

L-carnitine level in neonates – a large, retrospective analysis

GREGOR SELIGER^{1,4}, EVA KANTELHARDT¹, CAROLIN VAN DER WAL¹, UTA KELLER², KLAUS EDER²,
SIMONE PÖTZSCH³, FRIEDRICH RÖPKE¹, CERRIE SCHELER¹

Abstract

L-carnitine has an essential role in the transport of long-chain fatty acids through the mitochondrial membrane in order to ensure efficient β -oxidation of fatty acids. During ongoing pregnancy levels of L-carnitine gradually decrease. Metabolic diseases like diabetes mellitus often come with secondary shortage of L-carnitine. We investigated blood from 3652 neonates taken on the third day of life. Free L-carnitine was detected by tandem mass spectrometry. L-carnitine levels of very premature neonates (< 32/0 gestational age (GA)) were significantly higher than term babies (< 32/0 GA: 23.5 $\mu\text{mol/l}$, $n = 151$; 32/0-36/6 GA: 19.5 $\mu\text{mol/l}$, $n = 425$; 37/0-37/6 GA: 18.8 $\mu\text{mol/l}$, $n = 232$; 38/0-38/6 GA: 17.7 $\mu\text{mol/l}$, $n = 545$; 39/0-39/6 GA: 17.4 $\mu\text{mol/l}$, $n = 723$; 40/0-40/6 GA: 18.0 $\mu\text{mol/l}$, $n = 869$; 41/0-41/6 GA: 18.7 $\mu\text{mol/l}$, $n = 512$; >41/6 GA: 19.7 $\mu\text{mol/l}$, $n = 66$). Male neonates showed significantly higher L-carnitine levels by average of 2 $\mu\text{mol/l}$ than female neonates (19.4 $\mu\text{mol/l}$, $n = 1885$ versus 17.5 $\mu\text{mol/l}$, $n = 1767$; $p < 0.001$). There was no significant difference of L-carnitine levels in preterm neonates with respiratory distress syndrome (RDS) compared to neonates without RDS (21.9 $\mu\text{mol/l}$, $n = 113$ versus 21.0 $\mu\text{mol/l}$, $n = 162$; $p = 0.54$). There was no correlation between L-carnitine levels and birth weight. Neonates whose mothers had gestational diabetes showed higher L-carnitine level than neonates whose mothers did not develop gestational diabetes (18.9 $\mu\text{mol/l}$, $n = 120$ versus 17.3 $\mu\text{mol/l}$, $n = 2266$; $p = 0.03$). This analysis should contribute to a more solid scientific basis for the controversial discussion about the effects of L-carnitine in pregnancy to give the ground for further clinical studies in this area.

Key words: L-carnitine, neonates, pregnancy

Introduction

Carnitine (γ -Trimethylamino- β -hydroxybutyrate) is a derivative of gamma-aminobutyric acid and is synthesised endogenously in liver and kidney from lysine and methionin. There are two substantial physiological functions of L-carnitine. On the one hand long-chained fatty acids are transported through the inner mitochondrial membrane to ensure an efficient β -oxidation of fatty acids. On the other hand L-carnitine buffers the cytoplasmatic pool of Coenzyme A in the intermediary metabolism.

A disturbed metabolism of L-carnitine leads to energy deficit and disturbance of fat metabolism. Underlying causes for L-carnitine deficiency can be triggered primarily by a defect in L-carnitine metabolism or secondary as a result of diseases or certain physiologic conditions [1]. The L-carnitine needs of the human body are covered by one third through autogenous synthesis, the rest is received exogenously [2].

Up to now studies have shown that levels of total and free L-carnitine drop significantly during pregnancy. Plasma levels of free L-carnitine < 20 $\mu\text{mol/l}$ or a ratio free L-carnitine/total L-carnitine of < 0.7 are characteristic of secondary deficiency [3].

Since the fetus is not capable of completely synthesising L-carnitine himself, he is dependant on L-carnitine from the maternal circulation. L-carnitine can pass the placenta easily [4].

Whether a nutritional lack of L-carnitine leads to gestational metabolic diseases has not been sufficiently investigated, so far.

Gestational diabetes and hyperlipidemia can occur due to metabolic disorders and cause increased risk for mother and fetus. Some studies show that such metabolic diseases are

accompanied by secondary L-carnitine deficiency (free L-carnitine < 20 $\mu\text{mol/l}$) and L-carnitine insufficiency (acetyl-/free carnitine > 0.4) [5, 6].

The transplacental glucose supply is suddenly interrupted at birth for the neonate; the glycogen storage is rapidly used up. Intensified oxidation of fatty acids is used for energy supply as compensation. Sufficient concentration of L-carnitine in the tissue is prerequisite to mobilise the endogenous fat storage as well as oxidation of fatty acids (liver, heart & skeletal muscle) and production of ketone bodies [7, 3, 8].

