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REVIEW ARTICLE

Mass Spectrometric Analysis of L-carnitine and its Esters: Potential Biomarkers of Disturbances in Carnitine Homeostasis

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Abstract: ***Purpose*:** After a golden age of classic carnitine research three decades ago, the spread of mass spectrometry opened new perspectives and a much better understanding of the carnitine system is available nowadays. In the classic period, several human and animal studies were focused on various distinct physiological functions of this molecule and these revealed different aspects of carnitine homeostasis in normal and pathological conditions. Initially, the laboratory analyses were based on the classic or radioenzymatic assays, enabling only the determination of free and total carnitine levels and calculation of total carnitine esters' amount without any information on the composition of the acyl groups. The introduction of mass spectrometry allowed the measurement of free carnitine along with the specific and sensitive determination of different carnitine esters. Beyond basic research, mass spectrometry study of carnitine esters was introduced into the newborn screening program because of being capable to detect more than 30 metabolic disorders simultaneously. Furthermore, mass spectrometry measurements were performed to investigate different disease states affecting carnitine homeostasis, such as diabetes, chronic renal failure, celiac disease, cardiovascular diseases, autism spectrum disorder or inflammatory bowel diseases.

***Results*:** This article will review the recent advances in the field of carnitine research with respect to mass spectrometric analyses of acyl-carnitines in normal and various pathological states.

***Conclusion*:** The growing number of publications using mass spectrometry as a tool to investigate normal physiological conditions or reveal potential biomarkers of primary and secondary carnitine deficiencies shows that this tool brought a new perspective to carnitine research.

Keywords: L-carnitine, acylcarnitines, mass spectrometry, carnitine homeostasis, cardiovascular diseases, diabetes, autism spectrum disorder, chronic renal failure.

1. INTRODUCTION

Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is a vitamin-like water soluble small molecule that is found in almost all mammalian species, several micro-organisms and plants. This compound has a long, more than one hundred years old history. It was discovered in 1905 [1] in muscle extract and although its chemical structure was elucidated already in 1927 [2], its primary physiological function, and its importance in fatty acid metabolism, was established only around 30 years later [3].

Since carnitine possesses a chiral carbon, it has two enantiomers: D- and L-carnitine, however, only the L-isomer is physiologically active [4]. Carnitine is present in the body as free and esterified form (acylcarnitines), which make up the carnitine system together with the cellular proteins required for their metabolism and transport. Most of the endogenous carnitine pool is distributed in the skeletal and cardiac muscle (approximately 98%) and only less than 1 % is located within the plasma.

Over the past 30-40 years, due to the intensive research on carnitine metabolism, several physiological functions of this molecule were established and different aspects of carnitine homeostasis in normal and pathological conditions have been revealed [5]. Initially, the measurements of carnitine were based on classic or radioenzymatic assays [6], which provided only the determination of free and total carnitine levels and calculation of the amount of the total carnitine esters without any information of the composition of the acyl groups. Introducing mass spectrometry, a novel

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Table 1. Types of human carnitine acyltransferases and their cellular localization.

Enzyme Group	Substrate Specificity	Localization
Carnitine Acetyltransferase	Short-chain acyl groups (C1-C4)	Nucleus, mitochondrium, peroxisomes, microsomes
Carnitine Octanoyltransferase	Medium-chain acyl groups (C5-C12)	Peroxisomes, microsomes
Carnitine Palmitoyltransferase I, II	Long-chain acyl groups (>C12)	Mitochondrium

and highly sophisticated technique [7] facilitated the expansion of the measurements from free carnitine to specific and sensitive determination of different carnitine esters and thus this tool brought a new perspective to carnitine research.

2. BIOCHEMICAL PROPERTIES AND FUNCTIONS OF CARNITINE

A well-known biochemical property of carnitine is related to its ability to form acyl-carnitine esters with organic acids in certain enzymatic reactions. Its classic physiological role can also be derived from this chemical property since neither the free long-chain fatty acids, nor their Coenzyme-A esters can cross the inner mitochondrial membrane alone; the transport is possible exclusively in carnitine ester form. Therefore, carnitine plays an indispensable role in the mitochondrial oxidation of long-chain fatty acids and in ketogenesis [8, 9].

The formation of acylcarnitines catalyzed by carnitine acyltransferases (Table **1**), a family of a protein which has a wide and overlapping carbon chain-length specificity, various cellular localization and metabolic functions (eg mitochondrial and peroxisomal fatty acid oxidation, cellular CoASH homeostasis). The detailed structures and functions of these enzymes are discussed in a recent review [10].

The transport itself is achieved by the carnitine cycle, which consists of three steps. The first step, in which the long-chain acyl moieties are transferred between the Coenzyme-A and carnitine by the reversible reactions catalyzed by carnitine palmitoyltransferase I (CPTI), takes place in the outer mitochondrial membrane. (Fig. **1**). The second step is the direct transfer of the carnitine esters across the membrane, which is catalyzed by the carnitine translocase (CACT). In the last step, carnitine palmitoyltransferase II (CPTII) reconverts acylcarnitines back to acyl-CoA species and carnitine at the matrix side of the inner mitochondrial membrane. [9].

It is clear nowadays that the biological function of the carnitine cannot be reduced to the role of fatty acid oxidation. Among others, carnitine modulates the acyl-CoA/CoA ratio, which in turn regulates many mitochondrial enzymes involved in tricarboxylic acid (TCA) cycle, gluconeogenesis, urea cycle and fatty acid oxidation [11]. It contributes to energy storage in the form of acetyl carnitine [4] and modulates the toxicity of partially metabolized acyl groups by promoting their excretion in carnitine ester form [12]. Furthermore, it is involved in the reesterification of triacylglycerol in the endoplasmic reticulum before its excretion in the form of very-low-density lipoproteins, in the stimulation of the oxidative metabolism of pyruvate and branched chain amino acids, in the deacylation and reacylation processes during the transformation of membrane phospholipids in erythrocytes, in the transmission of fatty acids between phospholipids and triglycerides in neurons, in the synthesis and elongation of multiply unsaturated fatty acids as well as stabilization of proteins and membranes [13].

3. HOMEOSTASIS OF CARNITINE IN HUMANS

Since the function of L-carnitine is vital in the intermediary metabolism it is not surprising that its endogenous plasma and tissue concentrations are maintained within a relatively narrow homeostatic range. Processes involved in this homeostasis include carrier-mediated gastrointestinal absorption from dietary sources, endogenous biosynthesis, extensive renal tubular reabsorption and compartmentalization through carrier-mediated transport between plasma and tissue [14].

The carnitine pool comprises extracellular and intracellular compartments and it can be found in the body as free and esterified form (acylcarnitine). The various esters of carnitine with short-, medium- and long-chain fatty acids are all present in biological systems. The whole body carnitine content is in a fairly dynamic state. Migration of carnitine and acylcarnitines occurs among the gastrointestinal tract, the liver, the kidney and carnitine dependent tissues such as heart or skeletal muscle. Any metabolic change is accompanied by redistribution between the carnitine and acylcarnitine pools of the tissues involved [15]. Although 99% of the carnitine amount has an intracellular localization, mainly found in the skeletal and cardiac muscle (98 %), the relationship between serum acylcarnitine (AC) and free carnitine (FC) is highly sensitive to intramitochondrial alterations, therefore the measurement of AC and FC can reflect metabolic alterations in normal and abnormal situations as well, and the acylcarnitine profile analysis could be a promising tool for the investigation of these circumstances. Such normal conditions include fasting, pregnancy and neonates [16], pathological situations with altered carnitine ester profile include several inborn errors of metabolism, malabsorption, medical interventions such as haemodialysis and treatment with valproate, pivalic acid-containing antibiotics and some chemotherapeutic agents (*e.g.*, cisplatin, doxorubicin

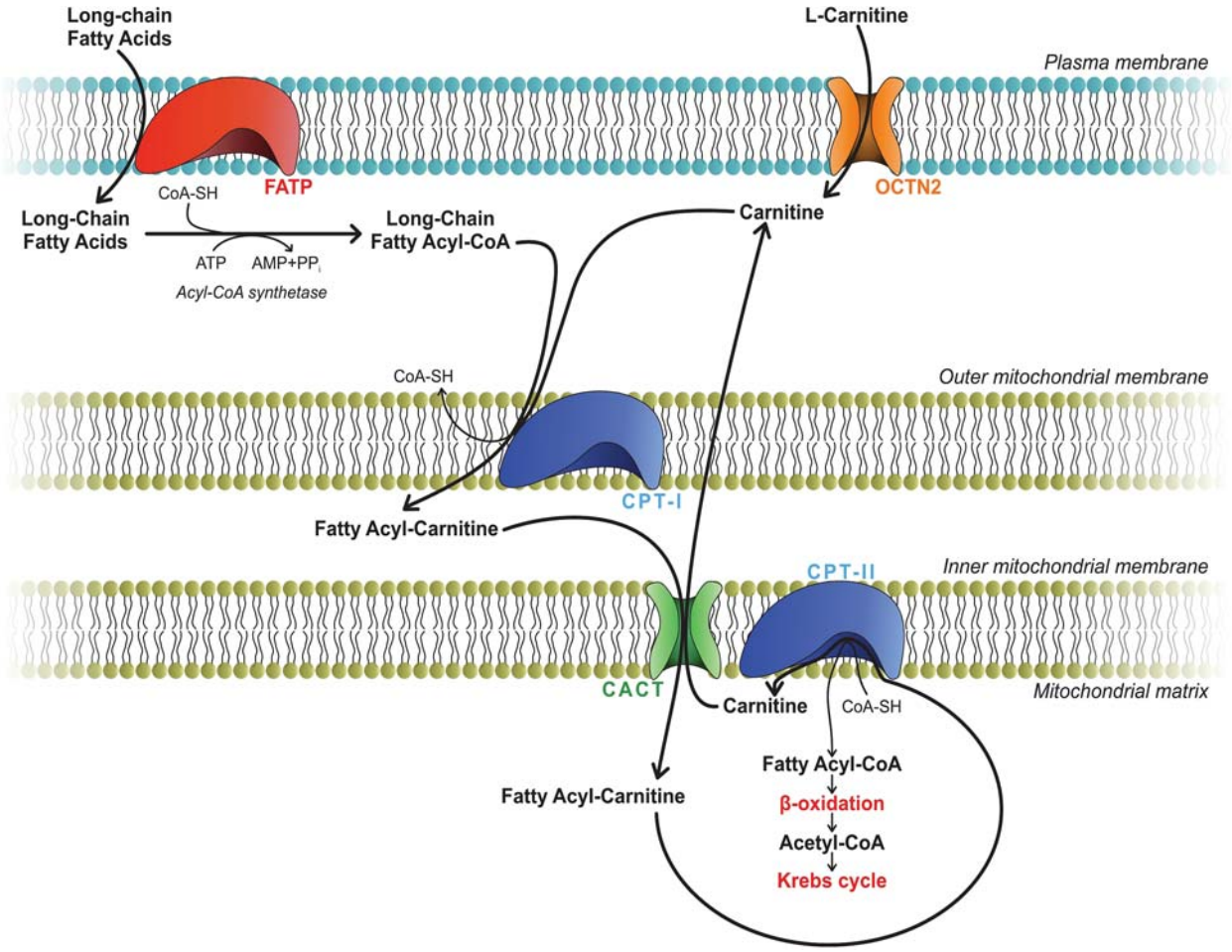

Fig. (1). The Carnitine System. Role of carnitine in the transport of long chain fatty acids from the cytosol into the mitochondrial matrix. FATP: fatty acid transporter protein; OCTN2: organic cation transporter 2; CPT-I: carnitine palmitoyl transferase I; CPT-II: carnitine palmitoyl transferase II; CACT: carnitine acyl carnitine translocase.

and ifosfamide) [17]. Recently, plasma ACs have been proposed as biomarkers of insulin resistance and metabolic inflexibility in adults, as well as that of autism [11, 18].

4. CARNITINE DEFICIENCIES

Carnitine deficiency represents a heterogeneous group of diseases with widely varying clinical symptoms. Since carnitine is necessary not only for fatty acid oxidation but also plays a crucial role in cellular homeostasis of free and acyl-CoA, it is not surprising that carnitine deficiency has a wide variety of clinical manifestations. Moreover, it adds to the complexity that several other factors affect the carnitine requirement such as age, diet, metabolic conditions (stress, fed versus fasting state, and rest versus exercise) and tissue dependence on fatty acid oxidation [19, 20]. The definition of carnitine deficiency is reduced tissue carnitine concentration that is below the requirement for normal cellular metabolism. Data from clinical and biochemical studies imply that tissue carnitine levels may have to fall to less than 10% to 20% of normal before the biologic effects can be clinically significant [21]. Carnitine deficiency can be primary or secondary (Fig. **2**). In primary systemic carnitine deficiency mostly a disturbed cellular uptake of carnitine can be observed, while in secondary carnitine deficiency the carnitine depletion is the secondary cause of other conditions or disease.

