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Review

Biological roles of L-carnitine in perinatal metabolism

Joaquín Arenas*, Juan C. Rubio, Miguel A. Martín, Yolanda Campos

Centro de Investigación Hospital Universitario 12 de Octubre, Avda de Andalucía, km 5.4., 28041 Madrid, Spain

Abstract

Carnitine performs a crucial role in the energy supply of tissues during fetal life and in the neonatal period by controlling the influx of fatty acids into mitochondria. Carnitine also facilitates the oxidation of pyruvate and branched chain amino acids, and contributes to the protection of cells from the deleterious actions of acyl CoAs. Carnitine further acts as a secondary antioxidant, favouring fatty acid replacement within previously oxidatively damaged membrane phospholipids. Availability of L-carnitine is essential in the developing fetus for processes underlying fetal maturation. L-carnitine is also essential for development of hepatic ketone synthesis, a central pathway for neonatal energy metabolism. Ketone bodies inhibit the oxidation of both glucose and lactate, sparing these metabolic substrates for biosynthetic functions.

Keywords: L-carnitine; Beta oxidation; Fetal maturation; Energy metabolism; Perinatal metabolism; Antioxidant

1. Carnitine metabolism

About 75% of the carnitine source for the body stores comes from the diet: red meat in adults and human milk in infants are the main sources. In man the liver and the kidney synthesize the remaining 25% from the immediate precursor gamma butyrobetaine: protein-bound lysine and methionine are required for carnitine biosynthesis [12]. Carnitine is present in tissues and biological fluids in free and esterified forms. In humans, acylcarnitine esters account for some 25% of total

*Corresponding author. Tel.: + 34-1-3908598; fax: + 34-1-3908358; e-mail: jarenas@h12o.es

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carnitine in serum and for about 15% of total carnitine in liver and skeletal muscle [8,9,12]. Total carnitine concentration in human tissues is higher in the heart and skeletal muscle than in the liver and the brain. These values reflect the higher rates of fatty acid oxidative metabolism in the former tissues [12]. Because of the bulk of skeletal muscle and its great carnitine concentration, most of the total body carnitine is stored in muscle [8,12]. The remaining body carnitine is in the heart, liver and kidney, with less than 1% in biological fluids. Carnitine in blood is much less concentrated than in tissues [12].

Consequently carnitine, either introduced in the diet or synthesized de novo in the liver and kidney, must be actively concentrated from the blood into fatty acid-metabolizing organs, such as skeletal muscle [12]. Cell receptors with high affinity for carnitine have been identified in muscle and heart cells, and cultured fibroblasts [12]. The liver and the brain have low-affinity receptors for carnitine, while the intestinal epithelial cells and the renal tubular cells have intermediate-affinity receptors for carnitine [12]. The kidney, besides its contribution carnitine biosynthesis, is crucial in regulating the plasma and tissue levels of carnitine. The renal threshold for free carnitine is similar to the concentration of carnitine in plasma, and the proximal tubule actively transports carnitine across its membrane, thus minimizing the loss of carnitine from the body [3].

Therefore, a complex metabolic equilibrium exists between the various carnitine fractions in the different body compartments, and between the carnitine pool of tissues and blood, as well as the fraction excreted in the urine.

2. Old and new biological roles of L-carnitine

An overview of the main physiological roles of L-carnitine is shown in Table 1. Carnitine is a quaternary amine, synthesized from the amino acid lysine, which performs a crucial role in energy supply by controlling the influx of long-chain fatty acids into mitochondria. L-carnitine, two carnitine palmitoyl transferases, CPT I and II, located respectively in the outer and inner mitochondrial membrane, and a carnitine-acylcarnitine translocase embedded in the inner mitochondrial membrane, are required in mammalian tissues to transfer long-chain acyl CoAs across the inner

