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Review article

Carnitine: A novel health factor-An overview

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ABSTRACT

Carnitine term comprises L-carnitine, acetyl –L-carnitine and Propionyl –L-carnitine. Carnitine in greater amount obtained from animal dietary sources as compared to plant sources. The endogenous synthesis of carnitine takes place in animal tissues like liver, kidney and brain using precursor amino acid lysine and methionine by iron, vitamin C, niacin, pyridoxine dependent pathway. This is the basis of vegans generally depending on carnitine in larger proportion through in vivo synthesis than omnivorous subjects. The concentration of Tri methyl lysine residues and the tissue specificity of Butyro betaine Dehydrogenase will play a significant role in regulating the carnitine biosynthesis. Carnitine transport from the site of synthesis to target tissue occurs via blood. Therefore, the measurement of normal plasma carnitine concentration represents the balance between the rate of synthesis and rate of excretion through specific transporter proteins. The cellular functional role of carnitine depends on the uptake in to cells through carnitine transport proteins and transport in to mitochondrial matrix. The function of carnitine is to traverse Long chain Fatty Acids across inner mitochondrial membrane for β-oxidation for rapid production of ATP. The carnitine level in plasma or tissue is done by spectro photometric, HPLC, or Tandem Mass Spectro photometry methods. Carnitine deficiency results in muscle disorders, there are two types of deficiency states such as primary and secondary deficiency. The primary is of systemic or myopathic, characterized by defect of high affinity organic cation transporter protein present on the plasma membrane of liver and kidney and also due to dysfunction of carnitine reabsorbtion through similar transport proteins in renal tubules. However, secondary carnitine deficiency associated with mitochondrial disorders and also defect of β-oxidation such as CPT-II and acyl CoA Dehydrogenase, several other causes are vitamin c deficiency, valaproate therapy, fancony syndrome, liver dysfunction, and kidney disease. Both these conditions results in recurrent muscle cramp, muscle weakness and fatigue, non ketotic hypoglycemia, encephalopathy, hepatomegaly, muscle necrosis, etc. Therefore, In view of the life threatening events of carnitine deficiency, Food drug administration considered L-carnitine as a drug to treat the primary and secondary carnitine deficiency. In recent times, carnitine has been extensively studied in various research activities to explore the therapeutic benefit. Thus, carnitine justifies as a novel health factor.

Key words: Acetyl Carnitine, Trimethyl lysine, β-oxidation, Carnitine palmitoyl transferase, L-Carnitine, Myopathy

1. INTRODUCTION

Carnitine ($C_7H_{15}NO_3$) is a naturally occurring dipolar amino acid like compound widely distributed in nature. In human body, it is particularly stored in skeletal muscles, heart, brain and sperm. In 1905, this quaternary ammonium compound was discovered in meat, and was extracted by Russian scientists *Gulewitsch* and *Krimberg*. The term carnitine is derived from the Latin term *carnis* means meat. Carnitine is chemically known as β-hydroxy-γ-Trimethyl ammonium butyrate or 4-N-trimethyl-3-hydroxy butyrate and it is a betaine derivative, structurally resembles choline.

In 1957, Fraenkel and Friedmann identified an essential factor required for the growth of the meal worm Tenebrio molitor named as vitamin B_T means biological B complex group of vitamins for the Tenebrio meal worm. The same was later on resolved as Carnitine [1]. The physical

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properties of Carnitine are water solubility, white crystalline powder, and possess melting point 210-212°C with a Molecular mass 161.2 g per molecule. Carnitine has less ultra violet absorption property but its natural degradant crotonyl betaine has many fold ultra violet absorption capacity due to two conjugated double bonds.

Carnitine biosynthesis takes place in living cells of liver and kidney. The precursors required for synthesis are essential amino acid lysine, methionine [2] and a cofactor L-ascorbic acid [3-4]. Therefore, it is not an essential dietary component due to in vivo synthesis. The biological effect of Carnitine depends on dietary supply of good quality proteins or complete proteins with high biological value. This has led to conditional supply of Carnitine in relation to Carnitine deficiency [5].

Carnitine exists as two types of stereoisomers, amongst them, L-Carnitine is naturally occurring and found to be biologically active, where as D-Carnitine is biologically inactive and does not occur naturally [6]. However, D-carnitine used to deplete tissue L-carnitine level partially [7]. Generally, the term Carnitine comprises number of different compounds such as L-carnitine, Propionyl-L-carnitine and acetyl –L-Carnitine [8].

Metabolically, Carnitine plays an important role in energy generation by fatty acid oxidation in the mitochondrial matrix [9]. It participated in the transport of activated fatty acid (acyl Co A) from cytoplasm across inner mitochondrial membrane through Carnitine transport Proteins termed as Carnitine shuttle. Generally, fatty acid containing more than fourteen carbon atoms needs Carnitine transport; they constitute either dietary fatty acids or fatty acids released from adipose tissues in response to hormone sensitive Tri acyl glycerol lipase action [10].

As the liver and kidney synthesize sufficient amount of Carnitine, that is enough to meets the daily body need. Food and Nutritive Board of the National academy of sciences concluded that Carnitine is not an essential nutrient due to adequate synthesis in the body under good health and growth condition [11]. Therefore, it is known as "Conditional health supplement" in relation to Carnitine deficiency which is perhaps linked to protein malnutrition, malabsorption and liver disorder.

The dietary sources of Carnitine are plant and animal sources, plant sources are poor sources, where as animal sources are good sources. Few plant sources include nuts, seeds, legumes, vegetables, cereals etc. In animal sources, the higher concentration present in red meat [12], beef streaks, pork, fish, chicken breast, lambs, sheep and diary by products like poultry eggs milk and cheese etc.

There has been extensive research work done in the field of carnitine since few decades. This overview article comprises the brief perspectives, biosynthesis, functions, and metabolic disorders of carnitine. Considerable effort is made to present the results obtained in human studies using secondary data source.