4.1. Primary Carnitine Deficiency

The systemic primary carnitine deficiency (SPCD) is a rare hereditary disorder of fatty acid oxidation caused by mutations in the *SLC22A5* gene encoding the high-affinity carnitine transporter, OCTN2 [22]. In the disease, inherited in an autosomal recessive manner, the affected transporter is not able to take up free carnitine from the circulation, which is accompanied by urinary loss of free carnitine because of the impaired renal tubular plasmalemmal uptake defect finally leading to tissue carnitine deficiency. Systemic primary carnitine deficiency shows a broad clinical spectrum from a metabolic decompensation in infancy, cardiomyopathy in childhood to fatigability in adulthood, or even asymptomatic adults [23]. In the classical form there are two main presentations with the onset in

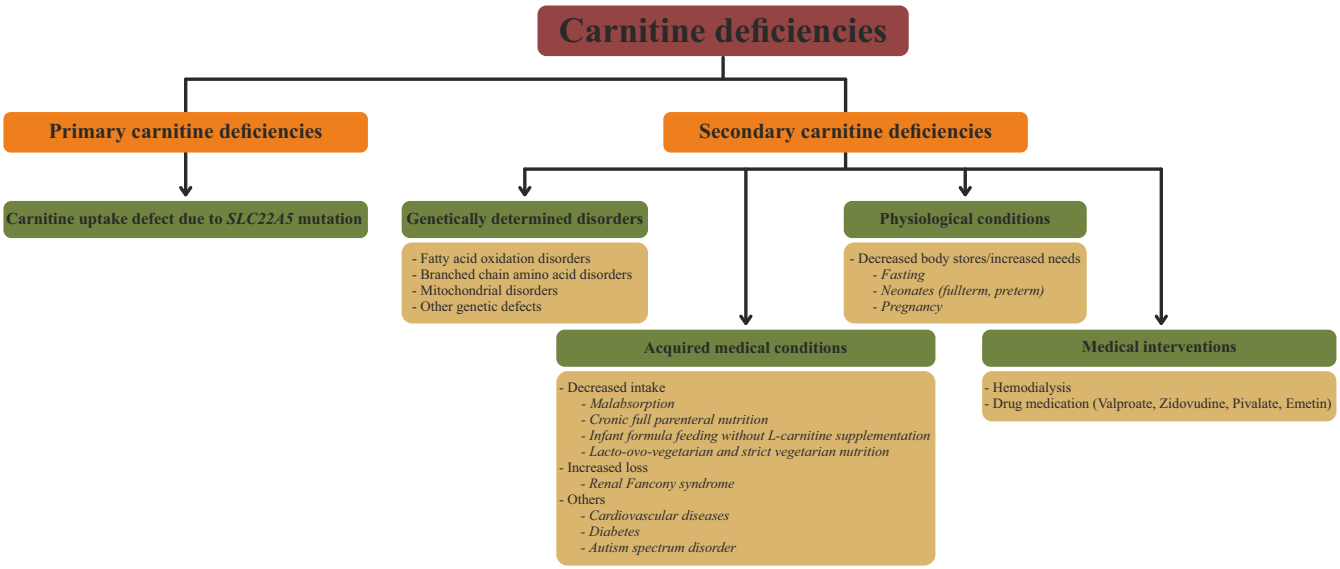

Fig. (2). Etiology of carnitine deficiencies.

infancy with acute nonketotic hypoglycemic episodes, poor feeding, failure to thrive, hypertrophic cardiomyopathy, hepatomegaly, elevated liver transaminases, hyperammonemia triggered by fasting or common illnesses such as upper respiratory tract infection or gastroenteritis, hypotonia, lethargy or encephalopathy associated with hypoglycemia, Reye syndrome and a delay in gross motor development due to muscle weakness. Primary systemic carnitine deficiency can lead to sudden infant death syndrome [23, 24]. The second presentation is onset in childhood (1 to 7 years) with progressive cardiomyopathy and muscle weakness, hepatomegaly and steatosis of the liver. Diagnosis is based on the detection of very low levels of plasma free carnitine followed by the confirmation with diminished carnitine transporter activity in skin fibroblast [25] or the verification of biallelic pathogenic variants in the *SLC22A5* gene [26, 27]. Infantile metabolic and childhood myopathic presentations of SPCD can be fatal if untreated. Carnitine supplementation can prevent the majority of the symptoms and preserve cardiac function [23]. The administration of 100-400 mg/kg/day oral levocarnitine (L-carnitine) improves metabolic decompensation and skeletal and cardiac muscle functions if started before the irreversible organ damage. Hypoglycemic episodes are treated with intravenous dextrose infusion. The long-term prognosis of SPCD is favorable as long as affected individuals continue with carnitine supplements [26].

While previously, in many cases, the variable clinical symptoms only subtly implied carnitine deficiency to the clinicians, introducing tandem mass spectrometry technique into the newborn screening program nowadays enables the easy identification of primary carnitine deficiency in infants through the detection of low levels of free carnitine [28-30]. Interestingly, several studies reported women whose SPCD diagnosis was only revealed after newborn screening identified low carnitine levels in their infants. Almost half of the mothers were asymptomatic, the others complained about decreased stamina, easy fatigability with exercise and fasting intolerance [31, 32]. Since there is a lifetime risk of morbidity and risk of sudden death of primary carnitine deficiency, the identification of adult patients with this condition is an added benefit of expanded newborn screening programs [31].

4.2. Secondary Carnitine Deficiency

In secondary carnitine deficiency, also referred to as carnitine insufficiency, the carnitine depletion can be associated with physiological conditions, such as fasting or pregnancy or could be the consequences of pathological conditions such as genetically determined metabolic disorders, acquired medical conditions or other external factors [33, 34]. Although these secondary carnitine deficiencies have less severe clinical consequences than the primary form, their prevalence is more common. In secondary carnitine deficiency the carnitine pool is shifted towards AC from free carnitine, since carnitine is utilized to eliminate acyl groups accumulating due to the underlying metabolic dysfunction. Thus in secondary carnitine deficiency normal or slightly decreased plasma FC levels and increased AC/FC ratio can be observed typically [15].

Carnitine disturbances may also develop due to several acquired medical conditions. Among others decreased intake (malnutrition, malabsorption such as in celiac disease, cystic fibrosis), decreased body stores (HIV/AIDS) or increased loss (renal Fanconi syndrome) could be responsible for these disturbances [14, 33].

The secondary carnitine deficiency can be induced by medical interventions like haemodialysis or some drug medication as well. Such a drug is pivaloic acid (trimethyl-acetic acid), the chemical structure of this

prodrug makes the entrance of this metabolite into the β oxidation impossible. Pivalate liberated from drugs forms ester with carnitine, and the sustained urinary loss of pivaloylcarnitine causes carnitine depletion [35, 36]. Other drugs, like the valproic acid (dipropyl acetic acid) causes also carnitine depletion, however, the mechanism is totally different since the valproate has poor carnitine ester forming capacity [36].

5. PHYSIOLOGICAL CONDITIONS WITH CARNITINE DEFICIENCY

5.1. Fasting

Altered dietary conditions result in a change of free carnitine and acylcarnitine concentrations. During carbohydrate depletion, as during starvation or after eating a high-fat diet, the proportion of acetylated carnitine in the liver and kidney significantly increases, while a high carbohydrate diet leads to acetylcarnitine decrease in the liver [37, 38]. It was observed that during fasting or during diabetic ketosis there is a delayed decrease in L-carnitine and a fast increase in acylcarnitines with various carbon-length [37]. It is concluded from animal studies that a redistribution of carnitine and its relative esters to the brain takes place during fasting, with the purpose to use acylcarnitines for energy production and for the delivery of acetyl-groups [37].

5.2. Neonates (Full Term, Preterm)

In healthy neonates, there is a strong correlation between total acylcarnitine concentration measured in cord blood and birth weight, moreover, lower umbilical artery pH caused accumulation of long-chain acylcarnitines [16]. Therefore, acylcarnitine profile may serve as a further useful parameter for identifying perinatal asphyxia and other metabolic disturbances in utero.

Regarding the postnatal changes of carnitine homeostasis in healthy newborns, blood levels of nearly all acylcarnitine species, compared with cord blood, were significantly higher on the postnatal day 5, whereas free carnitine remained unchanged. Total acylcarnitine/free carnitine-ratio increased, whereas the free carnitine/total carnitine-ratio further decreased. A relative deficiency of free carnitine in the early neonatal period may affect fatty acid oxidation rates and thus be of potential pathophysiological relevance under conditions with higher energy demands, *e.g*. in sepsis [16]. Moreover, the longitudinal study of acylcarnitines in preterm infants showed that postnatal carnitine levels are higher in very immature preterm infants compared with full-term infants, but become lower on day 28 [39]. Also, other studies implied, that particularly premature infants are not able to maintain their carnitine reserves without exogenous supply [40, 41]. Regarding the functional consequences, controversial data have been presented, however, a consensus has been achieved that favored the use of carnitine containing oral feeding, or supplementation when parenteral nutrition was needed. Indeed, the carnitine reserves of the neonates did not increase without oral intake, the human milk was considered as the best source. It is supported by a recent research as well, which demonstrated that the formula feeding of preterm infants is metabolically not equivalent to the breast-feeding [42]. There are still several uncertainties around the functions of the carnitine esters in the neonatal period, however, a study investigating the carnitine ester profile in mothers during pregnancy, after delivery and simultaneously in the cord- and neonatal blood of the neonates, revealed very dynamic profile changes in association with these events [43]. Albeit the explanation of the dynamic changes remains to be elucidated, the observation clearly demonstrates a very active involvement of the carnitine molecule in the complex metabolic processes associated with the profound metabolic alterations of pregnancy, the delivery and the neonatal adaptation.

6. PATHOLOGICAL CONDITIONS WITH CARNITINE DEFICIENCY

6.1. Inborn Errors of Metabolism: Clinical Applications of Acylcarnitine Profiling

As discussed in detail in the biochemical properties of carnitine the ability to esterify and transport metabolites implies that the acylcarnitine profile may be a useful indicator of metabolic changes, both related to physiological conditions and to disease states [37, 44]. Moreover, since the broad spectrum of esterified carnitine-metabolites differ both chemically and metabolically, changes in individual acylcarnitines may suggest changes in specific metabolic pathways [37]. The meticulous monitoring of dynamic changes in acylcarnitines may lead to a better understanding of disease pathomechanism but also has implications for the design of better treatment approaches.

The acylcarnitine profile has been shown to be very useful and successful in the identification of inborn errors of metabolism in neonatal screening using tandem mass spectrometry (MS/MS) based analysis as well as in selective screening and monitoring for inborn errors of metabolism [45, 46].

Inborn errors of metabolism can lead to a build-up of toxic intermediate metabolites and can be fatal or result in serious organ damage early in life. Therefore, early comprehensive neonatal screening is used to detect abnormalities to avoid major physical and neurological effects. Mass spectrometry-based analysis of amino acids and acylcarnitines is used to diagnose inborn errors of metabolism in newborns by identifying and quantifying specific metabolites [46] and providing early treatment to infants with disorders of mitochondrial β-oxidation, aminoacidopathies, organic acidemias, disorders of the urea cycle, and rare disorders of metabolism before the onset of symptoms. In addition complementation of tandem mass spectrometry with liquid chromatography is used to explore creatin metabolism and bile acid disorders [47]. Tandem mass spectrometry profiling of acylcarnitines and amino acids has been used as a first-tier screening

method, however, only amino acid screening has been validated routinely by amino acid analysis as a second-tier validation. Due to the lack of standard second-tier acylcarnitine validation methods results of acylcarnitine profiling have not been routinely confirmed by an independent second method. A recent publication of Hoppel *et al*. [48] described the successful application of a validated UHPLC-MS/MS second-tier method for the quantification of total carnitine, free carnitine, butyrobetaine, and acylcarnitines with the ability to separate constitutional isomers and diastereomeric acylcarnitines and give values with a high level of accuracy and precision.

In quantifying targeted metabolites measured by tandem mass spectrometry, many factors may have an effect on the results of acylcarnitines and amino acids. The concentration of metabolites can vary due to the disturbed biological metabolic process induced by medical treatment or influenced by specimen status based on various external factors [49]. Age-related variations in acylcarnitine and free carnitine concentrations have been observed and should be taken into consideration when diagnosing and managing inborn errors of metabolism [45].

A wide spectrum of organic acidurias is associated with secondary carnitine deficiency. In those diseases, the organic acids are accumulated due to a metabolic blockade, and the organic acid overload can deplete the carnitine reserves *via* urinary carnitine ester loss. On the other hand, this phenomenon can also be used for diagnostics, in many organic aciduria patients, secondary free carnitine deficiency occurs with the elevation of specific acylcarnitines. Thus several tandem mass carnitine ester profiles are characteristic for a specific disease. Recently metabolomics methodology has been used to identify patients with methylmalonic and propionic acidemia.

Other important examples are fatty acid oxidation defects, such as very long-chain acyl-CoA dehydrogenase deficiency (VLCAD) and medium-chain acyl-CoA dehydrogenase deficiency (MCAD) [50].

Experience with newborn screening (NBS) for disorders of fatty-acid oxidation (FAOD) is now becoming available worldwide due to the increasing number of programs [45]. Fairly distinct FAOD spectrum can be observed among various ethnic groups. Incidence calculations from international reports calculated a combined incidence of all FAOD of approximately 1:9,300. Consequently, there is a strong evidence and a significant prevalence for a clear benefit of NBS for medium-chain acyl-CoA dehydrogenase deficiency (MCAD) only in countries with a high percentage of Caucasians, with very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD) and long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency (LCHAD) being additional candidates.

In addition to identifying inborn errors of metabolism, the acylcarnitine profile may also be useful in identifying other metabolic perturbations such as biochemical alterations in diabetes and atherosclerosis [51, 52]. The metabolome and the targeted carnitinome or acylcarnitine profile are becoming increasingly popular tools.

Besides the diagnostic yield of acylcarnitine profiling the secondary carnitine deficiency in the inborn errors of metabolism raises the question of carnitine supplementation. Carnitine supplementation is used in the treatment of primary carnitine deficiency, and also where the deficiency is a secondary complication of several inborn errors of metabolism, such as organic acidaemias and fatty acid oxidation defects in children and adults [53]. A recent comprehensive meta-analysis [54] found that there are no published or ongoing randomized controlled clinical trials for carnitine supplementation in inborn errors of metabolism. In the absence of any high level evidence both on the effectiveness and safety of carnitine and on the necessary dose and frequency to be prescribed, decisions on supplementation should be based on clinical experience and in conjunction with preferences of the individual where appropriate.

