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Chapter

Coenzyme Q_{10} and L-Carnitine Disturbances in Children with Mitochondrial Diseases

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Abstract

Coenzyme Q10 (CoQ10) and L-carnitine are very important biologically active compounds involved in energy metabolism. L-carnitine and coenzyme Q10 disturbances in mitochondrial diseases require the correction. Patients and methods: The levels of coenzyme Q10 and L-carnitine (total carnitine, free carnitine, and acylcarnitines) were determined in children with mitochondrial diseases (25 children and 16 children, respectively). High-performance liquid chromatography with UV detection (chromatograph Shimadzu Nexera LC-30) and chromatography-mass spectrometry (Agilent 6410 QQQ, USA) were used. As an additional parameter of possible coenzyme Q10 and carnitine insufficiency, the coenzyme Q10/cholesterol ratio and acylcarnitines/free carnitine ratio were calculated. Results: A significantly low ratio of coenzyme Q10/cholesterol in children with mitochondrial diseases was revealed—0.10 ± 0.01 vs. 0.19 ± 0.01 in the control group ($p < 0.001$). A lower absolute level of coenzyme Q10 and tendency toward a more pronounced decrease in the Q10/cholesterol ratio in older patients (6–16 years) were shown. The free carnitine blood level was within the normal range and averaged at 29.8 ± 2.6 μmol/l; however, the level was lower than that in the control group (44 ± 5.2 μmol/l, $p < 0.05$). A pronounced significant increase in the acylcarnitines/free carnitine ratio was determined—1.5 ± 0.05 (the normal range < 0.6).

Keywords: children, mitochondrial diseases, coenzyme Q10, carnitine, treatment

1. Introduction

Mitochondrial diseases are a large heterogeneous group of pathological conditions caused by genetically determined defects in the mitochondria's structure and function in the release of the energy of organic substances and its accumulation in the form of macroergic phosphate compounds by the generation of adenosine triphosphate [1]. These disorders can be due to mutations in mitochondrial DNA or due to mutations in nuclear DNA. Some mitochondrial diseases are rare. However, in general, mitochondrial encephalopathy is the most common neurometabolic disorder [2]. Defects in the respiratory chain and oxidative phosphorylation are the basis of the pathogenesis of these severe diseases.

Mitochondrial diseases have a wide range of clinical presentations with a generally poor prognosis: failure to thrive, encephalopathy, respiratory insufficiency,

hypotonia, ataxia, seizures, cardiac involvement, hepatopathy and nephropathy, sensorineural hearing loss, retinopathy, lesions of the basal ganglia, and others. The common laboratory signs are lactic acidosis, hypo- or hyperglycemia (diabetes), elevated creatine kinase and aminotransferases, and urine organic acid/amino acid abnormalities [3].

Current therapies are frequently inefficient and mostly palliative. The treatment strategy for mitochondrial diseases is to improve the efficiency of biological processes in the respiratory chain and oxidative phosphorylation. Patients are prescribed complex treatment, including drugs that affect different stages of energy metabolism. This treatment approach shows a higher positive effect than monotherapy [4]. Coenzyme Q10 and L-carnitine are very important biologically active substances involved in energy metabolism. So, coenzyme Q10 and L-carnitine are often recommended for the treatment of mitochondrial diseases [5]. However, some authors acknowledge the lack of rationale behind these recommendations since the data from randomized clinical trials are still lacking.

2. Functions of CoQ10 and its biological role

Coenzyme Q10 is the most common ubiquinone in the human body. Its structure contains a quinoid ring and 10 isoprenyl groups. Coenzyme Q10 is structurally similar to vitamins E and K. Coenzyme Q10 exists in oxidized (ubiquinone) and reduced (ubiquinol) forms and is known to be a constituent of the biological membranes [6]. Coenzyme Q10 is one of the main components of the electron transport chain of mitochondria. In the form of ubiquinone, it acts as an electron transporter from Complex I and Complex II to Complex III. In this process, the formation of the reduced form—ubiquinol—occurs. Ubiquinol is a powerful antioxidant, which has a protective effect on biological membranes, regulates their permeability, inhibits peroxidation of plasma lipoproteins, and provides a recovery of tocopherol activity [6, 7]. According to the recent data, coenzyme Q10 is reported to be involved in the regulation of some gene expression and inflammatory mediators, in particular, by influencing the transcription factor NFkappaB1; its participation in DNA replication and repair was shown [8, 9].

