Serum carnitine concentration is decreased in patients with Lyme borreliosis*

Stężenie karnityny w surowicy jest obniżone u pacjentów chorych na boreliozę z Lyme

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Summary

Lyme borreliosis (LB) is a serious infectious disease. Carnitine plays a crucial role in metabolism and inflammatory responses. Carnitine may be important in improving neuronal dysfunction and loss of neurons.

To evaluate serum carnitine concentration in adult patients with various clinical types of LB.

Groups: 1) patients with erythema migrans (EM, n=16), 2) neuroborreliosis (NB, n=10), 3) post-Lyme disease (PLD, n=22) and healthy controls (HC, n=32). Total (TC) and free (FC) carnitine were determined with the spectrophotometric method.

TC levels (44.9±10.4, 28.0±8.4, 35.9±15.6 µmol/L) in the EM, NB and PLD patients were lower than in HC (54.0±11.4 µmol/L), p < 0.001. FC levels (32.7±7.7, 23.6±6.8, 26.3±11.2 µmol/L) in the EM, NB and PLD patients were lower than in HC (40.5±7.6 µmol/L), p < 0.001. AC levels (12.2±5.2, 4.4±2.6, 9.6±7.4 µmol/L) in the EM, NB and PLD patients were lower in the NB and PLD patients than in HC (13.5±8.40 µmol/L), p <0.001. AC/FC ratio was 0.31±0.14, 0.18±0.09, 0.39±0.33 in the EM, NB and PLD patients.

LB patients exhibit a significant decrease of their serum carnitine concentrations. The largest changes were in the NB and PLD patients. To prevent late complications of the disease a possibility of early supplementation with carnitine should be considered. Further studies are required to explain the pathophysiological significance of our findings.

Keywords: Lyme borreliosis - erythema migrans - neuroborreliosis - post-Lyme disease - carnitine

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Introduction

Lyme borreliosis (LB), although rarely lethal, is a severe infectious disease. It results from hard tick (arthropod from the genus *Ixodes*) bite transfaction of bacteria belonging to the genus *Borrelia* from infected animals (e.g., deer, mice, birds). Among ten species of the *Borrelia* genus three of them are pathogenic: *Borrelia burgdorferi sensu stricto* (s.s.), *B. afzelii* and *B. garinii*, frequently classified as *B. burgdorferi sensu lato* (s.l.). In North America the cause of LB is *B. burgdorferi s.s.*, whereas in Europe *B. afzelii* and *B. garinii* are involved. Apart from *Borrelia* species, the ticks may transmit more than one pathogen (e.g., tick-borne encephalitis virus) that may complicate diagnosis and treatment of tick-borne disease(s). In the tick’s saliva there are analgesic and anti-inflammatory substances; therefore the tick bite is painless and may be unnoticed by the patient [6,7,16,17,20].

The earliest manifestation of LB is the appearance of characteristic, single or multiple, skin lesions known as erythema migrans (EM), often accompanied by flu-like symptoms. In the second stage (early dissemination that may occur via the circulation and/or the neural pathway) of the disease multifocal EM, arthritis, early neuroborreliosis, including auto-immunological reactions [2,3,10,16]. Lyme borreliosis, although rarely lethal, is a severe infectious disease. It results from hard tick (arthropod from the genus *Ixodes*) bite transfaction of bacteria belonging to the genus *Borrelia* from infected animals (e.g., deer, mice, birds). Among ten species of the *Borrelia* genus three of them are pathogenic: *Borrelia burgdorferi sensu stricto* (s.s.), *B. afzelii* and *B. garinii*, frequently classified as *B. burgdorferi sensu lato* (s.l.). In North America the cause of LB is *B. burgdorferi s.s.*, whereas in Europe *B. afzelii* and *B. garinii* are involved. Apart from *Borrelia* species, the ticks may transmit more than one pathogen (e.g., tick-borne encephalitis virus) that may complicate diagnosis and treatment of tick-borne disease(s). In the tick’s saliva there are analgesic and anti-inflammatory substances; therefore the tick bite is painless and may be unnoticed by the patient [6,7,16,17,20].

