diseases, therapy with L-car is used to promote the reduction of the acyl Coenzyme A toxic accumulation, releasing Coenzyme A for other essential oxidative pathways and restoring normal concentrations in cases where there is a deficiency (Chalmers et al 1984). We believe that, based in these studies, the effect associated to L-car could be occurring in MSUD, by promoting the excretion of the metabolites in urine of the patients.

In conclusion, our results demonstrated that there are damage to protein and lipids in urine from MSUD patients, as well as alterations in the antioxidant capacity. The supplementation with L-car could be involved in the prevention of oxidative damage to protein and lipid in MSUD since we detected a decrease of di-tyrosine and isoprostanes levels in urine from treated patients, as well as an improvement on the urinary antioxidant capacity. Furthermore, we suggest that L-car could be used in MSUD patients to help in the excretion of toxic metabolites, representing a new approach to the current treatment, which consists of protein restrict diet. In addition, the use of urine, easily collected, can be an option for monitoring such patients by evaluating the parameters of oxidative stress.

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## 6. Conflict of interest

The authors declare that there are no conflicts of interest.

## 7. References

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**Legends**

**Figure 1.** Free L-carnitine measurement in blood spots from MSUD patients and controls by liquid chromatography electrospray tandem mass spectrometry (LC/MS/MS). Group A represents MSUD patients before treatment with L-car. Group B and Group C represent MSUD patients after 1 and 2 months of L-car supplementation, respectively. Data represent the mean ± SD. Number of MSUD patients = 7-4. Number of controls = 6. * $P<0.05$ compared to controls, # $P<0.05$ compared to Group A, § $P< 0.05$ compared to Group B.

**Figure 2**. **(A)** Di-tyrosine (Di-Tyr), **(B)** isoprostanes and **(C)** antioxidant capacity measurements in urine from MSUD patients and controls. Group A represents MSUD patients before treatment with L-car. Group B and Group C represent MSUD patients after 1 and 2 months of L-car supplementation, respectively. Data represent the mean ± SD. Number of MSUD patients = 7-4. Number of controls = 6. * $P<0.05$ compared to controls, # $P< 0.05$ compared to Group A, § $P< 0.05$ compared to Group B.

**Figure 3.** Correlation between Di-tyrosine vs. Free L-carnitine levels in MSUD patients. Graphs show the Pearson correlation coefficient and probabilities.

**Figures**

Figure 1.

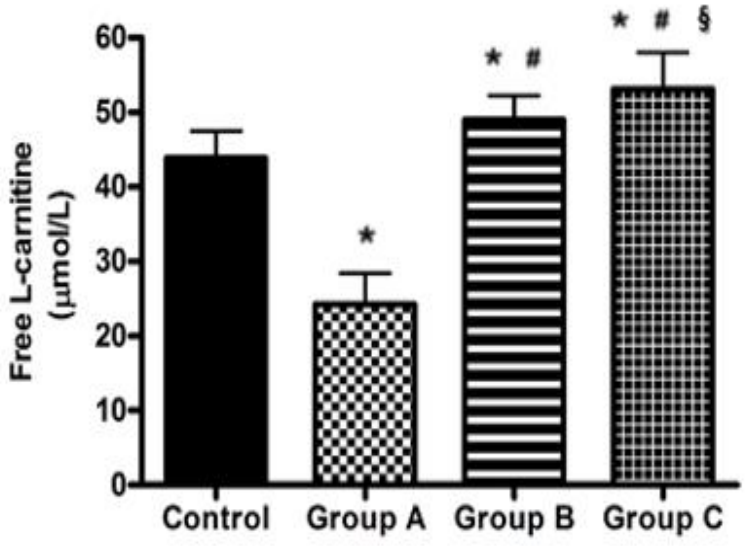


Figure 2.