Table 1
Physiological roles of L-carnitine

1	Mitochondrial long-chain fatty acid oxidation
2	Activation of aerobic glycolisis: Stimulation of pyruvate dehydrogenase complex
3	Enhancement of respiratory chain function
4	Buffering of the mitochondrial acyl CoA/CoA couple
5	Scavenger system for acyl groups
6	Peroxisomal fatty acid oxidation, intracellular communication
7	Branched-chain amino acid metabolism
8	Membrane stabilization
9	Donor of acetyl groups for biosynthesis (e.g. acetyl choline)
10	Antioxidant network

membrane for beta oxidation in the matrix [7]. Furthermore, intramitochondrial carnitine and the matrix enzyme carnitine acetyltransferase (CAT) can react with short- and medium-chain acyl CoAs to produce acylcarnitines, which can be shuttled out of mitochondria [7,12,26]. Through this mechanism, carnitine is able to modulate the intracellular concentrations of free CoA and acetyl CoA via the reversible formation of acetylcarnitine. Therefore, besides shuttling long-chain fatty acids into mitochondria, carnitine facilitates the oxidation of branched-chain ketoacids, and by preventing their accumulation it contributes to the protection of cells from the potentially membrane-destabilizing acylCoAs [26]. Carnitine may also shuttle acyl moieties, shortened by the peroxisomal beta oxidation system, from peroxisomes to mitochondria for further oxidation [12].

The relevance of carnitine in intermediary metabolism is supported by its role in the control of ketogenesis. Malonyl CoA, the first intermediate of fatty acid biosynthesis, is a potent inhibitor of CPT I, the acyltransferase located in the outer membrane in mitochondria, therefore suggesting a reciprocal control of hepatic fatty acid biosynthesis and oxidation by malonyl CoA and carnitine in normal and ketotic states [4]. With a carbohydrate diet, when the plasma ratio of glucagon to insulin is low, the malonyl CoA concentration rises with concomitantly enhanced fatty acid synthesis and suppression of fatty acid oxidation [13]. Conversely, in the fasting state or uncontrolled diabetes, where the plasma glucagon to insulin ratio is high, the malonyl CoA levels fall and carnitine levels increase in the liver. Fatty acid synthesis is then diminished and CPT becomes depressed, favouring fatty acid oxidation and ketogenesis [13].

In two recent reports, we have demonstrated that carnitine plays a pivotal role in the final steps of carbohydrate metabolism as well as in the regulation of the mitochondrial respiratory chain [2,3]. Carnitine facilitates the oxidation of pyruvate via pyruvate dehydrogenase complex (PDH) stimulation [2]. Moreover, carnitine induces an increase of the respiratory chain enzyme activities in muscle, probably by mechanisms involving mitochondrial DNA [19].

An emerging and fascinating role of L-carnitine is related to its capacity as an antioxidant. Carnitine and carnitine palmitoyl transferase can be considered integral components of the membrane phospholipid fatty acid turnover in human cells [1] (Fig. 1). Since this pathway is essentially related to the secondary antioxidant response to oxidatively damaged membrane phospholipids, it may be considered that carnitine takes part in the antioxidant network as a member of the secondary defence line. Moreover, we have documented an alteration in the acyl-flux regulated by CPT and carnitine in membrane erythrocytes from uremic patients [11]. In the latter, there is a marked increase in free radical formation, and therefore in cell membrane peroxidation. This process must be followed by proper membrane phospholipid repair, in which carnitine exerts a pivotal role. We found that L-carnitine supplementation improved membrane phospholipid fatty acid turnover, thus supporting the role of carnitine as a part of the secondary antioxidant system.

