Charles University in Prague

Faculty of Medicine in Pilsen

METABOLIC EFFECTS OF L-CARNITINE SUPPLEMENTATION IN HEMODIALYZED PATIENTS

(PhD Thesis Summary)

MUDr. Roman Cibulka

Pilsen 2007

This PhD thesis was elaborated within Postgraduate Studies in Biomedicine at the Institute of Clinical Biochemistry and Hematology, Faculty of Medicine in Pilsen, Charles University in Prague, and was supported by grant IGA MH CR No NB/7350-3 and by research project MSM No 0021620819.

Author: MUDr. Roman Cibulka

Address: Institute of Clinical Biochemistry and Hematology of Charles University Medical Faculty and Faculty Hospital in Pilsen,
Alej Svobody 80, 304 60 Pilsen
Czech Republic
Tel: +420 37 710 42 66, +420 37 710 4234
Fax: +420 37 710 4234
e-mail: cibulkar@fnplzen.cz

Commission: Biochemistry and Pathobiochemistry

Supervisor: Prof. MUDr. Jaroslav Racek, DrSc.

Oponents: Prof. MUDr. Tomáš Zima, DrSc., MBA
Doc. MUDr. Jaromír Eiselt, Ph.D.

PhD Thesis Report sent:

Defence of PhD Thesis:

Place of Defence:

Chairman of the Commission Biochemistry and Pathobiochemistry:
Prof. MUDr. Jiří Kraml, DrSc.

CONTENTS

1. INTRODUCTION

Chronic kidney disease (CKD) is a very real and growing problem, as indicated by demographic trends. The total number of treated patients has markedly increased during the last 30 years, and CKD currently affects approximately 19 million adult Americans, with an incidence that is increasing rapidly [Snyder & Pendergraph, 2005]. This trend is caused by a growing percentage of elderly people in the population as well as by technical progress and broader availability of dialysis therapy. An increasing number of diabetic patients is also an important factor.

CKD is characterized by progressive deterioration of kidney function, which develops eventually into a terminal stage of chronic kidney failure (CKF). CKF has traditionally been categorized as mild, moderate, or severe. Other poorly defined terms like uremia and end-stage renal disease (ESRD) have commonly been applied. During the last few years, an international consensus has emerged categorizing CKF into 5 stages according to the glomerular filtration rate (GFR) and presence of signs of kidney damage: stage 1: GFR > 90 ml/min and signs of kidney damage; stage 2: GFR = 60–89 ml/min and signs of kidney damage; stage 3: GFR = 30–59 ml/min; stage 4: GFR = 15–29 ml/min; and stage 5: GFR < 15 ml/min [Levey *et al.*, 2005].

Stage 5 represents the total inability of kidneys to maintain homeostasis, and this metabolic state is incompatible with life. Thus, at this stage, it is necessary to use methods that substitute for kidney function to ensure patient survival; these methods include peritoneal dialysis, hemodialysis (HD), and other extracorporeal purifying procedures, or kidney transplantation.

CKF is associated with many kinds of metabolic changes caused by the kidney disease and also attributable to dialysis treatment. Phenomena such as accumulation or deficit of various substances and dysregulation of metabolic pathways combine in the pathogenesis of these changes [Cibulka *et al.*, 2005]. In the process of accumulation, decreased urinary excretion plays a crucial role and leads to retention of metabolites in the organism (e.g., creatinine, urea, electrolytes, water). The increased formation of metabolites through catabolic processes and alternative metabolic pathways also wields an influence. Regular dialysis treatment partly decreases this accumulation but cannot avert the overall deficit.

This deficit of some important substances in CKF can be caused by deficient intake in diet, impaired intestinal absorption, or increased losses during dialysis sessions. Disturbed

synthesis of some crucial metabolic regulators (e.g., erythropoietin, active vitamin D) in kidneys also plays an important role.

All of the abovementioned factors lead to many serious complications for CKD patients during the course of predialysis and dialysis. All accelerate development of atherosclerosis, malnutrition hand in hand with inflammation, anemia, hyperparathyroidism, and other serious problems that markedly affect prognosis and the quality of life of patients with CKF [Cibulka *et al.*, 2005; Lindner *et al.*, 1974; Durak *et al.*, 1994; Silver, 2000].

1.1. Deficit of L-carnitine in hemodialyzed patients

One of the metabolic disorders, which we studied in more detail, is a deficit of L-carnitine (CAR). CAR is a substance which plays an essential role in beta-oxidation of fatty acids by catalyzing their transport into the mitochondrial matrix. It enables obtaining of energy, namely in muscle cells including myocardium [Cibulka *et al.*, 2005].

Patients with chronic kidney failure treated by HD are known to have decreased CAR concentration in plasma and tissues due to its impaired synthesis in kidneys and the great loss across the HD membrane during dialysis sessions. A single HD session reduces plasma free-CAR concentrations to about one-third of their predialysis values because of small molecule of free-CAR [Bohmer *et al.*, 1978]. On the other hand, renal elimination of acylcarnitine, which is physiologically ineffective, may be impaired in chronic kidney failure, leading to increased blood concentrations of acylcarnitine. Although these subnormal free-CAR levels with elevated acylcarnitine fractions may result in normal or elevated total plasma CAR levels in HD patients, the functional CAR pool in tissues is decreased [Golper & Ahmad S, 1992].

Considerable evidence suggests that CAR deficiency and abnormalities of CAR metabolism result in a number of clinical conditions that are associated with dialysis, including muscle weakness, hypotension, fatigue, muscle cramps, poor exercise tolerance, anemia, left ventricular dysfunction, and higher incidence of arrhythmias [Cibulka *et al.*, 2005; Goral, 2001]. Recent studies have demonstrated that CAR supplementation can restore the abnormal metabolism in HD patients and may alleviate some of the symptoms mentioned above [Ahmad *et al.*, 1990; Bellinghieri *et al.*, 2003; De Felice *et al.,* 1996; Golper *et al.*, 2003; Veselá *et al.*, 2001].

The American National Kidney Foundation has recently approved “Practice Recommendations for the Use of L-carnitine in Dialysis-Related Carnitine Disorder”, which manifests itself as a syndrome of clinical problems and symptoms, most notable of which are anemia that is hyporesponsive to erythropoietin therapy, intradialytic hypotension,

cardiomyopathy, and skeletal muscle dysfunction manifested as generalized fatigability [Eknoyan *et al.*, 2003].

2. AIMS

We linked our research project to our pilot study which had occurred several years ago. At that time 12 chronically HD patients had been observed for 9 months, whereof the CAR supplementation had lasted for 6 months three times weekly after each HD in the dose of 15 mg/kg. Effects of CAR on red blood cell count, lipid metabolism, parameters of nutrition, oxidative stress and calcium-phosphate (Ca-P) metabolism had been investigated [Veselá *et al.*, 2001]. As this pilot study brought many positive and unexpected findings (e.g., positive influence of hyperphosphatemia of HD patients), we decided to verify these primary findings in a larger study.

The main goal of this project was also to investigate possible effects of CAR supplementation on metabolic parameters which could be positively influenced by the support of intramitochondrial beta-oxidation and energy metabolism. These are markers of nutrition, lipid metabolism, red blood cell count and, according to our previous findings, also parameters of oxidative stress and Ca-P metabolism. The purpose is finding ways to prevent from the development of atherosclerosis and renal bone disease in chronically HD patients.

The consequential goal was to adapt an enzymatic photometric method for free-CAR determination and its automation for the Olympus AU 400 analyzer.

3. PATIENTS AND METHODS

3.1. Description of study population

We included into the study 112 chronically HD patients (78 men and 34 women) in the dialysis centers EuroCare in Pilsen (52 patients) and in Teplice (60 patients). Any special inclusion criteria were used; all patients on HD who signed an informed consent form were included. The average age of these patients at the beginning of the study was 62.0 ± 12.5 years (median = 61.0 years), the median dialysis treatment period was 17 months (minimum = 3, maximum = 189). All patients were under regular HD therapy and were dialyzed for 4 hours three times weekly. The study was approved by the local ethic committee. The causes which led to chronic renal failure were: chronic glomerulonephritis (17.6 % of patients), interstitial nephritis (29.6 % of patients), diabetic nephropathy (29.6 % of patients), polycystic kidneys (6.5 % of patients) and other reasons (16.7 % of patients).

These subjects were randomly divided by computer into two groups and then a half year supplementation period began. One group was supplemented by CAR in the dose of 15 mg/kg in a short intravenous infusion after each HD session (i.e., three times weekly), while the other one was a control group which took a placebo (isotonic solution of sodium chlorid). During the study, some persons from the primary included 112 patients must have been excluded. The causes of exclusion were: death (16 patients), kidney transplantation (6 patients), non-compliance (2 patients), change of residence (1 patient) and restitution of kidney function (1 patient). Thus 26 patients were excluded prematurely and 86 passed the study under all rules. Results were not evaluated in patients who had accidentally any changes in their therapeutic regime during the study.

3.2. Design of study

Our study began in October of 2003 and passed until July of 2004 (i.e., 9 months). CAR from Fresenius Kabi Austria GmbH (Graz, Austria) was used for supplementation. Heparinized samples of venous blood were collected before the study, after 3 and 6 months of CAR supplementation. Some routine laboratory tests were performed monthly, while some other special tests were performed only twice, at the beginning of the study and after 6 months of the study. The main reason was a relatively high price of these special laboratory examinations (see below). In case of CAR, pre- and postdialysis values were measured; all other parameters were measured only before the HD session. Except the laboratory parameters, some other data were recorded (e.g., causes of CKF, the date of inclusion into the

HD program, types of HD membranes and solutions, duration of HD treatment, weight of patients, native renal function, doses of erythropoietin, vitamin D treatment, incidence of intradialytic hypotension, arrhythmias, muscle cramps, subjective status of patients, etc.). Parameters of HD were not changed during the study. The treatment was modulated only in extraordinary cases of possible health hazard of a patient.