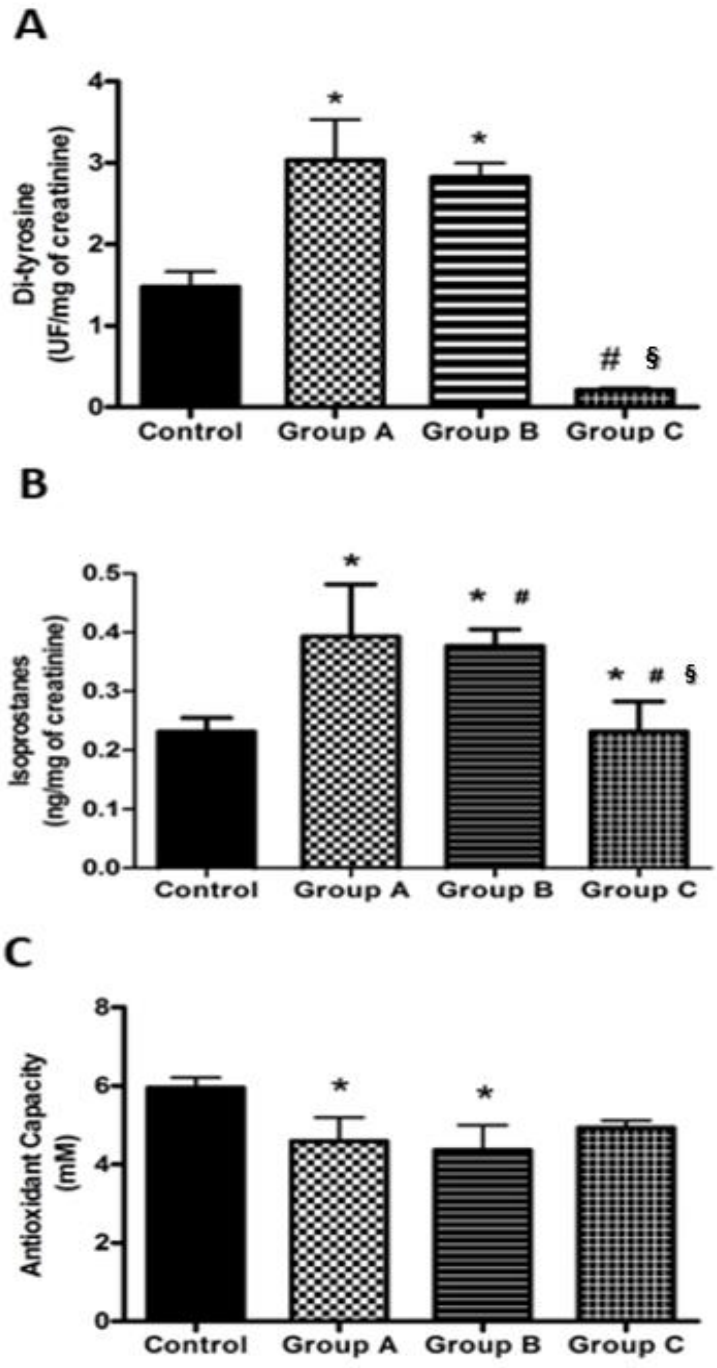
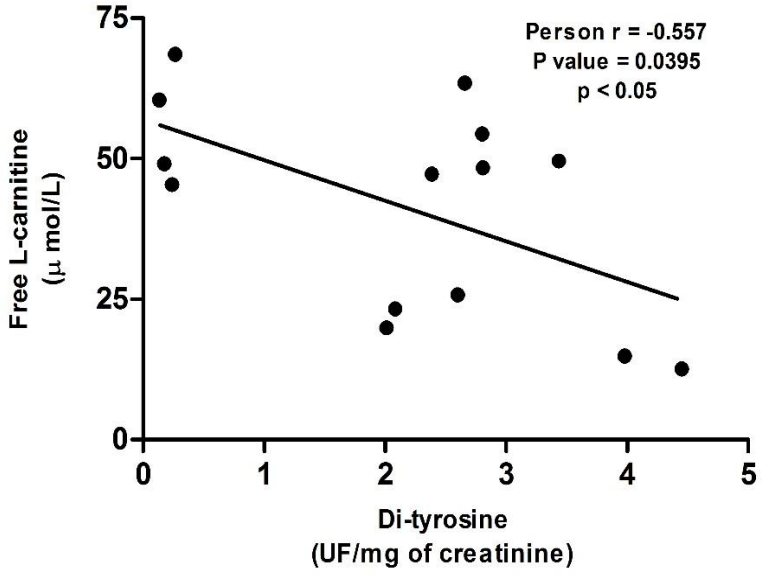

A
Di-tyrosine (UF/mg of creatinine)
0
1
2
3
4
*
*
# §
Control
Group A
Group B
Group C
B
Isoprostanes (ng/mg of creatinine)
0.0
0.1
0.2
0.3
0.4
0.5
*
* #
* # §
Control
Group A
Group B
Group C
C
Antioxidant Capacity (mM)
0
2
4
6
8
*
*
Control
Group A
Group B
Group C

Figure 3.