Furthermore, carnitine, via acetylcarnitine formation by CAT, represents a physiological means of providing acetyl groups for biosynthesis of some compounds [26]. This is particularly relevant in the formation of the neurotransmitter acetylcoline in

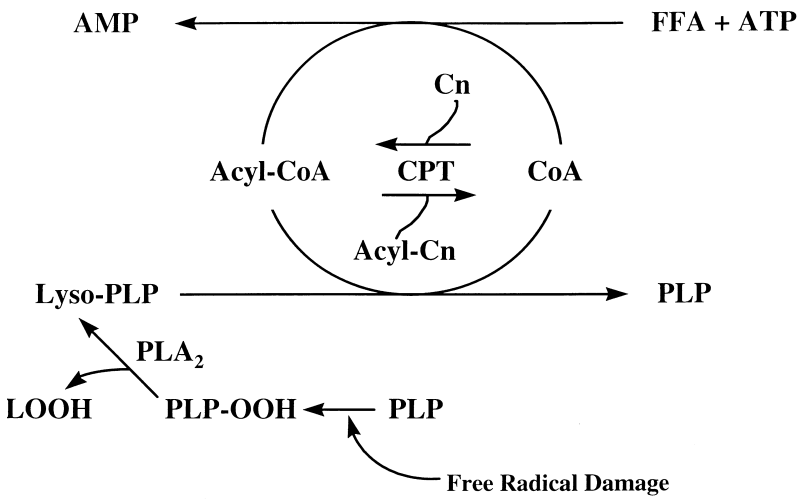

Fig. 1. The carnitine system and membrane phospholipid repair process. PLP: phospholipids; PLP-OOH: phospholipid hydroperoxide; Cn: carnitine; acyl Cn: acyl carnitine; CPT: carnitine palmitoyl transferase; LAT: lysophospholipid acyl CoA transferase; ACS: acyl CoA synthetase.

neurons [30]. Acetylcarnitine exerts, therefore, a cholinomimetic activity, and through this function may be critical for normal brain development in mammals.

3. Carnitine in perinatal metabolism

In fetal tissues of mammals, metabolic energy is obtained mainly from the oxidative metabolism of carbohydrates. Birth represents a sudden increase in energy requirements. In addition to ongoing needs for growth and differentiation, there are new requirements to generate metabolic energy for breathing, movement and maintenance of body temperature [24]. After delivery, the normal neonate adapts to fatty acids as a major source of calories. Within hours, accelerated lipolysis elevates free fatty acid concentrations and fatty acids are supplied by a high fat diet of milk [24].

The mammalian carnitine pool is heavily dependent on exogenous intake. The transplacental transport of carnitine or precursors from the maternal plasma appears important for the formation of carnitine stores in fetal tissues [6,27]. In term placenta, the maximal carnitine transfer is far in excess of the estimated carnitine requirements [24]. However, it is unknown whether carnitine biosynthesis in the fetus is a significant contributor to its carnitine status. It seems that transplacental transport may ensure sufficient amounts of carnitine even in the absence of carnitine biosynthesis in fetal tissues [24]. Carnitine is stored in fetal tissue in increasing amounts during the last part of gestation, and tissue carnitine stores at birth are directly related to gestational age [23].

Availability of L-carnitine is essential in the developing fetus, where L-carnitine is

essential for processes underlying fetal maturation. Given that in fetal tissues of mammals the metabolic energy is obtained mainly from the oxidative metabolism of carbohydrates, the strong dependence on L-carnitine is apparently puzzling. As stated above, carnitine regulates the activity of PDH, an enzyme that plays a central role in the final steps of aerobic catabolism of glucose [2]. Moreover, carnitine is a potent stimulator of the activities of the mitochondrial respiratory chain [19]. In fulfilling these two biological roles, carnitine provides the energy supply necessary to meet the energetic demands of fetal tissues. This fact could be particularly relevant in the developing brain, where beta-oxidation accounts for 25% of energy production [14], the remainder being dependent upon the oxidative metabolism of glucose.