6.2. Acquired Medical Conditions

6.2.1. Celiac Disease

Secondary carnitine deficiency may emerge due to acquired medical conditions, too. Besides the number of other conditions, decreased intake due to diets with low carnitine content or decreased reabsorption in malabsorption syndromes may induce carnitine deficiency [33]. Celiac disease (CD) is a representative example of the malabsorption syndromes, in which the intestinal mucosa is injured in a reversible, gluten dependent manner, resulting in decreased intestinal absorptive surface. Disturbed lipid and lipoprotein metabolism can be observed in this complex autoimmune type of gastrointestinal disorder [55-57].

The therapy includes withdrawal of the alimentary gluten and the introduction of the diet usually results in dramatic clinical improvement and normalization of numerous metabolic deteriorations [55, 58]. There is limited number of studies in the literature dealing with carnitine status in CD patients. In their study, Lerner *et al*. investigated the carnitine concentrations in sera of pediatric CD subjects, and found that the total serum carnitine concentration is decreased in patients with active disease as compared with normal subjects, while it was unchanged in CD patients with gluten withdrawal - associated non-active disease. The decrease of carnitine reserves in active disease is likely secondary to the mucosal injury associated with damage to the absorption [59]. In another research, Yuce and colleagues compared the serum carnitine levels in 30 children with CD to 30 controls using mass spectrometry. However the mean serum free carnitine levels were significantly lower in the patient group, no difference was observed between patients with and without diarrhea [60]. Carnitine insufficiency leading to inhibition of oxidative metabolism of fatty acids could be responsible for several rare extraintestinal manifestations which can be observed among CD patients, such as cardiomyopathy, encephalopathy,

myopathy with hypotonia and hyporeflexia, or disturbed liver function. Until recently, two CD patients with dilated cardiomyopathy [61, 62] and one with encephalopathy [63] were published in the literature. All of them presented disturbed carnitine homeostasis presumably due to their malabsorption disorder. L-carnitine supplementation was introduced in the treatment of fatigue present in adult CD patient [64] after establishing a low level of carnitine. Our mass spectrometry measurements of the carnitine ester profile confirmed that carnitine homeostasis is disturbed in this type of disorder [65]. Albeit the level of free carnitine did not differ in the celiac disease patients compared to healthy controls, markedly lower amounts could be detected in most carnitine esters in clinically asymptomatic adult celiac disease patients. These data represent that gluten withdrawal alone does not necessarily normalize all elements of the disturbed carnitine homeostasis.

6.2.2. Inflammatory Bowel Diseases

The inflammatory bowel diseases (IBD) are chronic relapsing inflammatory conditions of the gastrointestinal tract resulting from an inappropriate immunological response to the commensal intestinal microbiota in genetically susceptible individuals. Crohn's disease (CrD) and ulcerative colitis (UC) represent the two common forms of IBD [66]. The inflammation of CrD is transmural and discontinuous, the ileum and the colon are involved mainly, but it can affect any region of the gastrointestinal tract. Contrary to CrD ulcerative colitis is characterized by superficial colonic inflammation that progresses continuously from the rectum for a varying distance proximally [67].

Short-chain fatty acids (SCFAs), mainly butyrate and propionate are the major energy sources of the epithelial cells of the distal colon, however, these cells are able to metabolize other types of fuels, such as glucose and glutamine, but only at a much lower rate [68]. SCFAs are originated from carbohydrates by bacterial degradation, they are readily absorbed by the colon and serves as energy fuels for the colonocytes and other tissues, such as the skeletal muscle [69]. Increased or moderately decreased stool SCFA concentrations can be observed in patients with distal UC, reflecting their altered absorption [70, 71]. UC can, therefore, be considered essentially as an SCFA oxidation failure-associated disease, where the energy deficiency is a primary event in the development of the disease [68].

L-carnitine has an indispensable role in energy metabolism. Furthermore, it is capable to form esters with several medium-chain fatty acids and SCFA of both endogenous and exogenous origins [8, 35]. The circulating carnitine ester spectrum can reflect affected cellular metabolism of short-, medium-, and long-chain fatty acids [72]. Mass spectrometric analyses of carnitine esters in UC patients showed significantly lower levels of fasting propionyl-, butyryl-, and isovalerylcarnitine esters, resulting in lower levels of SCFA carnitine esters. Although the pathogenesis of UC is still unidentified, the pivotal role of the diminished availability of SCFAs for the enteral cells is in the focus of a widely accepted hypothesis. Moreover, higher levels of octanoyl-, decanoyl-, myristoyl-, palmitoyl-, palmitoleyl- and oleylcarnitine were detected in the patients' group compared to control subjects [73]. Based on current knowledge, it is difficult to explain these changes due to the limited nature of data. Only a few studies are available reporting alterations of fatty acid metabolism. However, the data are inconsistent, but suggest the involvement of LCFA metabolism in UC [74, 75]. Evaluation of cardiac function in IBD patients asymptomatic for cardiovascular diseases and to find a correlation between cardiac data and plasma carnitine ester profile were in the focus of another research. In this study, IBD patients displayed significantly lower levels of isovaleryl-carnitine, tiglyl-carnitine, octenoylcarnitine and decanoyl-carnitine. Although significant correlations were observed between some carnitine esters and echocardiographic parameters. The authors supposed that the deficiency of SCFA carnitine esters may play an essential role in cardiac involvement in the course of IBD and could lead to dilated cardiomyopathy [76]. Serum carnitine, acetylcarnitine and palmitoylcarnitine concentrations were determined in CrD and UC patients with malnutrition along with in patients maintained on enteral (EN) or total parenteral nutrition (TPN). Although the mass spectrometric measurement demonstrated carnitine deficiency among long-term EN and TPN subjects, no significant difference was detected in the levels of the three compound between CrD or UC and the corresponding control subjects [77].

In 2004, Peltekova *et al*. identified two novel polymorphisms in the carnitine/organic cation transporter gene cluster (*SLC22A4* and *SLC22A5*, encoding OCTN1 and OCTN2, respectively), that conferred risk for CrD [78]. Although the functional variants in general, may influence the carnitine homeostasis, surprisingly the plasma carnitine ester profile analyses of the CrD patients according to the different *SLC22A4* C1672T and *SLC22A5* G-207C genotypes demonstrated that the genotype variations were not associated with altered carnitine ester profiles [79]. In another study, the effect of an intronic variant (IGR2230a_1) of the *SLC22A5* gene on carnitine homeostasis was examined. Unfortunately, no association could be established between the genotype and the carnitine ester profile in CrD and UC patients [80].

Nevertheless, very limited data are available on the carnitine status in CrD; these studies are from the enzymatic radiochemical carnitine determination era. Using this approach, Demirkol *et al*. observed plasma free and total carnitine levels were significantly lower in paediatric patients with CrD [81]. In another study, a dramatically decreased free carnitine and significantly increased long- and short-chain acylcarnitine concentrations were detected, leading to a net increase in total carnitine. However, in that study patients with different types of inflammatory disorders after multiple trauma or head injury were examined [82]. Mass spectrometry study of the carnitine homeostasis in CrD

patients revealed complex changes in the carnitine esters, involving a lower amount of butyrylcarnitine, in a mixed population of CrD-affected patients [79].

Proinflammatory cytokines are essential in the pathogenesis of CrD. Activation of NF-kB, which plays a central role in immune and inflammatory responses and is involved in the proinflammatory cytokine gene transcription, is increased in the intestinal mucosa of CrD patients [83]. Butyrate inhibits the translocation of NF-kB transcription factor to the nucleus, thereby preventing the activation of proinflammatory genes [84]. Thus, the observed butyrylcarnitine level can be an element of the development of inflammatory processes.

6.2.3. Cardiovascular Diseases

Cardiovasular diseases (CVDs) are the leading cause of death and disability worldwide according to WHO reports. The number of people suffering from CVDs is still increasing as a consequence of an aging population and changes in lifestyle. CVDs are disorders of heart and blood vessels including coronary heart disease, myocardial infarction (MI), heart failure (HF), hypertension, stroke, hypercholesterolemia, diabetes, chronic kidney disease, peripheral arterial disease and vascular dementia. The underlying process in almost all CVDs is atherosclerosis and it is most commonly diagnosed in elderly patients. Although each disorder has different symptoms, they share common risk factors, such as: smoking, unhealthy diet, lack of physical activity leading to obesity, elevated blood pressure and high levels of blood glucose. The pathogenesis of most CVDs is still not fully understood and intensive research efforts have been made to identify not only risk factors but also biomarkers for early diagnosis and disease prognosis. Recently, a growing number of publications appeared in the field of metabolomics, which is a key technology of modern systems biology that focuses on obtaining an integral depiction of the current metabolic status of an organism, associated with physiological and pathophysiological processes [85-88]. As part of metabolomic studies carnitine and its esters are in the focus of these investigations since they have an indispensable role in beta oxidation of fatty acids and earlier it has been shown that acylcarnitines accumulate in the aortic tissues during the development of atherosclerotic lesions [89]. Moreover, recently a link between the supplementation of L-carnitine and the accelerated development of atherosclerosis has been described [90].

Rizza *et al* performed a targeted mass spectrometry based profiling of 49 metabolites including free carnitine and 30 acylcarnitines in sera in order to identify biomarkers predicting major cardiovascular events (MACEs) in elderly people. Differences in the levels of four distinct acylcarnitines (C10:2, C14, C16, C18:1) were observed between event (cardiovascular deaths, nonfatal myocardial infarction, nonfatal strokes and peripheral artery surgeries) and no event subjects. Principal Component Analysis and Random Survival Forest analysis revealed that medium- and long-chain acylcarnitines became an independent predictor of MACEs [91].

In another study, the primary goals of Shah and colleagues were to assess the predictive power of metabolite profiles to anticipate the presence of coronary artery disease (CAD) and to explore the relation of metabolite profiles with subsequent cardiovascular events. To achieve this goal mass spectrometric measurements of 69 metabolites, including acylcarnitine species, were performed in two cohorts: an initial and a replication one. Concentrations of several acylcarnitines were different between cases and controls in the initial group, including C16 acylcarnitines (C16:1, C16:1-OH/C14:1-DC, C16:2, C18-OH/C16-DC), C8, C8:1-OH/C6:1-DC and C10:1 acylcarnitines. For most metabolites, these differences remained after adjustment for CAD risk factors. In the replication group after adjustment only C14:1-OH and C16:1-OH/C14:1-DC long-chain ACs were significantly different. Principal Component Analysis revealed that among others medium-chain ACs were significantly different between cases and controls in the initial group, however, this factor was only weakly significant in the replication group. Furthermore, a signature composed of dicarboxylacylcarnitines was predictive of cardiovascular events in individuals with CAD [92]. Another study from the same research group was aimed to investigate whether metabolic profiles were capable to predict adverse events in patients undergoing coronary artery bypass grafting (CABG). The authors reported that elevated levels of short-chain dicarboxylacylcarnitines, ketone-related metabolites and short-chain acylcarnitines were predictive of a composite endpoint of MI, repeat revascularization or death at any point after CABG [93].

Global perturbations of acylcarnitine metabolism were manifested in plaque tissue. Vorkas *et al*. observed the accumulation of medium- and long-chain ACs and finally, they hypothesized that beta-oxidation is truncated, consequently reducing the production of short-chain ACs and leading to the recruitment of unsaturated fatty acylcarnitines in order to compensate metabolic requirements. These findings are indicative of dysregulations in metabolic oxidation [94].

Heart failure (HF) is a complex syndrome, characterized by perturbations in energy homeostasis and metabolism. The reversibility and prognostic value of circulating markers associated with these changes remain unclear. Ahmad *et al*. performed a metabolomic study to characterize circulating metabolites associated with poor outcomes in chronic systolic HF patients and assess whether these prognostic profiles can be modified with mechanical circulatory support for end-stage HF with long-term left ventricular assist device (LVAD) support. In chronic HF patients, the observed elevated circulating long-chain acylcarnitine metabolite levels (C16 and C18) in plasma were independently associated with impaired cardiorespiratory capacity and also increased the risk of all adverse clinical outcomes. Since the abnormalities were modifiable with LVAD support in end-stage HF patients, the authors suggest

that these metabolites may serve as potential targets for new diagnostics or therapeutic interventions [95]. Similarly to Ahmad's results, Gupte and colleagues reported that mechanical support with LVAD improved metabolic and transcriptional defects in heart failure tissue. They performed a targeted metabolomic and transcriptomic studies of failing human left ventricular tissue obtained during LVAD insertion (heart failure samples) and at heart transplant (post-LVAD samples). As a comparison, nonfailing left ventricular wall samples procured from explanted hearts of patients with right heart failure were used. Mass spectrometry based metabolomic analyses revealed a 2.6-fold increase in pyruvate concentrations coupled with reduced Krebs cycle intermediates and short-chain acylcarnitines in heart failure tissue, suggesting a global reduction in substrate oxidation. Moreover, the authors observed that the detected metabolic changes were associated with decreased transcript levels for enzymes that catalyze fatty acid oxidation and pyruvate metabolism and for key transcriptional regulators of mitochondrial metabolism and biogenesis. Finally, they concluded that the observed reversibility of the severely defective metabolism in heart failure by LVAD suggests metabolic resilience of the human heart [96].

6.2.4. Diabetes

Diabetes mellitus is a complex heterogeneous group of metabolic disorders with an underlying absolute or relative insulin deficiency. Affected carbohydrate and lipid metabolism are well established, moreover, numerous studies demonstrated an association between the proper metabolic control and the development of complications in this disorder [97, 98].

Although carnitine represents a link between carbohydrate and fatty acid metabolism, divergent results of carnitine metabolism in diabetes mellitus have been reported so far. Carnitine examination studies in type 1 diabetes (T1D) are extremely rare and profoundly radioisotopic methods were used with its inherent limitation that enables the determination of the levels of free and esterified carnitine only [99]. Decreased plasma levels of carnitine in T1D compared to controls were reported in these studies. Besides the decreased free and total carnitine levels significantly higher level of acylcarnitines and acyl/free ratio were observed in another study. The authors concluded that the relative deficiency of free and total carnitine is time related and may have potential interactions with long-term complications in T1D [100].