## Tables

**Table 1.** Urinary concentrations of amino acids (μmol/g creatinine), branched chain α-keto-acids and α-hydroxy acids (μmol/L) in MSUD patients and controls.

| | **Amino acids** | | | **α-Keto-acids** | | | **α-Hydroxy acids** | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leu | Ile | Val | KIC | KMV | KIV | HIC | HMV | HIV |
| **Control** | 32±7 | 17±3 | 50±14 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| **Group A** | 78±105 | 99±118 | 139±101 | 8.1±8.1 | 1.22±2.99 | 0.42±0.77 | 7±17$^{*}$ | 23±32 | 74±114 |
| **Group B** | 88±58 | 56±55 | 60±15 | 1.7±2.5 | 0.15±0.39 | 0.67±0.85 | 142±337$^{*\#}$ | 10±18 | 166±289 |
| **Group C** | 256±224$^{*}$ | 76±37$^{*}$ | 104±36 | 78±53$^{*\S}$ | 2.11±4.23 | 14±27 | 63±146$^{*\#\S}$ | 118±50$^{*\#\S}$ | 6993±8198 |

Values represents mean ± SD. Number of MSUD patients = 7-4. Number of controls = 6. * different to control P<0.05, # different to A P<0.05, § different to B P<0.05.

**ANEXOS**

# JIMD – Journal of Inherited Metabolic Disease

## Aims and Scope

The JIMD is the official journal of the Society for the Study of Inborn Errors of Metabolism, SSIEM. By enhancing communication between workers in the field throughout the world, the JIMD aims to improve the management and understanding of inherited metabolic disorders. It publishes results of original research and new or important observations pertaining to any aspect of inherited metabolic disease in humans and higher animals. This includes clinical (medical, dental and veterinary), biochemical, genetic (including cytogenetic, molecular and population genetic), experimental (including cell biological), methodological, theoretical, epidemiological, ethical and counselling aspects. The JIMD also reviews important new developments or controversial issues relating to metabolic disorders and publishes reviews and short reports arising from the Society's annual symposia. A distinction is made between peer-reviewed scientific material that is selected because of its significance for other professionals in the field, and non-peer-reviewed material that aims to be important, controversial, interesting or entertaining ("Extras").

The Journal of Inherited Metabolic Disease exists as two sister publications which are served by a single Editorial Team and a single manuscript submission and review process: the traditional print and online journal "JIMD" and the online-only "JIMD Reports". The latter publishes scientifically sound research findings or clinical observations that warrant communication in the peer-reviewed literature but are of more limited interest to the readers. In addition to full electronic publication as "JIMD Reports", the abstracts of these articles are also printed in the non-online section of the "JIMD" to reach the widest possible readership. All other types of articles are published electronically and in print in the JIMD.

## Scientific contributions

### *Full Articles*

The JIMD welcomes scientific contributions for publication as printed full articles in the following categories:

- **Original Articles:** Important manuscripts that may be expected to influence or change clinical or research practice with regard to inherited metabolic disorders. Original articles may include comprehensive studies on disease features in groups of patients, important novel information on a disease or relevant research findings. Exceptional case reports that are judged to be of general interest to the readers may also be accepted as original articles. The editors may reject submitted manuscripts as original articles but invite revision or resubmission for publication as Reports in "JIMD Reports". Anecdotal observations may also be submitted as "Extras".
- **Rapid Communications:** Highly competitive and timely manuscripts; please discuss this with the editors: editor@jimd.org.
- **Reviews:** Concise summaries of metabolic pathways, specific disorders, methods, treatment options etc.