In mammals, the largest amount of coenzyme Q10 is found in the heart and skeletal muscles. In the peripheral blood, coenzyme Q10 is bound to lipoproteins, and its level is positively correlated with total cholesterol [10, 11].

Most of the body's daily coenzyme Q10 requirement is derived from endogenous synthesis; small amounts of coenzyme Q10 are obtained from foods such as meat, fish, and nuts. Biosynthesis is a multi-step process, taking place on the inner mitochondrial membrane. Vitamins B2, B3, B6, B12, and C and folic and pantothenic acids are known to participate in the coenzyme Q10 biosynthetic pathway under the control of a dozen genes. The intensity of biosynthesis declines substantially with age [12, 13].

3. Coenzyme Q10 deficiency in disorders: the possibility of diagnosis

Primary coenzyme Q10 deficiency is due to a defect in its biosynthesis. These diseases form a separate group of mitochondrial diseases and are associated with mutations in multiple genes including PDSS1, PDSS2, CoQ2, CoQ6, CoQ9, and ADCK3. These diseases are characterized by a decrease in the level of coenzyme Q10 in tissues and in fibroblasts whereas the blood levels can be normal [11, 14, 15]. Secondary coenzyme deficiency with low plasma and tissue coenzyme Q10 levels

can occur in patients taking anticancer agents and statins. The hypocholesterolemic effect of statins is due to the inhibition of 3-hydroxy-3-methylglutaryl-COA reductase and a disruption of the synthesis of mevalonic acid, which is one of the precursors of not only cholesterol but also ubiquinone. Approximately a half of patients receiving statins show a decrease in coenzyme Q10 in the muscle tissue; myalgia and myoglobinuria may also be observed [16, 17].

Secondary coenzyme Q10 deficiency and low levels of coenzyme Q10 in plasma and tissues are found in certain diseases of older age (Parkinson's and Alzheimer's disease, atherosclerosis, diabetes mellitus, etc.) [13, 18, 19], in some hereditary diseases, including inborn errors of metabolism— mevalonic aciduria, phenylketonuria, glutaric acidemia II, ataxia-oculomotor apraxia 1, and cardiofaciocutaneous syndrome [11, 20]. Coenzyme Q10 deficiency in mevalonic aciduria and in phenylketonuria can be explained by insufficient cholesterol production: the decrease in the activity of mevalonate kinase and the inhibition of 3-hydroxy-3-methylglutaryl-COA reductase with high levels of phenylalanine, respectively. In phenylketonuria, ubiquinone deficiency may well be exogenous due to dietary restrictions such as avoidance of animal products [10].

A decrease in coenzyme Q10 was reported in the peripheral blood and muscles in some (20–40%) patients with mitochondrial pathology associated with mutations and depletion of mitochondrial DNA [21, 22]. Of interest, in children with myopathy due to other causes, there were no changes in the content of coenzyme Q10 in the muscles, except for the patients with Duchenne muscular dystrophy [23].

The coenzyme Q10 deficiency can be detected in biological fluids (plasma or serum), fibroblasts, and muscle tissue. However, the blood level of coenzyme Q10 is not considered as a reliable indicator of its state in the body. There is no clear correlation between the levels of ubiquinone in plasma and muscle tissue. This parameter is influenced by the lipid intake from foods and the blood levels of cholesterol and low-density lipoproteins [7, 10, 11]. Therefore, the ratio of coenzyme Q10 to cholesterol and low-density lipoproteins is proposed for clinical use. Apparently, the measurement of coenzyme Q10 in the peripheral blood mononuclear cells appears to be a promising detection method.

4. Low blood level of coenzyme Q10 as a diagnostic marker of mitochondrial encephalomyopathy and the rationale for therapy

In the Research and Clinical Institute of Pediatrics, an examination of 16 children (group 1) aged 1–16 years (average age 8.3 ± 1.5 years) with mitochondrial diseases was performed.

In nine children, the disease was caused by deletions or point mutations of mitochondrial DNA: Kearns-Sayre syndrome (common deletion of mitochondrial DNA) in three; mitochondrial encephalomyopathy with pyramidal-extrapyramidal syndrome (*MTND1* mutation) in two; mitochondrial encephalomyopathy with cardiomyopathy (*MTTK* mutations) in two; mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (*MTTL1* mutation) in 1; and maternal inherited Leigh syndrome (*MTND3* mutation) in 1.