Pulmonary surfactant production is an important process in fetal maturation. Carnitine has been shown to be more effective in inducing pulmonary surfactant production and lung maturation in both rats and humans than betamethasone, a drug commonly administered to women in preterm labor to induce fetal lung maturation [21]. The mechanisms whereby L-carnitine acts as an inductor of pulmonary surfactant production are unknown. It is widely accepted that membrane phospholipid fatty acid turnover is related to the production of pulmonary surfactant production. Since carnitine is an integral component of the membrane phospholipid fatty acid turnover in human cells [11], it is possible that carnitine causes lung maturation via membrane phospholipid repair activity.

After delivery, the neonate is faced with marked metabolic challenges. Many vital functions are assumed by the mother during prenatal life. With the interruption of the continuous supply of placentally transported nutrients, the neonate depends on the utilization of metabolic fuels derived from endogenous sources [15].

The release of energy-yielding substrates from stores accumulated prenatally is initiated by hormonal changes at birth. Glycogen from muscle, liver and other tissues represents a minor source of metabolic fuel for the newborn. Glycogen stores are exhausted within hours after birth. Although fat constitutes some 15% of the body weight, the proportion may vary in newborn infants after altered gestation [24].

The rapid increase in free glycerol and free fatty acids (FFA) in plasma of newborn infants give the first evidence of an accelerated release of these substrates from adipose tissue. However, the interpretation of plasma concentration is complicated; it may be high because of increased release or decreased uptake by tissues [24].

Adipose tissue has been shown to be influenced by the adaptive mechanisms necessary for the neonate to cope with the extrauterine environment. L-carnitine is high in brown fat, which is important in thermogenesis [16], and is high in all body tissues of cold acclimatized animals [28], suggesting a role in adaptation to the extrauterine environment.

Beta oxidation of FFA becomes increasingly important for energy production in adipocyte cells after birth [15]. The accumulation of FFA occurs when the glycogen stores become depleted. The increase in carnitine-dependent fatty acid oxidation is related to energy requirements. The availability of carnitine may affect the regulatory processes by which adipose tissue provides metabolic fuels for other tissues [24].

Liver glycogen stores accumulate during late fetal life in all mammals, but are rapidly mobilized at birth to provide glucose for tissues and cells that are entirely

dependent on glucose to maintain their function. The supply of FFA to the liver depends on the amount of adipose tissue stores and fatty acid release. Several regulatory mechanisms operate for the control of hepatic fatty acid oxidation [18]. Just after birth, there is a transient hypoglycemia, an increase in plasma glucagon and catecholamines, and a fall in plasma insulin. The production of ketone bodies in the liver is accompanied by an increase in carnitine content and seems to be regulated by CPT-1. This enzyme controls the rate of activated FFA into mitochondria [18]. The increase in the capacity for FFA oxidation and ketogenesis comes from a decrease in lipogenesis and malonyl CoA concentration. This mechanism may ensure a sensitive response to decreased availability of carbohydrates and provide control of ketone body production as alternative fuels in the fasting status [15,18].

At birth, the fetoplacental transfer of carnitine is interrupted. Before the onset of feeding the neonate heavily depends on fetal carnitine stores and on endogenous carnitine biosynthesis. Early postnatal ketogenesis is stimulated by carnitine from milk [15,24]. Fatty acid oxidation in the liver provides cofactors (e.g. Acetyl CoA, NADH and ATP) for gluconeogenesis and ketogenesis [4]. Early neonatal hypoglycemia may derive in part from reduced gluconeogenesis and may relate to a lower capacity to oxidize FFA. After birth there is a period in which FFA accumulate in tissues to a point where oversaturation of mitochondrial beta-oxidation is provoked. Then, FFA may be increasingly metabolized in peroxisomes where their chain-lengths are shortened. These shortened fatty acids are converted to acylcarnitines and may be transferred into mitochondria for further oxidation [4].