- **Metabolic Dissertations:** The JIMD invites all researchers who have completed a Ph.D. or M.D. thesis in the field of inborn errors of metabolism to submit a comprehensive review of the topic of their thesis. The article should not focus solely on the research findings but should cover all relevant information in the respective field. Such reviews preferably (but not necessarily) have a single author (other contributors should be acknowledged) and will be published with a photograph of the investigator.

All authors are invited to provide a colour picture that may be used for the front cover of the issue in which the article appears.

### *Reports (Online Articles)*

Some manuscripts present scientifically sound research findings or clinical observations that are worth communicating but are of more limited interest to the readers of JIMD and may be sufficiently summarised in an abstract of 250 words. In order to facilitate publication of these types of manuscripts, **"JIMD Reports"** has been introduced as a sister publication of the traditional "JIMD". It is an independent periodical with its own ISSN number.

All manuscripts submitted as Reports to the JIMD website will be considered for "JIMD Reports" rather than for the traditional journal. They will undergo the same review process as Original Articles (and in exceptional cases may be reassigned for publication in "JIMD"). In addition, the Editorial Team (based on the advice of reviewers and Communicating Editors) may reject Original Articles for publication in the traditional "JIMD" but offer publication in "JIMD Reports". After acceptance, articles in "JIMD Reports" are professionally typeset in the same manner as articles in "JIMD", and full documents are available online to SSIEM members and institutional subscribers via the Springer website. JIMD Reports are submitted to PubMedCentral and are listed in PubMed as well as in other abstracting and indexing services. After an embargo period of 12 months all paperspublished in JIMD Reports are available as free access articles on PubMed Central, thereby ensuring widest possible readership. In addition, the titles and abstracts of all Reports are printed in the print-only "Extras" section of "JIMD". It is recommended that authors make use of the full allowance of 250 words for the abstract of their Reports to convey the message of the article to the readers of "JIMD".

Reports follow the same rules as Full Articles; they should not be used as a form of preliminary communication. They may take the form of **Research Reports**, with content similar to that of original articles**,** or **Case Reports.** Case reports will only be considered when they highlight some unusual or previously unrecorded feature relevant to the disorder, or serve as an important reminder of clinical or biochemical features of a Mendelian disorder. Chance associations of two conditions or sporadic cases from new geographical locations (as opposed to systematic epidemiological studies) are not in themselves of sufficient scientific merit to justify publication.

### *Images in Metabolic Medicine*

The Editors will consider clear and interesting clinical pictures or other types of images (e.g. laboratory results or observations) submitted with a descriptive paragraph of up to 250 words. Prints, slides, or electronic copy are all acceptable. Authors must obtain informed consent for publication of patient-related materials. Case reports or additional information may be added as supplementary material. Images will be fully printed; title and author(s) will be listed in bibliographical databases such as Medline.

### *Editorials*

The JIMD invites communicating editors and reviewers of articles that have been accepted for publication in the JIMD to provide an editorial that places the article in a broader context. Editorials have no abstract, may be comprised of up to 500 words and should contain no more than two (if any) references. Additional material can be added as supplementary material online. Editorials will be fully printed; title and author(s) will be listed in bibliographical databases such as Medline.

### *Letters, Clinical/Research Observations*

The JIMD invites comments on previously published articles in the journal which should reach the editorial office within 4 weeks of publication of the original item. Correspondence may be subjected to peer-review and counter-replies are usually invited from the authors of the original publication.

The concise form of a letter may also be used to report exceptionally important clinical or research observations unrelated to a previous JIMD publication that merit communication but do not fulfil the requirements for scientific articles or short reports. These items will be peer reviewed and if accepted will be pubished under the heading “Observation”.

Letters should have no more than five authors. They have no abstract, are limited to a maximum of 500 words and should contain no more than two (if any) references. Additional material can be added as supplementary material online. Letters will be fully printed; title and author(s) will be listed in bibliographical databases such as Medline.

## Extras in the JIMD

The Editors of the JIMD invite submission of short items that are interesting, stimulating, important or entertaining to professionals working in the field of inborn errors of metabolism. These items will not usually be reviewed outside the editorial board and usually will not be referenced in bibliographic databases. All items of this type should be submitted by Email to the editorial office (editor@jimd.org); please provide full personal details for all authors of each contribution.