Mass spectrometric measurements are used as a tool for analyzing the acylcarnitine profile of type 2 diabetes (T2D) in several studies to clarify the pathogenesis of this metabolic disorder [98]. Investigation of urine samples of diabetes patients revealed that these patients excrete more long-chain acylcarnitines (C12-C16) than the controls, supposedly caused by a disrupted fatty acid metabolism [101]. In another study significantly increased plasma acetyl-, medium-chain (C6, C8, C10) and long-chain (C14, C18:1) carnitine ester levels and decreased propionylcarnitine concentration were observed in T2D African-American women. The detected plasma acylcarnitine profiles suggested an incomplete long-chain fatty acid oxidation and altered tricarboxylic acid cycle activity [102]. To find out the site of derangements in fatty acid oxidation (FAO) and electron transport chain (*ETC*) activity in obesity and T2D the plasma acylcarnitine profiles were characterized in patients with obesity and T2D during fasting and insulin-stimulated conditions. In this study, T2D patients appeared to have increased levels of short- and medium-chain acylcarnitines, both saturated and hydroxyl, as well as C4-dicarboxylcarnitine which correlated with an index of poor glycemic control. Furthermore, free carnitine and long-chain acylcarnitines levels were elevated as well. Insulin infusion leads to a significant decrease in every acylcarnitine species between carbon length of 2 and 18. The elevated levels detected for long-chain acylcarnitines could be the results of increased flux of fatty acids into the mitochondria, whereas the accumulation of many shorter species in T2D suggesting a generalized complex oxidation defect [103]. Mass spectrometric analysis detected elevated levels of 8 amino acids (among others leucine/isoleucine and valine) and elevated levels of C3 and C5 acylcarnitines in obese versus lean subjects. The authors hypothesized that increased levels of branched-chain amino acids are a contributing factor to insulin resistance, moreover, the increased branched-chain amino acid catabolic flux may contribute to increased gluconeogenesis and glucose intolerance *via* glutamate transamination to alanine [104]. Investigation of serum acylcarnitine profiles in different glucose tolerance states was in the focus of Zhang's study. The higher amount of free carnitine and long-chain acylcarnitine was observed among the newly diagnosed T2D patients compared to controls. It may suggest different degrees of involvement of dysregulated mitochondrial function and incomplete long-chain fatty acid oxidation pathways in the natural course of T2D [105]. The observed circulating acylcarnitine profiles in type 1 diabetes, type 2 diabetes and metabolic syndrome patients in our study implied that carnitine homeostasis could have similarities in these metabolic disorders. It is supposed that an inhibited carnitine palmitoyltransferase-1 (CPT1)-mediated entry of free fatty acids into mitochondria could be responsible for the observed reduced levels of both long-chain and medium-chain carnitine ester metabolites in our patients [106]. Acylcarnitine measurements were performed as part of a targeted metabolic profiling approach to reveal whether the levels of these species could serve as early biomarkers in type 2 diabetes. Sun and colleagues found a significant association between a panel of acylcarnitines, especially long-chain acylcarnitines and future risk of type 2 diabetes. Furthermore, it supposed a substantially improved predictive ability for incident diabetes beyond conventional risks, including BMI and fasting glucose [107]. In another prospective nested case-cohort study short-chain and long-chain

acylcarnitine species were found to be significantly associated with the incidence of T2D in individuals at high cardiovascular risk. [108].

A recent review raises the question whether acylcarnitines reflect or inflict insulin resistance. As a result of several animal and human studies it is supposed that lipotoxicity plays a crucial role in the induction of insulin resistance and due to the theory of the role of impairments of FAO in insulin resistance, more and more attention is turning toward the acylcarnitines, which have distinct functions in the mitochondrial lipid metabolism. Acylcarnitines are suggested to prevent the accumulation of noxious acylCoAs, and reduce CoA trapping as well. Furthermore, the metabolism of short-chain acylcarnitines and the interaction of acetyl-CoA and acetylcarnitine through carnitine acetyl transferase may have an effect on the regulation of the pyruvate dehydrogenase complex, thus influencing the glucose oxidation. However, there is an emerging theory on the role of increased, though incomplete, FAO by disproportional regulation of FAO, TCA and respiratory chain in the development of insulin resistance. It was suggested that acylcarnitines may simply reflect the FAO flux and do not play a role in the induction of insulin resistance itself [109].

A number of human studies revealed currently that accumulation of acylcarnitines is associated with insulin resistance, therefore plasma acylcarnitines have been proposed as biomarkers of insulin resistance [103, 110].

6.2.5. Autism Spectrum Disorder

Autism spectrum disorders (ASD) represent a clinically heterogeneous group of neurodevelopmental disorders characterized by impairments in reciprocal social interaction and communication along with restrictive, repetitive and stereotyped patterns of behaviors [111]. ASD encompasses autistic disorder, Asperger syndrome, pervasive developmental disorder—not otherwise specified. The etiology of ASD is still unclear nowadays, only 6-15 % of individuals with ASD have an identifiable genetic background such as a Mendelian condition, a specific genetic syndrome, rare chromosomal abnormalities or rare copy number variations (CNV) [112]. Although several cognitive and behavioral features of ASD were assumed to arise from a central nervous system dysfunction (CNS) earlier, a number of investigations have implicated recently that multiple non-CNS physiological and metabolic abnormalities are associated with ASD [113, 114]. Some studies have shown that carnitine metabolism was also affected in certain ASD patients. Filipek *et al*. measured the decreased levels of free and total carnitine in children with autism. The observed relative carnitine deficiency in these patients, accompanied by slight elevations in lactate and significant elevations in alanine and ammonia levels, implies mild mitochondrial dysfunction. Finally, they hypothesized that the mitochondrial defect could be responsible for the observed carnitine deficiency [115]. In another study, Clark-Taylor presented a case of an 8-year-old male patient with ASD whose acylcarnitine profile analysis showed a prominent elevation of unsaturated fatty acid metabolites C14:1 and C14:2. The detected AC profile alteration was similar to that observed in experimental mice with long chain acyl-CoA dehydrogenase (LCAD) deficiency [116], so an LCAD deficiency was suspected in this patient [117]. The purpose of Frye and colleague was to reveal whether the same pattern of biomarkers of abnormal fatty acid metabolism are present in children with ASD as it was seen in a rodent ASD model “treated” with propionic acid (PPA) to induce mitochondrial disease. Consistent elevations in short-chain and long-chain, but not medium-chain acyl-carnitines were observed in 17% of a large cohort of children with ASD. The pattern of acyl-carnitine abnormalities shows a similar increase in brain acyl-carnitines seen in the PPA rodent model of ASD [118]. In ASD the incidence of antibiotic use is high, which can promote the overgrowth of PPA producing bacterial population in the gut. Moreover, beta lactam antibiotics commonly used in pediatric treatment of infections directly impair carnitine reabsorption as well [119]. Since both phenomena can be detrimental to mitochondrial metabolism the authors supposed that the antibiotic overuse can cause these two effects to act synergistically to induce an acquired mitochondrial disorder, especially in genetically susceptible individuals [120].

Metabolic studies performed among the ASD patients suggested the involvement of acquired mitochondrial disease in the pathogenesis of a subgroup of autism, therefore the observed unique AC profiles can be potential biomarkers reflecting the acquired mitochondrial disease in ASD [121].

6.3. Medical Interventions

6.3.1. Haemodialysis

In healthy individuals, the plasma and tissue levels of carnitine are maintained within a relatively narrow homeostatic range that is controlled by carrier-mediated gastrointestinal absorption from the dietary sources, endogenous biosynthesis, extensive renal tubular reabsorption, and compartmentalization through carrier-mediated transport between plasma and tissue. In the regulation process, kidney has an essential role primarily through extensive and saturable tubular reabsorption of L-carnitine by the glomerulus and preferential excretion of acylcarnitines [14].

In patients with end-stage renal disease (ESRD) not receiving dialysis treatment an altered carnitine homeostasis can be observed, showing higher plasma concentrations of L-carnitine and its acyl derivatives than in healthy individuals [122, 123]. This alteration reflects the chronic metabolic derangements associated with this condition. Dramatic redistribution of the plasma carnitine pool toward acylcarnitines is characteristic for ESRD [124], reflecting incomplete oxidation of endogenous substrates and their accumulation in the coenzyme A and carnitine pools [125].

Several research studies among ESRD patients revealed that haemodialysis (HD) therapy has a further impact on the carnitine pool [126-128]. Due to the small molecular weight and very low binding to plasma protein, carnitine is easily dialysed, in addition, the dialysis membrane demonstrates differential selectivity for carnitine and acylcarnitines, these leading to diminished free carnitine levels in these patients, finally resulting in an abnormal plasma acyl to free carnitine ratio. Although, a typical HD session can induce as much as a 75% decline in plasma free carnitine level, by 8 hours after dialysis the concentrations have returned to predialysis values [5]. Time-course analysis of carnitine depletion in patients on regular HD revealed a progressive decline in plasma and muscle carnitine with time, the rate of decline, however, was faster in the plasma than in the muscle [15, 127, 129]

In earlier studies, only the L-carnitine depletion and acylcarnitine accumulation could be detected with the classic radioenzymatic assays. The improvement in the analytical methodology, with special respect to the appearance of sophisticated techniques like mass spectrometry, enabled a more detailed examination of the impact of HD on carnitine homeostasis. Mass spectrometry methods allowed the accurate and precise measurement of not only free carnitine but also individual acylcarnitines, thus the measurement of short-, medium- and long-chain carnitine esters. Mass spectrometry measurements revealed that the short- and medium-chain esters are removed by HD, and the dialytic removal of ACs is inversely related to the carbon chain length of the acyl groups [130, 131].

Disturbances in carnitine homeostasis in ESRD patients receiving long-term hemodialysis may be responsible for certain clinical problems, such as skeletal myopathies, cardiomyopathy, poor exercise performance, anemia and dialytic complications like hypotension, cramps, weakness and fatigue [132, 133]. These symptoms have already been recognized as carnitine-responsive events [134, 135], and pharmacokinetic studies have provided convincing evidence that repeated dosing of L-carnitine after each dialysis session results in a new steady-state plasma carnitine concentration in about 6 to 8 weeks that remains constant for at least 6 months. To replenish tissue carnitine stores, however, longer supplementation up to 9-12 months may be required [15, 136-138].

6.3.2. In vitro Fertilization

Normal oocyte maturation, fertility and embryonic development are in close relationship with energy metabolism [139, 140]. Although numerous studies established the prominent role of fatty acids in energy supply providing developmental competence to oocytes and early embryos, growing evidence suggests that excessive non-esterified fatty acid supply is leading to fatty acid accumulation that may compromise oocyte maturation and developmental capacity of the early embryo [141, 142]. Metabolism of free fatty acids *via* beta-oxidation takes place with the involvement of the carnitine cycle. Since carnitine has a regulatory function on the intracellular acyl-CoA/free CoA ratio, therefore the accumulation of its esters (acylcarnitines) is regarded as an indicator of mitochondrial dysfunction and impaired cellular fatty acid metabolism. Several animal and human studies demonstrated the beneficial effect of L-carnitine on oocyte quality and reproductive performance [143, 144].

Identification of potential biomarkers in follicular fluid (FF) in order to predict *in vitro* fertilization (IVF) outcome is in the focus of recent studies. FF serves as a dynamic, physiological environment of maturing oocytes and embryos, therefore, it is assumed to reflect metabolic changes that occur during maturation. It has been demonstrated to contain hormones, growth factors, reactive oxygen species, cytokines, apoptotic factors and several metabolic intermediates [145]. Targeted analysis of specific biomarkers or certain combination of biomarkers has revealed an important correlation with oocyte quality and/or related embryo [146].

Due to the pivotal role of carnitine in energy metabolism which has an impact on oocyte and embryo development carnitine and acylcarnitines in plasma or in follicular fluid could be a promising biomarker for the expectable reproductive outcome. Although there are numerous animal studies where the effect of L-carnitine supplementation on oocyte quality and preimplantation embryo development was investigated, very limited data are accessible for the level of carnitine and its esters in human ovarian follicular samples. No correlation has been found between the total carnitine content of the follicular fluid and either the circulating estradiol content of the serum or the outcome of the IVF procedure [147]. In another study, L-carnitine levels were examined in follicular fluid samples from women treated with human chorionic gonadotropin. Whether the L-carnitine level is regulated during the menstrual cycle or with gonadotropin stimulation is not yet known [148]. In order to further explore the relationship between IVF parameters and the composition of endogenous carnitine pool we performed the mass spectrometric analysis of FC and 20 major AC esters in serum and FF samples obtained from patients undergoing IVF. Interestingly, the levels of individual ACs in FF proved to be dependent on maternal serum concentrations and on the carbon chain length of acyl groups. These findings are consistent with the notion that FC and AC esters cross the blood-follicular barrier but the passage through this barrier is reduced as molecular weight and lipophilicity increases with the increasing carbon chain length. Furthermore, carnitine profiling of serum and FF revealed a marked reduction in total carnitine, FC and AC levels in IVF patients with oocyte number of >9 and/or with embryo number of >6 as compared to those with the respective values of <9 and/or <6. Based on these results, it is suggested that the L-carnitine/AC pathway is upregulated and the actual carnitine pool is depleted in patients with better reproductive potential [146]. Since the follicular fluid represents a favorable milieu for proper oocyte development Gervais *et al* tried to determine whether

the metabolic and inflammatory factors present in follicular fluid were associated with fertility outcomes. Although they did not directly measure the quality of the oocyte, among others no correlation was observed between the follicular fluid acylcarnitines and the maturity of the oocytes. However, in this study, only the C16 and C3, as well as C16/C3 ratio was determined [149].