In recent years, a role of carnitine (C) and its esters, acyl- and acetyl-carnitines (AC), in the inflammatory reactions in various diseases has been considered. Carnitine is synthesized from lysine and methionine in the liver, kidney and brain but not in the cardiac and skeletal muscles, although they are the main “users” of the substance. About 75% of the carnitine originates from the diet (meat, milk), whereas 25% is synthesized endogenously. Interestingly, vegetarians exhibit normal levels of serum carnitines. In the tissues, nearly all carnitine is present inside the cells. In the circulation and tissues it remains in dynamic equilibrium with acyl-carnitines, with the ratio <0.6 being assumed normal [8,9]. Carnitine plays a basic role in the transport of activated, long-chain fatty acids from the cytoplasm into the mitochondria. Intracellular β-oxidation of fatty acids involves their interaction with CoA, which results in the formation of acyl-CoA that further reacts with carnitine to form acyl-carnitine. Acyl-carnitine freely passes through the mitochondrial membrane, where it reacts with intra-mitochondrial CoA to rebuild acyl-CoA and the free carnitine pool. Acyl-CoA is oxidized and acetyl-CoA, which may react with part of the free carnitine pool to form acetyl-carnitine (free carnitine and acetyl-carnitine are transported outside the mitochondrion) and free CoA, is formed. An excess of acetyl-CoA in the mitochondrion inhibits β-oxidation, whereas deficit of CoA limits the Krebs cycle and induces glycolysis, resulting in toxic lactic acid accumulation in the muscles [1,11].

Lowering of the carnitine concentrations has been demonstrated in patients suffering a wide spectrum of diseases such as impaired immune reactions (e.g., sepsis, infection with immunodeficiency virus), various metabolic and cardiovascular disorders, and chronic fatigue syndrome. However, carnitine homeostasis in the course of Lyme borreliosis has not been studied so far [4,5,9,12,13].

The aim of the study was to evaluate serum carnitine concentration in adult patients with various clinical types of Lyme borreliosis.

Material and methods

**Material**

Clinical forms of LB were classified according to Asbrink and Hovmark [2]. Patients with diagnosed Lyme borreliosis were divided into three groups. Group 1 consisted of patients with erythema migrans (EM), n=16, 6 male and 10 female, aged from 20 to 75 years (47±15 years). Group 2 consisted of patients with neuroborreliosis (NB), n=10, 7 male and 3 female, aged from 24 to 83 years (57±16 years). Seven of them presented Lyme meningitis and facial nerve palsy. Four patients had moderate to severe cranial nerve paralysis. Group 3 consisted of patients with post-Lyme disease (PLD), n=22, 12 male and 10 female, aged from 29 to 67 years (52±11 years). Six patients presented fibromyalgia, 10 patients had arthral-
gias, 2 exhibited headache and 4 patients showed chronic fatigue syndrome. Blood sampling was performed at the time of the patients’ admission to hospital. They were without specific treatment yet. The control group consisted of 32 apparently healthy males (n=17) and females (n=15) aged from 22 to 60 years (43±11 years). The inclusion criteria were: 1) expanding EM with a diameter of >5 cm and characteristic clinical symptoms lasting from several days to weeks, 2) presence of antibodies against *B. burgdorferi s.l.* in the serum and/or cerebrospinal fluid, Lyme meningitis and/or cranial nerve palsy and other neuroborreliosis symptoms, 3) patients with symptoms of chronic/late Lyme borreliosis lasting for at least 1 year, with or without a distant history of Lyme meningitis, EM or specific LB treatment. The exclusion criteria were: primary carnitine deficiency, tick-borne encephalitis virus co-infection, genetic and metabolic disorders, heart, renal and hepatic failure.

**METHODS**

**Serological determinations**

Titers of IgM and IgG antibodies against *B. burgdorferi s.l.* in the serum and cerebrospinal fluid samples were assayed using ELISA Borrelia IgG+VlsE and Borrelia IgM 14 kDa+OspC kits, and using Western blot analysis with Anti-Borrelia IgG Line Immunobassay and Anti-Borrelia IgM Line Immunobassay kits, all purchased from DRG GmbH, Marburg, Germany.

In the patients with suspected Lyme neuroborreliosis, intrathecal synthesis of anti-*Borrelia s.l.* antibodies in cerebrospinal fluid and in the serum was assayed using Borrelia IgG/IgM Line Immunoblot from Sekisui Virotech GmbH, Rüsselsheim, Germany.

**Carnitine determination**

Total (TC) and free (FC) carnitine were determined as previously described [12]. Briefly, 0.5 mL of serum was centrifuged (2000 g, 40 min, at room temp.) through the Centricon YM-30 filter (Millipore, Bedford, MA, USA, cut-off 30 kDa). FC was measured in the serum filtrates, without hydrolysis of acyl-carnitine esters: short-, medium-, and long-chain acylcarnitines, (AC). TC (TC=FC+AC) was assayed applying the method of Cederblad [5]. FC determination is based on the reaction of FC with added acetyl-CoA and carnitine acetyltransferase. As a result of the reaction CoA-SH is formed and further reacts with the added 5,5’-dithiobis-2-nitrobenzoic acid. Absorbance of the product was measured spectrophotometrically at 412 nm. TC concentration was quantified using 200 µL of serum filtrate after its incubation with 20 µL of 1 M KOH for 1 h at 56°C in order to hydrolyze carnitine esters. After incubation, the mixture was neutralized to pH ~7.0 with 4 µL of 5 M HCl and assayed for the TC as described above. AC concentration was calculated by subtracting FC from the TC concentration. The ratio of AC/FC was evaluated as described by Schmidt-Sommerfeld [19]. Sensitivity of the method was 4.0 µmol/L. Intra- and inter-assay coefficients of variation (CV%) were 2.0 and 7.0%, respectively.