2. BIOSYNTHESIS OF CARNITINE

Carnitine homeostasis is maintained by endogenous synthesis, absorption from dietary foods, and tubular reabsorption by the kidney. Carnitine is essential metabolite and plays various indispensable roles in intermediary metabolism. Its anabolism occurs in liver and kidney using precursor lysine and methionine together with vitamin C, pyridoxine, niacin, and iron. Vegetarians obtain little Carnitine in their diet, therefore, the carnitine biosynthesis is highly significant in them in comparison to omnivorous humans due to their major source of Carnitine is meat [13]. However, in omnivorous humans, Carnitine is obtained about 75% from the diet and 25% from the de novo synthesis [14].

In mammals, certain proteins like actin, myosin, calmodulin, cytochrome-c and histones contains L-lysine amino acid residues, which during their post translational modifications event undergoes transmethylation reactions [15]. This reaction is catalyzed by specific methyl transferases using Active methionine ie S-Adesonosyl Methionine as methyl group donor [16]. The product produced in this reaction is Protein bound N6-trimethyl-L-lysine residues and S-Adenosyl Homocysteine. Lysosomal proteolytic enzymes cleave the protein part and liberate the free 6-N-trimethyl lysine residues, which is the initial metabolite in Carnitine biosynthesis [17-18].

The tissue distributions of Carnitine-biosynthetic enzymes in humans have been investigated [19]. N6-trimethyl lysine residues hydroxylated by N6-trimethyl lysine Dehydrogenase, which is non heme ferrous-iron dioxygenase present on outer mitochondrial membrane that catalyze the hydroxylation reaction in presence of L-ascorbic acid that require to maintains the iron atom in reduced state, Iron atom (Fe^{2+}), oxygen molecule, and 2-oxoglutarate undergoes oxidative decarboxylation to succinate and carbon dioxide [20]. The product formed is 3-hydroxy-N6-trimethyl lysine. N6-trimethyl lysine Dehydrogenase activity is highest in Kidney, heart, muscle, and brain.

The cytosolic enzyme 3-hydroxy-N6-trimethyllysine aldolase, which catalyse aldolytic or non hydrolytic cleavage of 3-hydroxy-N6-trimethyl lysine to release glycine molecule and produce the product 4-N-trimethyl amino butyraldehyde. This enzyme activity was found highest in liver, which is identical to Serine hydroxyl methyltransferase. In humans, from the two isoenzymes (cytosolic and mitochondrial) the cytosolic serine hydroxyl methyltransferase is expressed predominantly in the kidney, liver, and skeletal muscle. This cytosolic Serine hydroxyl methyltransferase possess 3-hydroxy-N6-trimethyl lysine aldolase activity. Like many aldolase, this enzyme also depends on the co-enzyme pyridoxal phosphate [19]. However, mitochondrial Serine hydroxyl methyltransferase is expressed ubiquitously [21].

4-N-trimethyl amino butyraldehyde Dehydrogenase or deoxy carnititne aldehyde dehydrogenase, a liver cytoplasmic enzyme catalyze the oxidation reaction using niacin reducing equivalents to produce Deoxy Carnitine or Butyrobetaine

[22].Most of these tissues are able to produce this product efficiently. But, the conversion of Butyrobetaine in to L-carnitine only takes place in humans particularly in liver, kidney, brain and testes. Therefore only liver and kidney can transport carnitine to the blood. Butyrobetaine Dehydrogenase a dioxygenase catalyses the stereo specific hydroxylation of Butyrobetaine to L-Carnitine and oxidative decarboxylation of 2-oxoglutarate in to succinate and carbon dioxide. This enzyme also requires molecular oxygen, iron atom in ferrous state and L-ascorbic acid. Butyrobetaine Dehydrogenase activity expressed differentially in tissues such as three fold higher in kidney than liver [23] brain, testis, and epididymis. Butyrobetaine Dehydrogenase activity is age dependent, its activity low at birth, increases to adult values during puberty, however in kidney activity is unaltered in birth [24].

The biosynthetic pathway is summarized here in. The release of 6-N-trimethyllysine (TML) residues from certain proteins by lysosomal protein degradation undergoes hydroxylation by N6-trimethyl lysine Dehydrogenase (TMLD) in to 3-hydroxy-N6-trimethyllysine (HTML), which is cleaved by specific aldolase 3-hydroxy-N6-trimethyllysine aldolase (HTMLA) in to 4-N-trimethyl amino butyraldehyde [TMABA] and glycine, subsequently TMABA oxidized by 4-N-trimethyl amino butyraldehyde Dehydrogenase (TMABADH) to form Butyrobetaine (BB) Finally, stereospecific Butyrobetaine Dehydrogenase [BBD] yields L-carnitine from Butyrobetaine as shown in Fig.1.

3. REGULATION OF CARNITINE BIOSYNTHESIS

The supply of exogenous Carnitine precursors increases the Carnitine biosynthesis through increased rate of formation of epsilon Tri methyl lysine [TML] residues in proteins, therefore, the availability of TML is the rate limiting step for carnitine synthesis. Similarly, total turnover of proteins also furnish adequate substrate for Carnitine biosynthesis [25]. Peroxisomal Proliferators substances (Clofibrate) bind to Peroxisome proliferator's receptor-a and gets activated. This activation enhances the hepatic synthesis and regulated [26]. The hormones such as insulin, glucagon, nor epinephrine, thyroid hormones, dopamine and androgen also regulate the synthesis [27]. Dietary intake of Carnitine is more in case of non vegetarians in comparison to vegetarians; therefore the relay on biosynthesis of Carnitine is less when compared to vegans. Dietary Carnitine is absorbed in small intestine and enters the blood. Plasma concentration of Carnitine is 30-89 μM/L with normal mean value in males is 59.3 ± 11.9 μM/L, in female is 51.5 ± 11.6 μM/L [28].