6.4. Xenobiotic Function of Carnitine

The carnitine molecule is capable to form an ester with exogenous organic acids, too. A classic example is the valproic acid (VPA), a branched chain carboxylic acid (dipropyl acetic acid), which is widely used as an effective broad-spectrum antiepileptic drug [150, 151] and more recently for the prophylaxis of migraine and in patients with bipolar disorder as well [152, 153]. It has serious adverse effects, even Reye-like syndrome can develop during the course of therapy. A number of clinical studies demonstrated low blood level of free carnitine during VPA treatment [154, 155], since the valproate's ester forming capacity with carnitine is poor, so the carnitine deficiency caused by this drug is not due to the sustained urinary loss of carnitine ester. These carnitine measurements were based on radiochemical assay and it turned out that the valproate also interferes with these assays. Meanwhile, VPA decreases the muscle carnitine reserves, and this has also biochemical consequences: in epileptic patients with chronic valproate treatment a shift was observed in the metabolic fuel consumption towards the preferential use of carbohydrates determined by indirect calorimetry measurements, and administration of oral carnitine supplementation partially reversed this trend by increasing the partition of fats oxidized. Although the mass spectrometric measurement of acylcarnitines is a promising tool to investigate the effect of VPA treatment on carnitine status and its related metabolic pathway, but there are only a few studies in this field and the results are contradictory to the earlier data [156, 157]. Silva *et al*. failed to detect decreased plasma free carnitine during VPA monotherapy in adult patients and their findings are in agreement with some other reports [158, 159] that suggest that VPA has no effect on serum free carnitine concentrations of patients with epilepsy on a normal diet. In contrast, VPA was found to affect the levels of C5-OH, C14:2, C8DC and C18-OH acylcarnitines [157]. In line with this study, VPA monotherapy does not result in a decrease in free carnitine or in the accumulation of long-chain acylcarnitines in Nakajima *et al* study. In addition, they found a significant positive correlation between blood VPA concentrations and the C6, C12, C14:1, C16:1, C18:1 acylcarnitines in all VPA-treated children [160]. In their study, Werner and colleagues investigated the effect of VPA monotherapy, VPA therapy combined with another antiepileptic drug (AED) and carbamazepine (CBZ) monotherapy on serum acylcarnitine profile. Moreover, they compared the results of early treatment intervals (12-66 days) versus late treatment intervals (90-260 days). Interestingly, the VPA treatment resulted in the decrease of free, C2, C16, C18 and total carnitine and increase of C5OH and C8 esters in the early treatment interval, but the effect remained unchanged only for C16, C18 and C5OH species in the late treatment interval. The VPA and AED combined therapy resulted in the decrease of C18 level only and the CBZ therapy had no effect on the AC levels [156]. Morand *et al*. performed mass spectrometry measurements to reveal the molecular mechanisms leading to hypocarnitinemia in patients treated with valproate. After an initial drop in the levels of carnitine and acetylcarnitine a slow recovery was observed. The authors concluded that the patients develop hypocarnitinemia at the beginning of the valproate therapy most probably due to an impairment of proximal steps of carnitine biosynthesis. Furthermore, the observed normalization of the plasma carnitine levels in most patients during long-term treatment is attributable to a higher renal reabsorption of carnitine and short-chain acylcarnitines [161]. The aim of Celik and colleagues was to investigate the effect of six month VPA monotherapy on serum free and AC levels and left ventricular systolic function in pediatric patients with idiopathic epilepsy. A significantly decreased levels of C0 and C5:1 were detected whereas the amount of C2, C3, C5-OH, C8:1 and C4-DC were increased. However, the VPA treatment had no influence on left ventricular systolic function [162].

Contrary to the valproate, the pivalate (trimethylacetate) readily forms ester with carnitine. Pivalate is used in pharmacology as a helper to achieve better gastrointestinal absorption of a prodrug (like the pivampicillin, which is the pivaloyl ester of ampicillin). After absorption, the pivalate is released from the complex, and it readily forms ester with the carnitine molecule. Ingestion of drugs containing pivalate ester causes immediate and sustained loss of carnitine, administration of pivampicillin with equal molar carnitine further increases the fraction of pivaloyl-carnitine excreted into the urine. There are a very limited number of studies in the literature using mass spectrometry to reveal the effect of pivalate-conjugated antibiotic (PCA) therapy on carnitine homeostasis. Decreased plasma free carnitine concentration and increased levels of pivaloylcarnitine were detected mainly in these studies, therefore long-term administration of PCA should be avoided particularly in children [163, 164].

The pivampicillin treatment was used as a model for the study of the metabolic consequences of the carnitine loss and then the replacement therapy; administration of the drug resulted in a shift in the metabolic fuel consumption from fats to carbohydrates, while the co-administration of oral carnitine supplementation partially reversed this shift likely *via* the aiding the excretion of pivaloylcarnitine end replacement of the carnitine reserves of the body.

7. MASS SPECTROMETRIC MEASUREMENTS

The mass spectrometric results presented in this article are mainly based on the following procedure.

Starting materials were dry blood spots, plasma or serum or urine samples depending on the tested group of patients. Free carnitine and all the acylcarnitines were measured mainly after derivatization as butyl-esters using isotope dilution mass spectrometry method, that means that for calibration and quantification stable-isotope internal standards were used. Since reference standards are not available for all acylcarnitine species, the quantitation of these compounds based on an extrapolation of the calibration for the nearest species with a similar structure. In some instances, the analysis was performed in underivatized specimens. For MS/MS-based testing, acylcarnitines were analyzed without chromatographic separation in the positive-ion mode, using either precursor-ion (also called parent-ion) scan or multiple-reaction monitoring (MRM).

CONCLUSION

The role of carnitine is essential in energy metabolism since it facilitates the transport of long chain fatty acids through the mitochondrial membrane and controls the rate of beta-oxidation of long-chain fatty acids with subsequent energy production. During the past decades, a large number of investigations were published establishing several physiological functions of this molecule and revealing different aspects of carnitine homeostasis in normal and pathological conditions. The improvement in the analytical methodology, with special respect to the appearance of sophisticated techniques such as mass spectrometry, enabled a more detailed examination of carnitine homeostasis in different disease states. Mass spectrometric measurements facilitated the accurate and precise determination of not only free carnitine but also individual acylcarnitines and thus this tool brought a new perspective to carnitine research in the last decade. Unique acylcarnitine profiles can be observed in several various disease states therefore it could be a promising biomarker for primary and secondary carnitine deficiencies.

LIST OF ABBREVIATIONS

TCA	=	Tricarboxylic acid
AC	=	Acylcarnitine
FC	=	Free carnitine
SPCD	=	Systemic primary carnitine deficiency
MS	=	Mass spectrometry
VLCAD	=	Very long-chain acyl-CoA dehydrogenase deficiency
MCAD	=	Medium-chain acyl-CoA dehdrogenase deficiency
NBS	=	Newborn screening
FAOD	=	Disorders of fatty-acid oxidation
LCHAD	=	Long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency
CD	=	Celiac disease
IBD	=	Inflammatory bowel diseases
CrD	=	Crohn's disease
UC	=	Ulcerative colitis
SCFA	=	Short-chain fatty acid
EN	=	Enteral nutrition
TPN	=	Total parenteral nutrition
CVD	=	Cardiovasular diseases
MI	=	Myocardial infaction
HF	=	Heart failure
MACE	=	Major cardiovascular event
CAD	=	Coronary artery disease
CABG	=	Coronary artery bypass grafting
LVAD	=	Left ventricular assist device
T1D	=	Type 1 diabetes
T2D	=	Type 2 diabetes
FAO	=	Fatty acid oxidation
ETC	=	Electron transport chain
CPT1	=	Carnitine palmitoyltransferase-1
ASD	=	Autism spectrum disorders
CNV	=	Copy number variations
CNS	=	Central nervous system
LCAD	=	Long chain acyl-CoA dehydrogenase
PPA	=	Propionic acid
ESRD	=	End-stage renal disease
HD	=	Haemodialysis
FF	=	Follicular fluid
IVF	=	*In vitro* fertilization
VPA	=	Valproic acid
AED	=	Antiepileptic drug
CBZ	=	Carbamazepine
PCA	=	Pivalate-conjugated antibiotic

AUTHORS' CONTRIBUTIONS

JB, KK and BM conceived and designed research; ASZ prepared figures; JB and ASZ drafted manuscript; JB KK and ASZ edited and revised manuscript; JB and BM approved final version of manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Gulewitsch W, Krimberg R. Zur kenntnis der extraktivstoffe der muskeln. II. Mitteilung. Über das carnitin. Hoppe Seylers Z Physiol Chem 1905; 45: 326-30. [http://dx.doi.org/10.1515/bchm2.1905.45.3-4.326]

[2] Tomita M, Sendju Y. Über die oxyaminoverbindungen, welche diebiuretreaktion zeigen. III. Spaltung der g-amino-b-oxy-buttersäure in die optisch-aktiven komponenten. Hoppe Seylers Z Physiol Chem 1927; 169: 263-77. [http://dx.doi.org/10.1515/bchm2.1927.169.4-6.263]

[3] Fritz I. The effect of muscle extracts on the oxidation of palmitic acid by liver slices and homogenates. Acta Physiol Scand 1955; 34(4): 367-85. [http://dx.doi.org/10.1111/j.1748-1716.1955.tb01256.x] [PMID: 13282744]

[4] Bremer J. Carnitine--metabolism and functions. Physiol Rev 1983; 63(4): 1420-80. [http://dx.doi.org/10.1152/physrev.1983.63.4.1420] [PMID: 6361812]

[5] Hoppel C. The role of carnitine in normal and altered fatty acid metabolism. Am J Kidney Dis 2003; 41(4)(Suppl. 4): S4-S12. [http://dx.doi.org/10.1016/S0272-6386(03)00112-4] [PMID: 12751049]

[6] Brass EP, Hoppel CL. Carnitine metabolism in the fasting rat. J Biol Chem 1978; 253(8): 2688-93. [PMID: 632294]

[7] Millington DS, Kodo N, Norwood DL, Roe CR. Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. J Inherit Metab Dis 1990; 13(3): 321-4. [http://dx.doi.org/10.1007/BF01799385] [PMID: 2122093]

[8] Bieber LL. Carnitine. Annu Rev Biochem 1988; 57: 261-83. [http://dx.doi.org/10.1146/annurev.bi.57.070188.001401] [PMID: 3052273]

[9] McGarry JD. The mitochondrial carnitine palmitoyltransferase system: its broadening role in fuel homoeostasis and new insights into its molecular features. Biochem Soc Trans 1995; 23(2): 321-4. [http://dx.doi.org/10.1042/bst0230321] [PMID: 7672352]

[10] Adeva-Andany MM, Calvo-Castro I, Fernández-Fernández C, Donapetry-García C, Pedre-Piñeiro AM. Significance of l-carnitine for human health. IUBMB Life 2017; 69(8): 578-94. [http://dx.doi.org/10.1002/iub.1646] [PMID: 28653367]

[11] Evangeliou A, Vlassopoulos D. Carnitine metabolism and deficit--when supplementation is necessary? Curr Pharm Biotechnol 2003; 4(3): 211-9. [http://dx.doi.org/10.2174/1389201033489829] [PMID: 12769764]

[12] Duran M, Loof NE, Ketting D, Dorland L. Secondary carnitine deficiency. J Clin Chem Clin Biochem 1990; 28(5): 359-63. [PMID: 2199597]

[13] Reda E, D'Iddio S, Nicolai R, Benatti P, Calvani M. The carnitine system and body composition. Acta Diabetol 2003; 40(Suppl. 1): S106-13. [http://dx.doi.org/10.1007/s00592-003-0040-z] [PMID: 14618447]

[14] Reuter SE, Evans AM. Carnitine and acylcarnitines: pharmacokinetic, pharmacological and clinical aspects. Clin Pharmacokinet 2012; 51(9): 553-72. [http://dx.doi.org/10.1007/BF03261931] [PMID: 22804748]

[15] Calvani M, Benatti P, Mancinelli A, *et al.* Carnitine replacement in end-stage renal disease and hemodialysis. Ann N Y Acad Sci 2004; 1033: 52-66. [http://dx.doi.org/10.1196/annals.1320.005] [PMID: 15591003]

[16] Meyburg J, Schulze A, Kohlmueller D, Linderkamp O, Mayatepek E. Postnatal changes in neonatal acylcarnitine profile. Pediatr Res 2001; 49(1): 125-9. [http://dx.doi.org/10.1203/00006450-200101000-00024] [PMID: 11134502]

[17] Sayed-Ahmed MM. Role of carnitine in cancer chemotherapy-induced multiple organ toxicity. Saudi pharmaceutical journal: SPJ : the official publication of the Saudi Pharmaceutical Society 2010; 18(4): 195-206. [http://dx.doi.org/10.1016/j.jsps.2010.07.008]

[18] Ramos-Roman MA, Sweetman L, Valdez MJ, Parks EJ. Postprandial changes in plasma acylcarnitine concentrations as markers of fatty acid flux in overweight and obesity. Metabolism 2012; 61(2): 202-12. [http://dx.doi.org/10.1016/j.metabol.2011.06.008] [PMID: 21820684]

[19] Rebouche CJ. Carnitine function and requirements during the life cycle. FASEB J 1992; 6(15): 3379-86. [http://dx.doi.org/10.1096/fasebj.6.15.1464372] [PMID: 1464372]

[20] Almannai M, Alfadhel M, El-Hattab AW. Carnitine Inborn Errors of Metabolism. Molecules 2019; 24(18): E3251. [http://dx.doi.org/10.3390/molecules24183251] [PMID: 31500110]

[21] Stanley CA. New genetic defects in mitochondrial fatty acid oxidation and carnitine deficiency. Adv Pediatr 1987; 34: 59-88. [PMID: 3318304]

[22] Nezu J, Tamai I, Oku A, *et al.* Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. Nat Genet 1999; 21(1): 91-4. [http://dx.doi.org/10.1038/5030] [PMID: 9916797]