Seven children were diagnosed with mitochondrial diseases of nuclear origin: leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (*DARS2* mutations) in four and Leigh syndrome (*SURF1* mutations) in three.

The comparison group (group 2) consisted of 13 children with neurodegenerative diseases aged 1–15 years (mean age 6.4 ± 1.3 y): 6 children with neuronal ceroid

lipofuscinosis 2 (*TPP1* mutations), 2 with sialidosis type 2 (*NEU1* mutations), and 5 with progressive ataxia not genetically confirmed. The control group (group 3) consisted of 29 healthy children aged 1–16 years (mean age 7.4 ± 0.8 y) who underwent a routine examination at the health center (an informed consent of the parents was obtained) [24]. The ratio of boys:girls is 16:13. There was no statistically significant difference in the average age between the three groups.

A detailed work-up of the patients of the first two groups included clinical (pedigree, neurological, cardiological, and other examinations) examination and biochemical tests. In all children, the plasma level of coenzyme Q10 was determined by high-performance liquid chromatography with UV detection (chromatograph Shimadzu Nexera LC-30). The blood cholesterol level was determined photometrically (analyzer Konelab Prime 60i), followed by the calculation of the coenzyme Q10/cholesterol ratio. The DNA diagnosis of diseases was carried out in the laboratory of inborn errors of metabolism of Research Centre for Medical Genetics (Moscow, Russia).

Statistical data processing was carried out by methods of variation statistics and correlation analysis (Statistica, Excel 7.0). Student's t-test was used to assess the statistical significance of the data, and the differences were considered statistically significant at $p < 0.05$.

The blood levels of coenzyme Q10 in children with mitochondrial diseases (group 1) were 0.56 ± 0.05 μmol/l (**Table 1**) and did not differ from that in the control group but was significantly lower ($p < 0.01$) than that in children with neurodegenerative diseases (group 2). The blood level of coenzyme Q10 in children with neurodegenerative diseases was 1.53 ± 0.23 μmol/l and significantly exceeded that in healthy children ($p < 0.01$).

The blood cholesterol level in patients of the first and second groups was significantly higher than that in healthy children ($p < 0.01$). The ratio of coenzyme Q10/cholesterol was severely impaired compared with that in healthy children (see **Table 1**). This parameter was significantly decreased in patients with mitochondrial diseases (0.10 ± 0.01 vs. 0.19 ± 0.01, $p < 0.001$) but increased in those with neurodegenerative diseases (0.31 ± 0.04, $p < 0.002$).

For the subgroup analysis, children aged 1–5 and 6–16 years were analyzed separately within each group (**Table 2**). In healthy children (group 3), there were no age-related differences.

In the group of older patients with mitochondrial diseases, the blood level of coenzyme Q10 was significantly lower than that in the younger subgroup ($p < 0.05$) and was significantly different from its level in children in the second

Groups of children	Levels in the blood		
	Coenzyme Q10, μmol/l	Cholesterol, μmol/l	Coenzyme Q10/cholesterol
Group 1, n = 16	0.56 ± 0.05	5.7 ± 0.32	0.10 ± 0.01
Group 2, n = 13	1.53 ± 0.23	4.8 ± 0.31	0.31 ± 0.04
Group 3, n = 29	0.67 ± 0.04	3.5 ± 0.12	0.19 ± 0.01
p	$^{1-2}p < 0.001$ $^{2-3}p < 0.001$	$^{1-3}p < 0.001$ $^{2-3}p < 0.001$	$^{1-2}p < 0.001$ $^{1-3}p < 0.001$ $^{2-3}p < 0.002$

^{1-2}p, ^{1-3}p, and ^{2-3}p - significance of differences between groups (1-2, 1-3, and 1-3)

Table 1.
Coenzyme Q10 and cholesterol levels (M ± m) in the blood of the examined children.

