*Jacek Płonka[1], Halina Woś[2], Lidia Hyla-Klekot[3]

Carnitine concentration levels in serum of children with nephrotic syndrome

Stężenie karnityny w surowicy krwi u dzieci z zespołem nerczycowym

[1]Pediatric Ward of the Regional Medical Centre, Opole
Head of Ward: Janusz Zaryczański, MD, PhD
[2]Pediatric Clinic of the Silesian Medical University, Katowice
Head of Clinic: prof. Halina Woś, MD, PhD
[3]Technical University in Opole, Department of Physiotherapy
Head od Department: prof. P.O. Krzysztof Wronecki, MD, PhD

S u m m a r y

Introduction. Carnitine is a quaternary ammonium compound. Its key function in organism is based on taking an active part in lipids' metabolism. Disorders appearing in lipid distribution are one of the main symptoms of nephrotic syndrome in children. In result, such disorders may later lead to metabolic syndrome. The recent, however not numerous, studies on carnitine levels and their behaviour in children suffering from nephritic syndrome, showed disorders concerning carnitine. Unfortunately, the results seem not to be very consistent.

Aim. The main objective of the study was to assess the concentration levels of carnitine and its fractions in serum of children with nephrotic syndrome and also take an attempt to determine the relationship between carnitine and its fractions levels change and the lipid and protein distribution parameters.

Material and methods. The subjects of the study were 37 children (the age range between 17 months and 15 years 8 months) who were all diagnosed with nephrotic syndrome. All these children had their carnitine and its fractions levels in serum determined as well as the concentration level of cholesterol, triglycerides and total protein. The tests were done in both, the acute period of nephrotic syndrome and in its remission phase.

The method used to determine carnitine and its fractions levels in serum was the one invented by Cederblat with its later modifications introduced by Rössler, and based on the radio-enzymatic combined test.

Results. Average carnitine concentration in the acute period of nephrotic syndrome was 46.55 +/- 15.65 μmol/L, however, after proteinuria remission it decreased and came to 40.72 +/- 10.9 μmol/L (statistically typical value). The average free carnitine concentration level observed in the acute period was also higher than in the remission period: 38.09 +/- 13.53 vs 33.03 +/- 9.62. Similar correlations were observed with regards to acyl-carnitine and free acyl-carnitine ratio. Carnitine distribution disorders were found in the majority of patients.

Conclusions:

1. Children with nephrotic syndrome show disorders of carnitine metabolism, expressed either in its high concentration or its deficiency. However, clinical features of carnitine deficiency have not been observed.
2. Higher levels of carnitine observed in the acute period of the disease can be associated with hyperlipidaemia's stimulating influence on carnitine's metabolism.
3. A normalization process of the lipid and protein distribution parameters in the remission period of the disease is accompanied by the decrease of concentration level of total carnitine as well as free and acyl-carnitine.
4. Carnitine's participation and its role in complex metabolic disorders observed in nephrotic syndrome requires further studies and analyses.

Key words: L-carnitine, nephrotic syndrome, cholesterol, triglycerides, protein

S t r e s z c z e n i e

Wstęp. Karnityna jest czwartorzędową aminą biogenną, której kluczowa funkcja w organizmie polega na udziale w metabolizmie lipidów. Zaburzenia gospodarki lipidowej to jeden z głównych objawów zespołu nerczycowego u dzieci, które w przyszłości prowadzić mogą do zespołu metabolicznego. Nieliczne dotychczas badania zachowania się stężenia karnityny w zespołach nerczycowych u dzieci wskazują na zaburzenia dotyczące karnityny, ale ich wyniki są niespójne.

Celem pracy była ocena stężenia karnityny i jej frakcji w surowicy krwi u dzieci z zespołem nerczycowym, próba określenia zależności między zmianą stężenia karnityny i jej frakcji a parametrami gospodarki lipidowej i białkowej.

Materiał i metody. Badaniem objęto 37 dzieci w wieku od 17 miesięcy do 15 lat 8 miesięcy z rozpoznanym zespołem nerczycowym, u których oznaczono poziom karnityny i jej frakcji w surowicy, stężenie cholesterolu, trójglicerydów i białka całkowitego w surowicy krwi, w okresie ostrym i w okresie remisji zespołu nerczycowego.