Excess Carnitine is excreted through urine, however the high affinity of Carnitine transport involved in tubular reabsorbtion of Carnitine in the kidney and thus it is highly conserved after glomerular filtration process. This tubular reabsorbtion occurs through sodium dependent Carnitine transport and organic cation transport-2(OCTN2) [29-30]. Decreased carnitine biosynthesis also occurs by malnutrition in premature neonates, disease of the biosynthesis organ, and use of valproate (31). Most of the cells have low Km except liver and brain contains high Km value 0.5 mM/L and more than 1.0 mM/L respectively. This indicates the low affinity of carnitine uptake due to carnitine biosynthesis. However, those cells with quite low Km indicate the higher affinity to carnitine and are able to uptake carnitine from circulatory system.

4. FUNCTIONS OF CARNITINE

Carnitine is synthesized in liver, kidney and in heart muscle because they are highly dependent on energy during aerobic conditions, thereby these tissues contains high concentration of carnitine which is more than seventy percent of plasma. The other tissue where complete enzyme system for carnitine synthesis are lacking needs to obtain it by specific active transport through high affinity plasma membrane bound carnitine transporter from blood. Similarly, in kidney tubular cells also carnitine transport system has high affinity to carnitine to maintain conservation. In regard to this, carnitine is a carrier molecule of long chain fatty acid across inner mitochondrial membrane in to mitochondrial matrix for oxidation [32-33].

Long chain fatty acid do not traverse Inner mitochondrial membrane freely, oxidative phosphorylation of reducing equivalents released from Krebs cycle, depends on the acetyl CoA pool obtained from oxidation of carbohydrates. Therefore, the acetyl CoA pool from fatty acid oxidation hampering. Thereby, in the light of this, the fatty acid in the cytoplasm gets activated upon binding of CoA with thioester bond catalysed by Acyl CoA synthetase or Thiokinase using ATP as energy molecule.

The organic cation transport 2 helps to accumulate carnitine with in cells. L-carnitine traverse activated fatty acid in to matrix across inner mitochondrial membrane. There are four Carnitine Palmitoyl transferase (CPT) isoforms found, they are CPT1a in liver, CPT1b in muscle and other tissues, CPT1c in brain and testis and CPT II (34). The two isoenzymes form of carnitine Palmitoyl Transport (CPT) are CPT-I and CPT-II, CPT-I localized in inner side of the outer mitochondrial membrane, where the catalytic site and regulatory domain of enzyme are facing toward the cytosolic site that catalyse esterification of acyl coA and carnitine in to acyl-carnitine complex. This CPT–I enzyme also sensitive for malonyl CoA inhibition [35-37] and allosterically inhibited by malonylCoA[38] Acyl carnitine complex transported across Inner mitochondrial membrane through specific transporter- Carnitine-acyl carnitine translocase (CACT). The inner side of mitochondrial membrane, CPT-II is located; it breaks the acyl bond of acyl-carnitine releases fatty acid. Simultaneously, Coenzyme A recycling takes place and carnitine enters for transport of another molecule of fatty acid. The transported acyl CoA from cytoplasm undergoes B-oxidation for rapid energy release (Fig.2). Acetyl carnitine

