THE PHYSIOLOGICAL ROLE OF L-CARNITINE

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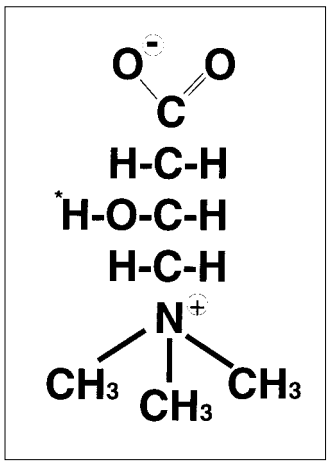
1. Chemistry

Pure L-carnitine is a white, highly water-soluble powder and is a substance with good thermostability (up to 200 °C). Its chemical name is β-hydroxy-γ-trimethyl-aminobutyrate (Fig. 1). It has very low toxicity with a LD_{50} in rodents of 9 g/kg bodyweight. In aqueous solution L-carnitine, being a zwitterion, is freely soluble in water as its ionisable groups (COO^- and $N^+(CH_3)_3$) are over 90 % dissociated at a physiological pH (~7.4) due to their pK values. The characteristic hygroscopicity of the substance limits its use in the chemically pure form, for example as a feed additive. For such applications it is used in granular forms containing about 50 % L-carnitine. In these preparations L-carnitine is embedded in the pores of a silicate matrix, which greatly reduces its tendency to combine with water.

Figure 1: L-carnitine, (β-hydroxy-γ-trimethyl-aminobutyrate)



* = binding site for acyl residues

2. Biochemistry

The hydroxyl group at the C_2 location of L-carnitine (Fig. 1) is virtually undissociated in solution, but is of great physiological significance because it determines one of its functions in the body. The β-hydroxyl group of L-carnitine reacts with activated fatty acids (acyl-CoA compounds) and, catalysed by L-carnitine acyltransferases (CAT), forms energy-rich acetyl-L-carnitine in the process. This occurs in principle by the following reaction:

$$\text{acyl-CoA + L-carnitine} \underset{\text{(CAT)}}{\rightleftharpoons} \text{acetyl-L-carnitine + CoA}$$

This reaction is reversible, as required, depending on cellular localisation (intra- and extra-mitochondrial) and chain length of the acyl residues. It is catalysed by various L-carnitine acyltransferases. The co-reactants, primarily activated fatty acids, which can participate in this reaction have chain lengths from C_2 to C_{22} and include branched chain residues, e.g. from the breakdown of branched chain amino acids (DiLISA et al., 1995).

The C-atom of the β-hydroxyl group is optically active due to its four different ligands and occurs in two isomeric forms, the D-form and the L-form. Of the two, only the L-form is encountered in the body, but the D-form can also exert effects in the body. It interacts with various transport proteins which carry L-carnitine from the plasma into the cells and competitively inhibits L-carnitine acyltransferases (CERRETELLI and MARCONI, 1990; MEIER, 1987). Overall, DL-carnitine is toxic and has no beneficial effects.

3. Discovery of L-carnitine, historical review

L-carnitine was discovered in 1905 as a constituent of muscle tissue (GULEWITSCH and KRIMBERG, 1905; KUTSCHER, 1905) (Table 1). It owes its name to the high concentration with which it occurs in meat. Its chemical structure was elucidated some 20 years later. The question as to which of the two antiomeric forms occurs in the body was not answered until the early 1960s. Some 15 years before its stereospecificity was clarified (1947), researchers had come across clues to its biological function. When breeding mealworms, which are popular with aquaculturists, as fish food, an essential nutritional factor was discovered in mealworm larvae, which one year later was given the name vitamin B_T (FRAENKEL and FRIEDMAN, 1957; FRAENKEL et al., 1948). The "T" in this abbreviation stands for the Latin name for the mealworm, ***Tenebrio molitor***. Soon afterwards the unknown compound (vitamin BT) was identified as L-carnitine. Mealworm larvae growing in a state of L-carnitine deficiency accumulate excessive amounts of fat in their cells and yet seem to die of starvation. This suggested that L-carnitine might play a role in the oxidation of fat. The function of L-carnitine in mammals, however, remained a mystery for a long time afterwards. In 1955 FRITZ discovered that adding L-carnitine to muscle extracts stimulates the oxidation of palmitate. This observation led to the discovery of the **mitochondrial carrier function** of L-carnitine and its important role in the burning of free fatty acids (BREMER, 1962). Many further biological functions of L-carnitine have become known since then. These will be considered only briefly in this article.

Table 1: History of L-carnitine research

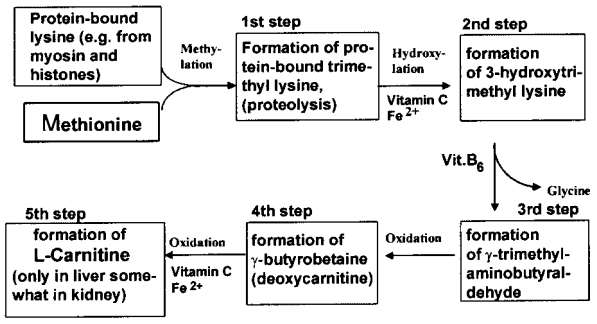
Discovery	1905 in muscle tissue, hence the name "L-carnitine"
Structure	Elucidated in 1927
Steric configuration	1962: the biologically active form was identified as a L-(R)(-)-β-hydroxy configuration
Biological function	1948: discovery of an essential nutritional factor for mealworm larvae, name: vitamin B_T1952: vitamin BT identified as L-carnitine
Function in mammals	1958: stimulation of the oxidation of fatty acids, "L-carnitine shuttle"
Clinical research	1973: discovery of major disorders of L-carnitine metabolism in humans

It was not long before the significance of these discoveries for clinical applications was recognised when in 1973 the first cases of congenital disorders of L-carnitine metabolism and L-carnitine transport were described in humans (ENGEL and ANGELINI, 1973). Today many different disorders of L-carnitine metabolism are known. These can be due to primary or secondary (acquired) L-carnitine deficiency (ANGELINI and VERGANI 1996, SCHOLTE and DE JONGE, 1987). Research into these conditions has considerably extended our knowledge of the function of L-carnitine.