Systemic carnitine deficiency develops when cellular uptake and renal excretion of carnitine is not balanced by endogenous synthesis and exogenous intake [12]. Endogenous synthesis of carnitine is limited in the neonate by low levels of gamma butyrobetaine hydroxilase, the enzyme which catalyzes the final step of the carnitine synthetic pathway. Critical co-factors for the carnitine biosynthetic pathway include S-adenosylmetionine, pyridoxal phosphate, iron, ascorbic acid, nicotinic acid and alpha-ketoglutarate. Deficiency of any of these compounds impairs carnitine biosynthesis [17].

Premature infants, who are born with the lowest stores of carnitine, cannot achieve adequate carnitine homeostasis without an exogenous supply [25]. Therefore, premature infants receiving unsupplemented infant formula, breast milk or parenteral hyperalimentation are at high risk of developing systemic carnitine deficiency [6,29]. Infants receiving total parenteral nutrition have been shown to exhibit biochemical signs of impaired fatty acid metabolism [5,10]. Poor carnitine availability in premature infants may hamper adequate extrauterine growth despite apparently adequate caloric intake through inefficient use of available substrates for energy production [22].

In a recent report [20] Iafolla and Roe suggested that premature infants suffer from systemic carnitine deficiency, which is clinically significant, and is correctable by supplementation. In addition, they reported that premature infants are intrinsically deficient, in that they are unable to adequately synthesize enough carnitine to meet their metabolic demands, and that apnea, hypotonia, poor weight gain, delayed development, and gastrointestinal reflux in very low gestation premature infants are clinical manifestations of an untreated systemic carnitine deficiency [20].

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References

[1] Arduini A, Mancinelli G, Radatti GL, Dottori S, Molajoni F, Ramsay RR. Role of carnitine and carnitine palmitoyl transferase as integral components of the pathway for membrane phospholipid fatty acid turnover in intact human erythrocytes. J Biol Chem 1992;267:12673–81.
[2] Arenas J, Huertas R, Campos Y, Díaz AE, Villalón JM, Vilas E. Effects of L-carnitine on the pyruvate dehydrogenase complex and carnitine palmitoyl transferase activities in muscle of endurance athletes. FEBS Lett 1994;341:91–3.
[3] Arenas J, Ricoy JR, Encinas AR, Pola P, D′Iddio S, Zeviani M, DiDonato S, Corsi M. Carnitine in muscle, serum, and urine of nonprofessional athletes: Effects of physical exercise, training, and L-carnitine administration. Muscle Nerve 1991;14:598–604.
[4] Bieber LL. Carnitine Ann Rev Biochem 1988;57:261–83.
[5] Bonner CM, DeBrie KL, Hug G, Landrigan E, Taylor BJ. Effects of parenteral L-carnitine supplementation on fat metabolism in premature neonates. J Pediatr 1995;126:287–92.
[6] Borum PR. Role of carnitine during development. Can J Physiol Pharmacol 1985;63:571–6.
[7] Bremer J. Carnitine: Metabolism and functions. Physiol Rev 1983;63:1420–80.
[8] Campos Y, Huertas R, Bautista J, Gutiérrez E, Aparicio M, Lorenzo G, Segura D, Villanueva M, Cabello A, Alesso L, Arenas J. Muscle carnitine deficiency and lipid storage in patients with mitochondrial myopathy. Muscle Nerve 1993;16:778–81.
[9] Campos Y, Huertas R, Lorenzo G, Bautista J, Gutiérrez E, Aparicio M, Alesso L, Arenas J. Plasma carnitine insufficiency and effectiveness of L-carnitine therapy in patients with mitochondrial myopathy. Muscle Nerve 1993;16:150–3.
[10] Christensen ML, Heims RA, Mauer EC, Storm MC. Plasma carnitine concentration and lipid metabolism in infants receiving parenteral nutrition. J Pediatr 1989;115:794–8.
[11] De los Reyes B, Pérez-García R, Liras A, Arenas J. Reduced carnitine palmitoyl transferase activity and altered acyl-trafficking in red blood cells from hemodialysis patients. Biochem Biophys Acta 1996;1315:37–9.
[12] Di Donato S. Disorders of lipid metabolism affecting skeletal muscle: Carnitine deficiency syndromes, defects in the catabolic pathway, and Chanarin disease. In: Engel AG, Franzini-Armstrong C, editors. Myology, 2nd ed., New York: McGraw Hill, 1994:1587–609.
[13] DiDonato S, Garavaglia B, Rimoldi M, Carrara F. Clinical and biomedical phenotypes of carnitine deficiencies. In: Ferrari R, DiMauro S, Sherwood G, editors. L-carnitine and its Role in Medicine: From Function to Therapy, New York and London: Academic Press, 1992:81–96.
[14] Edmond J, Auestad N, Robbins R, Bergstrom JD. Ketone body metabolism in the neonate: Development and effect of diet. Fed Proc 1985;44:2359–64.
[15] Hahn P, Novak M. How important are carnitine and ketones for the newborn infant? Feder Proc 1985;44:2369–73.
[16] Hahn P, Skala J. Carnitine and brown adipose tissue metabolism in the rat during development. Biochem J 1972;127:107–11.
[17] Helms RA. Possible carnitine deficiency in the neonate. In: Borum P, editor. Clinical Aspects of Human Carnitine Deficiency, New York: Pergamon Press, 1986:80–96.
[18] Hoppel CL. Carnitine and carnitine palmitoyltransferase in fatty acid oxidation and ketosis. Fed Proc 1982;41:2853–7.
[19] Huertas R, Campos Y, Díaz AE, Esteban J, Vechietti L, Montanari G, D'Iddio S, Corsi M, Arenas J. Respiratory chain enzymes in muscle of endurance athletes: Effect of L-carnitine. Biochem Biophys Res Commun 1992;188:102–7.