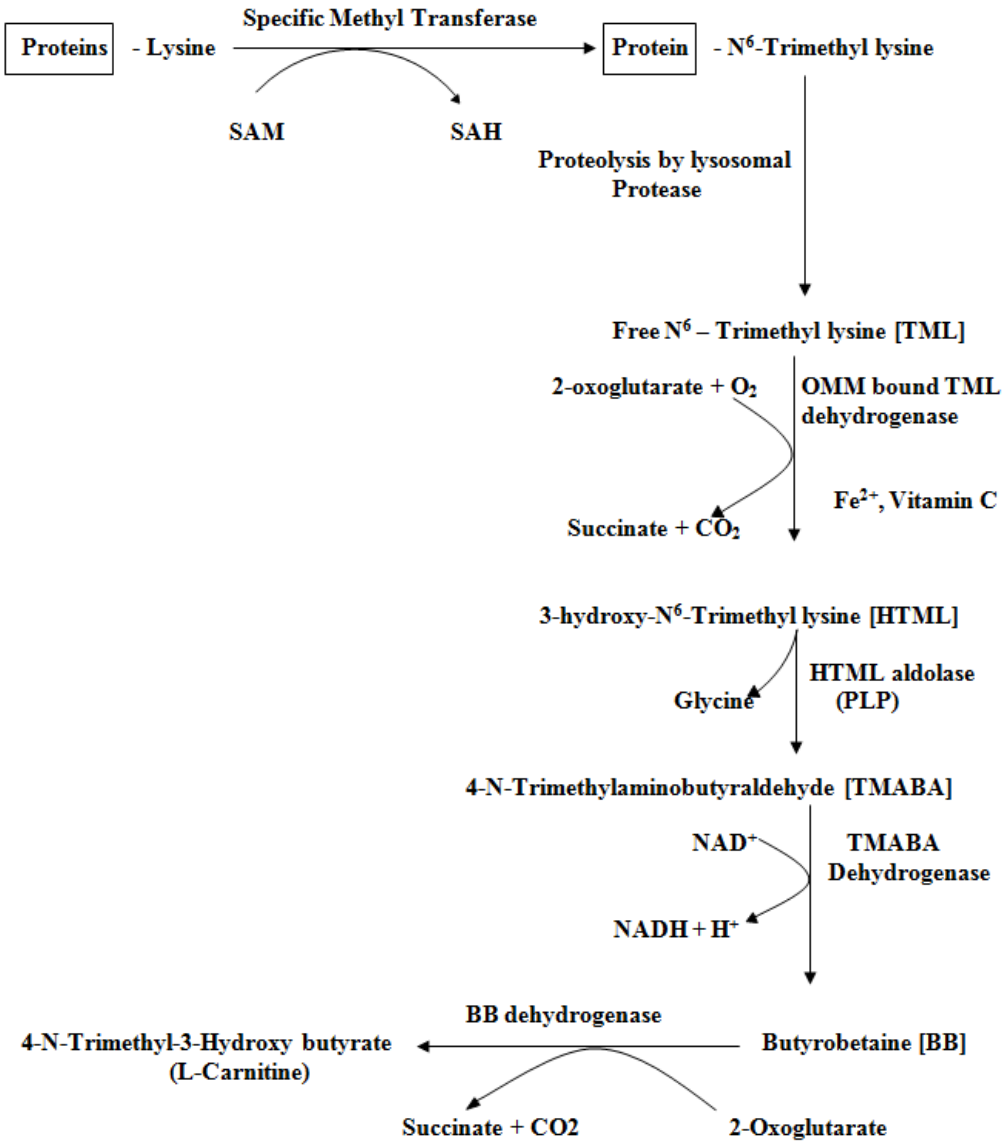

Fig.1. Biosynthesis of L-Carnitine from lysine

formed by acetyl CoA carboxylase transported in to cytoplasm where it serves as precursor for fatty acid or acetyl choline synthesis.

Human genetic defect disorders of enzymes and transporter protein affect the different steps involved in carnitine transport mechanism [39]. The carnitine uptake defect occurs due to the defect in cellular carnitine transporter (CT) that impairs the mechanism of fatty acid transport in to mitochondria for oxidation. This is reported to be rare kind of incidence occurs in population. The associated symptoms are hypoglycemia, and cardiomyopathy.

Several genetic disorders are well known due to genetic defect of CPT-I, CPT-II, CACT, amongst population [40]. These conditions associated with symptoms of liver disease, and recurrant hypoketotic hypoglycemia. The striking observation from the available literature is that heart and muscles are unaffected due to expression of genetically distinct isoforms and also they contain elevated carnitine level in plasma.

The Carnitine Palmitoyl transferase -I defect affects only liver, and causes a decrease in hepatic production of glucose and ketone bodies. Here, the affected have elevated plasma carnitine level [41].This condition has serious consequence in maternal illness during a pregnancy with associated symptoms like decreased release of glucose and decrease production of ketone bodies. The complications like Acute Fatty liver of Pregnancy (AFLP) and Hemolysis elevated liver enzymes level low platelet count (HELLP) are serious hepatic conditions in heterozygous women. Whose fetus later on going to have Long Chain hydroxyl Acyl CoA Dehydrogenase (LCHAD) deficiency.

The genetic defect of carnitine-acyl carnitine translocase (CACT) characterized by LCFA oxidation disorder, involves life threatening fatal multiorgan disorders occurs with rare