4. Biosynthesis of L-carnitine

L-carnitine is synthesised by plant and animal cells and is an ubiquitous substance in nature. It occurs in animals, fungi, bacteria and plants (KLEBER, 1996; GERHARD et al., 1995; PANTER and MUDD, 1969). The synthesis of L-carnitine in animals, described in a simplified form, involves five reaction steps (Fig. 2).

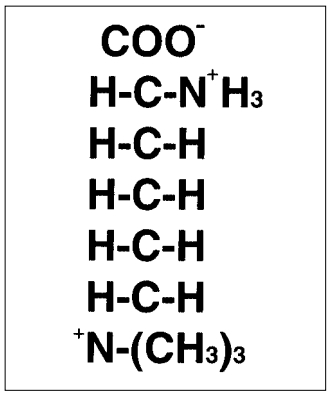
Figure 2: Reaction sequence in the biosynthesis of L-carnitine (schematic)



Starter substrates are protein-bound lysine and methionine. Also needed for the biosynthesis are vitamin C, vitamin B_6 and Fe^{2+}. In domestic animals the last step in the biosynthetic pathway, the formation of L-carnitine from γ-butyrobetaine, takes place almost execlusively in the liver.

1. The **first reaction product** is 6-N-trimethyl lysine (Fig. 3). It is formed by N-methylation of lysine. In this process S-adenosyl-methionine acts as a donor of methyl groups. Trimethyl lysine is initially bound in protein and may be found in myosin for example (HARDY et al., 1970). It is released in lysosomes through proteolysis.
2. The **second reaction product** (3-hydroxy-6-N-trimethyl lysine) is formed in the cytosol by hydroxylation of the C3 of 6-N-trimethyl lysine. This reaction requires the participation of vitamin C (NELSON et al., 1981) and bivalent iron (Fe^{2+}).
3. Following cleavage of a C2-body (glycine), the **third reaction product** (γ-trimethyl-aminobutyraldehyde), a C4-body, is formed. It is called deoxy L-carnitine aldehyde. The formation of this intermediate requires the presence of vitamin B_6.
4. Oxidation of the aldehyde to the carboxyl group results in the **fourth reaction product** (γ-butyrobetaine = deoxy L-carnitine). It is the immediate precursor of L-carnitine at the intermediate level.
5. Oxidation of deoxy L-carnitine eventually leads to the formation of L-carnitine, the **fifth reaction product**. This reaction, too, requires the presence of vitamin C and Fe^{2+}.

Figure 3: Structural formula of 6-N-trimethyl lysine



While the first four reaction steps can occur in all cells of the body, not least in muscle, the fifth and last reaction step can only take place in a few organs. In domestic animals it occurs almost exclusively in the liver and in man to some extent also in the kidneys. The enzyme mediating this reaction, deoxy L-carnitine hydroxylase, has only been found in these organs. It transfers an oxygen function to the β-C-atom of γ-butyrobetaine. The precursor (6-N-trimethyl lysine) and intermediate reaction products of L-carnitine synthesis, especially γ-butyrobetaine, are transported from other organs in the bloodstream to the liver and the kidneys. It has been estimated, based on the lysine content of the proteins involved in methylation, that about 30 g protein is needed for the synthesis of 1 g L-carnitine (e.g. for excretion with the milk).

5. Biological functions of L-carnitine

L-carnitine has many different functions in the body. Two of these are discussed below as they are of major practical significance. These are

1. the **catalytic** function of L-carnitine in the mitochondrial combustion of fatty acids and
2. the **metabolic** function of L-carnitine as a buffer for excess acyl residues.

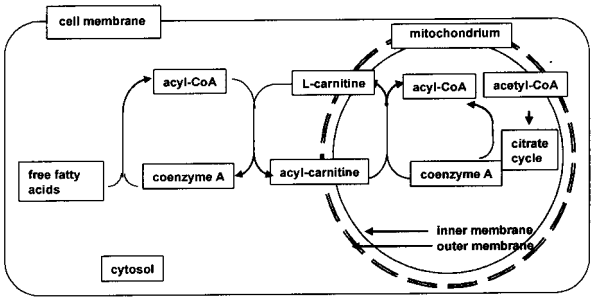
In the first-named function L-carnitine is only needed in relatively small amounts; it is not really used up and is available for this function over and over again during the process. In the second function free L-carnitine is converted to L-carnitine ester (mainly acetyl-L-carnitine); here it is needed in larger (metabolic) quantities and used up almost completely.

5.1 Mitochondrial combustion and oxidation of fatty acids, catalytic function of L-carnitine

The addition of muscle extracts (FRITZ, 1955) or L-carnitine to bovine liver bioptates increases the breakdown of palmitate two- to six-fold (DRACKLEY et al., 1991). At the same time the synthesis of triglycerides is severely inhibited. The metabolic rate of palmitoyl-CoA in heart and liver preparations of neonate piglets increased significantly in vitro through L-carnitine (HONEYFIELD and FROSETH, 1991). In liver preparations from early-weaned piglets supplemental L-carnitine raised lipid oxidation (COFFEY

et al., 1991). Generally speaking, L-carnitine promotes the burning of fat and inhibits lipogenesis. In body cells, such as muscle, fatty acids must be transported for combustion and energy generation into the mitochondria where they are burnt initially by β-oxidation and later in the citrate cycle. After entering the cells, fatty acids are activated and thereby bound to coenzyme A before entering the mitochondria. They form acyl-CoA compounds. The inner mitochondrial membrane is, however, impermeable to acyl-CoA compounds. The activated acyl residues are therefore transferred at the inner mitochondrial membrane from coenzyme A to L-carnitine (see reaction in chapter 2.) because the inner mitochondrial membrane possesses special transport proteins (translocases) for acyl L-carnitine (Fig. 4).

Figure 4: Carrier function of L-carnitine in the transport of activated fatty acids from the cytosol into mitochondria, (schematic), "carnitine shuttle"



Inside the mitochondrial matrix the reaction is reversed and the acyl L-carnitines are once more bound to coenzyme A. The released L-carnitine is transported back to the outside into the cytosol by the translocases in a 1:1 ratio with acyl L-carnitine. The L-carnitine content of the mitochondrial matrix does not change during this cycle and no L-carnitine is spent. The process is referred to as a "L-carnitine shuttle" and was the first biological function of L-carnitine described in warm-blooded animals.