Groups of children	Levels in the blood		
	Coenzyme Q10, μmol/l	Cholesterol, μmol/l	Coenzyme Q10/ cholesterol
Group 1, n = 16			
Age 1–5 years, n = 6	0.73 ± 0.15*	6.4 ± 0.81 $^{1-2}p < 0.05$ $^{1-3}p < 0.001$	0.12 ± 0.03 $^{1-2}p < 0.001$ $^{1-3}p < 0.05$
Age 6–16 years, n = 10	0.47 ± 0.04 $^{1-2}p < 0.001$ $^{1-3}p < 0.05$	5.5 ± 0.38 $^{1-3}p < 0.001$	0.09 ± 0.01 $^{1-2}p < 0.001$ $^{1-3}p < 0.001$
Group 2, n = 13			
Age 1–5 years, n = 7	1.18 ± 0.26 $^{2-3}p < 0.001$	4.4 ± 0.44 $^{2-3}p < 0.05$	0.26 ± 0.03 $^{2-3}p < 005$
Age 6–16 years, n = 6	1.93 ± 0.37 $^{2-3}p < 0.001$	5.3 ± 0.38 $^{2-3}p < 0.001$	0.37 ± 0.07 $^{2-3}p < 0.05$
Group 3, n = 29			
Age 1–5 years, n = 15	0.64 ± 0.04	3.5 ± 0.16	0.19 ± 0.02
Age 6–16 years, n = 14	0.69 ± 0.07	3.6 ± 0.18	0.20 ± 0.02

**Significant difference from the older age subgroup; $p < 0.05$. ^{1-2}p, ^{1-3}p, and ^{2-3}p - significance of differences between groups (1-2, 1-3, and 1-3)*

Table 2.
Coenzyme Q10 and cholesterol levels (M ± m) in the blood in children of different age subgroups.

($p < 0.001$) and third ($p < 0.05$) groups of the same age. In addition, older patients (6–16 years) tend to have lower cholesterol levels and lower coenzyme Q10/cholesterol ratios than younger patients (1–5 years). In the group of children with mitochondrial encephalomyopathies, there was a negative correlation between the blood level of coenzyme Q10 and cholesterol and the patient's age ($r = -0.54$ and $r = -0.48$, respectively; $p < 0.05$). There were no differences in the studied parameters between children with mitochondrial diseases caused by mutations of mitochondrial (n = 9) and nuclear (n = 6) DNA. By age, these subgroups were not different.

In the group of children with neurodegenerative diseases, the opposite tendency was observed toward higher rates of coenzyme Q10, cholesterol, and their ratios in patients of the older subgroup (see **Table 2**); unreliability of differences seems to be associated with a small number of patients. A positive correlation between the blood levels of coenzyme Q10 and cholesterol ($r = 0.66$; $p < 0.05$) was established. These parameters correlated positively with the age of the patients ($r = 0.37$ and $r = 0.41$, respectively; $p < 0.05$).

To sum up, our study demonstrated that the average level of coenzyme Q10 in patients with mitochondrial diseases did not differ from that in healthy children. However, these patients had a higher cholesterol level and, as a result, a reduced ratio of coenzyme Q10/cholesterol. In patients of the older subgroup (6–16 years), the changes were more pronounced: significantly lower levels of coenzyme Q10 and a tendency toward a lower Q10/cholesterol ratio than the younger subgroup (1–5 years).

In children with neurodegenerative diseases (not caused by primary mitochondrial dysfunction), the level of cholesterol (as well as in the first group) was higher than that in healthy children. Other test results differed considerably from those in patients with mitochondrial pathology. High blood level of coenzyme Q10 and an

increase in the coenzyme Q10/cholesterol ratio were revealed, and these changes increased with age.

Due to a small sample size, the obtained data were considered as preliminary. Nevertheless, our results revealed a coenzyme Q10 deficiency in children with mitochondrial diseases and emphasized the difference in the pathogenesis of primary mitochondrial diseases and neurodegenerative diseases of non-mitochondrial origin. In both patient groups, the age-related aggravation of these changes was noted to correspond to a progressive disease course. The similarity of clinical manifestations underlies the difficulties in the differential diagnosis between mitochondrial encephalomyopathies and neurodegenerative diseases. The detection of the plasma coenzyme Q10 level can be helpful in the differential diagnosis between these conditions.

In addition, a low coenzyme Q10/cholesterol ratio and a tendency toward a decrease in the coenzyme Q10 levels with age suggest its insufficiency in patients with progressive mitochondrial encephalomyopathy. This provides a rationale for the use of coenzyme Q10 in the treatment of patients with mitochondrial diseases.