Do oznaczenia w osoczu karnityny i jej pochodnych zastosowano metodę opracowaną przez Cederblata w modyfikacji Rösslera opartą na kombinowanym teście radio-enzymatycznym.

Wyniki. Średnie stężenie karnityny całkowitej w ostrym stanie ZN wynosiło 46,55 +/- 15,67 μmol/L, natomiast po ustąpieniu białkomoczu było niższe i wynosiło 40,72 +/- 10,9 μmol/L ($p < 0,05$). Również średnie stężenie karnityny wolnej w ostrym okresie choroby było wyższe niż w okresie zdrowienia: 38,09 +/- 13,53 vs 33,03 +/- 9,62. Podobne korelacje zachodziły w odniesieniu do karnityny zacylowanej oraz wskaźnika karnityny zacylowanej dowolnej. Zaburzenia gospodarki karnityną obserwowano u większości pacjentów.

Podsumowanie:

1. U dzieci z ZN występują zaburzenia w zakresie metabolizmu karnityny, wyrażające się bądź wysokim jej stężeniem, bądź niedoborem. Nie obserwuje się klinicznych cech niedoboru karnityny.
2. Wyższe wartości karnityny w ostrym okresie choroby mogą być związane ze stymulacyjnym wpływem hiperlipidemii na jej metabolizm.
3. Normalizacji parametrów gospodarki lipidowej i białkowej w okresie remisji zespołu nerczycowego towarzyszy spadek stężenia karnityny całkowitej, wolnej i zacylowanej.
4. Udział karnityny w złożonych zaburzeniach metabolicznych występujących w zespole nerczycowym wymaga dalszych analiz.

Słowa kluczowe: L-karnityna, zespół nerczycowy, cholesterol, trójglicerydy, białko

INTRODUCTION

Nephrotic syndrome is a multidimensional disorder of body homeostasis induced by a loss of protein due to a destroyed nephrons' filter membrane. In the light of recent studies, it has been found out that this phenomenon may have the acquired character and be the effect of the loss of balance between inhibitors and promoters of protein permeability through nephrons' membrane or be the conseqence of a genetically determined, primary defect of this microstructure (1, 2). Nephrotic syndrome is the most frequently observed clinical form of children's age glomerulopathy. Its chronic and recurrent character cause that incidence of its appearence in children's population is estimated to reach the level of 16/100 000 (1).

Nephrotic syndrome, developing on the basis of minimal change of chronic glomerulonephritis and focal segmental glomerulosclerosis, appears to be one of the most difficult diagnostic and therapeutic problem found in children's nephrology. The leading biochemical disorder in nephrotic syndrome is a loss of protein in the amount of 50 mg/kg/daily. Proteinuria results in dyslipidemia that has been observed in case of some patients even when proteinuria is in its remission stage. In spite of the fact that the lipid distribution disorder participation in nephrotic syndrome has been known for over 170 years, its exact pathomechanism is still unclear (3-5).

The lipid disorder found in nephrotic syndrome concerns all lipoprotein fractions, however the leading disorder is hypercholesterolemia and hypertriglyceridemia. HDL lipoprotein concentration may be decreased, increased or normal (5). Hyperlipidemia appearing in the course of nephrotic syndrome, is connected with a compensatory growth of protein synthesis, including apoprotein present in the liver, as the response to the loss of protein excreted with urine. Later, proteins combine with lipid elements of serum and create lipoproteins. Lipoprotein molecules are not excreted with urine and therefore their concentration in serum is above normal.

Hypercholesterolemia observed in nephrotic syndrome is the effect of an intensive cholesterol synthesis resulting from the increased activity of a key enzyme and its biosynthesis-reductase HMG-CoA, and also function disorder of other enzyms involved in lipid metabolism: lecithin-cholesterol acyltransferase (LCAT) and sterol O-acyltransferase acyl-CoA: cholesterol (ACAT). The latter one catalyzes the intracellular esterification of cholesterol and formation of cholesteryl esters in the cell. The intracellular estrification of cholesterol by ACAT leads to the decrease of free cholesterol in serum which activates the key enzyme cholesterologenesis-reductase HMG-CoA (4, 5). Hypertriglyceridemia appearing in nephrotic syndrome is caused by the increased VLDL synthesis in the liver and a defective lipoprotein catabolism, especially of a VLDL fraction. LDL molecules undergo direct synthesis in the liver through the alternative metabolic path.