Earlier studies led to the opinion that L-carnitine might primarily be involved in the transport of long-chain fatty acids into mitochondria. This referred mainly to fatty acids with chain lengths $>C_{14}$. Support for this view came also from studies of BENEVENGA et al. (1989); BENEVENGA et al. (1986) and PETTIGREW et al. (1986). In order to minimise piglet losses due to potential energy deficits early in life, these authors fed lipid preparations with a high content of medium chain fatty acids to piglets. The piglets receiving such preparations showed no further benefit when the diet was supplemented with additional L-carnitine. It was argued that L-carnitine might not be essential for the transport of activated fatty acids of medium chain length (from C_6 to C_{12}) into mitochondria (BREMER, 1983).

In more recent studies it was demonstrated that L-carnitine also influences oxidation of medium chain fatty acids. A number of newborn colostrum-deprived piglets were orogastrically lavaged with medium chain triglycerides (MCT) and L-carnitine. Administration of the MCT significantly increased the concentration of L-carnitine esters in plasma and urine and lowered the ratio of free L-carnitine:L-carnitine esters (HEO et al., 2001a; HEO et al., 2001b). As shown in respiration experiments with those piglets, L-carnitine also stimulated oxidation of intravenously administered octanoate (VAN KEMPEN and ODLE, 1992; VAN KEMPEN and ODLE, 1995). Stimulating effects of L-carnitine on oxidation rates of MCTs were also observed in infants receiving formula diets (REBOUCHE et al., 1990) and in adult humans (ROSSLE et al., 1990). These studies provided no information about the site of action of L-carnitine in MCT oxidation. Theoretically, L-carnitine could facilitate mitochondrial transport of MCTs or it could participate in regulating the transport of acetyl-CoA which is formed during β-oxidation.

The metabolic and functional effects of oral and parenteral administered L-carnitine have been examined in different animal species, including domestic animals. These experiments demonstrated that L-carnitine influences a remarkably wide range of body functions including performance and growth. In addition, some of the observed effects were accompanied by changes in composition of whole blood or blood plasma. However, the effects observed after L-carnitine supplementation, which are discussed in the following chapters, were not consistently observed in all experiments. Several observations, which are mentioned below, probably await further confirmation.

5.2 Symptoms of L-carnitine deficiency

Many of the symptoms occurring in congenital or experimentally induced L-carnitine deficiency can be attributed to a breakdown of the L-carnitine shuttle. These symptoms include the accumulation of fat in muscle in the form of lipid drops (myolipidosis), pronounced muscle weakness, rapid fatigue including the cardiac muscle and muscular pain (myalgia) (ANGELINI and VERGANI, 1996; FRITZ and ARRIGONI-MARTELLI, 1993). Progressive cardiomyopathy (weakness of the cardiac muscle) soon develops which determines the clinical picture. If the condition is caused by primary L-carnitine deficiency all these symptoms can be relieved by the administration of L-carnitine. Evidence of major disorders of L-carnitine metabolism has also been found in animals (VAN KEMPEN and ODLE, 1992).

5.3 L-carnitine as acetyl buffer, metabolic function

The second function of L-carnitine, as an acetyl buffer, is important for the muscle and liver metabolism. The performance of this function requires large amounts of L-carnitine.

Evidence of this second function of L-carnitine can be obtained by comparing the concentrations of L-carnitine in muscle with those of the substrates and intermediates involved in the burning of fatty acids (Table 2). The comparison shows that the concentration of free L-carnitine in muscle is 10 to 100 times higher than the concentration of the other intermediates involved in the energy metabolism. L-carnitine occurs in muscle in mmolar concentrations, whereas the concentration of the remaining intermediates involved in the energy metabolism is in the micromolar range (Fig. 5). This high concentration gives L-carnitine the ability to act as a store of acetyl-CoA and to react in a stoechiometric ratio with this substrate.

5.3.1 Role of L-carnitine as acetyl buffer, two metabolic conditions

Conditions can arise under which the amount of activated fatty acids and the amount of the resulting acetyl-CoA

Table 2: Concentration of some substrates and intermediates of the energy metabolism in resting muscle in µmol/kg wet weight

Citrate	250
α-ketoglutarate	130
Pyruvate	45
ATP	500
Coenzyme A	80
Acetyl-CoA	20
Free L-carnitine	4,500
Acetyl-L-carnitine	100

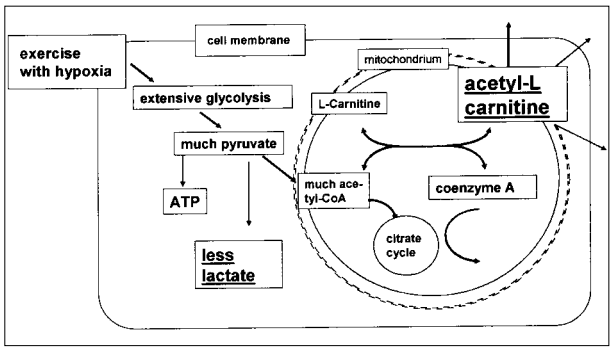
exceed both the oxidative requirement and the oxidative capacity of the cell. These include **supermaximal**, i.e. anaerobic, **exercise** and/or an extremely **high lipolysis rate**, as encountered for example in high yielding dairy cows and sheep in late pregnancy. In resting muscle of pigs, horses and dogs, about 98 % of the L-carnitine is present as free L-carnitine (own data) and the remainder as acyl L-carnitine. The free L-carnitine binds to acetyl groups if necessary.

5.3.2 Function of L-carnitine in working muscle

- **Function as acetyl store**

If the energy generated by oxidative processes in working muscle is not sufficient for the activity performed by the muscle, the ATP shortfall is supplied by glycolysis, depending on the activity level. This process ends with pyruvate which, in order to prevent end product inhibition of the glycolytic process, is converted either to lactate or acetyl-CoA. In a situation of increasing hypoxia, conversion to acetyl-CoA would seem to be futile at first glance because the energy utilisation in the citrate cycle fails due to lack of oxygen.