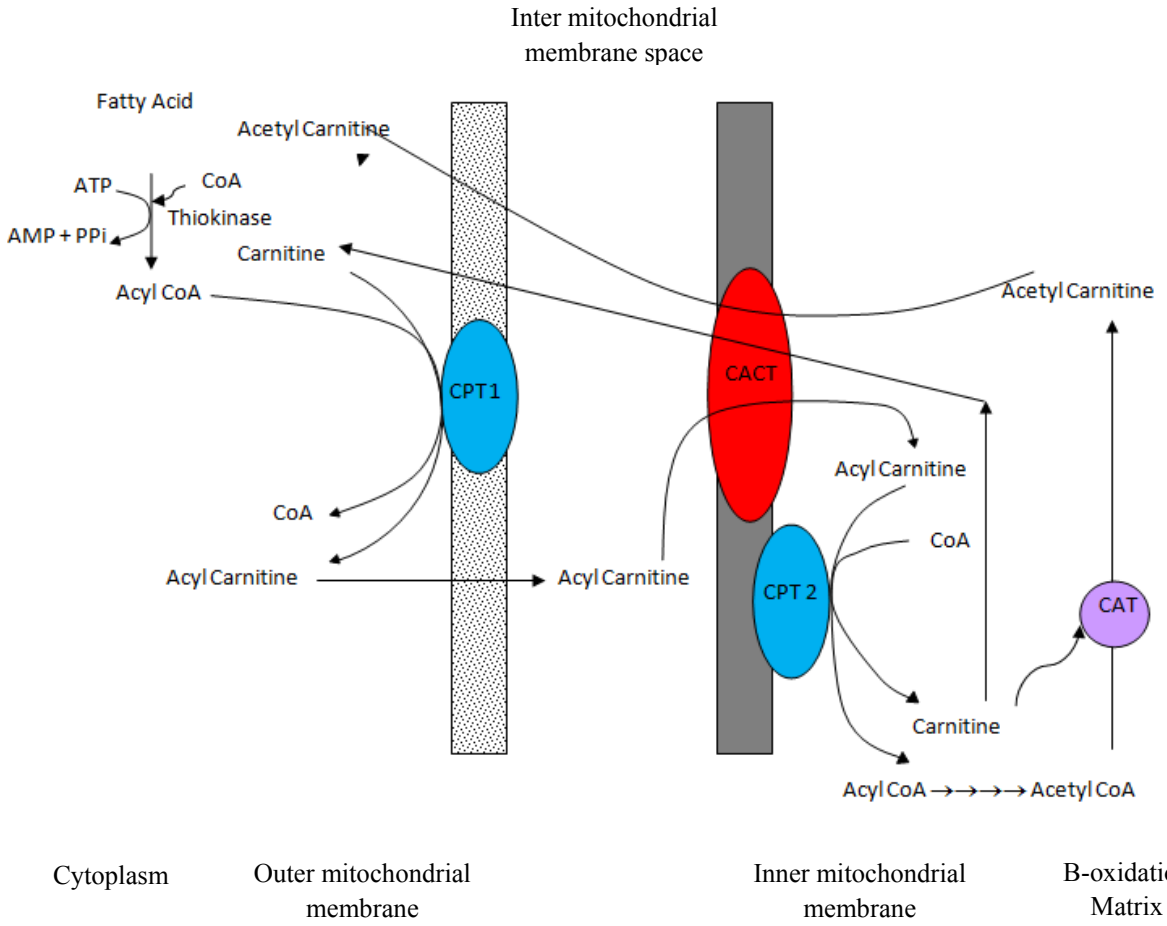

Fig.2. Role of L-Carnitine in transport of fatty acid in to matrix
CPT-I Carnitine Palmitoyl Transeferase -I; CPT-II Carnitine Palmitoyl Transeferase-II; CACT Carnitine Acyl Carnitine Translocase; CAT Carnitine Acetyl Transferase

frequency. The clinical features are symptoms like cardiomyopathy, intermittent hypoglycemia (liver disease) pregnancy eclampsia. In neonates, cardiomyopathy with arrhythmia leading to cardiac arrest Low carnitine levels in tissue and blood is common observation [42-44].

Generally, Carnitine Palmitoyl Transferase-II genetic defect reported as mild and severe form of defects. The mutation of CPT-II gene [45-47] at C4395 results altered activity of CPT-II enzyme in the mitochondrial matrix. That prevents oxidation of LCFA being transported in to mitochondrial matrix. CPT-II defect reported as mild form with loss of partial enzyme activity generally affects muscle results weakness during prolonged exercise [48], when muscle stands on energy source by fatty acid oxidation. Myoglobinuria due to exercise induced muscle breakdown are commonest symptoms of CPT-II deficiency. However, 1973, CPT-II deficiency as adult myopathic form first noticed by Dimauro and Dimauro [49]. However, mutation of CPT-II gene in severe form results with complete loss of enzyme activity is fatal multiorgan disorder in infants with cardiomyopathy, liver disease, and congenital anamolies. The serious consequences are hypoketotic hypoglycemia,cardiac malfunction and some time death due to Sudden Infant Death syndrome (SIDS) [50]. Thus finally CPT-II mild defect is rhabdomylosis like symptoms, severe defect with hypoketotic hypoglycemic cardiomyopathy followed by SIDS. Patients have low carnitine level but high acyl carnitine:free carnitine ratio than the normal 0.4 ratio, adults patients have serum and urine positive for myoglobin, serum Creatine kinase and elevated transaminase level.

As per the available information on all these enzymes such as CPT I, CPT 2, and CACT defect, a suggestion has been made to take the fat diet with less long chain fatty acids (LCFA), and with good amount of short chain fatty acids (SCFA) and medium chain fatty acids (MCFA) and also recommendation of carnitine supplementation. The avoid of fasting and exercise also advocated.

5. ESTIMATION OF CARNITINE

The major problem associated with carnitine deficiency is impairment in transport of fatty acid and oxidation. Therefore, the assay of carnitine concentration in biological samples and tissues is use full criteria to understand the

carnitine deficiency and related abnormalities. L-carnitine, acyl carnitine and total carnitine are measured by enzymatic or radio enzymatic methods. Propionyl-L-carnitine and other esters containing fatty acids with more than three carbon atoms can be measured by HPLC methods. Short, medium or long chain esters of carnitine can be measured by Tandem Mass spectrometry [51].

Several methods are available to measure carnitine level. The more convenient biological assay using yellow meal worm Tenebrio molitor larvae. The growth and survival of Tenebrio used as the criterion in assay for vitamin B_T (carnitine) [52]. The free and total carnitine are determined after removal of proteins by spectrophotometric method has been extensively adapted for carnitine assay and the procedure is conveniently extended for the use of automated analyzer [53-55]. In this method, sample containing L-carnitine allowed to react with the substance acetyl CoA catalyzed by carnitine-acetyl-transferase to form acetyl carnitine and CoA. Coenzyme-A reacts non enzymatically with 5,5'-Dithio bis 2-nitro benzoate (DTNB) to form 5-thio-2-nitrobenzoate (TNB). The concentration of TNB measured spectrophotometrically at 410nm [56-62]. This method has high precision, low reagent cost and less analysis time and also correlates with standard carnitine radio enzymatic assay.

Another spectrophotometric method for estimation of free carnitine is by using purified bacterial carnitine Dehydrogenase with NAD+. The acyl carnitine present is subjected for hydrolysis by bacterial acyl carnitine hydrolase before the determination of total carnitine. The measured free carnitine accounts for total carnitine [63]. Even though, spectrophotometric method is widely used, Tandem Mass spectrometry used to estimate the assay of carnitine and its short-chain, medium-chain and long-chain esters in biological fluids [64-66].

In Tandem mass spectrometry (TMS), carnitine can be measure by multiple stages of mass analysis separation. Accomplished with ions trapped in the same place with multiple separation steps taking place over time. In comparison to Radio enzymic assay, the Tandem mass spectrometry method is a precise method for determination of carnitine concentration in plasma that over comes the disadvantage of the radio chemical method. This procedure highly sensitive, free from radiological hazard and less volume of sample (100µl) required for analysis. The TMS measures carnitine and all types of acyl carnitine species developed for diagnostic screening purpose. Because the occurrence of acyl carnitine moiety in urine or blood is suggestive for the presence of inborn errors of metabolism and is of great value in addition to the Gas Chromatography-Mass spectroscopy (GC-MS) screening for the organic aciduria [65, 67-68].