Dyslipidemia appearing in nephrotic syndrome is a significant pathophysiological factor responsible for damaging nephrons. Receptors for lipoproteins have been found in mesangium as well as in podocytes. In nephrotic syndrome, lipoproteins are uncontrollably intercepted by mesangium cells and then they undergo a proliforation process. Lipoproteins accumulated in mensangium get oxidized and in this form they stimulate the process of antibodies creation. Complexes, i.e. an oxidized LDL antibody, are intercepted by macrophages which later undergo a transformation

process in order to become foam cells which are the source of numerous mediators of the inflammatory condition. The effect of their activity is the further proliferation process of mensangium cells, increased adhesion of monocytes into endothelium cells, creation of inflammatory intumescences, monocytes apoptosis and in result, dysfunction of nephrones' filtering membrane. The whole process is referred to as ateroglomerulosclerosis (7, 8). The lipid metabolism disorder in nephrotic syndrome constitutes a great risk factor of developing early atherosclerosis together with its clinical consequences, such as cardiovascular system related diseases.

The key function of carnitine as an essential nutricious substance, belonging to a group of natural elements possessed by all superior organisms is its active part in lipid metabolism, enabling transport of longchain fatty acids to mitochondrial matrix where they can undergo the process of β-oxidation. All the research, carried out in recent years, is trying to explain the role of carnitine in human organism and its significant role in lipid metabolism. Not numerous research concerning carnitine's behaviour in serum of children suffering from nephrotic syndrome show disorders in concentration levels of total and free carnitine as well as its esters. Unfortunately, the results of the research are not consistent.

AIM OF THE STUDY

The objective of the study is:

- to assess the concentration levels of carnitine and its fractions in serum of children with nephrotic syndrome,
- to take an attempt to determine the relationship between carnitine and its fractions levels change and the lipid and protein distribution parameters.

PATIENTS AND METHODS

The subjects of the study were 37 children at the age between 16 months old and 15.8 years old (average 4.8 years old) including 10 girls and 27 boys with a diagnosis of nephrotic syndrome. All these children had their carnitine and its fractions (total, free and acyl-carnitine) levels in serum determined as well as the concentration level of lipids (total cholesterol), triglycerides and total protein and the ratio of acyl-carnitine to free carnitine. The tests were done twice, in the acute period of nephrotic syndrome, i.e. when the children were taken to hospital (test 1) and in the disease remission phase, i.e. when proteinuria disappeared (test 2).

The results have been presented in table 1. Carnitine and its fractions concentration levels have been determined by means of Cederblat method with its later modifications introduced by Rössler. The standard values relating to carnitine and its fractions have been presented in table 2. The tests to determine carnitine and its esters concentration levels were done in the Radiodiagnostics and Nuclear Medicine Unit of the Radiology and Nuclear Medicine Departament of the Silesian Medical University in Katowice and the remaining tests were done in the Diagnostics Laboratory Unit of the Regional Medical Centre in Opole. The statistical analysis was made by means of classical methods in Rv2.10 platform (The R. Foundation for Statistical Comuting). The differences between carnitine concentration levels were assessed by means of the t-student

Table 1. Tests results.