**Statistical analysis**

The data, expressed as a mean ± SD, were analyzed by the statistical analysis program Statistica version 10.0 (StatSoft, Cracow, Poland), using Student’s t-test for independent sales. The statistical significance of differences was set at p <0.05.

**Results**

All of our patients with LB exhibited normal values (within the reference range) of their serum aminotransferases, lactate dehydrogenase, glucose, creatinine, cholesterol, triglycerides and blood test including leukocyte count. Erythrocyte sedimentation rate was slightly elevated (>30 mm/h) in 2 (20 %) patients with NB and in 1 (5 %) with PLD, whereas C-reactive protein was increased (>5.0 mg/L) in 3 (30%) NB patients and in 2 (9%) PLD patients. The basic urine test was normal in the studied groups (data not shown).

Titers of the antibodies against *B. burgdorferi s.l.* were not performed for the EM patients at the time of their admission. NB and PLD patients exhibited presence of *B. burgdorferi* IgM and/or IgG antibodies in their sera and cerebrospinal fluid. Intrathecal synthesis of the antibodies was further confirmed for the patients with LB meningitis using Borrelia Line IgG and Borrelia Line IgM Line ImmunobLOTS as detailed in Materials and Methods (data not shown).

Serum total carnitine (TC) concentration was 44.9±10.4, 28.0±8.4 and 35.9±15.6 µmol/L in the EM, NB and PLD patients, respectively, versus 54.0±11.4 µmol/L in the control group (Fig. 1). All of the TC values were significantly lower as compared with the control group values. The lowest TC concentrations were found among the patients with NB and PLD (p<0.001).

Serum free carnitine (FC) concentration was 32.7±7.7, 23.6±6.8 and 26.3±11.2 µmol/L in the EM, NB and PLD patients versus 40.5±7.6 µmol/L in the control group (Fig. 2). All of the FC values were significantly lower when compared to the values seen in the control group (p<0.001). The lowest FC concentrations were found in the NB and PLD patients (p<0.001).

Serum acyl-carnitines (AC) concentration was 12.2±5.2, 4.4±2.6 and 9.6±7.4 µmol/L in the EM, NB and PLD group, respectively, versus 13.5±8.40 µmol/L in the control group (Fig. 3). The AC levels of the NB and PLD patients, but not of the EM patients (p<0.05), were significantly lower as compared to those in the control group. The lowest AC concentrations were seen in the NB patients (p<0.001).
In this study, we found that in patients with various clinical types of Lyme borreliosis, the mean serum concentration of TC and FC was significantly lower as compared to the mean concentrations seen in the healthy controls. Interestingly, AC concentration in the EM patients was at a normal level. Values of the AC/FC ratio were normal in both the EM and PLD patients. EM is the early stage
Table 1. Extreme serum carnitine concentrations and acyl-carnitine to free carnitine ratio in patients with erythema migrans (EM), neuroborreliosis (NB) and post-Lyme disease (PLD)

<table>
<thead>
<tr>
<th>Carnitine (C) (µmol/L)</th>
<th>EM (n=16)</th>
<th>NB (n=10)</th>
<th>PLD (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>0</td>
<td>1 (10%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>20-38</td>
<td>4 (25%)</td>
<td>7 (70%)</td>
<td>13 (59%)</td>
</tr>
<tr>
<td>Free C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>0</td>
<td>3 (30%)</td>
<td>9 (41%)</td>
</tr>
<tr>
<td>20-34</td>
<td>7 (44%)</td>
<td>5 (50%)</td>
<td>9 (41%)</td>
</tr>
<tr>
<td>Acyl-C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>1 (6%)</td>
<td>6 (60%)</td>
<td>10 (45%)</td>
</tr>
<tr>
<td>&gt;22</td>
<td>0</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Acyl-C/Free C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.60</td>
<td>1 (6%)</td>
<td>0</td>
<td>5 (23%)</td>
</tr>
</tbody>
</table>