Radio isotopic methods based on the reaction with C^{14} labeled acetyl CoA and its conversion in to C^{14}–acetyl carnitine. This is purified on a column; the sulfhydryl group of coenzyme A formed is oxidized by N-ethyl maleimide [69-71]. L-Carnitine, acetyl-L-carnitine and total L-carnitine including all types can easily be assayed by radio enzyme methods [72-73]. Besides, Carnitine estimation can also be done by HPLC methods [74-75]. Sample preparation steps for these methods include filtration, concentration, solid phase extraction and dialysis chamber extraction etc. However, in every method prior to estimation, the subject needs to be kept under fasting condition before assay of carnitine since it is widely distributed in the meat and dairy products. Accordingly, fasting criteria for adults and children's is directed.

6. CARNITINE DEFICIENCY

The low level of carnitine from normal level in blood and tissues indicates the carnitine deficiency. It occurs due to many causes namely decrease intake of carnitine rich foods like meat and diary products, decreased in vivo synthesis of carnitine due to liver dysfunction, excessive loss through urine under diuresis, hemodialysis and diarrhea conditions. Impairment in function of carnitine is associated with the genetic defect of carnitine transport system and β-oxidation and also due to more demand of carnitine by the body during ketosis or high fat oxidation. Use of anti convulsant drug valaproic acid also lowers blood carnitine level by increase in urinary excretion. Vitamin C deficiency decrease carnitine biosynthesis and creates carnitine deficiency [76-77].

The two types of carnitine deficiency are Primary carnitine deficiency and Secondary carnitine deficiency. In Primary carnitine deficiency, low levels of carnitine in the blood and tissues noticed. It is a genetic disorder due to defect of the high affinity cellular plasma membrane carnitine transporter present in tissues like kidney and muscle. The primary carnitine deficiency subdivided in to a) Systemic carnitine deficiency b) Myopathic carnitine deficiency. Systemic refers to generalized carnitine deficiency where as myopathic affects only muscles.

In primary systemic carnitine deficiency, plasma carnitine level decreases due to the defect of high affinity Plasma membrane carnitine transporter in tissues like muscle, kidney, heart and fibroblast [78].The low level of plasma carnitine due to the loss in to urine due to defective reabsorbtion of carnitine through kidney carnitine transporter. The inheritance of Primary Systemic carnitine deficiency is an autosomal recessive pattern, caused by the mutation of the gene SLC22A5 located on chromosome 5q31.1. This produce defective carnitine transporter known as protein organic cation transporter-2 (OCTN2) that decreases carnitine transport in to cells [79]. As a result, there is shortage of intracellular carnitine. which needs to release energy from LCFA oxidation in mitochondria. Reduced energy release causes some symptoms. Where as in myopathic carnitine deficiency tissue carnitine decreases without altering normal plasma carnitine level.

In both the cases, causative factor is genetically defective high affinity Plasma membrane carnitine-transporter system that usually results the deficiency manifestations such as

recurrent muscle cramp, progressive to severe skeletal muscle weakness and fatigue, hypoglycemic hypoketotic encephalopathy, cardiomyopathy, lipid storage myopathy, fatty liver (hepatomegaly), hyperamonemia muscle ache. Muscle necrosis with myoglobinuria, stomach discomfort with decreased Gastro intestinal motility results abdominal pain and diarrhea, Hypochromic anemia [80]. The affected subjects should avoid fasting and intake of dietary LCFA but supplement of SCFA and MCFA are beneficial, besides high intake of carbohydrate.

Secondary carnitine deficiencies caused due to certain metabolic disorders in mitochondria such as inherited defect of carnitine palmitoyl transferase II and acyl CoA Dehydrogenase in β-oxidation pathway. This defect results fatigue due to accumulation of acyl carnitine in plasma and tissues which are excreted in urine. Acyl carnitine also inhibits tubular carnitine transporter and decrease the tubular reabsorbtion of carnitine and thus lowers carnitine pool by enhanced excretion. Particular conditions like chronic kidney failure, lysinuria decrease carnitine biosynthesis, malnutrition, Liver disorder, fanconi syndrome with excess loss of carnitine in urine, vitamin C deficiency and use of cetain drugs Valaproate that inhibit renal tubular carnitine transporter, muscle carnitine uptake, sequestration of acetyl CoA disturbs the intermediary metabolism. Bicampicillin emetine, Zidovudine used in AIDS patients cause mitochondrial impairement and reduce carnitine uptake in muscles leads to carnitine deficiency [81].

The genes for the defective enzymes in fatty acid oxidation that cause secondary carnitine deficiency have been identified. In disorders of fatty acid oxidation, particularly Medium chain acyl CoA Dehydrogenase (A985G mutation) is the most common in born error of metabolism manifests within two years of life with the symptoms vomiting, lethargy, hypoketotic hypoglycemia and dicarboxylic aciduria.

Ketone bodies are the energy source during starvation, therefore, the absence of starvation ketosis is an indication of defect in fatty acid oxidation in liver, this also slows down the gluconeogenesis, Both these blockage increases the glucose utilization produce hypoglycemia.the combined effect results the drastic reduction in the supply of energy causes brain function abnormalties is encephalopathy. enlarged and poor contraction of heart is cardiomyopathy. Blockage of β-oxidation, increases the accumulation of medium chain fatty acids diverts in to alternate omega oxidation produce medium chain dicarboxylic acids that accumulates in blood is organic academia and their excretion in to urine is organic aciduria. Besides medium chain esters of glycine and carnitine are also excreted.

Fatty acid oxiadation defect makes the accumulation of excess lipid in muscles causes skeletal myopathy, hepatomegaly, cardiomyopathy. Long chain Fatty acyl carnitine is toxic to cardiac tissues in neonate's causes cardiac arrhythmia and often cardiac arrest. In pre term newborns immature renal tubular functions with impaired carnitine biosynthesis develop carnitine deficiency. Patients with this acyl coA Dehydrogenase defect should consume high carbohydrate diet and avoid fasting. CPT-II genetic defect (43) with mild defect presents rhabdomylosis triggered by prolonged exercise, and severe defect with cardiomyopathy, liver disease, congenital anamolies and adult onset myopathy. Similarly, CACT defect also multiorgan disorder produce clinical features of intermittent hypoglycemia, cardiomyopathy conditions are proved severe in the age of three years. [39,42].