	Number of tested	Average	Median	Range of variability		Standard deviation
				Min	Max	
Age/months	37	56.41	42.00	17.00	176.00	42.83
Total protein in serum[1] g/l	37	44.60	42.0	31.80	70.40	9.50
Total protein in serum[2] g/l	29	56.20	56.80	35.60	79.0	8.70
Cholesterol[1] mmol/l	37	10.04	10.28	3.39	17.59	3.07
Cholesterol[2] mmol/l	31	6.48	5.85	4.12	10.59	1.72
Triglycerides[1] mmol/l	34	3.02	2.71	0.64	8.73	1.89
Triglycerides[2] mmol/l	20	2.16	1.79	0.12	5.21	1.27
Protein in urine[1] g/l	37	15.49	11.80	0.00	64.50	15.47
Protein in urine[2] g/l	34	0.79	0.00	0.00	9.70	2.32
Total carnitine[1] umol/l	37	46.55	44.05	22.49	101.06	15.67
Free carnitine[1] umol/l	37	38.09	35.41	16.76	77.33	13.53
Acyl-carnitine[1] umol/l	37	8.46	6.88	3.64	23.73	4.25
Acyl/free carnitine ratio[1] umol/l	37	0.23	0.21	0.06	0.62	0.11
Total carnitine[2] umol/l	37	40.72	39.27	19.81	77.04	10.90
Free carnitine[2] umol/l	37	33.03	31.56	15.99	55.26	9.62
Acyl-carnitine[2] umol/l	37	7.71	6.89	2.80	21.81	4.21
Acyl/free carnitine ratio[2] umol/l	37	0.25	0.22	0.07	0.74	0.16

[1]means the acute stage of a disease (the moment of taking a child to hospital)
[2]means the test done in the remission period of proteinuria

test for the combined data and statistical significance $p < 0.05$ was accepted as characteristic. The tests were done with the approval of the Bioethics Board of the Silesian Medical University in Katowice.

RESULTS

The average concentration level of total carnitine in test 1 came to 46.55 ± 15.65 μmol/L and was by 5.83 μmol/L ($p = 0.02$) higher in comparison to the test done in the remission period of proteinuria (40.72 μmol/L). The average concentration level of free carnitine in the initial test came to 38.09 ± 13.53 μmol/L and in the final test to 33.03 ± 9.62 μmol/L ($p = 0.016$). Whereas, the average concentration level of acyl-carnitine in test 1 came to 8.46 μmol/L and in test 2-7.71 μmol/L (not statistically important difference). The values of acyl-carnitine to free carnitine ratio determined in test 1 and 2 (0.23 and 0.25 respectively) did not appear to show a significant difference either.

Assessing the influence of lipid and total protein concentration levels on carnitine and its fractions concentration levels determined in the acute stage of the disease (test 1), significant positive correlations between total and free carnitine concentration levels and cholesterol and total protein concentration levels were demonstrated and additionaly a correlation between acyl-carnitine and total protein found in serum was shown. Assessing the influence of lipid and total protein concentration levels on carnitine and its fractions concentration levels in serum determined in the remission stage of the disease (test 2), significant correlations between total ($p = 0.0042$) and free ($p = 0.0054$) carnitine concentration levels and cholesterol concentration levels, and a correlation between acyl-carnitine concentration level ($p = 0.01656$) and triglycerides concentration level were demonstrated. Similarly to the acute stage, the correlation between total and free carnitine concentration and the concentration of total protein in serum ($p = 0.0056$ and $p = 0.00688$ respectively) was shown. Carnitine deficiency was observed in case of 14 children (38%) whereas the increased values only in 7 (18%).

DISCUSSION

Kidneys play a very important role in carnitine's metabolism as they are the place of its synthesis as well as its excretion. Disorders observed in carnitine's distribution may be also caused by the limited amount of protein contained in human diet, reduction of endogenic synthesis, decrease of nephron filration, loss of carnitine into dialysis liquid or eventually lipid metabolism disorder. In spite of the fact that the values of concentration level of total, free and acyl-carnitine remained within the standard limits, in both phases of the disease, disorders were observed in the majority of patients. Comparison of carnitine and its fractions concentration levels observed in the acute stage of the disease to those found in its remission stage, showed their lower concentration levels in the latter stage. Very similar observations were made by Zachwieja and Gousseinow who studied these parameters in children with nephrotic syndrome (9, 10). Total carnitine concentration levels determined in serum in the acute period of the disease fluctuated between 22.49 μmol/L and 111.12 μmol/L and in the remission period it turned to be between 19.81 μmol/L and 77.04 μmol/L. The decreased concentration levels of total carnitine were observed in case of 14 examined kids (38%), in 7 (19%) cases during the acute period and in other 7 (19%) cases in the remission one. On the other hand, in case of 4 (11%) patients examined in the acute stage of the disease and in 3 (8%) examined in the remission stage, total carnitine concentration level was above standard values. Carnitine dificiency in serum in children with nephrotic syndrome was also observed by Birkan (11). In two cases, where concentration level of free carnitine determined in both, the acute and the remission stage, was decreased, the course of the disease was referred to as the most serious. It was characterized by high values of cholesterol and triglycerides, huge loss of protein and albumins excerted with urine and clinically, by long-lasting oedema – these children were diagnosed with steroid-resistant nephrotic syndrome. This observation is in accordance with the one made by Zachwieja who, in his research, also found out that patients with low carnitine concentration level in serum, showed relatively worse response to steroid treatment (9). In this study one case seems to be particularly worth mentioning. It concerns the youngest patient, a 16-month-old boy whose total and free carnitine concentration levels determined in test 1 were high: 101.06 μmol/L and 77.33 μmol/L respectively. The values determined in the final test showed that both, total and free carnitine concentration levels fluctuated around the lowest limits and the course of the illness was of mild character. The recurrence of the disease was not observed during further, a few years long, observation period. In case of 2 children, the value of acyl-carnitine to free carnitine ratio, determined in the acute period, exceeded 0.4. Such a result allows to diagnose a relative carnitine dificiency, however no clinical characteristics of such deficiency were observed, i.e. muscle tension weakening, alimentary canal peristalsis disorder, cardiomyopathy, liver defect expressed by increased activity of aminotranferases and hypoglycemia. The above mentioned symptoms