Following laboratory parameters were tested during the study (frequency of examinations is stated in brackets; for example 0, 3, 6 mean that the particular examination was performed at the beginning of the study and after 3 and 6 months of the supplementation):

- free-CAR (0, 3, 6; 7 and 8 – only in supplemented patients);
- red blood cell count and weekly doses of erythropoietin (0, 1, 2, 3, 4, 5, 6);
- parameters of lipid metabolism: total cholesterol, cholesterol in high-density lipoproteins (HDL) and low-density lipoproteins (LDL), lipoprotein(a), triglycerides (0, 3, 6);
- parameters of nutrition and inflammation: albumin (0, 1, 2, 3, 4, 5, 6), prealbumin, transferin (0, 3, 6), C-reactive protein (0, 6);
- parameters of oxidative stress: antioxidative enzymes superoxiddismutase and glutathionperoxidase, reduced glutathione in erythrocytes, malondialdehyde (thiobarbituric acid reactive substances respectively), oxidized LDL particles, advanced glycation end-products, advanced oxidation protein products (0, 6);
- parameters of Ca-P metabolism and markers of bone turnover: Ca, P, Ca-P product (0, 1, 2, 3, 4, 5, 6), parathormone, bone-specific alkaline phosphatase, osteocalcin and osteoprotegerin (0, 6).

Statistical analysis was performed using the Statistical Analysis Software, version 8.02; Statistica 98 Edition and MedCalc, version 8.1. Means, medians, standard deviations, variances, frequencies and other basic statistical measurements were computed in the given groups. The comparison of variables between the two studied groups at the beginning of the study and during the supplementation, allowing consideration for the distribution of the variables, was performed by the Mann-Whitney test. The multivariate stepwise logistic regression analysis was used for the explanation the relations among parameters of Ca-P metabolism (concretely for CAR, Ca, P and Ca-P product because these parameters were measured three times). The paired Wilcoxon test was used for the comparison of changes of the variables in time (levels of measured parameters at the beginning in comparison with the levels at the end of the trial). Relations among these changes were evaluated using the Spearman correlation analysis. The multiple regression analysis was used for explanation of

relations among observed changes in the field of Ca-P and bone metabolism. Previously, a stepwise algorithm had served to select significant dependent variables for the multiple regression model. $P < 0.05$ was considered as statistically significant.

The statistical evaluation of results was performed in following blocks:

- changes of free-CAR levels during a HD procedure and in the long term in dependence on the CAR supplementation;
- effect of CAR supplementation on red blood cell count and weekly doses of erythropoietin;
- effect of CAR supplementation on lipid metabolism;
- effect of CAR supplementation on parameters of nutrition and inflammation;
- effect of CAR supplementation on parameters of oxidative stress and antioxidative defence;
- effect of CAR supplementation on parameters of Ca-P and bone metabolism in particular focus on a possible influence of secondary hyperparathyroidism.

3.3. Laboratory methods

3.3.1. Determination of L-carnitine

One of our goals was establishment a method for free-CAR determination and its automation using an Olympus (Mishima, Japan) analyzer. We used an enzymatic photometric test from Roche (Mannheim, Germany) intended for manual processing and the Olympus AU 400 automat analyzer.

The principle of detection described in the fly sheet from the test is the following: free CAR is acetylated to acetylcarnitine by acetyl-CoA in the presence of the enzyme carnitine acetyl transferase. The resulting CoA is acetylated back to acetyl-CoA in the presence of adenosine-5'-triphosphate (ATP) and acetate, catalyzed by the enzyme acetyl-CoA synthetase. This results in the formation of adenosine-5'-monophosphate (AMP) and inorganic pyrophosphate. In the presence of ATP, supported by myokinase, AMP forms at twice the amount of adenosine-5'-diphosphate (ADP). This is converted in the following reaction with phosphoenol pyruvate in the presence of pyruvate kinase. The pyruvate formed is reduced to L-lactate by reduced nicotinamide adenine dinucleotide (NADH) in the presence of lactate dehydrogenase. The amount of NADH consumed during the reaction is equivalent to half the amount of CAR. NADH is the parameter which is quantified according to its absorbance at 340 nm.

We followed instructions of the producer with a few of our own modifications. Venous blood samples were collected into test tubes with K_3EDTA (ethylenediaminetetraacetic acid). Plasma was prepared in the usual way and deproteinized

using a perchloric acid solution (0.6 mol/l) at the ratio of 1:1 (0.5 ml + 0.5 ml). After mixing the solution was kept in an ice-bath for 10 minutes. Then it was centrifuged at 3000 g for 10 minutes and 1.2 mol/l potassium carbonate solution (0.5 ml) was added to the supernatant (0.5 ml) to adjust pH. This mixture was kept in an ice-bath for 20 minutes and then centrifuged at 3000 g for 5 minutes. The supernatant was frozen and stored at -80 ºC until the analysis (approx. 3 months).

As far as the method of CAR detection itself, the fly sheet from the test is intended for manual processing. It is necessary to work with relatively high amounts of samples and reagents. The reaction is time-consuming (about 40 minutes), too laborious and complicated. It is a multi-step process in which 3 types of samples and reagents must be mixed gradually. Absorbance is measured repeatedly during the course of this process, whereby the concentration of CAR is finally calculated from the absorbance difference of the blank (the sample is replaced by deionized water), the absorbance difference of the sample and other physical values [Roche Diagnostics GmbH].

Because we wanted to apply this method employing the Olympus AU 400 analyzer, it was necessary to modify it for using only two reagents in smaller volumes according to the analyzer's options. The commercial test contains three bottles No. 1 followed by bottles No. 2, 3, 4 and 5. Exact composition of particular solutions is stated on the web sites of Roche [Roche Diagnostics GmbH] or it is described in our article [Cibulka *et al.*, 2006].

In accordance with the manufacturer's directions, the entire amount of the Bottle No. 1 should be dissolved in 10 ml of deionized water and mixed with 1 ml of detergent from the Bottle No. 5. This solution (Solution No. 1) is stable for 4 days at 2-8º C. Suspensions in Bottles No. 2, 3 and 4 are ready to use; they are stable for one year at 2-8º C. They are gradually and separately added to the reaction mixture whose final volume is 2.205 ml.

Our modification of this method for the Olympus AU 400 analyzer was the following: Solution No. 1 was mixed with Solution No. 2 (suspension from the Bottle No. 2 and deionized water in the rate of 1:10) in the ratio of 1:1. This final solution was used as the R1 solution in the Olympus analyzer. Its stability was confirmed by controls always before and after a series of measurements. The R2 solution was prepared as suspension from the Bottle No. 3 diluted by the 0.1 M Tris buffer in the rate of 1:50. 75 µl of the R1 solution was automatically diluted with 75 µl of water and added to 25 µl of sample or standard solution. This mixture was incubated with the starting solution R2 at 37° C for 5 minutes. A decrease in absorbance at 340 nm was measured kinetically between the 12^{th} and 27^{th} photometric points.

We tested some analytical parameters of our modified method for free-CAR determination using samples obtained from the external quality control system ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism) and further with the help of the manufacturer's controls from Roche and also on the basis of our own plasma samples. Except the samples of HD patients (see above the paragraph Description of study population), we examined also samples of 68 healthy blood donors from Transfusion department of Charles University Hospital in Pilsen (46 men, 22 women; mean age 37.9 ± 11.6 years). We determined own reference values from their free-CAR concentrations as mean ± 2 SD (standard deviation) with respect to a normal data distribution; this was 40.1 ± 17.8 μmol/l. Mean bias was acquired by measurement of four external quality control samples (at different CAR concentrations) from ERNDIM; it was 5.1 %. Sensitivity of our method was calculated as a limit of quantification (the value of mean absorbance of 10 blank samples + 10 x SD) at 2.6 μmol/l. Intra-assay coefficient of variation was 2.4 %, inter-assay coefficient of variation was 8.3 %. Analytical recovery was determined using standard addition of synthetic CAR (25, 50 and 100 μM) from Leopold Pharma (Graz, Austria) to a sample of known CAR concentration (58.5 μmol/l). Each sample was measured three times and the mean ± SD and recovery (%) were calculated. The analytical recovery was 101.8 %, 99.5 % and 95.4 % recovery was determined to be 101.8, 99.5 and 95.4 % [Cibulka *et al.*, 2006].

The advantages of the automated method in comparison with the original manual method are:

1. The possibility to work with much smaller amounts of samples, reagents and diluents in the automatic analyzer. The total volume of the reagent mixture is reduced from 2.205 ml to 0.175 ml.
2. The total analysis time is much shorter. CAR determination in one series of 100 samples only took approximately 1 h. Similar determination using the manual technique on a photometer with one cuvette would take approximately 10 h.
3. The price of analysis is mainly dictated by the amounts of reagents consumed. As the automated technique uses volumes approximately 10-fold lower than for the manual technique, the price should also be approximately 10-fold lower. Under our conditions these costs are € 13/sample for manual processing, in contrast to € 1.3/sample for automated processing.

Another advantage of our method is that it shows good sensitivity for measurement of low CAR concentrations, which can occur in patients immediately after a HD session.

3.3.2. Determination of red blood cell count

Red blood cell count was examined on a Sysmex 9000 R (Shanghai, Japan) analyzer.

3.3.3. Determination of parameters of lipid metabolism

Determination of parameters of lipid metabolism (total cholesterol, HDL- and LDL-cholesterol, triglycerides) was performed using routine enzymatic photometric methods on the Olympus AU 400 analyzer. Total cholesterol and triglycerides (TG) were detected with kits from Dialab (Vienna, Austria), HDL-cholesterol by a direct method with kits from Human Diagnostics (Wiesbaden, Germany); LDL-cholesterol concentration was estimated by Friedewald formula. Lipoprotein(a) was assessed by an immunoturbidimetric method from Dako (Glostrup, Denmark).