5. Functions of carnitine and its biological role

Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is a low molecular weight compound, which is crucial for energy metabolism in the human body. As an L-stereoisomer, carnitine is present in various tissues. Endogenous carnitine formation occurs in the liver, kidney, and brain cells through the transformation of the amino acids lysine and methionine, with a glycine involvement. Vitamins C, B6, and B3 and iron ions are the cofactors for carnitine biosynthesis. The biosynthesis steps are under the control of mitochondrial enzymes (trimethyl-lysine deoxygenase, 3-OH-trimethyl-lysine aldolase, 4-trimethylaminobutanal dehydrogenase, butyrobetaine dioxygenase) [25]. However, biosynthesis provides only a part of carnitine daily requirements, and the main source of its intake is animal foods including red meat, fish, and dairy products. Carnitine absorption in the gastrointestinal tract, tubular reabsorption, and delivery to the tissue are provided by transport proteins, with OCTN2 being the main carnitine transporter. Carnitine is transported to the skeletal muscles and myocardium—these tissues contain the main reserves of carnitine, due to their high activity of lipid metabolism [26]. The importance of carnitine for the body is evidenced by its almost complete reabsorption in the renal tubules.

Studies have established the value of carnitine for the processes of biological oxidation and maintenance of mitochondrial functions in the human body. Carnitine is crucial in conditions of a high energy consumption. These conditions such as intercurrent diseases, increased physical activity, starvation, etc. are characterized by increased catabolism. After depletion of carbohydrate reserves, lipids become the main sources of the ATP synthesis in the body.

One of the main vital functions of carnitine is bioenergetics. Carnitine is involved in lipid catabolism, providing its initial stages—an activation and a transfer of long-chain fatty acids from the cytoplasm through the outer and inner mitochondrial membranes to the mitochondrial matrix, thereby making them available for subsequent β-oxidation to form acetyl-CoA. In addition, fatty acid oxidation is the main pathway of ketogenesis, and ketone bodies are an additional energy substrate for peripheral tissues and the brain [26, 27].

The effect of carnitine on fat metabolism occurs through its participation in the cytoplasmic synthesis of fatty acids. Carnitine provides the reverse transfer of acetyl groups of mitochondrial acetyl-CoA through the mitochondrial membrane into the cytoplasm.

An important function of carnitine is due to its ability to bind acyl radicals. Thus, carnitine regulates tissue energy metabolism, affecting the ratio of acyl-CoA/free CoA in the mitochondria. Likewise, the detoxifying role of carnitine is achieved through binding of organic acid derivatives (intermediates in oxidative processes) and its excretion from the cell. These organic acid derivatives, accumulating in the mitochondria and cytoplasm, have an adverse effect, by inhibiting the enzyme activity.

Carnitine appears to play an important role in the permeability of mitochondrial membranes. The protective impact of carnitine relies on the prevention of a negative membranotropic action of toxic agents, inhibitors of complexes of a mitochondrial respiratory chain (3-nitropropionic acid, methylphenylpyridine, and others), and inducers of apoptosis (long-chain fatty acid radicals). Carnitine supplementation in experimental animals prevents these disorders or significantly reduces their severity and prevents a degenerative damage to the nervous tissue [28–31].

In addition, a favorable impact of carnitine on the cytokine production and on vascular endothelium was revealed. Carnitine is capable of restoring endothelial function and preventing of vascular remodeling caused by a decrease in nitric oxide production [25, 32, 33]. Apparently, the carnitine functions require further research.

6. Carnitine deficiency in diseases

There are primary and secondary carnitine deficiencies. The primary deficiency is due to an autosomal recessive defect of the gene SLC22A5, which is expressed in the skeletal muscles, heart, and kidneys. The gene SLC22A5 encodes a transport protein OCTN2, the sodium-dependent organic cation transporter. The genetic defect disrupts the transport of carnitine into the tissues and reabsorption in the renal tubules. The clinical manifestations of the disease include cardiomyopathy, skeletal myopathy, fatty liver, and kidney dystrophy [34].

The causes of secondary carnitine deficiency are diverse and associated with an interruption of endogenous synthesis and disturbance of absorption from food and of retention in the body, as well as with an increased excretion through the kidneys or gastrointestinal tract [35]. The activity of endogenous carnitine biosynthesis depends on the function of the liver and kidneys. Biosynthesis decreases with malnutrition due to a protein deficiency. Carnitine removal is enhanced in stress, intercurrent diseases, and impaired renal tubular function. Low blood carnitine level is determined in children with epilepsy treated with valproate, in patients with heart failure, and in patients on hemodialysis [36–38].