### *Fillers*

Small texts that are used to fill gaps, e.g. at the end of original articles, have been a long and cherished tradition in some journals. They usually have the added advantage of entertaining readers and stimulating thought. The Editors invite interesting stories or personal experiences of up to a few hundred words on topics such as:

- A patient / paper / experience that changed my practice
- A memorable patient / experience
- An error that proved educational or informative for lab operation or clinical care
- How I embarked on this career path, and lessons learned along the way
- Any other story conveying instruction, pathos, or humour

If the filler refers to an identifiable person, written consent for publication from that person

or an appropriate relative is required.

### *Book Reviews*

Instructive reviews of up to 400 words are invited on new books published in the field of inborn errors of metabolism, or closely affiliated areas.

### *Obituaries*

The Editors of the JIMD strongly encourage submission of obituary notices for all recently deceased SSIEM members or other persons in the field of inborn errors of metabolism. Obituary notices should be mailed to the editorial office. Please give your name and contact details, including a phone number and email address. Obituaries will be considered by the editorial board and may be shortened; they will be published (without proofs) with the name of the person(s) who submitted the notice.

Please provide:

1. The full **name** of the deceased
2. A photograph
3. A summary of **Important data:**
    a. *(Last) professional position and title, place of work*
    b. *Date and place of birth*
    c. *Primary degree with university and year when obtained*
    d. *Additional professional qualifications with university and year when obtained*
    e. *Date of death, Cause of death*
4. The **main text** summarising important contributions and personal characteristics of the deceased. The last sentence should state the remaining relatives such as spouse and/or the number of children and grandchildren.

## *Instructions for Submission*

Material submitted to the JIMD (incl. JIMD Reports) must conform to the uniform requirements for manuscripts submitted to biomedical journals as outlined by the International Committee of Medical Journal Editors (http://www.icmje.org/index.html); see also International Committee of Medical Journal Editors (1999) *Med Educ* 33: 66-78.

### Online Submission

All scientific contributions for publication in the JIMD (including JIMD Reports) must be submitted by the web-enabled online manuscript submission and review system. As the review process is also fully web-based, this system allows editors to keep review times as short as possible and offers authors the option to track progress of the review of their manuscripts. The online manuscript submission and review system for the Journal of Inherited Metabolic Disease offers easy and straightforward log-in and submission procedures. Please refer to:

www.editorialmanager.com/boli

The system supports a wide range of submission file formats for manuscripts (Word, WordPerfect, RTF, TXT and LaTex) and figures (TIFF, EPS, Microsoft® Office formats and

Postscript). PDF is not an acceptable file format.

If you encounter any difficulties while submitting your manuscript online, please contact the responsible Editorial Assistant by clicking on "CONTACT US" from the tool bar.

## General Rules

It is a condition of acceptance that all articles have not been and will not be published elsewhere in substantially the same form. The submitting author must have circulated the article and secured final approval of the version to be peer-reviewed from all co-authors prior to article submission. This includes confirmation of

- absence of previous similar or simultaneous publications,
- their inspection of the manuscript,
- their substantial contribution to the work (all authors should have been involved in (a) conception and design, or analysis and interpretation of data, and (b) drafting the article or revising it critically for important intellectual content)
- their agreement to submission.

It should be noted that these conditions are later confirmed in writing by the corresponding author in a copyright transfer form at the time of acceptance. Publication elsewhere, at any time, of a similar article perhaps only differing in some aspects of data, especially if the JIMD article is not cross-referenced, may justify formal retraction at a later date.

Supplementary (internet-only) material may be published for all articles; we encourage or request deposition of raw data when this appears appropriate.

**The following information will be required at the time of online manuscript submission and need to be summarized under a separate section on the third manuscript page under "*Compliance with Ethics Guidelines*".**

## Conflict of Interest:

The Conflict of Interest statements should list each author separately by name:
*John Smith declares that he has no conflict of interest.*
*Paula Taylor has received research grants from Drug Company A.*
*Mike Schultz has received a speaker honorarium from Drug Company B and owns stock in Drug Company C.*
If multiple authors declare no conflict, this can be done in one sentence:
*John Smith, Paula Taylor, and Mike Schultz declare that they have no conflict of interest.*
With regard to the mandatory submission of the *Conflict of Interest Disclosure Form*, please see the following section on "**Competing Interests**."