3.3.4. Determination of parameters of nutrition and inflammation

Concentration of albumin was detected photometrically with a kit ALBUMIN liquicolor from Human Diagnostics (Wiesbaden, Germany). Concentrations of C-reactive protein (CRP), transferin and prealbumin were assessed immunoturbidimetrically; CRP with a kit from K-Assay (Kamiya Biomedical Comp., Seattle, WA, USA), transferin and prealbumin with kits from Roche (Mannheim, Germany) on the Olympus AU 400 analyzer.

3.3.5. Determination of parameters of oxidative stress and antioxidative defence

Antioxidative enzymes superoxiddismutase (SOD) and glutathionperoxidase (GPx) were detected using kits from Randox (Crumlin, UK), reduced glutathione (GSH) in erythrocytes was assessed photometrically with a kit from Oxis (Portland, USA), malondialdehyde (MDA) was also tested photometrically as thiobarbituric acid reactive substances (TBARS). MDA and other degradation products of peroxidation react with two molecules of thiobarbituric acid, a colour product occurs and is extracted with n-butanol. This product is subsequently measured using AUTO-EIA II microplate reader from Labsystems Oy (Espoo, Finland). Oxidized LDL particles (oxLDL) were determined using ELISA method from Mercodia (Uppsala, Sweden). Advanced glycation end-products (AGEs) were assessed fluorimetrically and are expressed in arbitrary (fluorescence) units (FU) and as fluorescence per gram of protein (FU/g). Advanced oxidation protein products (AOPP) were measured spectrophotometrically according to Witko-Sarsat [Witko-Sarsat *et al.*, 1996] and are expressed in chloramine units (mmol/l) [Kalousová *et al.*, 2002].

3.3.6. Determination of parameters of calcium-phosphate and bone metabolism

Concentrations of Ca and P were detected by enzymatic photometric tests from Olympus on the Olympus AU 400 analyzer, intact parathormone (PTH) by a luminoimmunoassay on a Liason automated immunoanalyzer from DiaSorin (Saluggia, Italy) and bone-specific alkaline phosphatase (b-ALP) by an immunochemical chemiluminescent method from Beckman Coulter (Fullerton, USA) on an Access 2 (Fullerton, USA) analyzer. Osteocalcin (OC) was assessed by an immunoradiometric assay from Cis Bio International (Lyon, France) and osteoprotegerin (OPG) by an enzyme immunoassay from Biomedica (Vienna, Austria).

4. RESULTS

4.1. Changes in L-carnitine concentrations

The average concentration of free-CAR (shortcut CAR will be used in the following text) in healthy blood donors was 40.1 ± 8.9 μmol/l (43.4 ± 8.1 μmol/l in men and 33.1 ± 5.6 μmol/l in women). The average CAR concentration in HD patients before HD session was 30.5 ± 10.3 μmol/l ($p < 0.001$ for the difference between healthy persons and HD patients before HD) and 9.4 ± 4.6 μmol/l after it ($p < 0.001$ for the difference of values before HD and after HD). A difference between HD men and women was not statistically significant.

Both groups of HD patients had comparable serum CAR concentrations at the beginning of the study. The CAR concentration increased rapidly after 3 resp. 6 months of CAR supplementation (from 29.5 ± 8.7 μmol/l to 135.3 ± 39.9 μmol/l resp. 111.7 ± 35.7 μmol/l; $p < 0,001$), while the control group had a tendency to decrease (from 30.4 ± 11.5 μmol/l to 30.2 ± 12.5 μmol/l resp. 25.7 ± 10.0 μmol/l), see *Table 1.* The difference between the supplemented group and the placebo group was confirmed also on the basis on the logistic regression analysis.

The serum CAR concentration was observed in supplemented HD patients still two months after the supplementation had ended (i.e., 7th and 8th month after the study had begun). The CAR level leveled off at mean concentration about 65 μmol/l (64.6 ± 20.7 μmol/l resp. 65.0 ± 16.6 μmol/l; $p < 0,01$ vs. levels before the supplementation), see *Tab. 1.*

Table 1. *Comparison of serum free-CAR concentrations between the group of HD patients supplemented with L-carnitine (CAR) and the placebo group of HD patients without CAR supplementation (PL). Levels are expressed as mean ± standard deviation in μmol/l before HD session and after it.*

Group	Month 0		Month 3		Month 6		Month 7	Month 8
	before HD	after HD	before HD	after HD	before HD	after HD	before HD	before HD
CAR	**29.5** ± 8.7	**9.2** ± 4.4	**135.3** ± 39.9	**39.4** ± 12.5	**111.7** ± 35.7	**37.6** ± 12.6	**64.6** ± 20.7	**65.0** ± 16.6
PL	**30.4** ± 11.5	**9.7** ± 5.0	**30.2** ± 12.5	**10.8** ± 5.8	**25.7** ± 10.0	**11.1** ± 3.8	–	–

Abbreviations: CAR – carnitine, HD – hemodialysis, PL – placebo

According to the reference values for CAR in serum, which are stated in a book *Clinical Laboratory Diagnostics* from Thomas et al. [Thomas, 1998], 26.5 % of our HD patients had decreased levels before HD at the beginning of the study, while 73.5 % of our patients had their levels in the reference ranges. In comparison with our control group of blood donors, approximately 40 % of our patients had their CAR levels comparable with this control group, while approximately 60 % of them had their CAR concentration significantly lower. All HD patients had significantly decreased levels of CAR after HD session (approx. to one third of predialysis values), which corresponds with the findings of Goral [Goral, 2001].

The CAR concentration increased so markedly in the supplemented group that their CAR levels exceeded the lower reference limit even after HD session. It is interesting that the effect of CAR supplementation was long-lasted, higher levels of CAR remained two months after the supplementation had ended.

4.2. Changes of red blood cell count and doses of erythropoietin

We judged in this block an impact of CAR supplementation on hemoglobin (Hb) concentration and weakly doses of recombinant human erythropoietin (rHuEPO). Statistical description of these parameters is shown in *Table 2.* With respect to non-normally data distribution weakly doses of rHuEPO are expressed as median values.

Table 2. *Levels of hemoglobin and weakly doses of erythropoietin in the course of supplementation with L-carnitine in the supplemented group (CAR) and in the control group taking placebo (PL). Levels of hemoglobin are shown as mean ± standard deviation, weakly doses of erythropoietin are expressed as median values.*

Parameter	Group	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Hb (g/l)	CAR	**113.32** ± 18.33	**113.24** ± 17.03	**114.22** ± 16.62	**116.02** ± 16.88	**112.70** ± 16.43	**113.68** ± 16.45	**114.84** ± 17.02
	PL	**115.15** ± 17.49	**113.61** ± 18.12	**116.41** ± 17.38	**120.20** ± 15.69	**113.12** ± 13.41	**118.80** ± 15.93	**117.05** ± 17.26
Dose of rHuEPO (U/week)	CAR	**3000**	**4000**	**4000**	**3500**	**3500**	**4500**	**4500**
	PL	**2000**	**4000**	**4000**	**4500**	**4500**	**5000**	**4500**

Abbreviations: CAR – carnitine, Hb – hemoglobin, PL – placebo, rHuEPO – recombinant human erythropoietin, U – unit

We did not prove neither any significant changes in the Hb concentration nor changes in the rHuEPO doses in the given groups. Not any trends were observed between the patients supplemented with CAR and the controls taking placebo.

4.3. Changes of parameters of lipid metabolism

Statistical description of observed parameters of lipid metabolism is summarized in *Table 3.*

Table 3. *Parameters of lipid metabolism in the course of supplementation with L-carnitine in the supplemented group (CAR) and in the control group taking placebo (PL). Values are shown as mean ± standard deviation.*

Parameter	Group	Month 0	Month 3	Month 6
Total cholesterol (mmol/l)	CAR	**4.61** ± 1.05	**4.66** ± 1.02	**4.62** ± 1.29
	PL	**4.57** ± 1.10	**4.47** ± 0.98	**4.43** ± 0.95
HDL-cholesterol (mmol/l)	CAR	**1.04** ± 0.31	**1.04** ± 0.32	**1.06** ± 0.37
	PL	**1.13** ± 0.31	**1.17** ± 0.37	**1.14** ± 0.36
LDL-cholesterol (mmol/l)	CAR	**2.68** ± 0.82	**2.74** ± 0.76	**2.69** ± 0.77
	PL	**2.65** ± 0.93	**2.60** ± 0.77	**2.56** ± 0.83
Triglycerides (mmol/l)	CAR	**2.43** ± 1.18	**2.38** ± 1.76	**2.32** ± 1.04
	PL	**1.89** ± 0.93	**2.22** ± 1.19	**1.99** ± 0.95
Lipoprotein(a) (g/l)	CAR	**0.38** ± 0.54	**0.47** ± 0.69	**0.46** ± 0.69
	PL	**0.48** ± 0.59	**0.52** ± 0.70	**0.54** ± 0.66

Abbreviations: CAR – carnitine, PL – placebo

In respect the fact that CAR has an indispensable role for the appropriate function of intramitochondrial beta-oxidation of fatty acids, we supposed a correction mainly in the serum TG concentration. It was really a decreasing tendency in the supplemented group in contrast to an increasing tendency in the control group. However, these changes were not statistically significant.

Changes of other parameters of lipid metabolism, i.e., total cholesterol, HDL- and LDL-cholesterol and lipoprotein(a) did not show to be statistically significant in the given groups.

4.4. Changes of parameters of nutrition and inflammation

Statistical description of observed parameters of nutrition and inflammation is summarized in *Table 4.* With respect to non-normally data distribution the CRP concentrations are expressed as median values (minimum–maximum).