Secondary insufficiency occurs in inborn errors of metabolism. In particular, secondary carnitine insufficiency is characteristic for a large group of hereditary diseases of organic and fatty acid metabolism. In these diseases, low levels of carnitine in the peripheral blood and tissues result from the accumulation of acylcarnitines and their enhanced renal excretion [34, 39].

7. The carnitine insufficiency in children with mitochondrial encephalomyopathies

Some patients with mitochondrial diseases (about one-fourth patients) were reported to have a decrease in carnitine levels in the peripheral blood [40, 41]. Our study was designed to diagnose and treat carnitine insufficiency in patients with mitochondrial diseases; to achieve this, we analyzed the clinical parameters and

laboratory findings of 40 children aged 2–15 years: 25 with mitochondrial encephalomyopathies (group 1) and 15 with congenital myopathies (group 2). Group 1 consisted of 10 children with Kearns-Sayre syndrome (common deletion of mitochondrial DNA); 2 with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MTTL1 mutation); 1 with myoclonic epilepsy associated with ragged-red fibers (MTTK mutation); 5 with leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (DARS2 mutations); 1 with Barth syndrome (TAZ mutation); 3 with Leigh syndrome (SURF1 mutations); and 3 with POLG-related diseases (mitochondrial recessive ataxia syndrome in 2 and autosomal recessive progressive external ophthalmoplegia in 1). Group 2 consisted of 10 children with central core disease and 5 with minicore myopathy. The control group included 10 children without mitochondrial diseases or congenital myopathies, who attended the clinic.

The clinical examination included pedigree, neurological, cardiological, and other examinations. In all children, acid–base balance of the blood and the level of lactic and pyruvic acids were determined. The level of L-carnitine—total carnitine, free carnitine (C0), and acylcarnitines (AC)—in dry blood spots was measured by chromatography-mass spectrometry (Agilent 6410 QQQ, USA). As an additional parameter of a possible carnitine insufficiency, the ratio AC/C0 was calculated. The DNA diagnosis of mitochondrial diseases was carried out in the laboratory of inborn errors of metabolism of the Research Centre for Medical Genetics (Moscow, Russia). Structural myopathy was diagnosed using a morphological study of muscle tissue (light and electronic microscopy, histochemical methods).

Statistical data processing was carried out by methods of variation statistics and correlation analysis (Statistica, Excel 7.0). Student's t-test was used to assess the statistical significance of the data, and the differences were considered statistically significant at $p < 0.05$.

In children with mitochondrial diseases, the clinical presentations of the nervous and muscular system involvement prevailed: fast fatigability, low exercise tolerance, muscle weakness and hypotension, and a development delay. Severe psychomotor retardation was noted in three children with Leigh syndrome. Most patients had ataxia, ophthalmoplegia, headaches and vomiting, and heart damage (atrioventricular blockade, cardiomyopathy). Some patients had a short stature, seizures, retinitis pigmentosa, a hearing loss, and an impaired liver function with a moderate increase in blood aspartate and alanine aminotransferase.

Compensated metabolic acidosis was detected in 12 of 25 children. Elevated blood lactate levels were noted within the range 2.9–6.7 mmol/l in 20 children (the normal range 1.0–1.7 mmol/l). In 11 patients, the pyruvate level was elevated (0.19–0.39 mmol/l, norm 0.09–0.12 μmol/l).

In children with mitochondrial diseases, the total carnitine blood levels (**Figure 1**) ranged from 39.1 to 95.3 μmol/l, averaging (M ± m) 75.8 ± 6.2 μmol/l (in the control group—from 41.1 to 119.9 μmol/l; 73.7 ± 9.7 μmol/l). There was a negative correlation between the total carnitine and lactate in the peripheral blood of patients with mitochondrial diseases ($r = -0.65$; $p < 0.05$).

The free carnitine blood level was within the normal range (19–60 μmol/l) and averaged at 29.8 ± 2.6 μmol/l; however, the level was lower than that in the control group (44 ± 5.2 μmol/l, $p < 0.05$). In six patients, the free carnitine blood level was at the lower limit of the normal range, not exceeding 25 μmol/l.