[23] El-Hattab AW, Scaglia F. Disorders of carnitine biosynthesis and transport. Mol Genet Metab 2015; 116(3): 107-12. [http://dx.doi.org/10.1016/j.ymgme.2015.09.004] [PMID: 26385306]

[24] Melegh B, Bene J, Mogyorósy G, *et al.* Phenotypic manifestations of the OCTN2 V295X mutation: sudden infant death and carnitine-responsive cardiomyopathy in Roma families. Am J Med Genet A 2004; 131(2): 121-6. [http://dx.doi.org/10.1002/ajmg.a.30207] [PMID: 15487009]

[25] Frigeni M, Balakrishnan B, Yin X, *et al.* Functional and molecular studies in primary carnitine deficiency. Hum Mutat 2017; 38(12): 1684-99. [http://dx.doi.org/10.1002/humu.23315] [PMID: 28841266]

[26] Wang Y, Ye J, Ganapathy V, Longo N. Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. Proc Natl Acad Sci USA 1999; 96(5): 2356-60. [http://dx.doi.org/10.1073/pnas.96.5.2356] [PMID: 10051646]

[27] Lahrouchi N, Lodder EM, Mansouri M, *et al.* Exome sequencing identifies primary carnitine deficiency in a family with cardiomyopathy and sudden death. Eur J Hum Genet 2017; 25(6): 783-7. [http://dx.doi.org/10.1038/ejhg.2017.22] [PMID: 28295041]

[28] Mutlu-Albayrak H, Bene J, Oflaz MB, Tanyalçın T, Çaksen H, Melegh B. Identification of SLC22A5 gene mutation in a family with carnitine uptake defect. Case Rep Genet 2015; 2015: 259627. [http://dx.doi.org/10.1155/2015/259627] [PMID: 26075114]

[29] Sun Y, Wang YY, Jiang T. Clinical features and genotyping of patients with primary carnitine deficiency identified by newborn screening. J Pediatr Endocrinol Metab 2017; 30(8): 879-83. [http://dx.doi.org/10.1515/jpem-2017-0002] [PMID: 28753539]

[30] Rasmussen J, Hougaard DM, Sandhu N, *et al.* Primary carnitine deficiency: Is foetal development affected and can newborn screening be improved? JIMD Rep 2017; 36: 35-40. [http://dx.doi.org/10.1007/8904_2016_30] [PMID: 28105570]

[31] Schimmenti LA, Crombez EA, Schwahn BC, *et al.* Expanded newborn screening identifies maternal primary carnitine deficiency. Mol Genet Metab 2007; 90(4): 441-5.

[http://dx.doi.org/10.1016/j.ymgme.2006.10.003] [PMID: 17126586]

[32] El-Hattab AW, Li FY, Shen J, Powell BR, Bawle EV, Adams DJ, *et al.* Maternal systemic primary carnitine deficiency uncovered by newborn screening: clinical, biochemical, and molecular aspects. Genetics in medicine : official journal of the American College of Medical Genetics 2010; 12(1): 19-24.
[http://dx.doi.org/10.1097/GIM.0b013e3181c5e6f7]

[33] Pons R, De Vivo DC. Primary and secondary carnitine deficiency syndromes. J Child Neurol 1995; 10(Suppl. 2): S8-S24.
[http://dx.doi.org/10.1177/0883073895010002S03] [PMID: 8576570]

[34] Tanphaichitr V, Leelahagul P. Carnitine metabolism and human carnitine deficiency. Nutrition 1993; 9(3): 246-54.
[PMID: 8353366]

[35] Melegh B, Kerner J, Bieber LL. Pivampicillin-promoted excretion of pivaloylcarnitine in humans. Biochem Pharmacol 1987; 36(20): 3405-9.
[http://dx.doi.org/10.1016/0006-2952(87)90318-2] [PMID: 3675603]

[36] Melegh B, Kerner J, Jaszai V, Bieber LL. Differential excretion of xenobiotic acyl-esters of carnitine due to administration of pivampicillin and valproate. Biochem Med Metab Biol 1990; 43(1): 30-8.
[http://dx.doi.org/10.1016/0885-4505(90)90005-L] [PMID: 2106908]

[37] Jones LL, McDonald DA, Borum PR. Acylcarnitines: role in brain. Prog Lipid Res 2010; 49(1): 61-75.
[http://dx.doi.org/10.1016/j.plipres.2009.08.004] [PMID: 19720082]

[38] Sunny NE, Satapati S, Fu X, *et al.* Progressive adaptation of hepatic ketogenesis in mice fed a high-fat diet. Am J Physiol Endocrinol Metab 2010; 298(6): E1226-35.
[http://dx.doi.org/10.1152/ajpendo.00033.2010] [PMID: 20233938]

[39] Meyburg J, Schulze A, Kohlmueller D, *et al.* Acylcarnitine profiles of preterm infants over the first four weeks of life. Pediatr Res 2002; 52(5): 720-3.
[http://dx.doi.org/10.1203/00006450-200211000-00018] [PMID: 12409519]

[40] Melegh B, Kerner J, Sándor A, Vincellér M, Kispál G. Effects of oral L-carnitine supplementation in low-birth-weight premature infants maintained on human milk. Biol Neonate 1987; 51(4): 185-93.
[http://dx.doi.org/10.1159/000242650] [PMID: 3580423]

[41] Melegh B. Carnitine supplementation in the premature. Biol Neonate 1990; 58(Suppl. 1): 93-106.
[http://dx.doi.org/10.1159/000243304] [PMID: 2265223]

[42] Bene J, Komlósi K, Melegh BI, Decsi T, Koletzko B, Sauerwald U. Differences in circulating carnitine status of preterm infants fed fortified human milk or preterm infant formula. J Pediatr Gastroenterol Nutr 2013; 57(5): 673-6.
[http://dx.doi.org/10.1097/MPG.0b013e31829fad06] [PMID: 23783025]

[43] Talián GC, Komlósi K, Decsi T, Koletzko B, Melegh B. Determination of carnitine ester patterns during the second half of pregnancy, at delivery, and in neonatal cord blood by tandem mass spectrometry: complex and dynamic involvement of carnitine in the intermediary metabolism. Pediatr Res 2007; 62(1): 88-92.
[http://dx.doi.org/10.1203/PDR.0b013e3180676cca] [PMID: 17515842]

[44] Knottnerus SJG, Bleeker JC, Wüst RCI, *et al.* Disorders of mitochondrial long-chain fatty acid oxidation and the carnitine shuttle. Rev Endocr Metab Disord 2018; 19(1): 93-106.
[http://dx.doi.org/10.1007/s11154-018-9448-1] [PMID: 29926323]

[45] Lindner M, Hoffmann GF, Matern D. Newborn screening for disorders of fatty-acid oxidation: experience and recommendations from an expert meeting. J Inherit Metab Dis 2010; 33(5): 521-6.
[http://dx.doi.org/10.1007/s10545-010-9076-8] [PMID: 20373143]

[46] Lehotay DC, Hall P, Lepage J, Eichhorst JC, Etter ML, Greenberg CR. LC-MS/MS progress in newborn screening. Clin Biochem 2011; 44(1): 21-31.
[http://dx.doi.org/10.1016/j.clinbiochem.2010.08.007] [PMID: 20709048]

[47] Briand G, Lemaire-Ewing S, Parente F, Garnotel R. Mass spectrometry and inherited metabolic diseases diagnosis. Ann Biol Clin (Paris) 2015; 73(1): 93-106.
[http://dx.doi.org/10.1684/abc.2014.1022] [PMID: 25582726]

[48] Minkler PE, Stoll MS, Ingalls ST, Kerner J, Hoppel CL. Quantitative acylcarnitine determination by UHPLC-MS/MS--Going beyond tandem MS acylcarnitine “profiles”. Mol Genet Metab 2015; 116(4): 231-41.
[http://dx.doi.org/10.1016/j.ymgme.2015.10.002] [PMID: 26458767]

[49] Yoon HR. Screening newborns for metabolic disorders based on targeted metabolomics using tandem mass spectrometry. Ann Pediatr Endocrinol Metab 2015; 20(3): 119-24.
[http://dx.doi.org/10.6065/apem.2015.20.3.119] [PMID: 26512346]

[50] Van Hove JL, Kahler SG, Feezor MD, *et al.* Acylcarnitines in plasma and blood spots of patients with long-chain 3-hydroxyacyl-coenzyme A dehydrogenase defiency. J Inherit Metab Dis 2000; 23(6): 571-82.
[http://dx.doi.org/10.1023/A:1005673828469] [PMID: 11032332]

[51] Vilskersts R, Kuka J, Liepinsh E, *et al.* Methyl-γ-butyrobetaine decreases levels of acylcarnitines and attenuates the development of atherosclerosis. Vascul Pharmacol 2015; 72: 101-7.
[http://dx.doi.org/10.1016/j.vph.2015.05.005] [PMID: 25989106]

[52] Anderson SG, Dunn WB, Banerjee M, *et al.* Evidence that multiple defects in lipid regulation occur before hyperglycemia during the prodrome of type-2 diabetes. PLoS One 2014; 9(9): e103217.
[http://dx.doi.org/10.1371/journal.pone.0103217] [PMID: 25184286]

[53] Spiekerkoetter U, Lindner M, Santer R, *et al.* Treatment recommendations in long-chain fatty acid oxidation defects: consensus from a workshop. J Inherit Metab Dis 2009; 32(4): 498-505.
[http://dx.doi.org/10.1007/s10545-009-1126-8] [PMID: 19452263]

[54] Nasser M, Javaheri H, Fedorowicz Z, Noorani Z. Carnitine supplementation for inborn errors of metabolism. Cochrane Database Syst Rev 2012; 2(2): CD006659.
[http://dx.doi.org/10.1002/14651858.CD006659.pub3] [PMID: 22336821]

[55] Capristo E, Addolorato G, Mingrone G, *et al.* Changes in body composition, substrate oxidation, and resting metabolic rate in adult celiac disease patients after a 1-y gluten-free diet treatment. Am J Clin Nutr 2000; 72(1): 76-81.
[http://dx.doi.org/10.1093/ajcn/72.1.76] [PMID: 10871564]

[56] Vuoristo M, Kesäniemi YA, Gylling H, Miettinen TA. Metabolism of cholesterol and apolipoprotein B in celiac disease. Metabolism 1993; 42(11): 1386-91.
[http://dx.doi.org/10.1016/0026-0495(93)90187-S] [PMID: 8231831]

[57] Rosenthal E, Hoffman R, Aviram M, Benderly A, Erde P, Brook JG. Serum lipoprotein profile in children with celiac disease. J Pediatr Gastroenterol Nutr 1990; 11(1): 58-62.
[http://dx.doi.org/10.1097/00005176-199007000-00012] [PMID: 2388134]

[58] Green PH, Jabri B. Coeliac disease. Lancet 2003; 362(9381): 383-91.
[http://dx.doi.org/10.1016/S0140-6736(03)14027-5] [PMID: 12907013]

[59] Lerner A, Gruener N, Iancu TC. Serum carnitine concentrations in coeliac disease. Gut 1993; 34(7): 933-5.
[http://dx.doi.org/10.1136/gut.34.7.933] [PMID: 8344581]

[60] Yüce A, Demir H, Temizel IN, Koçak N. Serum carnitine and selenium levels in children with celiac disease. Indian J Gastroenterol 2004; 23(3): 87-8.
[PMID: 15250563]

[61] Curione M, Danese C, Viola F, *et al.* Carnitine deficiency in patients with coeliac disease and idiopathic dilated cardiomyopathy. Nutr Metab Cardiovasc Dis 2005; 15(4): 279-83. [http://dx.doi.org/10.1016/j.numecd.2005.01.002] [PMID: 16054552]

[62] Uslu N, Demir H, Karagöz T, Saltik-Temizel IN. Dilated cardiomyopathy in celiac disease: role of carnitine deficiency. Acta Gastroenterol Belg 2010; 73(4): 530-1. [PMID: 21299168]

[63] Karakoç E, Erdem S, Sökmensüer C, Kansu T. Encephalopathy due to carnitine deficiency in an adult patient with gluten enteropathy. Clin Neurol Neurosurg 2006; 108(8): 794-7. [http://dx.doi.org/10.1016/j.clineuro.2005.10.012] [PMID: 16325996]

[64] Ciacci C, Peluso G, Iannoni E, *et al.* L-Carnitine in the treatment of fatigue in adult celiac disease patients: a pilot study. Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver 2007; 39(10): 922-8.