Table 4. *Parameters of nutrition and inflammation status in the course of supplementation with L-carnitine in the supplemented group (CAR) and in the control group taking placebo (PL). Levels of albumin, prealbumin and transferin are shown as mean ± standard deviation, levels of C-reactive protein are expressed as median values (minimum–maximum).*

Parameter	Group	Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Albumin (g/l)	CAR	**39.05** ± 3.87	**38.26** ± 3.33	**38.41** ± 2.87	**38.98** ± 2.75	**38.72** ± 3.03	**39.10** ± 3.32	**41.32** ± 5.68
	PL	**38.69** ± 3.20	**38.10** ± 3.37	**38.85** ± 3.45	**39.29** ± 3.03	**39.15** ± 3.03	**39.21** ± 2.75	**40.53** ± 3.50
Prealbumin (g/l)	CAR	**0.31** ± 0.10	–	–	**0.35** ± 0.10	–	–	**0.27** ± 0.09
	PL	**0.30** ± 0.10	–	–	**0.33** ± 0.12	–	–	**0.28** ± 0.11
Transferin (g/l)	CAR	**1.72** ± 0.44	–	–	**1.75** ± 0.40	–	–	**1.45** ± 0.48
	PL	**1.68** ± 0.32	–	–	**1.69** ± 0.28	–	–	**1.43** ± 0.45
CRP (mg/l)	CAR	**4.0** (1.0–75.0)	–	–	–	–	–	**5.0** (1.0–160.0)
	PL	**4.0** (1.0–50.0)	–	–	–	–	–	**5.0** (1.0–186.0)

Abbreviations: CAR – carnitine, CRP – C-reactive protein, PL – placebo

We did not prove any significant changes of measured parameters in the given groups. Not any trends were observed between the patients supplemented with CAR and the controls taking placebo.

4.5. Changes of parameters of oxidative stress and antioxidative defence

Levels of observed parameters of oxidative stress and antioxidative defence are summarized in *Table 5.* Results are expressed as median values (minimum–maximum) because of non-normally distribution of most data.

Table 5. *Levels of parameters of oxidative stress and antioxidative defence in the course of supplementation with L-carnitine in the supplemented group (CAR) and in the control group taking placebo (PL). Results are shown as median values (minimum–maximum) because of non-normally distribution of most data. P expresses a statistic significance of the change (month 6 – month 0); *p<0.05, **p<0.01, ***p<0.001, NS – statistically not significant change.*

Parameter	Group	Month 0	Month 6	*P*
SOD (U/g)	KAR	**977.5** (489.0–1601.0)	**1081.0** (743.0–1477.0)	*
	PL	**874.0** (488.0–1582.0)	**1077.0** (688.0–1582.0)	*
GPx (U/g)	KAR	**51.2** (24.2–82.4)	**50.8** (21.4–105.7)	NS
	PL	**54.2** (27.2–75.7)	**54.6** (28.3–80.2)	NS
GSH (mmol/l)	KAR	**2.03** (1.35–82.40)	**1.85** (0.93–2.67)	*
	PL	**2.06** (1.41–2.85)	**1.87** (0.84–3.06)	*
MDA (TBARS) (µmol/l)	KAR	**2.15** (1.12–5.58)	**1.89** (1.37–8.21)	*
	PL	**2.14** (1.49–4.43)	**2.01** (1.18–3.59)	NS
OxLDL (IU/l)	KAR	**157.0** (64.0–299.1)	**120.6** (53.0–359.0)	***
	PL	**144.3** (43.0–292.0)	**98.0** (55.0–259.0)	*
AGE (RLU/g bílkoviny séra)	KAR	**25.7** (14.1–44.6)	**28.5** (17.3–43.5)	***
	PL	**25.9** (15.7–39.4)	**27.3** (13.9–44.9)	***
AOPP (µmol/l)	KAR	**101.5** (8.0–246.0)	**94.0** (51.0–253.0)	NS
	PL	**83.0** (52.0–181.0)	**85.0** (46.0–161.0)	NS

Abbreviations: AGE – advanced glycation end-products, AOPP – advanced oxidation protein products, CAR – carnitine, GSH – reduced glutathione, GPx – glutathionperoxidase, MDA – malondialdehyde, oxLDL – oxidized LDL particles, PL – placebo, SOD – superoxiddismutase, TBARS – thiobarbituric acid-reactive substances

Initial values of measured parameters did not differ significantly between given groups of HD patients. Although some changes occurred in the course of a half year supplementation period, it was mostly in both groups together. Our groups also did not differ neither in the dynamics of antioxidative enzymes SOD and GPx nor in levels of GSH in erythrocytes, AGE

and AOPP. The MDA (resp. TBARS) concentration decreased significantly in supplemented patients ($p < 0.05$), while a drop was only insignificant in controls. The oxLDL concentration decreased significantly in both groups, but the change was more significant in the supplemented group ($p < 0.001$) in comparison with the control group ($p < 0,05$).

4.6. Changes of parameters of calcium-phosphate and bone metabolism

Levels of observed parameters of Ca-P metabolism and bone turnover in HD patients supplemented with CAR in contrast to controls taking placebo are summarized in *Table 6.* Results are expressed as median values (minimum–maximum) because of non-normally distribution of most data.

The multivariate stepwise logistic regression analysis showed that serum CAR concentration was only the variable which was different between both groups of HD patients. Both groups did not differ in concentrations of Ca, P and Ca-P product. Levels of these parameters oscillated and therefore it was not possible to notice any trend. In case of PTH, the median concentration had a decreasing tendency in the supplemented group in contrast to an increasing tendency in the control group. However, these changes were not statistically significant because of a very wide dispersion of PTH concentrations among all patients. We did not find any differences in dynamics of b-ALP between both groups. We noted a significant decrease after the supplementation period, but it occurred in both groups simultaneously, so that we cannot attribute this effect to CAR supplementation. The change in the concentration of OC can be characterized as a decreasing tendency of bone turnover in the supplemented patients, although it was only an insignificant change. A significant increase was, on the contrary, noted in the controls ($p < 0.05$).

The normal range for OPG in serum is 18 – 42 ng/l. Our patients had at the beginning of the study the average concentration 172.0 ± 91.4 (median = 142.0) ng/l. The OPG concentration increased significantly after CAR supplementation ($p < 0.05$), while an increase was only insignificant in the controls.

Table 6. *Levels of parameters of Ca-P metabolism and bone turnover in the course of supplementation with L-carnitine in the supplemented group (CAR) and in the control group taking placebo (PL). Results are shown as median values (minimum–maximum) because of non-normally distribution of most data. P expresses a statistic significance of the change (month 6 – month 0); *p<0.05, **p<0.01, ***p<0.001, NS – statistically not significant change.*

Parameter	Group	Month 0	Month 3	Month 6	*P*
Ca (mmol/l)	CAR	**2.30** (1.65–2.97)	**2.28** (1.52–2.78)	**2.32** (1.87–3.18)	NS
	PL	**2.35** (1.91–2.85)	**2.37** (2.03–3.12)	**2.36** (2.03–2.80)	NS
P (mmol/l)	CAR	**1.74** (0.71–3.71)	**1.86** (0.94–3.43)	**1.76** (0.57–3.30)	NS
	PL	**1.71** (1.02–3.50)	**2.11** (1.10–3.63)	**1.84** (0.69–4.30)	NS
Ca x P ($mmol^2/l^2$)	CAR	**4.08** (1.55–7.26)	**4.35** (2.14–8.12)	**4.22** (1.52–7.59)	NS
	PL	**4.14** (2.42–8.68)	**4.70** (2.35–8.86)	**4.15** (1.51–8.99)	NS
PTH (ng/l)	CAR	**186.0** (12.0–646.0)	–	**135.5** (11.0–627.0)	NS
	PL	**148.0** (10.0–1294.0)	–	**207.0** (10.0–685.0)	NS
b-ALP (µg/l)	CAR	**13.9** (4.8–80.3)	–	**13.2** (4.4–51.7)	*
	PL	**15.2** (5.8–47.8)	–	**13.2** (5.4–49.3)	*
OC (µg/l)	CAR	**78.3** (19.8–259.2)	–	**68.8** (6.5–381.9)	NS
	PL	**62.7** (16.6–409.1)	–	**79.8** (20.7–408.2)	*
OPG (ng/l)	CAR	**144.0** (56.0–388.0)	–	**182.0** (56.0–450.0)	*
	PL	**140.0** (48.0–448.0)	–	**164.0** (48.0–460.0)	NS

Abbreviations: b-ALP – bone-specific alkaline phosphatase, Ca – calcium, Ca x P – Ca-P product, CAR – carnitine, OC – osteocalcin, OPG – osteoprotegerin, P – inorganic phosphate, PL – placebo, PTH – parathormon

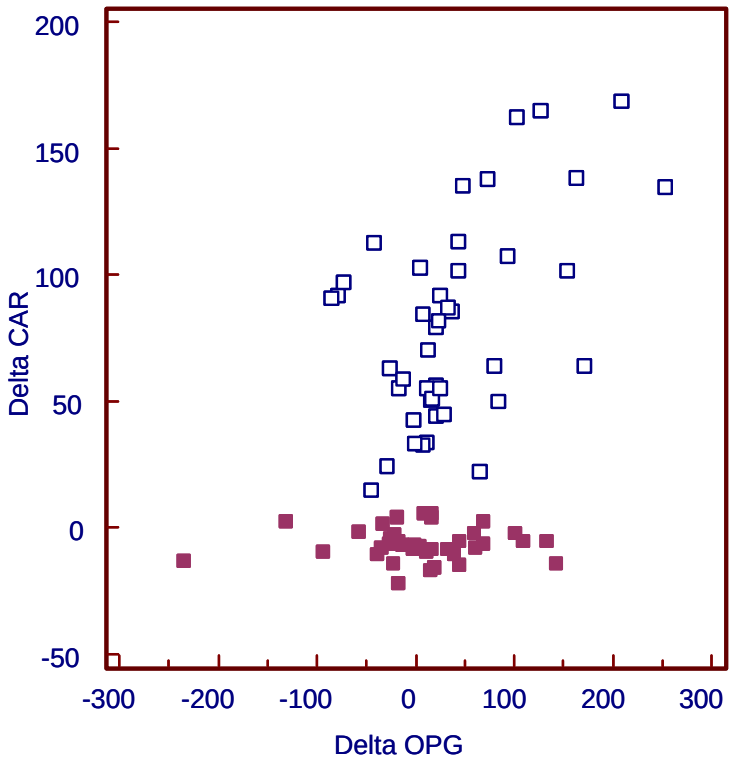
A significant correlation was found by the Spearman correlation analysis between changes of CAR (CAR before HD 6 – CAR before HD 0) and OPG (OPG 6 – OPG 0) levels in the supplemented group ($r = 0.51$, $p < 0.001$), while no correlation occurred in the controls ($r = 0.03$, $p = 0.88$). Stepwise regression analysis (including changes of CAR, PTH, Ca, P; age and dialysis treatment period as input independent variables and OPG change as a dependent variable) revealed the CAR change, PTH change and age as the most significant variables for the multiple regression analysis. It showed that the CAR change was the most significant factor influencing the OPG change in the supplemented group (adjusted $R^2 = 0.38$, $p < 0.001$). In the same regression model, no relation was observed between these variables in the control group without CAR supplementation. The results are shown in *Table 7* and *Figure 1*.