## Informed Consent

For studies with human subjects include the following:
*All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.*
Proof that informed consent was obtained must be available upon request

If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach, and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

If any identifying information about patients is included in the article, the following sentence should also be included:
*Additional informed consent was obtained from all patients for which identifying information is included in this article.*

## Animal Rights

For studies with animals include the following sentence:
*All institutional and national guidelines for the care and use of laboratory animals were followed.*

**For articles that do not contain studies with human or animal subjects performed by any of the authors, please include the following sentence:**
*This article does not contain any studies with human or animal subjects performed by the any of the authors.*

**Details of the contributions of individual authors**
Please make clear who has contributed pertinent aspects of the planning, conduct, and reporting of the work described in the article.

## Competing Interests

*Conflict of interest exists when an author (or the author's institution), reviewer, or editor has financial or personal relationships that inappropriately influence (bias) his or her actions (such relationships are also known as dual commitments, competing interests, or competing loyalties). These relationships vary from those with negligible potential to those with great potential to influence judgment, and not all relationships represent true conflict of interest. The potential for conflict of interest can exist whether or not an individual believes that the relationship affects his or her scientific judgment. Financial relationships (such as employment, consultancies, stock ownership, honoraria, paid expert testimony) are the most easily identifiable conflicts of interest and the most likely to undermine the credibility of the journal, the authors, and of science itself. However, conflicts can occur for other reasons, such as personal relationships, academic competition, and intellectual passion.*

*Expert reviewers approached for assessment of submitted articles are also requested to declare conflicts of interest that may impede on their judgment of that article. This specifically includes competing research in the same area that could be negatively affected by publication of the submitted article.*

*For additional information see also "uniform requirements for manuscripts submitted to biomedical journals" at [www.icmje.org](http://www.icmje.org).*

***Please note:** it is mandatory to fill out completely and submit the **Conflict of Interest***

***Disclosure Form*** *with the first of any revisions submitted, major or minor. This form can be found at the JIMD website, at [http://www.springer.com/medicine/internal/journal/10545](http://www.springer.com/medicine/internal/journal/10545). Any revised papers submitted without this form will not be reviewed and thus will not be published.*

## Manuscript Structure

The first page should include:

- *Title* of the article
- *Authors' names and institutional affiliations* set out as in a current issue of the JIMD
- Name, email address and full postal address, including postal (ZIP) code, of the author who will be dealing with correspondence and proofs.
- *Word counts* for the text (excluding summary, acknowledgments, references and figure legends) and the summary. *Number of figures and tables;* please also state whether a colour picture is provided that may be used for the *front cover of the issue in wh*ich the article appears.

The second page should include

- A *summary* (= *abstract)* of not more than 250 words (Medline allows a maximum of 4096 characters and will truncate longer abstracts).
- A *concise 1 sentence take-home message* (synopsis) of the article, outlining what the reader learns from the article (this is usually printed on the (inside) back cover of JIMD)

The third page should include

- *Compliance with Ethics Guidelines* as described above under "General Rules".

## Recommendations for Manuscript Length

Competition for publication in all scientific journals has become increasingly intense, and the JIMD is no exception. We strongly encourage prospective authors to consider brevity in their presentation and, if needed, to avail themselves of the on-line supplementary material for those figures and tables that could be accommodated in that venue. In order for the Editorial Board to accommodate the broadest spectrum of submissions, and to maximize the access for prospective authors to both JIMD and the on-Line JIMD Reports, the following recommendations for length have been formulated:

***Full Articles: Total word count 3000, including 500 words for the Introduction and a maximum of 4 combined figures/tables.***

***Reports: Total word count 2250, including 400 words for the introduction and a maximum of 3 total figures/tables.***

***It is expected that more comprehensive reviews will exceed these limits, but the authors of such reviews are again encouraged to work for brevity and succinctness in presentation. In all instances, literature citations should be reasonable and appropriate for the presentation, but should not exceed 30 citations for articles and 25 citations for reports. Appropriate use of the cited literature is one way in which prospective authors can control the length of their submissions.***