Figure 5: Function of L-carnitine in working muscle during hypoxia. L-carnitine acts as an intermediate store of activated acetyl groups.



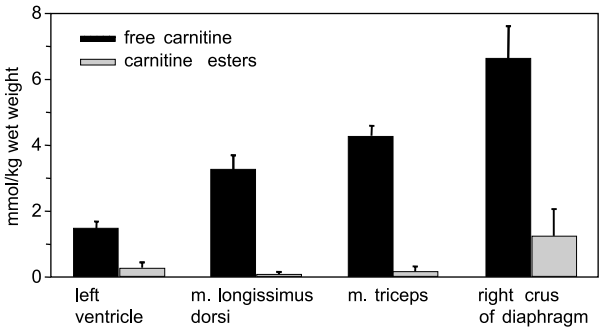
Yet even in this type of situation the conversion of pyruvate to acetyl-CoA can still be useful because it can continue to react with free L-carnitine, which is relatively abundant, to yield acetyl-L-carnitine and is thus removed from the reaction pathway (Fig. 5). For this function L-carnitine is required in larger than catalytic amounts. Experiments have shown that L-carnitine does in fact perform this function. In muscle bioptates of horses obtained after intensive exercise on the treadmill, the concentration of free L-carnitine had fallen from 98 % to about 10 % and the concentration of acetyl-L-carnitine had risen correspondingly (CERRETELLI and MARCONI, 1990; FOSTER and HARRIS, 1987; HARRIS et al., 1987). The blood also shows increased concentrations of L-carnitine esters relative to free L-carnitine after physical exertion (SAHLIN, 1990).

- **Function as stabiliser of a low acyl-CoA/CoA concentration ratio**

L-carnitine performs a further function in mitochondrial energy production which is also related to its role as a reservoir for the binding of activated acetyl groups, namely the maintanance of a low acetyl-CoA/CoA concentration ratio. The transfer to activated acetyl residues from acetyl-CoA to L-carnitine lowers the acetyl-CoA concentration and raises that of free coenzyme A. A high CoA/acetyl-CoA ratio drives the citrate cycle (BROCKHUYSEN et al., 1965) since free coenzyme A is required for the formation of succinyl-CoA from α-ketoglutarate.

We conducted tests measuring the ratio of free L-carnitine to L-carnitine esters in bioptates from different muscles in resting horses (Fig. 6). The lowest concentration of L-carnitine esters was found in skeletal muscle, followed by cardiac muscle and the diaphragm (crus).

Figure 6: Content of free L-carnitine and L-carnitine esters in muscle bioptates of horses (- ± SD, N = 5) (HARMEYER and BIRCH unpublished)



The data show that the content of L-carnitine esters is higher in muscles which perform some work even when the body is at rest, such as the heart and diaphragm, than in skeletal muscle. Moreover, the concentration of total L-carnitine in the crus of the diaphragm was four times higher than in cardiac muscle. This finding may reflect the special ability of the diaphragm for hypoxic energy generation. The cardiac muscle with its relatively low L-carnitine content is known for its very limited ability to supply energy in hypoxia. Skeletal muscle is better at doing this, depending on the fibre type, and according to our research the muscles of the diaphragm seem to possess this facility to an even greater extent. This feature would seem to be desirable as a safety mechanism enabling the body to maintain respiration even in severe hypoxia.

5.3.3 L-carnitine and fat mobilisation

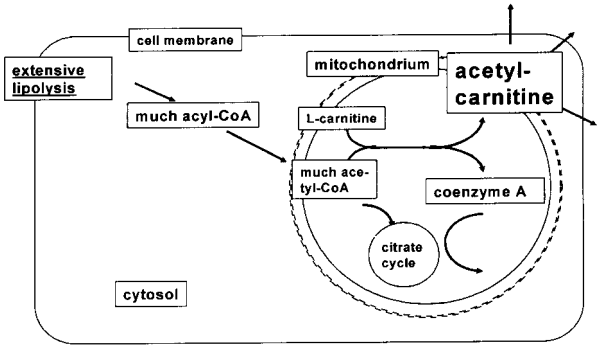
- **Perinatal period of ruminants**

In rabbits (GILLIES and BELL, 1976) and monkeys (BELL and DeLUCIA, 1983) fed high-fat diets plasma triglyceride levels rise. This is accompanied by an increase in the concentration of free L-carnitine by up to 100 % (BELL et

al., 1987). In rabbits with congenital hyperlipidaemia supplemental L-carnitine lowers plasma triglyceride levels by 5 to 40 % (BELL et al., 1987).

In situations of increased lipolysis, for example in cows in peak lactation, L-carnitine is also of significance as an acetyl buffer. Under these circumstances it is not the muscles but the liver which dominates the proceedings. The acetyl group of acetyl-CoA, which is abundantly produced from lipolysis and breakdown of fatty acids, is increasingly transferred to L-carnitine as required. The resulting acetyl-L-carnitine can be passed by the liver to the blood (Fig. 7). In diabetes and hunger the concentration of total L-carnitine in plasma rises.

Figure 7: Function of L-carnitine as acetyl buffer in the liver during lactation in late pregnancy



- **Ketotic states**

The buffer function of L-carnitine is of greatest significance in ketotic states. Ketotic cows have a higher concentration of acetyl-L-carnitine in the blood and the milk than healthy, non-ketotic cows (ERFLE et al., 1970). Own studies conducted in collaboration with the Medizinische Klinik in Leipzig showed that the content of total plasma L-carnitine of dairy cows consists of about 90 % free L-carnitine and 10 % L-carnitine esters. As the calving date approached the concentration of free L-carnitine fell by 40 % and remained low during the first few weeks of lactation (CITIL et al., 1999; FÜRLL et al., 1999; FÜRLL et al., 1997). At the same time the proportion of L-carnitine esters rose to 40 - 50 % of the total L-carnitine content. In cows with symptoms of ketosis the concentration of L-carnitine esters in the plasma was two to four times higher than in dairy cows without ketotic symptoms (Fig. 8). These findings support the statement that L-carnitine acts as an acetyl buffer under conditions of increased fat mobilisation as exists in dairy cows during the perinatal period.