Table 7. *Multiple regression analysis of the OPG change as dependent variable and the CAR change, PTH change and age as independent variables. RC means regression coefficient, SE is standard error, P expresses statistical significance.*

Independent variable	*RC*	*SE*	*P*
CAR change	0.75	0.23	0.003
PTH change	0.20	0.08	0.010
Age	1.82	0.73	0.020
Adjusted R2		0.38	

Abbreviations: CAR – carnitine, OPG – osteoprotegerin, PTH – parathormone

Figure 1. *Correlation between changes of serum free-CAR and OPG levels in HD patients supplemented with L-carnitine (open squares) and controls taking placebo (full squares).*



Abbreviations: CAR – carnitine, HD – hemodialysis, OPG – osteoprotegerin

4.7. Changes of clinical status

The CAR administration was not accompanied by any adverse effects. None of the patients left the study because of any negative subjective symptoms. The patients who died in the course of the study originated equally from the supplemented group (7 patients) and the control group (9 patients). On the other hand, no difference was noticed in clinical status and incidence of complications of HD treatment between both groups.

5. DISCUSSION

Some authors describe that CAR administration can influence positively the nutritional status of HD patients. Argani et al. supplemented 500 mg CAR perorally for two months and observed an increase of albumin concentration from 37.0 ± 4.0 to 42.0 ± 5.0 g/l [Argani *et al.*, 2005]. Savica et al. administered CAR intravenously for six months in a dose of 20 mg/kg (n = 48) or placebo (n = 65) three times weekly et the end of each HD session. The supplemented patients had a significant increase in serum concentrations of albumin, transferin and hemoglobin along with a decrease of CRP concentration. An opposite trend of these parameters was noted in the control group. Authors explained these changes by an antiinflammatory effect of CAR accompanied by an improve of energy metabolism after its supplementation [Savica *et al.*, 2005].

No significant effect of CAR on nutritional parameters was observen in our study. If we look on these parameters before the supplementation, we can see that only a little percentage of patients had a true protein malnutrition. Concretely only 8.7 % of included patients had their prealbumin concentration lower than the lower reference limit (< 0.2 g/l); 10.9 % of patients had their albumin concentration lower than the lower reference limit (< 35.0 g/l). It seems that most of HD patients did not suffer from protein malnutrition at the beginning of the study and therefore an effect of CAR could not have manifest.

Some studies suggest that CAR may treat anemia by increasing erythrocyte survival time, thereby serving as an adjunct to EPO for HD patients [Boran *et al.,* 1996; Bérard & Iordache, 1992]. We confirmed these findings in our pilot study [Veselá *et al.*, 2001], but not in the current study. We cannot explain this discrepancy convincingly.

In respect of possible effects of CAR on lipid metabolism, we supposed mainly some influence of intramitochondrial beta-oxidation and subsequently correction of serum TG concentration. Simultaneously, we had a hypothesis that an increased removing of superfluous fatty acids from cytosol could lead to modulation of oxidative stress. The facts that HD patients often suffer from CAR deficiency as well as positive results of ou pilot study [Veselá *et al.*, 2001] were good background for validity of our hypothesis. Some authors even attribute to CAR direct antioxidative effects [Ames & Liu, 2004; Sener *et al.*, 2004].

The opposite trend in serum TG concentration between the supplemented and the placebo group along with the significant drop of MDA (resp. TBARS) concentration in supplemented patients in contrast to controls maintain our hypothesis, in spite of the fact that

other parameters of lipid metabolism, oxidative stress and antioxidative defence were not so striking as we had expected.

In case of Ca-P metabolism, we were also surprised at a relatively good state of our study population at the beginning of the trial. Levels of Ca, P, Ca-P product and PTH did not deviate from standard of recommended values issued by the National Kidney Foundation [National Kidney Foundation, 2003]. We supposed more severe hyperphosphatemia in HD patients in concordance with literary data [Giachelli *et al.*, 2005]. Our hypothesis was based on a potencial reduction of phosphatemia due to improved energy metabolism during CAR supplementation. The superfluous P should be used in a larger degree for phosphorylation reactions including ATP synthesis in the mitochondria, and therefore the concentration of P in serum should decrease. This hypothesis was also supported by results of other investigations [Ahmad *et al.,* 1990; Veselá *et al.*, 2001]. In our pilot study, we found a decreasing tendency of P concentrations from 2.10 ± 0.52 mmol/l to 1.98 ± 0.40 mmol/l, but these changes were not significant. The principal difference between these studies and our current study was that the studies mentioned above were performed on relatively seriously hyperphosphatemic patients. It could be one of the reasons why we cannot confirm our primary hypothesis.

Although we did not prove any significant changes in the concentrations of Ca and P, we did find a decreasing tendency of PTH in the supplemented group along with an increasing tendency in the control group. We cannot explain convincingly this finding; nevertheless it is possible to speculate on a direct effect of CAR on parathyroid glands. It has not been investigated yet, as there has been no published evidence up to now. The potential influence of CAR on PTH synthesis and secretion and, in this way, correction of secondary hyperparathyroidism should be more deeply researched.

As beta-oxidation of fatty acids yield an important deal of energy for bone cells [Adamek *et al.*, 1987], we focused on a potential effect of CAR supplementation on the metabolism of osteoblasts. We observed b-ALP and OC levels mainly as markers of bone turnover. In spite of its retention and accumulation in HD patients, OC is considered as a very good marker of bone turnover [Charhon *et al.,* 1986; Sebert *et al.*, 1987]. Normal values for OC determined in our laboratory were 6.0 – 31.0 μg/l in men and 8.0 – 36.0 μg/l in women. With regard to these values, 91.7 % of HD patients in our study had an increased OC concentration at the beginning of the study while only 8.3 % were in the reference ranges; the median concentration was 65.2 μg/l. B-ALP concentration was also near the upper limit of normal [Ureña & De Vernejoul, 1999]. Therefore we presumed a higher bone turnover in most of HD patients at the beginning of the study. The changes of b-ALP, OC and PTH in HD

patients which were supplemented with CAR can be described as a tendency to decrease of bone turnover and secondary hyperparathyroidism.

The identification of the OPG/RANKL/RANK system as the dominant, final mediator of osteoclastogenesis represents a major advance in bone biology [Khosla, 2001; Kostenuik, 2005]. RANKL (ligand for receptor activator of nuclear factor kappa B) expression is inducible on the osteoblast cell membrane, while its receptor (RANK) is found on cells of an osteoclastic lineage. Binding of RANKL to RANK promotes osteoclasts differentiation, activation and survival, thereby increasing the pool of mature osteoclasts available to undertake bone resorption. OPG (osteoprotegerin) is also an osteoblastic product, but is secreted rather than membrane bound. Once released, it acts as a decoy receptor for RANKL, preventing its interaction with RANK and slowing the recruitment of osteoclasts. In this way, bone remodeling becomes intimately related to changes in expression of OPG and RANKL [Khosla, 2001]. In HD patients, the bone mineral density was positively correlated with the OPG level in the study of Nakashima et al. [Nakashima *et al.*, 2006]. Authors concluded that OPG might be an independent factor that prevents bone loss in HD patients.

The normal range for OPG in serum is 18 – 42 ng/L. Circulating OPG levels have been found to be elevated in uremic patients in many other studies, but the factors regulating OPG levels in dialysis patients remain unclear. Avbersek-Luznik et al. say that it is a compensating production which protects bones against intensive bone loss during dialysis treatment [Avbersek-Luznik *et al.*, 2002].

Remarkable changes in the OPG concentration were registered in our study after 6 months of CAR supplementation. While the significant increase was noted in the supplemented group, virtually no change occurred in the control group. This effect could be explained via PTH action. As noted earlier, PTH had a decreasing tendency in the patients supplemented with CAR contrary to an increasing tendency in the controls. It was proved that PTH is a factor which decreases OPG/RANKL ratio. Lee and Lorenzo have described that PTH was increasing RANKL and decreasing OPG expression by osteoblasts [Lee & Lorenzo, 1999]. Another explanation could subsist on a direct effect of CAR on the metabolism of osteoblasts. This hypothesis shows to be more probable with respect to the results of Spearman correlation and multiple regression analysis mentioned above. Colucci et al. proved as well that CAR administration positively affect human osteoblast proliferation and differentiation in vitro [Colucci *et al.*, 2005], which also favours this hypothesis.