[20] Iafolla AK, Roe CR. Systemic carnitine deficiency in premature infants: Results of a randomized trial of treatment, J Pediatr, in press.
[21] Lohninger A, Bock P, Dadak C, Feiks A, Kaiser E. Effect of carnitine on foetal rat lung dipalmitoyl phosphatidylcholine content and lung morphology. Carnitine and lung surfactant. Eur J Clin Chem Clin Biochem 1990;28:313–8.
[22] Melegh B, Kerner J, Sandor A, Vinceller M, Kispal G. Effect of oral L-carnitine supplementation in low birth weight premature infants maintained on human milk. Biol Neo 1987;51:185–93.
[23] Nakano C, Takashima S, Takeshita K. Carnitine concentration during the development of human tissues. Early Hum Devel 1989;19:21–7.
[24] Novak M. Carnitine in perinatal metabolism of lipids. In: Ferrari R, DiMauro S, Sherwood G, editors. L-carnitine and its Role in Medicine: From Function to Therapy, London and New York: Academic Press, 1992:104–12.
[25] Penn D, Ludwigs B, Schmidt-Sommerfeld E, Pascu F. Effect of nutrition on tissue carnitine concentrations of tissues of different gestational ages. Biol Neo 1985;47:130–5.
[26] Siliprandi N, Sartorelli L, Ciman M, Di Lisa F. Carnitine: Metabolism and clinical chemistry. Clin Chim Acta 1989;183:3–11.
[27] Schmidt-Sommerfeld E, Penn D, Wolf H. The influence of maternal fat metabolism on fetal carnitine levels. Early Hum Devel 1981;5:233–42.
[28] Theirrault DG, Mehlman MA. Metabolism of carnitine in cold acclimatized rats. Can J Biochem 1965;43:1437–43.
[29] Warshaw JB, Curry E. Comparison of serum carnitine and ketone body concentration in breast and formula fed newborn infants. J Pediatr 1980;97:122–5.
[30] White HL, Scates PW. Acetyl L-carnitine as a precursor of acetylcholine. Neurochem Res 1990;15:597–601.