6. L-carnitine in body tissues, distribution in different organs

L-carnitine occurs in gram quantities in the body and is distributed very unevenly across the various organs. A pig weighing 100 kg for example has a L-carnitine pool of about 24 g (Fig. 9). About 80 % of this is present in muscle and about 5 to 10 % in the gastrointestinal tract (FLORES et al., 1996). The liver contains only about 3 % of the body's L-carnitine and the blood with just 0.25 % has a negligible fraction of the total. This uneven distribution means that concentration gradients of 1:1000 occur between individual tissues and organs. A very high concentration of L-carnitine is found in mature bull spermatozoa which contain up to 100 mmol/kg of wet weight, equivalent to 16 g/kg

Figure 8: Free L-carnitine (top) and L-carnitine esters (centre) in µmol/l and in % (bottom) of total L-carnitine in plasma of dairy cows during the perinatal period (-±SEM, N = 19)

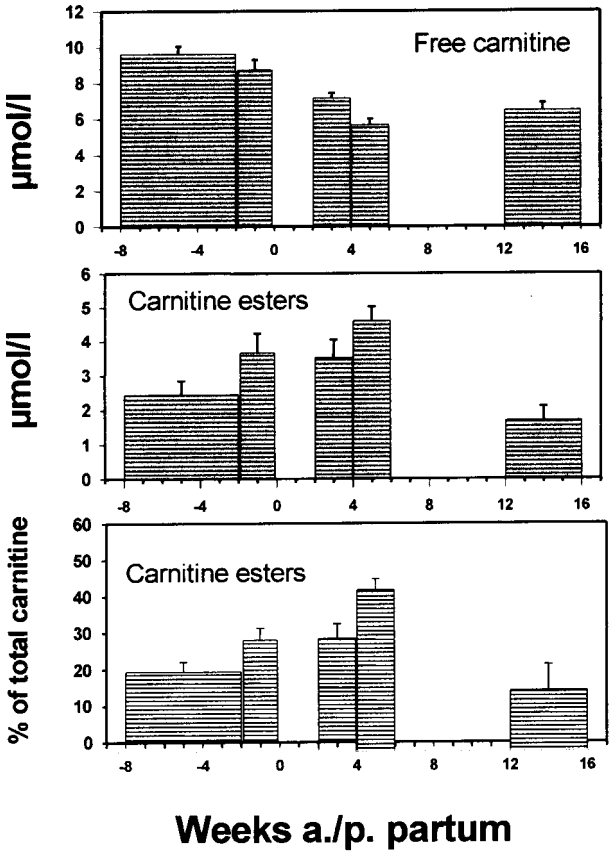
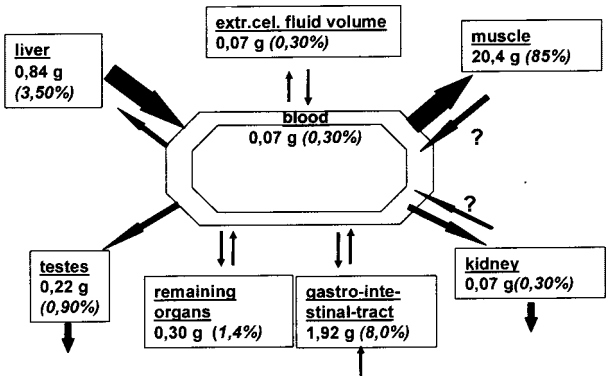


Figure 9: L-Carnitine pool and L-carnitine flux between blood and tissues, (schematic). The quantities refer to a pig of abaout 100 kg with a body pool of L-carnitine of about 24 g.



(CARTER et al., 1980) and in the luminal fluid of rat cauda epididymidis (53 mmol/l) (HINTON et al., 1979). This constitutes a concentration more than 2000 times higher than in rat blood plasma. The lowest L-carnitine concentrations are found in plasma, blood cells and urine (Table 3). Table 3 shows that muscle belongs to the L-carnitine-rich tissues. The L-carnitine content of muscle is about 100 to 300 times higher than that of plasma. Milk, which contains about 10 times more L-carnitine than plasma, is also relatively rich

in L-carnitine. The L-carnitine content in the milk of dairy cows declines significantly during the course of lactation and also with the number of lactations (ROOS et al., 1992). The mammary gland, like most other body cells, accumulates L-carnitine from the blood. In this way suckling offspring are supplied with sufficient amounts of L-carnitine. This would appear to be necessary since the L-carnitine content in neonates is very low in all tissues. The liver of neonates is still limited in synthesising L-carnitine in the quantities needed. It is true to say that neonates are unable to meet their L-carnitine requirement from endogenous synthesis.

The entire pool of L-carnitine in the tissues has to be transported there from the liver via the blood. The blood is therefore a sensitive indicator of L-carnitine status. Effects of L-carnitine supplementation can be easily monitored by changes in the plasma L-carnitine concentration. The plasma L-carnitine level responds to supplementation with increases of over 100 % depending on the dosage (LaCOUNT et al., 1995; BENAMOU and HARRIS, 1993; FOSTER et al., 1989a; FOSTER et al., 1989b). Increases in plasma L-carnitine induced by supplementation also raise the L-carnitine concentration in target tissues, as measurements of tissue bioptates have shown (IBEN and MEINART, 1997; NEGRAO et al., 1987).

7. The L-carnitine balance

L-carnitine is absorbed actively (GUDJONSSON et al., 1985a; LI et al., 1990) and passively (MARCIANI et al., 1991) in the intestine. The transport capacity is low when compared with that for glucose and amino acids (REBOUCHE and CHENARD, 1991). L-carnitine is presumably partially esterified in the intestine before being released into the blood (GUDJONSSON et al., 1985b).