6. CONCLUSIONS

Because CAR is essential for appropriate functioning of metabolism and its deficit, which was repeatedly approved in HD patients, can participate in development of serious complications of HD treatment, many authors recommend its supplementation in these patients. In accordance with our results, a large part of HD patients has their CAR level comparable with healthy population. An effect of supplementation may be in persons with a normal serum CAR concentration questionable. On the other hand, we are aware of the fact that the serum CAR level doesn't need to inform exactly about intracellular stores. On the contrary, positive effects are expected in patients with proved lower CAR concentrations. To date, the so called “dialysis-related carnitine disorder” has been diagnosed only on the basis of clinical symptoms which cannot serve as exact and objective evidence of CAR deficiency. Therefore, we decided to develop an automated method for CAR determination using an Olympus AU 400 analyzer, which would be easy to use and inexpensive. This enzymatic photometric method could be a useful tool for a more accurate diagnosis of CAR deficiency and thereby to complete clinical symptoms. Main advantages of our method in comparison to other methods of CAR determination are smaller amounts of required samples, reagents and diluents and a shorter analysis time. Furthermore, this method showed good sensitivity for measurement of low CAR concentrations, which can occur in patients immediately after HD session.

In terms of our findings during the trial, we draw the following conclusions:

1. The concentration of CAR in serum of chronically HD patients before HD session is decreased approximately in 40 % of patients; approximately 60 % of HD patients have their predialysis CAR levels which are comparable with healthy persons.
2. A single HD session reduces serum CAR concentration to about one-third of its predialysis value.
3. The supplementation with CAR leads to a high increase of serum CAR concentration, so that the values of CAR after HD exceed the lower reference limit for healthy population.
4. The higher CAR concentration remains in the long term after the end of supplementation.
5. A decrease of TG concentration along with a significant decrease of MDA and oxLDL concentrations in patients supplemented with CAR reflects an improvement of fatty acid metabolism and reduction of lipid peroxidation. As HD patients have a higher degree of oxidative stress a lower intracellular antioxidative defence, this effect could be relatively important.

6. The supplementation with CAR suppresses the activation of osteoclasts due to an increasing production of OPG which acts as an important inhibitor of osteoclastogenesis. In connection with reduced bone turnover and PTH levels, it seems like a very positive metabolic effect because secondary hyperparathyroidism is considered to be one of serious complications of HD treatment.
7. Any improvements in parameters of nutrition and inflammation as well as an influence of red blood cell count weren't noted. We explain these findings by a relatively good metabolic state of our patients at the beginning of the study. Parameters of nutrition and also levels of hemoglobin were for the most part of them surprisingly in reference ranges for healthy population before the CAR supplementation had started.
8. The administration of CAR was not accompanied by any adverse effects. On the other hand, no difference was noticed in clinical status and incidence of complications of HD treatment between both groups.
9. In conclusion, the supplementation with CAR can influence some metabolic disorders which occur in connection with its deficit in HD patients. We suppose positive effects mainly in patients with decreased serum CAR levels.
10. To date, the diagnosis of CAR deficiency was based only on clinical symptoms, which cannot be fully objective. We offer an enzymatic photometric method of CAR determination which we adapted on the Olympus AU 400 analyzer. This method can be helpful for the diagnosis verification and for selection of appropriate patients for supplementation with CAR.

7. SUMMARY

Chronic kidney failure is associated with many kinds of metabolic disorders caused by the kidney disease and also attributable to dialysis treatment. Phenomena such as accumulation or deficit of various substances and dysregulation of metabolic pathways participate in the pathogenesis of these changes.

One of these disorders, which we studied in more detail, is a deficit of carnitine. Carnitine is a substance which plays an essential role in beta-oxidation of fatty acids by catalyzing their transport into the mitochondrial matrix. It enables obtaining of energy, namely in muscle cells including myocardium.

Patients with chronic kidney failure treated by hemodialysis are known to have decreased carnitine concentration in plasma and tissues due to its impaired synthesis in kidneys and the great loss across the hemodialysis membrane during dialysis sessions. A single hemodialysis session reduces plasma free-carnitine concentrations to about one-third of their predialysis values because of small molecule of free-carnitine. On the other hand, renal elimination of acylcarnitine, which is physiologically ineffective, may be impaired in chronic kidney failure, leading to increased blood concentrations of acylcarnitine. Although these subnormal free-carnitine levels with elevated acylcarnitine fractions may result in normal or elevated total plasma carnitine levels in dialysis patients, the functional carnitine pool in tissues is decreased.

Considerable evidence suggests that carnitine deficiency and abnormalities of carnitine metabolism result in a number of clinical conditions that are associated with dialysis, including muscle weakness, hypotension, fatigue, muscle cramps, poor exercise tolerance, anemia, left ventricular dysfunction, and higher incidence of arrhythmias. Recent studies have demonstrated that carnitine supplementation can restore the abnormal metabolism in dialysis patients and may alleviate some of the symptoms mentioned above.

The American National Kidney Foundation has recently approved “Practice Recommendations for the Use of L-carnitine in Dialysis-Related Carnitine Disorder”, which manifests itself as a syndrome of clinical problems and symptoms, most notable of which are anemia that is hyporesponsive to erythropoietin therapy, intradialytic hypotension, cardiomyopathy, and skeletal muscle dysfunction manifested as generalized fatigability.

The main goal of this project was to investigate possible effects of carnitine supplementation on metabolic parameters which could be positively influenced by the support of intramitochondrial beta-oxidation and energy metabolism. These are markers of nutrition, lipid metabolism, red blood cell count and, according to our previous findings, also parameters of oxidative stress and calcium-phosphate metabolism. The purpose is finding ways to prevent from the development of atherosclerosis and renal bone disease in chronically hemodialyzed patients.

The consequential goal was to adapt an enzymatic photometric method for free-carnitine determination and its automation for the Olympus AU 400 analyzer.

According to our results, a great deal of hemodialyzed patients has carnitine levels comparable with a healthy population. Hence, an effect of supplementation in these persons is questionable, although we realize that serum carnitine concentration may not accurately predicate of tissue saturation. Conversely, positive effects could be expected in patients with decreased carnitine levels.

Some authors describe that the supplementation with carnitine may positively influence nutritional-inflammation status of hemodialyzed patients. Changes should be happened due to anti-inflammatory effect of carnitine and general improvement of the energy metabolism after its administration. Furthermore, many papers describe positive effects of carnitine on the red blood cell count which manifest by reducing doses of erythropoietin in hemodialyzed patients treated for anemia. This influence should be enabled by increased stability of the erythrocyte membrane and thus reduced degree of hemolysis. In contrast to our pilot study, these effects were not proved in the current study. We cannot identify causes so exactly, but it seems that a health state of the majority of patients was fairly well-managed at the beginning of the supplementation period and therefore an effect of carnitine on this field of metabolism could not have enforced.

With respect to potential effects of carnitine on lipid metabolism and oxidative stress, we noted a decreasing tendency of serum triglycerides levels in supplemented patients in contrast to controls. Concentration of malondialdehyde, which is considered to be a marker of oxidative stress, decreased. Other parameters of lipide metabolism, oxidative stress and antioxidative defense did not significantly differ between both groups of patients.

Although we did not prove any significant changes in concentrations of calcium and inorganic phosphate, we did find a tendency to correction of secondary hyperparathyroidism

and reduction of bone turnover in the group of patients supplemented with carnitine along with an increasing tendency in controls. Concentration of osteoprotegerin increased significantly after six months of the supplementation, while levels of parathormone and osteocalcin had only a decreasing tendency which was not statistically significant. An opposite trend was noted in the control group without carnitine supplementation. As osteoprotegerin is an important factor which suppress activation of osteoclasts, its increased concentration might play a role in prevention of renal bone disease and osteoporosis in hemodialyzed patients.

In conclusion, the carnitine supplementation can influence some clinical and biochemical symptoms which occur in connection with its deficit in hemodialyzed patients. Concerning metabolis parameters, these are namely markers of nutrition and inflammation, lipid metabolism, red blood cell count and, according to our previous experience, also some parameters of oxidative stress and calcium-phosphate metabolism.

However, our results have shown that a great deal of hemodialyzed patients may have serum carnitine levels in reference ranges for healthy population. We can explain in this way, why observed metabolic changes were greater in our pilot study. At that time, from about 50 hemodialyzed patients were taken into the study only 12 patients with the lowest carnitine levels.

To date, the deficit of carnitine in hemodialyzed patients has been diagnosed only on the basis of clinical symptoms defined by the American Kidney Foundation. These symptoms are very important, but cannot serve as exact and objective evidence of carnitine deficiency. Therefore we developed the photometric method for free-carnitine determination which we modified and adapted for use on the Olympus AU 400 analyzer. This method may be helpful for selection of appropriate patients for carnitine supplementation.

8. REFERENCES

- Adamek G, Felix R, Guenther HL, Fleisch H: **Fatty acid oxidation in bone tissue and bone cells in culture. Characterization and hormonal influences.** *Biochem J* 1987, 248:129-137.
- Ahmad S, Robertson HT, Golper TA, Wolfson M, Kurtin P, Katz LA, Hirschberg R, Nicora R, Ashbrook DW, Kopple JD: **Multicenter trial of L-carnitine in maintenance hemodialysis patients. II. Clinical and biochemical effects.** *Kidney Int* 1990, 38:912-918.
- Ames BN, Liu J: **Delaying the mitochondrial decay of aging with acetylcarnitine.** *Ann N Y Acad Sci* 2004, 1033:108-116.
- Argani H, Rahbaninoubar M, Ghorbanihagjo A, Golmohammadi Z, Rashtchizadeh N: **Effect of L-carnitine on the serum lipoproteins and HDL-C subclasses in hemodialysis patients.** *Nephron Clin Pract* 2005, 101:c174-9.
- Avbersek-Luznik I, Malesic I, Rus I, Marc J: **Increased levels of osteoprotegerin in hemodialysis patients.** *Clin Chem Lab Med* 2002, 40:1019-1023.
- Bellinghieri G, Santoro D, Calvani M, Mallamace A, Savica V: **Carnitine and hemodialysis.** *Am J Kidney Dis* 2003, 41:S116-22.
- Bohmer T, Bergrem H, Eiklid K: **Carnitine deficiency induced during intermittent haemodialysis for renal failure.** *Lancet* 1978, 1:126-128.
- Boran M, Dalva I, Gönenç F, Cetin S: **Response to recombinant human erythropoietin (r-Hu EPO) and L-carnitine combination in patients with anemia of end-stage renal disease.** *Nephron* 1996, 73:314-315.
- Bérard E, Iordache A: **Effect of low doses of L-carnitine on the response to recombinant human erythropoietin in hemodialyzed children: about two cases.** *Nephron* 1992, 62:368-369.
- Charhon SA, Delmas PD, Malaval L, Chavassieux PM, Arlot M, Chapuy MC, Meunier PJ: **Serum bone Gla-protein in renal osteodystrophy: comparison with bone histomorphometry.** *J Clin Endocrinol Metab* 1986, 63:892-897.
- Cibulka R, Racek J, Veselá E: **Význam L-karnitinu u pacientů s chronickým renálním selháním léčených hemodialýzou.** *Vnitr Lek* 2005, 51:1108-1113.
- Cibulka R, Široká R, Trefil L, Racek J, Veselá E: **Measurement of carnitine in hemodialysis patients - adaptation of an enzymatic photometric method for an automatic analyzer.** *Clin Chem Lab Med* 2006, 44:983-986.