The average acylcarnitine level was 44.5 ± 3.7 μmol/l (see **Figure 1**); 87% of acylcarnitines were represented by acetylcarnitine (C2). Acetylcarnitine level was 38.7 ± 3.9 μmol/l (higher than that in control group; $p < 0.05$); its level in four children went beyond the limit of the normal range (56.3, 60.2, 64.5, 65.7 μmol/l at a rate of up to 50 μmol/l).

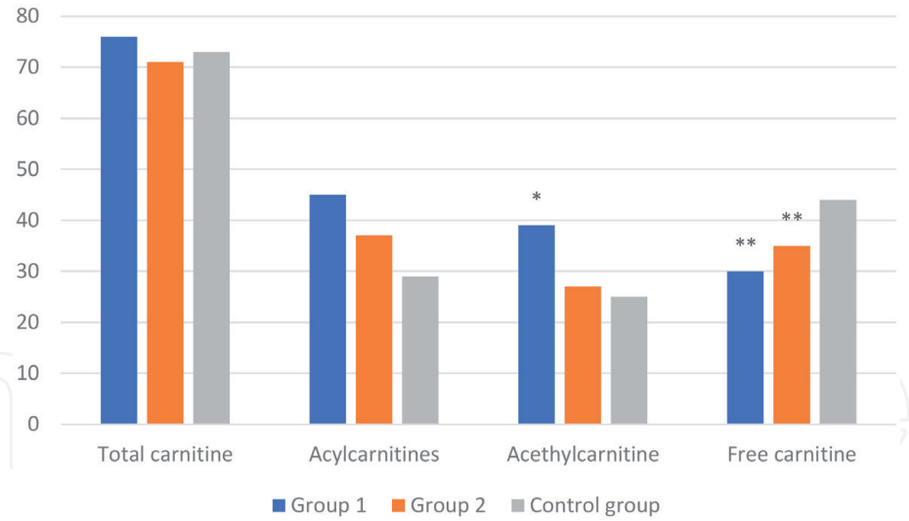


Figure 1.
*Comparison of carnitine levels (μmol/l) in dry blood spots in children of three groups. * significant difference (p < 0.05) with groups 2 and 3. ** significant difference (p < 0.05) with group 3.*

About a half (13) of the patients had elevated levels of other acylcarnitines: methylmalonyl-(C4DC), tiglyl-(C5: 1), glutaryl-(C5DC), hydroxybutyryl-(C4OH), hydroxyisovaleryl-(C5OH), and hexanoyl-(C6); there was an increase in the blood levels of alanine, glycine, and leucine. These metabolic changes are likely to be associated with the activation of ketogenesis, an impaired metabolism of lactate and pyruvate in mitochondrial diseases.

On average, the proportion of free carnitine was merely 39% of the total blood carnitine (the normal range is 70–80%). The proportion of acylcarnitines was 61%, significantly exceeding that of healthy subjects (20–30%).

The ratio of acylcarnitines and free carnitine AC/C0 was markedly increased to 1.3–1.8, reaching an average of 1.5 ± 0.05 (the normal range < 0.6). The increase in this ratio suggests the relative insufficiency of free carnitine and confirms the accumulation of esterified forms in the total carnitine pool.

In the patients with congenital myopathies, the clinical presentations predominantly included myopathic manifestations, impaired motor development, and increased fatigue. Moderately elevated blood lactate levels were found in eight children (1.9–2.8 mmol/l, the normal range 1.0–1.7 mmol/l). In 10 patients, the pyruvate level was increased (0.17–0.5 mmol/l, norm 0.09–0.12 mmol/l).

The total carnitine blood levels in children ranged from 46.7 to 106.1 μmol/l, averaging at (M ± m) 71.4 ± 3.5 μmol/l. There was no difference in total carnitine level between the patients with congenital myopathies and the control group.

The mean free carnitine level of 35.0 ± 1.9 μmol/l was within the normal range, but it was lower than that in the control group ($p < 0.05$). In two children with structural myopathy, free carnitine level was at the lower limit of the normal range.

The mean level of acylcarnitines was normal (36.9 ± 2.4 μmol/l). Likewise, the level of acetylcarnitine is not elevated. Of note, levels of other acylcarnitines were moderately elevated in three patients.