Secondary carnitine deficiency seen in when breast fed infants with insufficient milk produce hypoketotic hypoglycemia, later cardiac and skeletal muscle disease manifest. Altered consciousness followed by seizures, apnea, and cardio respiratory arrest. Patients with organic academia in secondary carnitine deficiency presents hypoglycemia, ketoacidosis and hyperammonemia. Carnitine deficiency in Patients with mitochondrial respiratory chain defects presents, lactic acidosis, encephalopathy, storage myopathy. Carnitine deficiency associated with urea cycle defects presents hyperammonemia.

Diagnosis of carnitine deficiency in neonates by measuring carnitine palmitoyl transferee activity evaluated by screening blood by mass spectrometry. The prenatal diagnosis by using amniotic villous cells. But in adults, carnitine deficiency detected by measuring acyl carnitine in serum, urine and tissues for understanding systemic and myopathic deficiency.

7. CURRENT RESEARCH UPDATES ON CARNITINE

Carnitine has been extensively used in various research activities in relation to obtain the beneficial effects under disease states. Some of the current research concepts are summarized in this issue. L-carnitine and Propionyl –L-carnitine improves the ability of heart to angina that causing chest pain. L-carnitine used therapeutically [82]. Acetyl –L-carnitine more efficiently used due to the good absorption in to Intestinal Mucosal cell and brain tissues [83].

The best evidence of angina is heart muscle cramp occurs when heart muscle does not receive enough oxygen from coronary arteries due to atherosclerosis poses the risk of heart attack. Nitroglycerine gives the immediate effect of relief. In addition to this medication alternative treatment provides usefulness along in association with standard medical care. In support of this, in a controlled study comprises 200 patients with angina were given a daily dose of L-carnitine along with used medication improves the heart conditions which was measured by various measures. Accordingly, it was evident that L-carnitine dose with medication exhibits the greater exercise without chest pain and also reduced the medication dosage and angina symptoms [84-87].

The beneficial role of L-carnitine supplementation helps cardiovascular disease and peripheral arterial disease. This observation was results of few studies where they demonstrate that L-carnitine supplementation prevents the

subsequent heart attack after heart attack [88]. It is also found beneficial in the management of cardiac ischemia and peripheral arterial disease [89]. Such as intermittent claudication [90] that results from an inadequate supply of oxygen rich blood to the legs and due to low level of carnitine in muscle. Supplementation of the L-carnitine results the increase of myocardium tissue carnitine. It acts as antianginal agent that decreases ST segment depression and left ventricular end-diastolic pressure. The supplemented carnitine increases the carnitine level and reduces the toxic effects of free fatty acids and also by improving carbohydrate metabolism.

The cardio protective effects and beneficial to peripheral arterial disease have been confirmed the therapeutic effect of L-carnitine and propionyl –L-carnitine on cardiovascular diseases study shown that L-carnitine reduces the myocardial injury after ischemia and prevents the toxic effect of Fatty acid accumulation that occurs in ischemia and improves the heart recovery. Thus clinically carnitine has anti ischemic properties. Thus, L-carnitine decreases FFA, increases cholesterol metabolism, decreases acetyl co-A, Co-A ratio in mitochondria, and increases Pyruvate dehydrogenase activity, increases tissue carnitine content. Prevents the loss of high energy compounds store, releases lactate, these are cardio protective actions of L-carnitine. Similarly, propionyl L-carnitine is high affinity to muscle carnitine transferase, stimulates better efficacy of kreb's cycle during hypoxia by providing intermediate succinyl coA from propionate [91].

"You are as young as mitochondria" implies that impairment in mitochondrial function progress the ageing process generally, normal carnitine concentration in tissue accounts for the integrity of the mitochondrial membrane. But in ageing, decrease of carnitine concentration cause impairment of mitochondria. Therefore, L-carnitine supplements prevent the mitochondrial decay [92]. Mitochondrial theory of aging explained by increase of Reactive oxygen species (ROS) production that in turn damage mitochondrial DNA and also Electron Transport Chain Components results Respiratory chain dysfunction. This kind of Increase oxidative stress eventually leads to cell death. Therefore, the balance between ATP production and ROS accumulation responsible for the impact of aging. L-carnitine increases the ATP production, decrease ROS accumulation and thus preventing aging [93-94]. L-carnitine translocates acetyl moiety from mitochondrial matrix in to cytoplasm for acetyl choline synthesis in the brain. L-ascorbic acid, glutathione, Vitamin E level decreases in aging process and increase lipofuschin level but after carnitine administration lipofuschin decreases. This explains the antioxidant role of carnitine in decreasing the lipofuschin. Frailty is a geriatric syndrome characterized by muscle weakness, sarcopenia, and fatigue. Carnitine supplementation minimizes the occurrence of frailty in geriatric cases [95].

The clinical use of L-carnitine in male infertility condition is well studied [96-97] and showed that carnitine concentration is important for the sperm metabolism, maturation and sperm motility [98-99]. Several reports also indicated that L-carnitine role in enhancement of mitochondrial fatty acid oxidation that intern supply energy for sperm action and prevents the sperm cell death in testis [100]. Similarly, L-carnitine and acetyl –L-carnitine dose concentration 3g/day facilitates the sperm motility and evince the antioxidant property of carnitine in asthenozoospermic cases [101]. More recently, a report indicated that relationship between varying concentrations of L-carnitine solution to sperm cell, in an in vitro study, the increase of sperm motility and also induction of carnitine gene expression in sperm cell with 100mmol/L carnitine is documented [102] and also the highest concentration repressed the gene expression. Propionyl–l-carnitine supplementation in men increase the sperm count and mobility, improves the erectile dysfunction.