- Colucci S, Mori G, Vaira S, Brunetti G, Greco G, Mancini L, Simone GM, Sardelli F, Koverech A, Zallone A, Grano M: **L-carnitine and isovaleryl L-carnitine fumarate positively affect human osteoblast proliferation and differentiation in vitro.** *Calcif Tissue Int* 2005, 76:458-465.
- De Felice SL, Lyons P, Caffar M C: **U.S.-Italy L-carnitine hemodialysis utilization survey.** *Dial Transplant* 1996, 25:368-373.
- Durak I, Akyol O, Başeşme E, Canbolat O, Kavutçu M: **Reduced erythrocyte defense mechanisms against free radical toxicity in patients with chronic renal failure.** *Nephron* 1994, 66:76-80.
- Eknoyan G, Latos L, Lindberg J: **Practice recommendations for the use of L-carnitine in dialysis-related carnitine disorder.** *Am J Kidney Dis* 2003, 41:868-876.
- Giachelli CM, Speer MY, Li X, Rajachar RM, Yang H: **Regulation of vascular calcification: roles of phosphate and osteopontin.** *Circ Res* 2005, 96:717-722.
- Golper TA, Ahmad S: **L-carnitine administration to hemodialysis patients: Has its time come?** *Semin Dial* 1992, 5:94-98.
- Golper TA, Goral S, Becker BN, Langman CB: **L-carnitine treatment of anemia.** *Am J Kidney Dis* 2003, 41:S27-34.
- Goral S: **Levocarnitine's role in the treatment of patients with end-stage renal disease: a review.** *Dial Transplant* 2001, 30:530-538.
- Kalousová M, Zima T, Tesař V, Škrha J, Štípek S: **Stanovení produktů pokročilé glykace a oxidace.** *Klin Biochem Metab* 2002, 10(31):11-16.
- Khosla S: **Minireview: the OPG/RANKL/RANK system.** *Endocrinology* 2001, 142:5050-5055.
- Kostenuik PJ: **Osteoprotegerin and RANKL regulate bone resorption, density, geometry and strength.** *Curr Opin Pharmacol* 2005, 5:618-625.
- Lee SK, Lorenzo JA: **Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation.** *Endocrinology* 1999, 140:3552-3561.
- Levey AS, Eckardt K, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G: **Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO).** *Kidney Int* 2005, 67:2089-2100.

- Lindner A, Charra B, Sherrard DJ, Scribner BH: **Accelerated atherosclerosis in prolonged maintenance hemodialysis.** *N Engl J Med* 1974, 290:697-701.
- Nakashima A, Yorioka N, Doi S, Takasugi N, Shigemoto K, Kohno N: **Osteoprotegerin and bone mineral density in hemodialysis patients.** *Osteoporos Int* 2006, 17:841-846.
- National Kidney Foundation: **K/DOQI clinical practice guidelines. Bone metabolism and disease in chronic kidney disease.** *Am J Kidney Dis* 2003, 42(Suppl 3):S1-S201.
- Roche Diagnostics GmbH: Roche Applied Science. http://www.roche-applied-science.com/pack-insert/11242008001a.pdf.
- Savica V, Santoro D, Mazzaglia G, Ciolino F, Monardo P, Calvani M, Bellinghieri G, Kopple JD: **L-carnitine infusions may suppress serum C-reactive protein and improve nutritional status in maintenance hemodialysis patients.** *J Ren Nutr* 2005, 15:225-230.
- Sebert JL, Ruiz JC, Fournier A, Fardellone P, Guéris J, Marié A, Morinière P, Codet MP, Renaud H: **Plasma bone Gla-protein: assessment of its clinical value as an index of bone formation in hemodialyzed patients.** *Bone Miner* 1987, 2:21-27.
- Sener G, Paskaloğlu K, Satiroglu H, Alican I, Kaçmaz A, Sakarcan A: **L-carnitine ameliorates oxidative damage due to chronic renal failure in rats.** *J Cardiovasc Pharmacol* 2004, 43:698-705.
- Silver J: **Molecular mechanisms of secondary hyperparathyroidism.** *Nephrol Dial Transplant* 2000, 15(Suppl 5):2-7.
- Snyder S, Pendergraph B: **Detection and evaluation of chronic kidney disease.** *Am Fam Physician* 2005, 72:1723-1732.
- Thomas L: **Clinical laboratory diagnostics.** TH-Books Verlagsgesellschaft mbH, Frankfurt, Germany 1998, :203.
- Ureňa P, De Vernejoul MC: **Circulating biochemical markers of bone remodeling in uremic patients.** *Kidney Int* 1999, 55:2141-2156.
- Veselá E, Racek J, Trefil L, Jankových V, Pojer M: **Effect of L-carnitine supplementation in hemodialysis patients.** *Nephron* 2001, 88:218-223.
- Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P, Descamps-Latscha B: **Advanced oxidation protein products as a novel marker of oxidative stress in uremia.** *Kidney Int* 1996, 49:1304-1313.

9. PUBLICATIONS OF THE AUTHOR

a) Original articles

- Široká R., Trefil L., Rajdl D., Racek J., Rusňáková H., Cibulka R., Eiselt J., Filipovský J.: **Asymmetric dimethylarginine, homocysteine and renal function - is there a relation?** *Clin Chem Lab Med* 2005, Vol. 43, No. 10: 1147-1150 (IF = 1,918).
- Cibulka R.: **Metabolické účinky karnitinu a jeho význam v medicíně.** *Klin Biochem Metab* 2005, Vol. 13 (34), No. 1: 24-28.
- Cibulka R., Racek J., Trefil L., Veselá E., Studenovská M., Rajdl D.: **Deficit L-karnitinu u hemodialyzovaných nemocných a význam jeho suplementace.** *Klin Biochem Metab* 2005, Vol. 13 (34), No. 1: 29-31.
- Cibulka R., Racek J., Veselá E.: **Význam L-karnitinu u pacientů s chronickým renálním selháním léčených hemodialýzou.** *Vnitr Lek* 2005, Vol. 51, No. 10: 1108-1113.
- Cibulka R., Racek J., Trefil L., Pikner R., Veselá E., Studenovská M.: **Suplementace L-karnitinem snižuje oxidační stres u hemodialyzovaných nemocných.** *Klin Biochem Metab* 2005, Vol. 13 (34), No. 4: 187-189.
- Cibulka R., Široká R., Trefil L., Racek J., Veselá E.: **The measurement of carnitine in hemodialysis patients - adaptation of enzymatic photometric method for an automatic analyzer.** *Clin Chem Lab Med* 2006, Vol. 44, No. 8: 983-986 (IF = 1,918).
- Široká R., Cibulka R., Rajdl D., Racek J.: **Asymetrický dimetylarginin – nový rizikový faktor kardiovaskulárních onemocnění.** *Vnitr Lek* 2006, Vol. 52, No. 3: 249-255.
- Široká R., Trefil L., Racek J., Cibulka R.: **Comparison of asymmetric dimethylarginine detection – HPLC and ELISA methods (technical brief).** *Klin Biochem Metab* 2006, Vol. 14 (35), No. 2: 111-113.
- Široká R., Trefil L., Rajdl D., Racek J., Cibulka R.: **Asymmetric dimethylarginine–comparison of HPLC and ELISA methods.** *J Chromatogr B Analyt Technol Biomed Life Sci* 2007, Vol. 850, 586-587 (IF = 2,391).

- Cibulka R., Široká R., Rajdl D., Racek J., Trefil L., Eiselt J.: **Asymmetric dimethylarginine (ADMA) as a novel independent risk factor for cardiovascular disease in haemodialysis patients.** *Klin Biochem Metab* 2007, Vol. 15 (36), No. 1: 39-42.
- Cibulka R., Rajdl D., Široká R., Eiselt J., Malánová L., Trefil L., Racek J.: **Asymetrický dimethylarginin (ADMA) jako nový nezávislý faktor přežití u hemodialyzovaných nemocných.** *Klin Biochem Metab* 2007, Vol. 15 (36), No. 3; přijato k publikaci.
- Cibulka R., Racek J.: **Metabolic disorders in patients with chronic kidney failure.** *Physiol Res* 2007; Epub ahead of print (IF = 1,806).
- Cibulka R., Racek J., Pikner R., Rajdl D., Trefil L., Veselá E., Studenovská M., Široká R.: **Effect of L-carnitine supplementation on secondary hyperparathyroidism and bone metabolism in hemodialyzed patients.** *Calcif Tissue Int* 2007; Epub ahead of print (IF = 2,487).

b) Abstracts

- Racek J., Veselá E., Cibulka R., Trefil L., Malánová L.: **Influence of supplementation with L-carnitine on parathyroid function in hemodialysis (HD) patients.** *Clin Chem* 2004, Vol. 50, No. 6, Suppl.: A101.
- Cibulka R., Racek J., Trefil L., Veselá E., Studenovská M., Rajdl D.: **Deficit of L-carnitine in hemodialysis patients and a potential role of its supplementation.** *Clin Chem Lab Med* 2004, Vol. 42, No. 9: A72-A73.
- Racek J., Cibulka R., Veselá E., Trefil L.: **L-carnitine supplementation decreases oxidative stress in haemodialysis patiens.** *Clin Chim Acta* 2005, Vol. 355, Suppl.: S194.
- Racek J., Cibulka R., Veselá E., Trefil L., Rajdl D., Studenovská M.: **L-carnitine concentrations in hemodialysis patients and a potential role of its supplementation.** *Clin Chem* 2005, Vol. 51, No. 6, Suppl.: A192-A193.
- Cibulka R., Široká R., Rajdl D., Racek J.: **Asymmetric dimethylarginine as a novel independent risk factor for cardiovascular disease in hemodialysis patients.** *Atherosclerosis Suppl* 2006, Vol. 7, No. 3: 298.