[65] Bene J, Komlósi K, Gasztonyi B, Juhász M, Tulassay Z, Melegh B. Plasma carnitine ester profile in adult celiac disease patients maintained on long-term gluten free diet. World J Gastroenterol 2005; 11(42): 6671-5. [http://dx.doi.org/10.3748/wjg.v11.i42.6671] [PMID: 16425363]

[66] Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347(6): 417-29. [http://dx.doi.org/10.1056/NEJMra020831] [PMID: 12167685]

[67] Petkau JM, Eksteen B. Selective biologics for ulcerative colitis and Crohn's disease - clinical utility of vedolizumab. Biologics 2016; 10: 33-52. [PMID: 27022240]

[68] Roediger WE. The colonic epithelium in ulcerative colitis: an energy-deficiency disease? Lancet 1980; 2(8197): 712-5. [http://dx.doi.org/10.1016/S0140-6736(80)91934-0] [PMID: 6106826]

[69] Gasbarrini G, Mingrone G, Giancaterini A, *et al.* Effects of propionyl-L-carnitine topical irrigation in distal ulcerative colitis: a preliminary report. Hepatogastroenterology 2003; 50(53): 1385-9. [PMID: 14571743]

[70] Roediger WE, Heyworth M, Willoughby P, Piris J, Moore A, Truelove SC. Luminal ions and short chain fatty acids as markers of functional activity of the mucosa in ulcerative colitis. J Clin Pathol 1982; 35(3): 323-6. [http://dx.doi.org/10.1136/jcp.35.3.323] [PMID: 7068924]

[71] Scheppach W, Sommer H, Kirchner T, *et al.* Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 1992; 103(1): 51-6. [http://dx.doi.org/10.1016/0016-5085(92)91094-K] [PMID: 1612357]

[72] Rashed MS. Clinical applications of tandem mass spectrometry: ten years of diagnosis and screening for inherited metabolic diseases. J Chromatogr B Biomed Sci Appl 2001; 758(1): 27-48. [http://dx.doi.org/10.1016/S0378-4347(01)00100-1] [PMID: 11482732]

[73] Bene J, Komlósi K, Havasi V, *et al.* Changes of plasma fasting carnitine ester profile in patients with ulcerative colitis. World J Gastroenterol 2006; 12(1): 110-3. [http://dx.doi.org/10.3748/wjg.v12.i1.110] [PMID: 16440427]

[74] Siguel EN, Lerman RH. Prevalence of essential fatty acid deficiency in patients with chronic gastrointestinal disorders. Metabolism 1996; 45(1): 12-23. [http://dx.doi.org/10.1016/S0026-0495(96)90194-8] [PMID: 8544768]

[75] Kinsella JE, Lokesh B, Broughton S, Whelan J. Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. Nutrition 1990; 6(1): 24-44. [PMID: 2135755]

[76] Danese C, Cirene M, Colotto M, *et al.* Cardiac involvement in inflammatory bowel disease: role of acylcarnitine esters. Clin Ter 2011; 162(4): e105-9. [PMID: 21912810]

[77] Iwamoto J, Honda A, Miyamoto Y, *et al.* Serum carnitine as an independent biomarker of malnutrition in patients with impaired oral intake. J Clin Biochem Nutr 2014; 55(3): 221-7. [http://dx.doi.org/10.3164/jcbn.14-77] [PMID: 25411530]

[78] Peltekova VD, Wintle RF, Rubin LA, *et al.* Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat Genet 2004; 36(5): 471-5. [http://dx.doi.org/10.1038/ng1339] [PMID: 15107849]

[79] Bene J, Komlósi K, Magyari L, *et al.* Plasma carnitine ester profiles in Crohn's disease patients characterized for SLC22A4 C1672T and SLC22A5 G-207C genotypes. Br J Nutr 2007; 98(2): 345-50. [http://dx.doi.org/10.1017/S0007114507705020] [PMID: 17391561]

[80] Talián G, Lakner L, Bene J, *et al.* Plasma carnitine ester profiles in Crohn's disease and ulcerative colitis patients with different IGR2230a_1 genotypes. Int J Immunogenet 2009; 36(6): 329-35. [http://dx.doi.org/10.1111/j.1744-313X.2009.00834.x] [PMID: 19735486]

[81] Demirkol M, Sewell AC, Böhles H. The variation of carnitine content in human blood cells during disease--a study in bacterial infection and inflammatory bowel disease. Eur J Pediatr 1994; 153(8): 565-8. [http://dx.doi.org/10.1007/BF02190659] [PMID: 7957402]

[82] Adlouni HA, Katrib K, Férard G. Changes in carnitine in polymorphonuclear leukocytes, mononuclear cells, and plasma from patients with inflammatory disorders. Clin Chem 1988; 34(1): 40-3. [PMID: 3338182]

[83] Schreiber S, Nikolaus S, Hampe J. Activation of nuclear factor kappa B inflammatory bowel disease. Gut 1998; 42(4): 477-84. [http://dx.doi.org/10.1136/gut.42.4.477] [PMID: 9616307]

[84] Segain JP, Raingeard de la Blétière D, Bourreille A, *et al.* Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. Gut 2000; 47(3): 397-403. [http://dx.doi.org/10.1136/gut.47.3.397] [PMID: 10940278]

[85] Psychogios N, Hau DD, Peng J, *et al.* The human serum metabolome. PLoS One 2011; 6(2): e16957. [http://dx.doi.org/10.1371/journal.pone.0016957] [PMID: 21359215]

[86] Demir Djekic R. Mehmed Novo, Michael Henein. Metabolomics in atherosclerosis. IJC Metab Endocr 2015; 8: 26-30. [http://dx.doi.org/10.1016/j.ijcme.2014.11.004]

[87] Rhee EP, Yang Q, Yu B, *et al.* An exome array study of the plasma metabolome. Nat Commun 2016; 7: 12360. [http://dx.doi.org/10.1038/ncomms12360] [PMID: 27453504]

[88] Zhong Z, Liu J, Zhang Q, *et al.* Targeted metabolomic analysis of plasma metabolites in patients with coronary heart disease in southern China. Medicine (Baltimore) 2019; 98(7): e14309. [http://dx.doi.org/10.1097/MD.0000000000014309] [PMID: 30762730]

[89] Gillies PJ, Bell FP. Arterial and plasma carnitine levels in rabbits: influence of age and dietary cholesterol. Exp Mol Pathol 1976; 25(3): 402-11. [http://dx.doi.org/10.1016/0014-4800(76)90048-4] [PMID: 1001407]

[90] Koeth RA, Wang Z, Levison BS, *et al.* Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013; 19(5): 576-85. [http://dx.doi.org/10.1038/nm.3145] [PMID: 23563705]

[91] Rizza S, Copetti M, Rossi C, *et al.* Metabolomics signature improves the prediction of cardiovascular events in elderly subjects. Atherosclerosis 2014; 232(2): 260-4. [http://dx.doi.org/10.1016/j.atherosclerosis.2013.10.029] [PMID: 24468136]

[92] Shah SH, Bain JR, Muehlbauer MJ, *et al.* Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. Circ Cardiovasc Genet 2010; 3(2): 207-14.

[http://dx.doi.org/10.1161/CIRCGENETICS.109.852814] [PMID: 20173117]

[93] Shah AA, Craig DM, Sebek JK, *et al.* Metabolic profiles predict adverse events after coronary artery bypass grafting. J Thorac Cardiovasc Surg 2012; 143(4): 873-8. [http://dx.doi.org/10.1016/j.jtcvs.2011.09.070] [PMID: 22306227]

[94] Vorkas PA, Shalhoub J, Isaac G, *et al.* Metabolic phenotyping of atherosclerotic plaques reveals latent associations between free cholesterol and ceramide metabolism in atherogenesis. J Proteome Res 2015; 14(3): 1389-99. [http://dx.doi.org/10.1021/pr5009898] [PMID: 25565173]

[95] Ahmad T, Kelly JP, McGarrah RW, *et al.* Prognostic implications of long-chain acylcarnitines in heart failure and reversibility with mechanical circulatory support. J Am Coll Cardiol 2016; 67(3): 291-9. [http://dx.doi.org/10.1016/j.jacc.2015.10.079] [PMID: 26796394]

[96] Gupte AA, Hamilton DJ, Cordero-Reyes AM, *et al.* Mechanical unloading promotes myocardial energy recovery in human heart failure. Circ Cardiovasc Genet 2014; 7(3): 266-76. [http://dx.doi.org/10.1161/CIRCGENETICS.113.000404] [PMID: 24825877]

[97] Tamamoğullari N, Siliğ Y, Içağasioğlu S, Atalay A. Carnitine deficiency in diabetes mellitus complications. J Diabetes Complications 1999; 13(5-6): 251-3. [http://dx.doi.org/10.1016/S1056-8727(99)00052-5] [PMID: 10764998]

[98] Bene J, Hadzsiev K, Melegh B. Role of carnitine and its derivatives in the development and management of type 2 diabetes. Nutr Diabetes 2018; 8(1): 8. [http://dx.doi.org/10.1038/s41387-018-0017-1] [PMID: 29549241]

[99] Winter SC, Simon M, Zorn EM, *et al.* Relative carnitine insufficiency in children with type I diabetes mellitus. Am J Dis Child 1989; 143(11): 1337-9. [PMID: 2816861]

[100] Mamoulakis D, Galanakis E, Dionyssopoulou E, Evangeliou A, Sbyrakis S. Carnitine deficiency in children and adolescents with type 1 diabetes. J Diabetes Complications 2004; 18(5): 271-4. [http://dx.doi.org/10.1016/S1056-8727(03)00091-6] [PMID: 15337500]

[101] Möder M, Kiessling A, Löster H, Brüggemann L. The pattern of urinary acylcarnitines determined by electrospray mass spectrometry: a new tool in the diagnosis of diabetes mellitus. Anal Bioanal Chem 2003; 375(2): 200-10. [http://dx.doi.org/10.1007/s00216-002-1654-7] [PMID: 12560963]

[102] Adams SH, Hoppel CL, Lok KH, *et al.* Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. J Nutr 2009; 139(6): 1073-81. [http://dx.doi.org/10.3945/jn.108.103754] [PMID: 19369366]

[103] Mihalik SJ, Goodpaster BH, Kelley DE, *et al.* Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity. Obesity (Silver Spring) 2010; 18(9): 1695-700. [http://dx.doi.org/10.1038/oby.2009.510] [PMID: 20111019]

[104] Newgard CB, An J, Bain JR, *et al.* A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009; 9(4): 311-26. [http://dx.doi.org/10.1016/j.cmet.2009.02.002] [PMID: 19356713]

[105] Zhang X, Zhang C, Chen L, Han X, Ji L. Human serum acylcarnitine profiles in different glucose tolerance states. Diabetes Res Clin Pract 2014; 104(3): 376-82. [http://dx.doi.org/10.1016/j.diabres.2014.04.013] [PMID: 24837145]

[106] Bene J, Márton M, Mohás M, *et al.* Similarities in serum acylcarnitine patterns in type 1 and type 2 diabetes mellitus and in metabolic syndrome. Ann Nutr Metab 2013; 62(1): 80-5. [http://dx.doi.org/10.1159/000345759] [PMID: 23296094]

[107] Sun L, Liang L, Gao X, *et al.* Early Prediction of Developing Type 2 Diabetes by Plasma Acylcarnitines: A Population-Based Study. Diabetes Care 2016; 39(9): 1563-70. [http://dx.doi.org/10.2337/dc16-0232] [PMID: 27388475]

[108] Guasch-Ferre M, Ruiz-Canela M, Li J, *et al.* Plasma acylcarnitines and risk of type 2 diabetes in a Mediterranean population at high cardiovascular risk. J Clin Endocrinol Metab 2019; 104: 1508-19. [PMID: 30423132]

[109] Schooneman MG, Vaz FM, Houten SM, Soeters MR. Acylcarnitines: reflecting or inflicting insulin resistance? Diabetes 2013; 62(1): 1-8. [http://dx.doi.org/10.2337/db12-0466] [PMID: 23258903]

[110] Bouchouirab FZ, Fortin M, Noll C, Dube J, Carpentier AC. Plasma palmitoyl-carnitine (AC16:0) is a marker of increased postprandial nonesterified incomplete fatty acid oxidation rate in adults with type 2 diabetes. Canadian journal of diabetes 2018; 42(4): 382-8 e1.

[111] Veenstra-Vanderweele J, Christian SL, Cook EH Jr. Autism as a paradigmatic complex genetic disorder. Annu Rev Genomics Hum Genet 2004; 5: 379-405. [http://dx.doi.org/10.1146/annurev.genom.5.061903.180050] [PMID: 15485354]

[112] Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder. Curr Opin Genet Dev 2012; 22(3): 229-37. [http://dx.doi.org/10.1016/j.gde.2012.03.002] [PMID: 22463983]

[113] Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Mol Psychiatry 2012; 17(3): 290-314. [http://dx.doi.org/10.1038/mp.2010.136] [PMID: 21263444]

[114] Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 2011; 25(1): 40-5. [http://dx.doi.org/10.1016/j.bbi.2010.08.003] [PMID: 20705131]

[115] Filipek PA, Juranek J, Nguyen MT, Cummings C, Gargus JJ. Relative carnitine deficiency in autism. J Autism Dev Disord 2004; 34(6): 615-23. [http://dx.doi.org/10.1007/s10803-004-5283-1] [PMID: 15679182]

[116] Cox KB, Hamm DA, Millington DS, *et al.* Gestational, pathologic and biochemical differences between very long-chain acyl-CoA dehydrogenase deficiency and long-chain acyl-CoA dehydrogenase deficiency in the mouse. Hum Mol Genet 2001; 10(19): 2069-77. [http://dx.doi.org/10.1093/hmg/10.19.2069] [PMID: 11590124]

[117] Clark-Taylor T, Clark-Taylor BE. Is autism a disorder of fatty acid metabolism? Possible dysfunction of mitochondrial beta-oxidation by long chain acyl-CoA dehydrogenase. Med Hypotheses 2004; 62(6): 970-5. [http://dx.doi.org/10.1016/j.mehy.2004.01.011] [PMID: 15142659]

[118] Thomas RH, Foley KA, Mepham JR, Tichenoff LJ, Possmayer F, MacFabe DF. Altered brain phospholipid and acylcarnitine profiles in propionic acid infused rodents: further development of a potential model of autism spectrum disorders. J Neurochem 2010; 113(2): 515-29. [http://dx.doi.org/10.1111/j.1471-4159.2010.06614.x] [PMID: 20405543]

[119] Fallon J. Could one of the most widely prescribed antibiotics amoxicillin/clavulanate “augmentin” be a risk factor for autism? Med Hypotheses 2005; 64(2): 312-5. [http://dx.doi.org/10.1016/j.mehy.2004.06.023] [PMID: 15607562]

[120] Frye RE, Melnyk S, Macfabe DF. Unique acyl-carnitine profiles are potential biomarkers for acquired mitochondrial disease in autism spectrum disorder. Transl Psychiatry 2013; 3e220