## *Units, symbols, database references*

At the time of first mention, diseases, enzymes or genes should be referenced to the appropriate classification, nomenclature or database:

- Inherited diseases to the OMIM catalogue number (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM)
- Enzymes to an Enzyme Commission (EC) number (http://www.chem.qmul.ac.uk/iubmb/enzyme/)
- Genes to the HUGO-approved gene symbol (http://www.gene.ucl.ac.uk/nomenclature/)

Authors should use SI units throughout the manuscript. Biochemical nomenclature should follow IUPAC-IUB recommendations (http://www.chem.qmul.ac.uk/iupac/jcbn/). Nomenclature of mutations or genetic variants should follow HGVS recommendations (http://www.hgvs.org/mutnomen/). At the time of first mention, genetic variants should be described with both protein designation and DNA designation (based preferably on cDNA reference numbers).

References to electronic databases (e.g. OMIM disorder/gene accession number(s), EC numbers, HUGO-approved gene symbol, GenBank Accession and version number(s) of the relevant wild-type gene sequence(s), locus-specific database(s) or other URLs of relevant databases)

Previously published material should be acknowledged, and written permission from copyright holders must be obtained to reproduce figures, tables or substantial sections of text. Where a paper relies on material that is under consideration by, or in press in another journal, a copy of this must be provided for the referees.

When writing the articles, please keep in mind the broad readership of the JIMD. For example, for methods that are widely reported or published it may be worthwhile to provide a brief two to three sentence description of the protocol to provide the reader with some insight into the methods used.

## *References*

Consult a current issue of the journal. Citations in the text should use authors' names then the date, e.g.: (Smith and Smith 1977); for 3 or more authors use et al, e.g. (Jones et al 1989).

The full references are listed in alphabetical order at the end of the paper. Authors are listed without 'and'. Give the first 3 authors plus et al when there are 7 or more authors. Both in the text and list use 'et al' without punctuation or italicization. Journal abbreviations follow Index Medicus or Chemical Abstracts. Examples are:

*Journals:*
Smith AL, Smith JD (1977) Hybridisation methods. *Nucl Acids Res* 8: 1095–1098.

*Chapter in an edited book:*
Weinstein L, Swartz MN (1974) Pathologic mechanisms of invading microorganisms. In Sodeman WA Jr, Sodeman WA, eds. *Pathogenic Physiology: Mechanisms of Disease.* Philadelphia: WB Saunders, 457–472.

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Reference to personal communications requires the explicit approval of the person quoted; written confirmation must be provided. Authors - not journal editors or copy editors - are responsible for the accuracy of all references, which includes verifying the source of email communications, before citing them as personal communications in manuscripts.

### *Research materials*

It is assumed that authors whose research is published by the JIMD will make antibodies, cloned DNA sequences, and similar materials available to other investigators in non-commercial institutions, so as to permit replication of the reported work.

### *After acceptance of a manuscript*

**Proofs** will be sent to the corresponding author by email. Responses, with or without corrections, should be sent within 72 hours. Please do not correct or edit the PDF file. Extensive corrections must be clearly marked on a printout of the PDF file and should be sent by first-class mail (airmail overseas). Minor corrections (+/- 10) may be sent via email attachment to proofscorrection@springer.com. Always quote the four-letter journal code (BOLI) and article number from your proof in the subject field of your Email.

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**Additional Information**

Additional information can be obtained from:

Dr. Verena Peters
Managing Editor, Journal of Inherited Metabolic Disease
University Children´s Hospital
Im Neuenheimer Feld 150
69120 Heidelberg
Germany

Tel: +49 (6221) 5631715
Fax: +49 (6221) 565565

Email: Verena.Peters@med.uni-heidelberg.de

Dr. Andrea Pillmann
Senior Editor, Biomedicine
Springer
Tiergartenstr. 17
69121 Heidelberg
Germany

Tel: +49 6221 487 8346
Fax: +49 6221 487 6 8346

Email: andrea.pillmann@springer.com

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