- Široká R., Cibulka R., Rajdl D., Racek J., Trefil L.: **Asymmetric dimethylarginine – comparison of chromatography and immunometric methods.** *Atherosclerosis Suppl* 2006, Vol. 7, No. 3: 590.
- Veselá E., Cibulka R., Malánová L., Racek J., Pikner R., Rajdl D., Studenovská M., Trefil L.: **Suplementace L-karnitinem zlepšuje metabolismus osteoblastů u hemodialyzovaných nemocných.** *Aktuality v nefrologii* 2006, Vol. 12, Suppl. 1: 18.
- Cibulka R., Rajdl D., Siroka R., Eiselt J., Malanova L., Trefil L., Racek J.: **Asymmetric dimethylarginine as a novel prognostic factor for survival in hemodialysis patients.** *Clin Chem Lab Med* 2007, Vol. 45, Spec. Suppl.: T048.
- Racek J., Eiselt J., Malánová L., Rajdl D., Široká R., Cibulka R., Trefil L.: **Asymmetric dimethylarginine as a novel prognostic factor for survival in hemodialysis patients.** *Clin Chem* 2007, Vol. 53, No. 6, Suppl.: A31.

c) Lectures and poster presentations

- Racek J., Veselá E., Cibulka R., Trefil L., Malanová L.: **Influence of supplementation with L-carnitine on parathyroid function in hemodialysis patients.** *56th Annual Meeting of the American Association of Clinical Chemistry,* 25.-29.7. 2004, Los Angeles, USA.
- Cibulka R., Racek J.: **L-karnitin a jeho význam ve zdraví a nemoci.** *Vitamíny*, 13.-15.9. 2004, Pardubice, Czech Republic.
- Cibulka R., Racek J., Trefil L., Veselá E., Studenovská M., Rajdl D.: **Deficit of L-carnitine in hemodialysis patients and a potential role of its supplementation.** *Swiss MedLab & 8th Alps Adria Congress,* 5.-9.10. 2004*,* Lucerne, Switzerland.
- Cibulka R., Racek J.: **L-karnitin a jeho význam ve zdraví a nemoci.** *Mezikrajský seminář pracovníků klinické biochemie a hematologie Plzeňského a Karlovarského kraje,* 31.3. 2005, Karlovy Vary, Czech Republic.
- Racek J., Cibulka R., Vesela E., Trefil L.: **L-carnitine supplementation decreases oxidative stress in haemodialysis patiens.** *16th IFCC – FESCC European Congress of Clinical Biochemistry and Laboratory Medicine – EUROMEDLAB,* 8.-12.5. 2005, Glasgow, Scotland.

- Cibulka R., Trefil L., Veselá E., Racek J.: **Metabolické účinky karnitinu u hemodialyzovaných nemocných s důrazem na ovlivnění oxidačního stresu.** *45. studentská vědecká konference,* 19.5. 2005, Plzeň, Czech Republic.
- Racek J., Cibulka R., Veselá E., Trefil L., Rajdl D., Studenovská M.: **L-carnitine concentrations in hemodialysis patients and a potential role of its supplementation.** *XIX International Congress of Clinical Chemistry IFCC/AACC Annual Meeting,* 24.-28.7. 2005, Orlando, USA.
- Cibulka R., Veselá E., Studenovská M., Racek J.: **Výživa při chronickém selhání ledvin: suplementace L-karnitinu u hemodialyzovaných nemocných.** *Pokroky ve farmakoterapii 2005 pro lékaře v praxi,* 7.-9.9. 2005, Zlín, Czech Republic.
- Cibulka R., Racek J., Trefil L., Veselá E.: **Suplementace L-karnitinem snižuje oxidační stres u hemodialyzovaných nemocných.** *VII. celostátní sjezd České společnosti klinické biochemie,* 11.-13.9. 2005, Olomouc, Czech Republic.
- Cibulka R., Racek J., Trefil L., Veselá E.: **Suplementace L-karnitinem snižuje oxidační stres u hemodialyzovaných nemocných.** *Atherosklerosa,* 14.-16.9. 2005, Praha, Czech Republic.
- Cibulka R., Pikner R., Trefil L., Veselá E., Racek J.: **Suplementace L-karnitinem pozitivně ovlivňuje metabolismus osteoblastů u hemodialyzovaných nemocných.** *46. studentská vědecká konference,* 11.5. 2006, Plzeň, Czech Republic.
- Rajdl D., Cibulka R., Široká R., Racek J., Trefil L., Eiselt J., Rubášová L., Schejbalová M., Michálková R.: **Asymetrický dimethylarginin u hemodialyzovaných nemocných.** *Konferencia učitel'ov chémie, biochémie a klinickej biochémie na lekárskych fakultách v ČR a SR,* 25.-28.5.2006*,* Vrútky, Slovakia.
- Cibulka R., Široká R., Rajdl D., Racek J.: **Asymmetric dimethylarginine as a novel independent risk factor for cardiovascular disease in hemodialysis patients.** *XIV International Symposium on Atherosclerosis,* 18.-22.6. 2006, Roma, Italy.
- Široká R., Cibulka R., Rajdl D., Racek J., Trefil L.: **Asymmetric dimethylarginine – comparison of chromatography and immunometric methods.** *XIV International Symposium on Atherosclerosis,* 18.-22.6. 2006, Roma, Italy.
- Veselá E., Cibulka R., Malánová L., Racek J., Pikner R., Rajdl D.: **Effect of L-carnitine supplementation on bone metabolism in haemodialysis patients.** *XLIII ERA-EDTA congress,* 15.-18.7. 2006, Glasgow, Scotland.
- Veselá E., Široká R., Eiselt J., Malánová L., Racek J., Trefil L., Rajdl D., Cibulka R.: **Asymmetric dimethylarginine as a novel independent risk factor for**

cardiovascular disease in haemodialysis patients. *XLIII ERA-EDTA congress,* 15.-18.7. 2006, Glasgow, Scotland.

- Cibulka R., Široká R., Rajdl D., Racek J.: **Asymmetric dimethylarginine (ADMA) as a novel independent risk factor for cardiovascular disease in hemodialysis patients.** *XX. biochemický zjazd,* 12.-16.9.2006, Piešťany, Slovakia.
- Široká R., Trefil L., Racek J., Cibulka R.: **Comparison of asymmetric dimethylarginine detection – HPLC and ELISA methods.** *XX. biochemický zjazd,* 12.-16.9.2006, Piešťany, Slovakia.
- Cibulka R., Široká R., Rajdl D., Racek J., Trefil L., Eiselt J., Rubášová L., Schejbalová M., Michálková R.: **Asymetrický dimethylarginin u hemodialyzovaných nemocných.** *Sympozium klinické biochemie FONS,* 17.-19.9.2006, Pardubice, Czech Republic.
- Cibulka R., Racek J., Pikner R., Trefil L., Veselá E.: **Suplementace L-karnitinem pozitivně ovlivňuje metabolismus osteoblastů u hemodialyzovaných nemocných.** *Sympozium klinické biochemie FONS,* 17.-19.9.2006, Pardubice, Czech Republic.
- Pikner R., Cibulka R., Racek J., Trefil L., Topolčan O.: **Renální osteodystrofie – patofyziologie a laboratorní diagnostika.** *Setkání pracovníků klinické biochemie a hematologie Plzeňského a Karlovarského kraje,* 21.3. 2007, Klatovy, Czech Republic.
- Racek J., Rajdl D., Eiselt J., Široká R., Cibulka R., Malánová L., Trefil L.: **Asymetrický dimethylarginin (ADMA) jako nový nezávislý faktor přežití u hemodialyzovaných nemocných.** *Imunoanalytické dny,* 1.-3.4. 2007, České Budějovice, Czech Republic.
- Cibulka R., Eiselt J., Opatrný K. Jr., Rajdl D., Opatrná S., Racek J.: **Asymetrický dimethylarginin u hemodialyzovaných (HD) a peritoneálně dialyzovaných (PD) nemocných.** 47. studentská vědecká konference, 23.5. 2007, Plzeň, Czech Republic.
- Cibulka R., Rajdl D., Siroka R., Eiselt J., Malanova L., Trefil L., Racek J.: **Asymmetric dimethylarginine as a novel prognostic factor for survival in hemodialysis patients.** *17th IFCC – FESCC European Congress of Clinical Biochemistry and Laboratory Medicine and 60th National Congress of the Netherlands Society for Clinical Chemistry and Laboratory Medicine (NVKC) – EUROMEDLAB,* 3.-7.6. 2007, Amsterdam, Netherlands.
- Racek J., Eiselt J., Malánová L., Rajdl D., Široká R., Cibulka R., Trefil L.: **Asymmetric dimethylarginine as a novel prognostic factor for survival in**

hemodialysis patients. *AACC Annual Meeting,* 15.-19.7. 2007, San Diego, California, USA.