Carnitine taken up by athletes for improvement of performance at 2-6 g/day for 1-28 days however, there is no evidence of increase of carnitine in muscles [103]. Patient's carnitine level intravenously may improve insulin sensitivity in diabetics by lowering blood glucose level due to increase rate of oxidation of glucose in cells. L-carnitine and acetyl carnitine are effective in improving insulin mediated glucose disposal either in healthy or type 2 diabetes mellitus [104].

HIV decreases lymphocytes and causes AIDS, hyperlipidemia, and insulin resistance together cause lipid dystrophy syndrome this syndrome represent mitochondrial toxicity either by HIV infection and the antiretroviral drugs. This induces carnitine deficiency that decreases mitochondrial oxidation. But, in 1998, Moretti and his co-workers reported Fas system involved in apoptosis of T-lymphocytes in HIV infected subjects, the signal transduced across membrane through Fas receptor, this signal activates the sphingomylinase that intern breakdown the sphingomylein and produce ceramide. L-carnitine supplementation at the dose level of 2-6 g/day for a period of weeks / months in HIV infected patient may slows down the lymphocyte death by reducing the Fas (death receptor present on surface of cell that leads to apoptosis)activation and ceremide production [105], reduce neuropathy [106], In 2001,Mouss reported from his pilot study that effect of L-carnitine at the dose level of 1gm bid for a 3 months does not significantly reduce blood lipid, however, reduction in cholesterol was significant [107].

Propionyl-L-carnitine reduces the symptoms of heart failure and improves the heart function during exercise. It reduces the symptoms of heart failure particularly sarcolemmal defects in congestive heart failure due to myocardial infarction, however, and improvement of the heart function observed in animals when they were treated with 100 mg/kg Propionyl- L-carnitine daily through intra peritoneal route for 4 weeks. The animals were assessed for their left ventricular function. Sarcolemmal membranes were examined for Na^+–K^+ ATPase, Na^+–Ca^{2+} exchange and adenylate cyclase activities. A marked improvement in the attenuated left ventricular function of the experimental

animals was seen upon treatment with propionyl -L-carnitine [108].

Peripheral vascular disease (PVD) is a manifestation of systemic atherosclerosis in the lower limbs, and these patients have three to five fold increased risk of cardiovascular mortality, the demonstration of the beneficial effects of certain nutrients like carnitine in the diet helps in the prevention of cardio vascular diseases. In the article by Hiatt.W.R.(2001) and Carrero(2006), presented an overview of how foods contains carnitine possibly reduce the risk factors of intermittent claudication and peripheral vascular disease [109-110].

In 2005, Anders and his co-workers, reported that Acetyl-L-carnitine improves chronic diabetic neuropathy, which is a painful complication results from damage to the nerves by high levels of blood glucose often can lead to cardiac arrhythmias, foot ulcers and even amputations. It was revealed that acetyl-L-carnitine not only improves the symptoms of diabetic neuropathy, but also helps regenerate nerve fibers and vibration perception. The researchers evaluated 1,257 patients of randomized trials with 0.5 or 1.0 grams acetyl-L-carnitine. In subjects, nerve conduction velocity and vibratory threshold used as parameter before treatment and at the end of the study. Neuropathy symptoms were assessed by comparing biopsy results. The result showed that treatment with acetyl-L-carnitine increased the number of nerve fibers and regenerating nerve fiber clusters. Patients demonstrated significant improvement in pain at the study's midpoint and conclusion. Thus Carnitine helps to improve the symptoms of diabetic neuropathy by increasing nerve function and also nerve regeneration [111]. According to various research workers, the carnitine has been experimented in various disorder conditions. Namely Acetyl –L-carnitine used to treat progression of Alzheimer's disease, decreases the depression and also improves the memory in elderly people [112]. Muscle fatigue caused by chemotherapy, radiation, and malnutrition is common observation in cancerous patients and also characterized by low level of carnitine. L-carnitine supplementation reduces the fatigue condition and improves the mood and sleep behavior [113-114].

Seldom data available on use of acetyl-L-Carnitine used to obtain promising results in peyronies disease, which is characterized by a curvature of the penis that leads to pain during an erection (erectile dysfunction) due to blocked blood flow and gives promising results. Preliminary studies suggest Propionyl-L-carnitine may help improve male sexual function. One study found that carnitine significantly improved the effectiveness of sidenafil in men with diabetes who had not previously responded to Viagra [115].

Thyroxine hormone concentration affects the carnitine metabolism, hyperthyroidism increases the urinary excretion of carnitine, where as hypothyroidism decreases the excretion of L-carnitine as reported by Maebashi and S. Benvega [116-117].

Cellulite is a condition of abnormal fat deposition in subcutaneous layer of respective area results the altered topography of the skin in post adolescent women. Skin change is characterized by dimpling and nodulanty of the skin. L-carnitine is effective cellulite reducer by inducing fat burning process when used through topical application as anti-cellulite cream [118].

8. CONCLUSIONS

In the fore going discussion, it is evident that the distribution of carnitine in various foods particularly from animal origin, the role of carnitine in intermediary metabolism in relation to energy generation, biosynthesis of carnitine and its possible regulation and genetic disorders along with the few recent research updates. Although, the biochemical role of carnitine in connection to transport of long chain fatty acid transport for energy release across mitochondrial inner membrane for β-oxidation, the various applications of carnitine - needs to be learnt precisely with large group of patients study. The various physiological events exerted in this current overview indicate that each and every functional aspect on the fatty acid oxidation and immediate energy release. Therefore, Food drug administration justifies the validity of the carnitine used to obtain the therapeutic benefits by scientific evidence and recommended L-carnitine as a drug to treat the primary and secondary carnitine deficiency.

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