of LB, and therefore the carnitine homeostasis was not substantially changed yet [2,10]. Indeed, our data indicate that numerous NB and PLD patients (but not those with EM) exhibited very severe deficiency of TC, FC (<20 µmol/L), and AC (<5 µmol/L). Moreover, even more patients (including in this case those with EM) had carnitine levels near or below the lower limit of the reference range. Intriguingly, the AC/FC ratio was increased >0.6 in only 1 (6%) and 5 (25%) EM and PLD patients, respectively. This ratio relates to the proportion of AC in the TC pool and therefore reflects the relation between acyl-CoA and free CoA and seems to be very sensitive to the rate of AC metabolism in mitochondria. AC accumulation in the circulation indicates mitochondrial dysfunction and impaired fatty acid oxidation [9,19]. The majority of our EM and PLD patients exhibited an AC/FC ratio much below 0.6, because their FC and AC levels were decreased concomitantly and proportionally. However, the ratio in the NB patients was significantly lower as compared to the ratio in EM or PLD patients, since their AC levels were decreased much more than their FC levels. The ratio >0.6 indicates accumulation of AC and/or decrease of FC level. It may be physiologically increased in neonates as a result of highly increased β-oxidation. Extremely high AC and low FC concentrations in the circulation may be due to deficiency of carnitine/AC translocase activity that is seen in primary carnitine deficit. In the case of our LB patients it seems that FC and AC decrease may be due to increased tissue availability of FC in order to enhance fatty acid supply to mitochondria to normalize energy metabolism [1,4].

Normal homeostasis of carnitine and its congeners is crucial for the energy metabolism. The substance provides long-chain fatty acids as well as products of their peroxisomal partial oxidation into the mitochondria for their β-oxidation. Carnitine removes an excess of toxic acyl-CoA metabolites from the mitochondrial matrix and regulates the intramitochondrial acyl-CoA/free CoA ratio, and AC provides acetyl moieties for the synthesis of acetylcholine and nuclear histone acylation in the central nervous system [11,14]. Also, carnitine plays several secondary roles as an immune system modulator, antioxidant and anti-inflammatory and anti-apoptotic agent, and regulator of cell volume and fluid balance in the tissues including nervous cells. It enhances synthesis and secretion of various pro-inflammatory cytokines and certain enzymes [9,10,15]. A substantial portion of the TC may be engaged in the above-mentioned functions, especially in the disease states including LB. Hypocarnitinemia may result from primary or secondary reasons. Primary carnitine deficiency is a rare, autosomal, recessive disorder due to a defect in the membrane OCTN2 carnitine transporter. Then the patients may exhibit an extremely high (>0.6) AC/FC ratio. Secondary carnitine deficit may be due to a poor diet, malnutrition, malabsorption, peritoneal dialysis, increased AC urinary excretion with certain drugs or organic acids. Carnitine deficiency has been observed in patients with liver and kidney diseases, diabetes, sepsis, myopathy, cardiomyopathy, malnutrition, malabsorption, cirrhosis, alcohol abuse, recurrent infections, fatigability and others [1,2,4,12,13,14]. Frequently, LB patients exhibit numerous symptoms that accompany the above-mentioned pathophysiological conditions. Carnitine is considered as a non-toxic, natural substance that may ameliorate and/or alleviate symptoms of various diseases. It has been shown that carnitine administration exhibits ameliorative actions in uremic patients, as well as in those with nerve conduction disturbances or with neuropathic pain. In sepsis carnitine supplementation may inhibit organ failure, hepatic lipogenesis, muscle wasting and a decreased rate of fatty acid metabolism. However, it has to be taken into account that it is poorly absorbed from the intestines and easily removed by renal clearance. Efficacy and clinical applicability of carnitine supplementation of patients with LB (at least those with severe carnitine deficiency) remain to be determined [4,18].

In summary, although our LB patients did not exhibit substantial changes in their basic biochemical (including cholesterol and triglycerides), hematological and urinary parameters, their serum carnitine levels were significantly decreased. In the patients with EM their TC and FC levels were not decreased as much as in the patients with NB or PLD. Characteristically, their mean AC level and AC/FC ratio remained within the reference ranges. NB patients exhibited the largest decrease of their AC and AC/FC ratio, and there were numerous patients with extremely low TC, FC and AC levels. The patients with PLD exhibited a large decrease of their TC, FC and AC levels, whereas their AC/FC ratio was within the reference range. Here, the patients with extremely low TC, FC and AC levels were most numerous. Whether
the characteristic spectra of the changes of carnitine levels and AC/FC ratios noted here really relate to the clinical types and severity of Lyme borreliosis remains to be determined. Despite numerous limitations of our study, such as its cross-sectional character and relatively small groups of patients, it seems that our results encourage further, systematic studies in this topic.

References


The authors have no potential conflicts of interest to declare.