Table 3: Total L-carnitine content in tissues and body fluids (figures in µmol per litre or per kg wet weight)

Plasma	8-40	increases with age
Muscle	2,000 to 5,000	especially high in sheep
Liver	200 to 300	capable of biosynthesis
Milk	100 to 500	tends to decline in the course of lactation; less in human milk
Seminal plasma	2,000 to 6,000	less in humans
Spermatozoa	40,000 to 100,000	
Urine	10 to 40	cattle: 1 to 4 mg/day

The liver receives some L-carnitine from the portal blood and releases it with a time delay back into the blood. L-carnitine esters are also released by the liver with the bile (enterohepatic cycle) (GUDJONSSON,1985b). L-carnitine and L-carnitine esters are readily filterable in the renal glomeruli. In animals with a normal L-carnitine status more than 98% of free L-carnitine is absorbed via the tubules (STADLER et al., 1993; LI et al., 1992). The absorption of L-carnitine esters is less efficient (BIEBER, 1988; CARLIN et al., 1986). Tubular absorption can, however, be modified depending on dietary supply, requirement and L-carnitine status (LI et al., 1992; REBOUCHE and CHENARD, 1991). During L-carnitine supplementation the renal excretion of L-carnitine increases considerably (SUZUKI et al., 1976; BAKER et al., 1993). L-carnitine is not metabolised in body cells (ODLE, 1996). Metabolites of L-carnitine present in urine are of microbial origin and result from the enterohepatic cycle of L-carnitine (REBOUCHE and CHENARD, 1991).

The L-carnitine requirement is considerably increased during growth and lactation. Cows excrete between 0.2 and 0.4 g L-carnitine per 10 litres of milk, depending on the stage of lactation (ROOS et al., 1992). If the amount synthesised by the body is insufficient to cover this demand, L-carnitine deficiency develops. The question whether such situations occur in high yielding animals is currently the subject of intensive research. But irrespective of this particular issue, there are conditions where L-carnitine deficiency exists.

7.1 L-carnitine deficiency

7.1.1 The neonatal phase in domestic animals

During the neonatal phase the L-carnitine requirement is usually covered through the milk (SCHIFF et al., 1979). This needs to be taken into account when formulating diets for early weaners or when using milk replacers.

7.1.2 Liver disease and stress on the liver

If the liver is diseased or under severe stress the hepatic L-carnitine biosynthesis is also restricted. Such situations can occur in high yielding livestock. While the milk output initially rises with each lactation, the L-carnitine content of the milk declines with the number of lactations and is negatively correlated with the milk yield (ROOS et al., 1992). It is still uncertain whether this should be interpreted as a sign of L-carnitine deficiency.

7.1.3 Differences between species

The current consensus is that carnivores (cats and dogs) are unable to meet their L-carnitine requirement through endogenous synthesis in the long term. This is not surprising since these animals have become adapted to an L-carnitine-rich diet in the form of meat in the course of evolution. As a consequence, the liver has probably lost its capacity for sufficient endogenous synthesis of L-carnitine.

8. Abstract

1. L-carnitine is a highly water-soluble substance of low toxicity which is synthesised endogenously in the body. It was first isolated from muscle and, as well as in mammals, also occurs in birds, fish, reptiles, insects, microbes and plants. L-carnitine reacts with activated fatty acids in the body.
2. The principal site of L-carnitine synthesis is the liver, although the starter substrate for its synthesis (L-lysine) originates mainly in muscle. Vitamin C, vitamin B_6 and Fe^{2+} are also needed for its synthesis.
3. L-carnitine is essential for the burning of fatty acids and acts as carrier in the transport of activated fatty acids into the mitochondria.

4. It also performs functions as a store of activated acetyl residues and maintains the concentration ratio of acetyl-CoA:free CoA at a low level. The storage function for activated acetyl residues is of significance in supermaximal exercise, ketotic states and hunger.
5. L-carnitine occurs in relatively large amounts in the body (about 24 g in a pig weighing 100 kg) and is distributed very unevenly in the body. Some 85 % of the total L-carnitine pool is present in muscle and less than 1 % in blood plasma. Milk is rich in L-carnitine.
6. In man, different forms of congenital primary and secondary L-carnitine deficiencies occur. Neonates rely on an exogenous supply of L-carnitine because their capacity for endogenous synthesis is still poorly developed. L-carnitine deficiency can occur in fast growing animals and in liver disease. Carnivores cannot cover their L-carnitine requirement by endogenous synthesis.
7. In broilers and fattening pigs, dietary L-carnitine supplements of about 50 mg/kg feed had beneficial effects on growth and feed conversion and led to an improvement of the meat:fat ratio. In laying hens, L-carnitine supplementation reduced the proportion of yolk in eggs and increased the proportion of albumen. Benefits of L-carnitine supplementation were also observed in beef cattle and young foals.

9. Literature

ANGELINI C, VERGANI L. Primary myopathic and systemic carnitine deficiency syndromes: new concepts from transport studies. Seim H, Loester H. (Eds.) Carnitine, Pathobiochemical Basics and Clinical Applications. Bochum: Ponte Press, Verlags-GmbH; 1996; 97-105, ISBN 3-920328-24-8.

BAKER H, FRANK O, DEANGELIS B, BAKER E R. Absorption and Excretion of L-Carnitine During Single or Multiple Dosings in Humans. Int. J. Vitam. Nutr. Res. 1993;63:22-26.

BELL F P, DELUCIA A. Plasma and liver carnitine (free and esterified) levels and their interrelationships in moderately hypercholesterolemic monkeys (Macaca arctoides). Can. J. Biochem. Cell Biol. 1983;61:328-332.

BELL F P, RAYMOND T L, PATNODE C L. The influence of diet and carnitine supplementation on plasma carnitine cholesterol and triglyceride in WHHL (Watanabe-Heritable Hyperlipidemic), Netherland Dwarf and New Zealand rabbits (Oryctolagus cuniculus). Comp. Biochem. Physiol. 1987; 587-591.

BENAMOU A E, HARRIS R C. Effect of Carnitine Supplement to the Dam on Plasma Carnitine Concentration in the Sucking Foal. Equ. Vet. J. 1993;25:49-52.

BENEVENGA N J, STEINMANN J K, CRENSHAW T D. Medium-chain triglycerides - A source of energy for the newborn piglets. Proc. Georgia Nutr. Conf. 1986; 13-18

BENEVENGA N J, STEINMANN-GOLDSWORTHY J K, CRENSHAW T D, ODLE J. Utilization of medium-chain triglycerides by neonatal piglets: I. Effects on milk consumption and body fuel utilization. J. Anim. Sci. 1989; 3331-3339.

BIEBER L L. Carnitine. Ann. Rev. Biochem. 1988;57:261-283.