Table 2. Standard values related to total, free, and acyl-carnitine and acyl-carnitine to free carnitine ratio for children over 1 year old.

Total carnitine μmol/L	Free carnitine μmol/L	Acyl-carnitine μmol/L	Acyl/free carnitine ratio
45.4 ± 9.6	35.7 ± 9.1	8.9 ± 5.4	< 0.4

are observed in patients suffering from other diseases, such as: type 1 diabetes, absorbing disorder or those treated with valproic acid (12-15).

Lipid disorder is one of the main symptom of nephrotic syndrome. In the group of tested subjects the concentration levels of cholesterol LDL and triglycerides significantly exceeded standard values. The increased values of cholesterol and triglycerides were observed in the acute as well as in the remission period of the disease, after proteinuria had disappeared. Such a situation may directly lead to later cardiac complications (16). Carnitine plays a key role in lipid metabolism, so that mutual correlation between lipids and carnitine's ingredients was analysed. Positive correlations of total and free carnitine concentration levels in relation to cholesterol concentration levels, in both, the acute and remission stages of the disease were demonstrated, and in the remission period the positive correlation between acyl-carnitine concentration level and triglycerides concentration level was shown. Similar results were presented by the authors of two works on carnitine's behaviour in children with nephrotic syndrome (9, 10). The hypothesis assuming a stimulating effect of a high concentration of lipids on the increased carnitine's synthesis, may explain the increased total and free carnitine concentration levels found in serum in the acute period of the disease, in case of high concentration of cholesterol. Proteinuria observed in nephrotic syndrome results in the reduced concentration of total protein and albumins found in serum. Hypoalbuminemia acting as a not specific stimulator of apoliprotein synthesis that takes place in the liver, leads to the increase of lipids and aminoacids, including lysine and methionine – the precursors of carnitine. Such situation was observed in the subjects of our research in the acute stage of their disease. On the other hand, the research conducted by Bircan (11) revealed a completely opposite situation. He found out that simultaneously with the decrease of albumins' concentration in serum and the increase of proteinuria, the values of carnitine concentration in serum showed statistically characteristic fall.

SUMMARY

The average concentration levels of total, free and acyl-carnitine determined in the acute stage of the disease as well as in its remission stage, remained within the standard limits, in spite of the fact that some carnitine distribution disorders were observed in the majority of the subjects. Nevertheless, no clinical symptoms of carnitine dificiency were found.

Significantly higher concentration levels of total and free carnitine were observed in the acute stage of the disease and they were accompanied by the increased lipid and protein distribution disorders, however cholesterol concentration which was getting normalized as well as hipoproteinemia remission were definitely connected with the decrease of carnitine and its fractions concentration levels.