[http://dx.doi.org/10.1038/tp.2012.143] [PMID: 23340503]
[121] Lv QQ, You C, Zou XB, Deng HZ. Acyl-carnitine, C5DC, and C26 as potential biomarkers for diagnosis of autism spectrum disorder in children. Psychiatry Res 2018; 267: 277-80. [http://dx.doi.org/10.1016/j.psychres.2018.06.027] [PMID: 29945069]
[122] Bartel LL, Hussey JL, Shrago E. Perturbation of serum carnitine levels in human adults by chronic renal disease and dialysis therapy. Am J Clin Nutr 1981; 34(7): 1314-20. [http://dx.doi.org/10.1093/ajcn/34.7.1314] [PMID: 7258121]
[123] Rodriguez-Segade S. Alonso dlP, Paz JM, Novoa D, Arcocha V, Romero R, *et al*. Carnitine deficiency in haemodialysed patients. Clinica chimica acta; international journal of clinical chemistry 1986; 159(3): 249-56.
[124] Golper TA, Wolfson M, Ahmad S, *et al.* Multicenter trial of L-carnitine in maintenance hemodialysis patients. I. Carnitine concentrations and lipid effects. Kidney Int 1990; 38(5): 904-11. [http://dx.doi.org/10.1038/ki.1990.289] [PMID: 2266674]
[125] Brass EP, Adler S, Sietsema KE, Hiatt WR, Orlando AM, Amato A. CHIEF Investigators. Intravenous L-carnitine increases plasma carnitine, reduces fatigue, and may preserve exercise capacity in hemodialysis patients. Am J Kidney Dis 2001; 37(5): 1018-28. [http://dx.doi.org/10.1016/S0272-6386(05)80019-8] [PMID: 11325685]
[126] Debska-Slizień, Kawecka A, Wojnarowski K, *et al.* Correlation between plasma carnitine, muscle carnitine and glycogen levels in maintenance hemodialysis patients. Int J Artif Organs 2000; 23(2): 90-6. [http://dx.doi.org/10.1177/039139880002300205] [PMID: 10741803]
[127] Steiber AL, Weatherspoon LJ, Spry L, Davis AT. Serum carnitine concentrations correlated to clinical outcome parameters in chronic hemodialysis patients. Clin Nutr 2004; 23(1): 27-34. [http://dx.doi.org/10.1016/S0261-5614(03)00085-2] [PMID: 14757390]
[128] Kamei Y, Kamei D, Tsuchiya K, Mineshima M, Nitta K. Association between 4-year all-cause mortality and carnitine profile in maintenance hemodialysis patients. PLoS One 2018; 13(8): e0201591. [http://dx.doi.org/10.1371/journal.pone.0201591] [PMID: 30133480]
[129] Evans A. Dialysis-related carnitine disorder and levocarnitine pharmacology. Am J Kidney Dis 2003; 41(4)(Suppl. 4): S13-26. [http://dx.doi.org/10.1016/S0272-6386(03)00113-6] [PMID: 12751050]
[130] Reuter SE, Evans AM, Faull RJ, Chace DH, Fornasini G. Impact of haemodialysis on individual endogenous plasma acylcarnitine concentrations in end-stage renal disease. Ann Clin Biochem 2005; 42(Pt 5): 387-93. [http://dx.doi.org/10.1258/0004563054889954] [PMID: 16168195]
[131] Csiky B, Bene J, Wittmann I, Sulyok E, Melegh B. Effect of hemodialysis session on the dynamics of carnitine ester profile changes in L: -carnitine pretreated end-stage renal disease patients. Int Urol Nephrol 2013; 45: 847-55. [PMID: 22684763]
[132] Ahmad S. L-carnitine in dialysis patients. Semin Dial 2001; 14(3): 209-17. [http://dx.doi.org/10.1046/j.1525-139X.2001.00055.x] [PMID: 11422928]
[133] Zhang YM, Zhuo L, Hu J, *et al.* Clinical significance of different carnitine levels for improving the prognosis of patients undergoing hemodialysis. Ren Fail 2016; 38(10): 1654-8. [http://dx.doi.org/10.1080/0886022X.2016.1229967] [PMID: 27758157]
[134] Kaneko M, Fukasawa H, Ishibuchi K, Niwa H, Yasuda H, Furuya R. L-carnitine Improved the Cardiac Function *via* the Effect on Myocardial Fatty Acid Metabolism in a Hemodialysis Patient. Intern Med 2018; 57(24): 3593-6. [http://dx.doi.org/10.2169/internalmedicine.1055-18] [PMID: 30146554]
[135] Maruyama T, Maruyama N, Higuchi T, *et al.* Efficacy of L-carnitine supplementation for improving lean body mass and physical function in patients on hemodialysis: a randomized controlled trial. Eur J Clin Nutr 2019; 73(2): 293-301. [http://dx.doi.org/10.1038/s41430-018-0348-y] [PMID: 30353121]
[136] Bene J, Csiky B, Komlosi K, Sulyok E, Melegh B. Dynamic adaptive changes of the serum carnitine esters during and after L-carnitine supplementation in patients with maintenance haemodialysis. Scand J Clin Lab Invest 2011; 71(4): 280-6. [http://dx.doi.org/10.3109/00365513.2011.560674] [PMID: 21366497]
[137] Bene J, Csiky B, Wittmann I, Sulyok E, Melegh B. Dramatic decrease of carnitine esters after interruption of exogenous carnitine supply in hemodialysis patients. Ren Fail 2012; 34(5): 555-8. [http://dx.doi.org/10.3109/0886022X.2012.664509] [PMID: 22417076]
[138] Suzuki A, Sakai Y, Hashimoto K, Osawa H, Tsuruoka S. Kinetics of carnitine concentration after switching from oral administration to intravenous injection in hemodialysis patients. Ren Fail 2018; 40(1): 196-200. [http://dx.doi.org/10.1080/0886022X.2018.1455587] [PMID: 29616582]
[139] Van Blerkom J, Davis PW, Lee J. ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. Hum Reprod 1995; 10(2): 415-24. [http://dx.doi.org/10.1093/oxfordjournals.humrep.a135954] [PMID: 7769073]
[140] Nagano M, Katagiri S, Takahashi Y. ATP content and maturational/developmental ability of bovine oocytes with various cytoplasmic morphologies. Zygote 2006; 14(4): 299-304. [http://dx.doi.org/10.1017/S0967199406003807] [PMID: 17266788]
[141] McKeegan PJ, Sturmey RG. The role of fatty acids in oocyte and early embryo development. Reprod Fertil Dev 2011; 24(1): 59-67. [http://dx.doi.org/10.1071/RD11907] [PMID: 22394718]
[142] Van Hoeck V, Sturmey RG, Bermejo-Alvarez P, *et al.* Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. PLoS One 2011; 6(8): e23183. [http://dx.doi.org/10.1371/journal.pone.0023183] [PMID: 21858021]
[143] Moawad AR, Tan SL, Xu B, Chen HY, Taketo T. L-carnitine supplementation during vitrification of mouse oocytes at the germinal vesicle stage improves preimplantation development following maturation and fertilization *in vitro*. Biol Reprod 2013; 88(4): 104. [http://dx.doi.org/10.1095/biolreprod.112.107433] [PMID: 23446455]
[144] Kitano Y, Hashimoto S, Matsumoto H, *et al.* Oral administration of l-carnitine improves the clinical outcome of fertility in patients with IVF treatment. Gynecological endocrinology: The official journal of the International Society of Gynecological Endocrinology 2018; 34(8): 684-8. [http://dx.doi.org/10.1080/09513590.2018.1431769]
[145] Baka S, Malamitsi-Puchner A. Novel follicular fluid factors influencing oocyte developmental potential in IVF: a review. Reprod Biomed Online 2006; 12(4): 500-6. [http://dx.doi.org/10.1016/S1472-6483(10)62005-6] [PMID: 16740225]
[146] Várnagy A, Bene J, Sulyok E, Kovács GL, Bódis J, Melegh B. Acylcarnitine esters profiling of serum and follicular fluid in patients undergoing *in vitro* fertilization. Reprod Biol Endocrinol 2013; 11: 67. [http://dx.doi.org/10.1186/1477-7827-11-67] [PMID: 23866102]
[147] Montjean D, Entezami F, Lichtblau I, Belloc S, Gurgan T, Menezo Y. Carnitine content in the follicular fluid and expression of the enzymes involved in beta oxidation in oocytes and cumulus cells. J Assist Reprod Genet 2012; 29(11): 1221-5.

[http://dx.doi.org/10.1007/s10815-012-9855-2] [PMID: 23054356]

[148] Dunning KR, Robker RL. Promoting lipid utilization with l-carnitine to improve oocyte quality. Anim Reprod Sci 2012; 134(1-2): 69-75. [http://dx.doi.org/10.1016/j.anireprosci.2012.08.013] [PMID: 22917873]

[149] Gervais A, Battista MC, Carranza-Mamane B, Lavoie HB, Baillargeon JP. Follicular fluid concentrations of lipids and their metabolites are associated with intraovarian gonadotropin-stimulated androgen production in women undergoing *in vitro* fertilization. J Clin Endocrinol Metab 2015; 100(5): 1845-54. [http://dx.doi.org/10.1210/jc.2014-3649] [PMID: 25695883]

[150] Beydoun A, Sackellares JC, Shu V. Safety and efficacy of divalproex sodium monotherapy in partial epilepsy: a double-blind, concentration-response design clinical trial. Neurology 1997; 48(1): 182-8. [http://dx.doi.org/10.1212/WNL.48.1.182] [PMID: 9008516]

[151] Marson AG, Al-Kharusi AM, Alwaidh M, *et al.* SANAD Study group. The SANAD study of effectiveness of valproate, lamotrigine, or topiramate for generalised and unclassifiable epilepsy: an unblinded randomised controlled trial. Lancet 2007; 369(9566): 1016-26. [http://dx.doi.org/10.1016/S0140-6736(07)60461-9] [PMID: 17382828]

[152] Bond DJ, Lam RW, Yatham LN. Divalproex sodium versus placebo in the treatment of acute bipolar depression: a systematic review and meta-analysis. J Affect Disord 2010; 124(3): 228-34. [http://dx.doi.org/10.1016/j.jad.2009.11.008] [PMID: 20044142]

[153] D'Amico D. Pharmacological prophylaxis of chronic migraine: a review of double-blind placebo-controlled trials. Neurological sciences: official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology 2010; S23-8. [http://dx.doi.org/10.1007/s10072-010-0268-7]

[154] Melegh B, Pap M, Morava E, Molnar D, Dani M, Kurucz J. Carnitine-dependent changes of metabolic fuel consumption during long-term treatment with valproic acid. J Pediatr 1994; 125(2): 317-21. [http://dx.doi.org/10.1016/S0022-3476(94)70218-7] [PMID: 8040784]

[155] Okumura A, Kurahashi H, Iwayama H, Numoto S. Serum carnitine levels of children with epilepsy: Related factors including valproate. Brain Dev 2019; 41(6): 516-21. [http://dx.doi.org/10.1016/j.braindev.2019.02.010] [PMID: 30827788]

[156] Werner T, Treiss I, Kohlmueller D, *et al.* Effects of valproate on acylcarnitines in children with epilepsy using ESI-MS/MS. Epilepsia 2007; 48(1): 72-6. [http://dx.doi.org/10.1111/j.1528-1167.2006.00833.x] [PMID: 17241210]

[157] Silva MF, Selhorst J, Overmars H, *et al.* Characterization of plasma acylcarnitines in patients under valproate monotherapy using ESI-MS/MS. Clin Biochem 2001; 34(8): 635-8. [http://dx.doi.org/10.1016/S0009-9120(01)00272-7] [PMID: 11849623]

[158] Kossak BD, Schmidt-Sommerfeld E, Schoeller DA, Rinaldo P, Penn D, Tonsgard JH. Impaired fatty acid oxidation in children on valproic acid and the effect of L-carnitine. Neurology 1993; 43(11): 2362-8. [http://dx.doi.org/10.1212/WNL.43.11.2362] [PMID: 8232957]

[159] Hirose S, Mitsudome A, Yasumoto S, Ogawa A, Muta Y, Tomoda Y. Valproate therapy does not deplete carnitine levels in otherwise healthy children. Pediatrics 1998; 101(5): E9. [http://dx.doi.org/10.1542/peds.101.5.e9] [PMID: 9565442]

[160] Nakajima Y, Ito T, Maeda Y, *et al.* Evaluation of valproate effects on acylcarnitine in epileptic children by LC-MS/MS. Brain Dev 2011; 33(10): 816-23. [http://dx.doi.org/10.1016/j.braindev.2010.12.003] [PMID: 21196091]

[161] Morand R, Todesco L, Donzelli M, Fischer-Barnicol D, Mullen PJ, Krähenbühl S. Effect of short- and long-term treatment with valproate on carnitine homeostasis in humans. Ther Drug Monit 2012; 34(4): 406-14. [http://dx.doi.org/10.1097/FTD.0b013e3182608e2f] [PMID: 22743351]

[162] Kulhas Celik I, Tasdemir HA, Ince H, Celik H, Sungur M. Evaluation ofserum free carnitine/acylcarnitine levels and left ventricular systolic functions in children with idiopathic epilepsy receiving valproic acid. Clin Neurol Neurosurg 2018; 170: 106-12. [http://dx.doi.org/10.1016/j.clineuro.2018.05.005] [PMID: 29772401]

[163] Nakajima Y, Ito T, Maeda Y, *et al.* Detection of pivaloylcarnitine in pediatric patients with hypocarnitinemia after long-term administration of pivalate-containing antibiotics. Tohoku J Exp Med 2010; 221(4): 309-13. [http://dx.doi.org/10.1620/tjem.221.309] [PMID: 20651467]

[164] Brass EP, Mayer MD, Mulford DJ, Stickler TK, Hoppel CL. Impact on carnitine homeostasis of short-term treatment with the pivalate prodrug cefditoren pivoxil. Clin Pharmacol Ther 2003; 73(4): 338-47. [http://dx.doi.org/10.1016/S0009-9236(02)17636-3] [PMID: 12709724]