BREMER J. Carnitine in intermediary metabolism. The metabolism of fatty acid esters of carnitine by mitochondria. J. Biol. Chem. 1962;237:2228-2231.

BREMER J. Carnitine - Metabolism and function. Physiol. Rev. 1983;63:1420-1480.

BROCKHUYSEN J, BAUDINE A, DELTOUR G. Effect of carnitine on acidosis and ketosis induced by lipid perfusions in dogs during starvation. Biochim. Biophys. Acta. 1965;106:207-210.

CARLIN J, REDDAR W, SANJAK M. Carnitine metabolism during prolonged exercise and recovery in humans. J. Appl. Physiol. 1986;55:489-495.

CARTER A L, STRATMAN F W, HUTSON S M, LARDY H A. The role of carnitine and its esters in sperm metabolism. Frenkel R A, McGarry J D. (Eds.) Carnitine, Metabolism, and Functions. New York: Academic Press; 1980; 251-263. ISBN 0-12-267060-4.

CERRETELLI P, MARCONI C. L-carnitine supplementation in humans. The effects on physical performance. Int. J. Sports Med. 1990;11:1-14.

CITIL M, FÜRLL M, HARMEYER J, TEUFEL E-M. Carnitine around parturition in healthy and sick cows (abstract). Wensing Th (Edit.), 10th Int. Conf. on Production Diseases in Farm Animals (ICPD), Wageningen Pers, The Netherlands, 1999; 344, ISBN 90-74134-60-2.

COFFEY M T, SHIREMAN R B, HERMAN D L, JONES E E. Carnitine Status and Lipid Utilization in Neonatal Piglets Fed Diets Low in Carnitine. J. Nutr. 1991;121:1047-1053.

DiLISA F., BARBATO R., MENABO R., SILIPRANDI N. Carnitine and carnitine esters in mitochondrial metabolism and function. De Jong J W, Ferrari R. (Eds.) The Carnitine System; A New Therapeutical Approach to Cardiovascular Diseases. Dordrecht, Boston: Kluwer Academic Publishers; 1995;162: Developments in Cardiovascular Medicine:21-38, ISBN 0-7923-3318-7.

DRACKLEY J K, BEITZ D C, YOUNG J W. Regulation of in vitro metabolism of palmitate by carnitine and propionate in liver from dairy cows. J. Dairy Sci. 1991;74:3014-3024.

ENGEL A O, ANGELINI C. Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy, - a new syndrome. Science. 1973;179:899-902.

ERFLE J D, FISHER L J , SAUER F. Carnitine and Acetylcarnitine in the Milk of Normal and Ketotic Cows. J. Dairy Sci. 1970;53:486-489.

FLORES C A, HU C, EDMOND J, KOLDOVSKY O. Milk carnitine affects organ carnitine concentration in newborn rats. Can. J. Physiol. Pharmacol. 1996;63:571-576.

FOSTER C V, HARRIS R C. Plasma carnitine concentration in the horse following oral supplementation using a triple dose regime. Equ. Vet. J. 1989a;21:376-377.

FOSTER C V L, HARRIS R C, POURET E J M. Effect of oral L-carnitine on its concentrations in the plasma of yearling thoroughbred horses. Vet. Rec. 1989b;125:125-128.

FOSTER C V, HARRIS R C. Formation of acetylcarnitine in muscle of horse during high intensity exercise. Eur. J. Appl. Physiol. 1987;56:639-642.

FRAENKEL, G, BLEWETT, M COLES, M. B_T, a new vitamin of the group and its relation to the folic acid group, and other anti-anaemia factors. Nature. 1948;161:981-983.

FRAENKEL G, FRIEDMANN S. Carnitine. Harris R S, Marrian G F, Thilmann K V. (Eds.) Vitamins and Hormons. Academic Press; 1957;XV:73-118.

FRITZ I B. The effects of muscle extracts on the oxidation of palmitic acid by liver slices and homogenates. Acta Physiol. Scand. 1955;34:367-385.

FRITZ I B, ARRIGONI-MARTELLI E. Sites of action of carnitine and its derivatives on the cardiovascular system. Interactions with membranes. Trends Pharmacol. Sci. 1993;14:355-360.

FURLL M, TEUFEL E-M, CITIL M, HARMEYER J. Zur Bedeutung von Carnitin bei Hochleistungskühen im Peripartalzeitraum. (About the role of carnitine in high performing dairy cows during the perinatal period). Schubert R, Flachowsky G, Bitsch R, Jahreis G. (Eds.) Vitamin und Zusatzstoffe in der Ernährung von Mensch und Tier. Verlag Gebrüder Franke KG, Gera; 1999; 223-228, ISBN 3-93-480500-0.

FUERLL M, HARMEYER J, TEUFEL E-M, and CITIL M. Carnitin-Konzentrationen im Blut bei Hochleistungskühen im peripartalen Zeitraum. (Concentrations of carnitine in blood plasma of high performing dairy cows during the perinatal period). Schubert R, Flachowski G, Bitsch R, Jahreis G. (Eds.) Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier. Verlag Buch- und Kunstdruckerei Keßler GmbH, Jena; 1997; 297-303, ISBN 3-00-002381-X.

GERHARDT B, FISCHER K, MAIER U. Effect of palmitoylcarnitine on mitochondrial activities. Planta. 1995;196:720-726.

GILLIES P J, BELL F P. Arterial and plasma carnitine levels in rabbits: influence of age and dietary cholesterol. Exp. Molec. Path. 1976;25:402-411.

GUDJONSSON H, LI B U K, SHUG A L. Studies of carnitine metabolism in relation to intestinal absorption. Amer. J. Physiol. 1985a;248:G313 - G319.

GUDJONSSON H, LI B U K, SHUG A L, OLSEN W A. In vivo studies of intestinal carnitine absorption in rats. Gastroenterology. 1985b;88:1880-1887.

GULEWITCH V S, KRIMBERG R. Zur Kenntnis der Extraktionsstoffe der Muskeln. 2.Mitteilung über das Carnitin. Hoppe-Seylers Z. Physiol. Chem. 1905;45:326-330.