CONCLUSIONS

1. Children with nephrotic syndrome show disorders of carnitine metabolism, expressed either in its high concentration or its deficiency. However, clinical features of carnitine deficiency have not been observed.
2. Higher levels of carnitine observed in the acute period of the disease can be associated with hyperlipidaemia's stimulating influence on carnitine's metabolism.
3. A normalization process of the lipid and protein distribution parameters in the remission period of the disease is accompanied by the decrease of concentration level of total carnitine as well as free and acyl-carnitine.
4. Carnitine's participation and its role in complex metabolic disorders observed in nephrotic syndrome requires further studies and analyses.

BIBLIOGRAPHY

1. Grenda R: Steroidooporne i steroidozależne submikroskopowe zapalenie nerek. Nefrologia i Dializoterapia Pol 2006; 10: 62-67.
2. Zwolińska D, Kiliś-Pstrusińska K: Białkomocz u dzieci i młodzieży – epidemiologia, patofizjologia i diagnostyka. Pediatr Med Rodz 2005; 1: 54-60.
3. Książek J, Ciechanowicz A, Wierzbicka A et al.: Is dyslipidemia sustained during remission of nephrotic syndrome genetically determined? Pol Arch Med Wewn 2008; 179(1-2): 11-17.
4. Kuźma E, Roszkowska-Blaim M: Zaburzenia lipidowe u dzieci z nawrotami zespołu nerczycowego. Przegląd Lekarski 2006; 63: 201-204.
5. Spadło A, Bodalski J: Zaburzenia gospodarki lipidowej w zespole nerczycowym. Przegląd Pediatryczny 1997; 27: 208-214.
6. Sheaer G, Kaysen PhD: Proteinuria and plasma compositional changes contribute to defective lipoprotein catabolism in the nephrotic syndrome by separate mechanisms. Am J Kidney Dis 2001; 37: 119-122.
7. Nowosińska E, Drobnik D, Czekalski S: Glomerulopatia lipoproteinowa. Nefrologia i Dializoterapia Polska 2000; 4: 205-207.
8. Dijkman H, Wetzels J, Baede J et al.: Glomerulal involution in children with frequently relapsing minimal change nephritic syndrome: an unrecognized form of glomerulosclerosis? Kidney International 2007; 71: 44-52.
9. Zachwieja J: Rola karnityny w zaburzeniach lipidowych u dzieci z pierwszym rzutem zespołu nerczycowego. Rozprawa doktorska, Poznańska Akademia Medyczna 1996.
10. Gousseinov A, Kantar M, Mir S: Free carnitine levels in children with steroid-sensitive nephrotic syndrome. Ped Int 2002; 44: 74-77.
11. Bircan Z, Kaplan A, Soker M: Serum levels of carnityne, apolipoprotein AI, and apolipoptotein B in children with nephrotic proteinuria. Pediatr Nephrol 1996; 10(5): 680.
12. Mamoulakis D, Galanakis E, Dionyssopoulou E et al.: Carnitine deficiency in children and adolescents type 1 diabetes. J of Diabetes and its complications 2004; 18: 271-274.

13. Ciacci C, Peluso G, Iannoni E et al.: L- carnitine in the treatment of fatigue in adult celiac disease patient. J Digestive and Liver Disease 2007; 39: 922-928.
14. Winter SC, Vance WH, Zorn EM et al.: Carnitine deficiency in paediatrics: experience at Valle Children's Hospital, Frenso, California.[In:] Ferrari R, Dimauro S, Sherwood G (eds.): L-carnitine and its role in medicine from function to therapy. Academic Press Inc San Diego 1992; 209-221.
15. Woś H: Badania nad rolą niedoboru karnityny w zespołach zaburzonego wchłaniania i trawienia u niemowląt i małych dzieci. Rozprawa habilitacyjna, Śląska Akademia Medyczna, Katowice 1996.
16. Filippo C, Taylor M, Mestroni L et al.: Cardiomyopathy and carnitine deficiency. J Molecular Genetics and Metabolism 2008; 94: 162-166.

received/otrzymano: 20.02.2013
accepted/zaakceptowano: 10.04.2013

Address/adres:
*Jacek Płonka
Pediatric Ward of the Regional Medical Centre
Al. Witosa 26, 45-418 Opole
tel.: +48 (77) 452-07-92, + 48 601-514-225
e-mail: jplonka@wcm.opole.pl