The proportion of free carnitine in the total carnitine was reduced—49% (the normal range 70–80%). The proportion of the esterified forms accounted for 51% of the total carnitine, which significantly exceeded the corresponding values of healthy individuals (20–30%). The ratio of acylcarnitines and free carnitine AC/C0 was increased to 0.9–1.7, averaging at 1.1 ± 0.08 (norm <0.6), which was consistent with carnitine insufficiency. In the control group of conditionally healthy children,

the ratio of AC/C0 was 0.66 ± 0.03 and was significantly different from that in groups 1 and 2 ($p < 0.01$).

The comparison of the carnitine parameters in patients with mitochondrial encephalomyopathies and congenital myopathies showed that in mitochondrial diseases, carnitine deficiency was more pronounced. Although the levels of acylcarnitines, free and total carnitine, were not significantly different, in the group of children with mitochondrial diseases, there was a tendency for an accumulation of bound carnitine and a lower level of free carnitine. Additionally, an acetylcarnitine level was significantly higher, and the impairment of total carnitine composition was more pronounced in children with mitochondrial diseases than in those with congenital myopathies. This was confirmed by a higher ratio of AC/C0 (1.5 ± 0.05 vs. 1.1 ± 0.08; $p < 0.01$).

The detection of carnitine deficiency underscores the need for L-carnitine therapy. L-carnitine per os in a dose of 30–50 mg/kg/day (depending on age) was included in the complex of energy treatment (coenzyme Q10, succinates, vitamin B) of patients with mitochondrial diseases. Within 1 year, three courses of therapy (for 2 months) were prescribed with a period off treatment for 1–2 months.

After 10–12 months of a follow-up of 18 children, a distinct improvement in one third of the patients was demonstrated: a reduction of fatigue, an improvement of exercise tolerance, and a reduction in the frequency of headache and nausea attacks. In one half cases, a stabilization with minimal positive dynamics was observed. These 15 children showed a decrease in the blood lactate level to 1.4–2.3 mmol/l. At the same time, in three children with Leigh syndrome, despite the treatment, a moderate progression of the disease with a persistent lacticidemia was observed.

The total carnitine remained at the same level (70.5 ± 5.1 μmol/l); a tendency ($p > 0.05$) toward an increase in the free carnitine level (36.2 ± 2.9 μmol/l) and a decrease in the acylcarnitine level (34.3 ± 4.1 μmol/l), including acetylcarnitine, was noted. A significant improvement in the AC/C0 ratio was revealed—a decrease to 0.9 ($p < 0.001$); the proportion of free carnitine in total carnitine increased significantly to 52% ($p < 0.01$). In general, the data indicate favorable changes: a reduction in carnitine deficiency, an improvement of its function, and a reduction in ketogenesis and in the severity of lactate metabolism disorders.

8. Conclusion

Coenzyme Q10 and carnitine are important components of energy metabolism that are involved in many biological processes in the human body. Our data suggest that there is an insufficiency of these compounds in patients with mitochondrial diseases. Our studies have not revealed the severe deficiency of these substances, while the evidence for a relative insufficiency were found. According to our laboratory data, coenzyme Q10 deficiency is manifested by a significantly low ratio of coenzyme Q10/cholesterol. Lower absolute level of coenzyme Q10 and tendency toward a more pronounced decrease in the Q10/cholesterol ratio in older patients (6–16 years), in our opinion, are consistent with the progressive course of mitochondrial pathology.

Carnitine deficiency is manifested by a tendency toward a decrease in the free carnitine blood level, a pronounced decrease in its proportion in total carnitine, and a significant increase in the ratio of bound and free carnitines.

The coenzyme Q10 and free carnitine insufficiency certainly adversely affects the course of the disease. The causes for these disorders remain unclear. Perhaps, defective mitochondria are not able to provide adequate biosynthesis of coenzyme Q10. Depletion of free carnitine is likely to occur as a result of the activation of

conjugation of acyl radicals, which accumulate in the disorders of respiratory chain and oxidative phosphorylation.

Given a crucial role of carnitine and coenzyme in mitochondrial energy processes, the insufficiency of these compounds should be treated. Clinical heterogeneity of mitochondrial diseases justifies further research in homogeneous patient groups in order to develop evidence-based recommendations and ensure higher treatment efficacy. Furthermore, a moderate carnitine deficiency in congenital structural myopathies provides indications for carnitine supplementation.

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