HARDY M F, HARRIS C I, PERRY S V, STONE D. Occurrence and Formation of the N´- Methyl-lysines in Myosin and the Myofibrillar Proteins. Biochem. J. 1970;120:653-660.

HARRIS R C, FOSTER C V L, HULTMAN E. Acetylcarnitine formation during intense muscular contraction in humans. J. Appl. Physiol. 1987;63:440-442.

HEO K N, ODLE J, LIN X, VAN KEMPEN T A T G, HAN IN K. Determination of carnitine renal threshold and effect of medium-chain triglycerides on carnitine profiles in newborn pigs. Asian-Aust. J. Anim. Sci. 2001a; 14:237-242.

HEO K, ODLE J, LIN X, Van KEMPEN T, HAN IN K. Effect of L-carnitine and medium-chain triglycerides on plasma and urinary carnitine in newborn piglets. Annual Swine Report, North Carolina State University, Coll. Agric. & Life Sci. 6 pg, 2001b.

HINTON B T, SNOSWELL A M, SETCHELL B P. The concentration of carnitine in the luminal fluid of the testis and epididymis of the rat and some other mammals. J. Reprod. Fertil. 1979; 56:105-111.

HONEYFIELD D C, FROSETH J A. Evaluation of Energy Sources With and Without Carnitine in Newborn Pig Heart and Liver. J. Nutr. 1991;121:1117-1122.

IBEN CH, MEINART S. Carnitin beim Masthuhn - Wirkung von L- und DL-Carnitin. Wien. Tieraerztl. Mschr. 1997;84:228-232.

KLEBER H P. Bacterial carnitine metabolism - starting point for biotechnological procedures of L(-)-carnitine synthesis. Seim H, Loester H. (Eds.) Carnitine, Pathobiochemical Basics and Clinical Applications. Bochum: Ponte Press Velags-GmbH; 1996; 33-46, ISBN 3-920328-24-8.

KUTSCHER F. Ueber Liebig´s Fleischextrakt. Mitteilung I. Z. F. Unters. D. Nahr. u. Genussm. 1905;10:528-537.

LaCOUNT D W, DRACKLEY J K, WEIGEL D J. Responses of Dairy Cows During Early Lactation to Ruminal or Abomasal Administration of L-Carnitin. J. Dairy Sci. 1995;78:1824-1836.

LI, B U K, LLOYD M L, GUDJONSSON H, SHUG A L, OLSEN W A. The Effect of Enteral Carnitine Administration in Humans. Amer. J. Clin.l Nutr. 1992;55:838-845.

LI B U K, BUMMER P M, HAMILTON J W, GUDJONSSON H, ZOGRAFI G, OLSEN W A. Uptake of L-Carnitine by Rat Jejunal Brush Border Microvillous Membrane Vesicles - Evidence of Passive Diffusion. Digest. Dis. Sci. 1990;35:333-339.

MARCIANI P, LINDI C, MARZO A, ARRIGONI M, CARDACE G, ESPOSITO G. L-Carnitine and carnitine ester transport in the rat small intestine. Pharmacol. Res. 1991;23:157-162.

MEIER P J. D-Carnitin, harmlos? Gitzelmann R, Baerlocher K, Steinmann B. (Eds.) Carnitin in der Medizin. Schattauer Verlag, Stuttgart, New York: 1987; 101-104, ISBN 3-7945-1115-8.

NEGRAO C E, JI L L, SCHAUER J E, NAGLE F J, LARDY H A. Carnitine supplementation and depletion: tissue carnitines and enzymes in fatty acid oxidation. J. Appl. Physiol. 1987;63:315-321.

NELSON P J, PRUITT R E, HENDERSON L-R L, JENNESS R, HENDERSON L M. Effect of ascorbic acid deficiency on the in vivo synthesis of carnitine. Biochim. Biophys. Acta 1981; 672:123-127.

ODLE J. Betaine and carnitine. Feed Management. 1996;47:25-27.

PANTER R A, MUDD J B. Carnitine levels in some higher plants. FEBS Lett. 1969;5:469-474.

PETTIGREW J E S G, CORNELIUS R L, MOSER T R, HEEG H, HANKE E, MILLER K P; HAGEN C D. Effect of oral doses of corn oil and other factors on preweaning survival and growth of piglets. J. Anim. Sci. 1996;62:601-612.

REBOUCHE C J, CHENARD C A. Metabolic fate of dietary L-carnitine in human adults - identification and qualification of urinary and faecal metabolites. J. Nutr. 1991; 121:539-546.

REBOUCHE C J, PANAGIDES D D, NELSON S E. Role of carnitine in utilization of dietary medium-chain triglycerides by term infants. Am. J. Clin. Nutr. 1990; 52:820-824.

ROOS N, DEVRESE M, SCHULTE-COERNE H, BARTH C A. L-Carnitin in Milch von monozygoten Zwillingskühen. (L-carnitine in milk of monozygotic twin cows). Kieler Milchwirtsch. Forschungsber. 1992;44:363-370.

ROSSLE C, CARPENTIER Y A, RICHELLE M, DAHLAN W, D'ATTELLIS N P, FURST P, ELWYN D H. Medium-chain triglycerides induce alterations in carnitine metabolism. Am. J. Physiol. 1990;258:E944-E947.

SAHLIN K. Muscle Carnitine Metabolism During Incremental Dynamic Exercise in Humans. Acta Physiol. Scand. 1990;138:259-262.

SCHIFF D., CHAN G., SECCOMBE D. Plasma carnitine levels during intravenous feeding of the neonate. J. Pediat. 1979;95:1043-1046.

SCHOLTE H R, DE JONGE P C. Metabolism, Function And Transport of Carnitine in Health and Disease. Gitzelmann R, Baerlocher F, Steinmannn B. (Eds.) Carnitin in der Medizin. Schattauer-Verlag, Stuttgart: 1987; 21-59.

STADLER D D, CHENARD C A, REBOUCHE C J. Effect of Dietary Macro Nutrient Content on Carnitine Excretion. Am. J. Clin. Nutr. 1993;58:868-872.

SUZUKI M, KANAYA M, MURAMATSU S, TAKAHASHI T. Effects of carnitine administration, fasting, and exercise on urinary carnitine excretion in man. J. Nutr. Sci. Vitaminol. 1976;22:169-174.