There is a correlation between L-carnitine levels in the umbilical cord and maternal blood. This shows that the newborn levels are dependant on the mothers' availabilities [8-11].

Therefore it can be concluded, that maternal supply of L-carnitine is essential for the development of fetal L-carnitine reservoir.

Our own animal experiments in pigs showed that supplementation of the sow with L-carnitine favours lactation and has a positive effect on milk composition. Also more life births were seen [12]. Up to now no comparable data from human studies are available.

Also other different effects (see above), which are attributed to L-carnitine supplementation, lack the proof through scientifically founded, sufficiently powered studies so far. We present now the world-wide largest study, which examines L-carnitine level in blood of neonates. Our investigation had the following goal: correlation between newborn L-carnitine levels and sex, gestational age, lung maturity, birth weight, gestational diabetes of the mothers and nicotine abuse of the mothers was examined on the basis of a large-scale longitudinal investigation.

¹ Department of Obstetrics and Gynecology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

² Institute of Nutrition Science, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

³ Malformation-Register, Otto von Guericke University, Magdeburg, Germany

⁴ Department of Obstetrics and Gynecology, St. Elisabeth Hospital, (Saale), Germany

Material and methods

Subjects. In the years 2002 to 2006 blood was examined from 3652 neonates. Informed consent was given by all mothers. All children born at the Department of Obstetrics at the Martin-Luther-University Halle-Wittenberg (Saxony-Anhalt, Germany) were included. The average birth weight was 3181 g (305-5410 g; SD \pm 749 g). The mean age of the mothers at the delivery was 28.6 years (14-44 years; SD \pm 5.8 years). The distribution of sex was almost equally distributed: 1767 female and 1885 male newborn. The average gestational age at birth was 38 weeks/5 days (23/0-44/0 weeks; SD \pm 3.0 weeks). None of the pregnant women participated in any other study during the pregnancy. No special L-carnitine diet was recorded. Early neonatal deaths within the first 48 hours were excluded.

Sample collection. The blood of the neonates was collected in the context of a routine blood investigation at the third day of life. For this the skin of the heel was punctured and a dried blood drop was taken.

Analysis. The free L-carnitine from full blood was determined. The detection was done by means of tandem mass spectrometry (PE Sciex API 365 electrospray ionization).

Statistics. The data was evaluated by means of the test statistics of a Student's t-test type 2. That is, two samples with same variance (homoscedastic) were examined.

Results

First the quantity of free L-carnitine in blood of the neonates was correlated with gestational age at birth. Significantly higher L-carnitine levels were seen in the blood of very premature neonates (< 32/0 GA) as compared to term babies (< 32/0 GA: 23.5 $\mu\text{mol/l}$, $n = 151$; 39/0-39/6 GA: 17.4 $\mu\text{mol/l}$, $n = 723$ ($p < 0.001$); 40/0-40/6 GA: 18.0, $n = 869$ ($p < 0.001$); see Figure 1). L-carnitine levels in the blood of neonates dropped with increasing gestational age up to term. When there

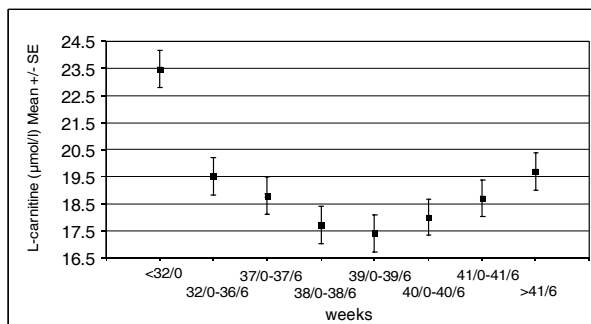


Fig. 1. L-carnitine level in neonates and gestational age

was prolonged pregnancy above term (> 40 weeks GA), L-carnitine levels rose again to the levels of neonates born between 32/0 and 36/6 GA (32/0-36/6 GA: 19.5 $\mu\text{mol/l}$, $n = 425$; 37/0-37/6 GA: 18.8 $\mu\text{mol/l}$, $n = 232$; 38/0-38/6 GA: 17.7 $\mu\text{mol/l}$, $n = 545$; 41/0-41/6 GA: 18.7 $\mu\text{mol/l}$, $n = 512$; > 41/6 GA: 19.7 $\mu\text{mol/l}$, $n = 66$).

The rise of the L-carnitine levels in neonates, which were born after 41/6 pregnancy weeks, is significant compared to neonates, which were born between 39/0 and 39/6 GA ($p = 0.01$).

Comparing L-carnitine levels of male neonates to female neonates showed a significant difference. On the average male

neonates showed approx. 2 $\mu\text{mol/l}$ higher L-carnitine levels in full blood up than female (19.4 $\mu\text{mol/l}$, $n = 1885$ versus 17.5 $\mu\text{mol/l}$, $n = 1767$; $p < 0.001$; see Figure 2).

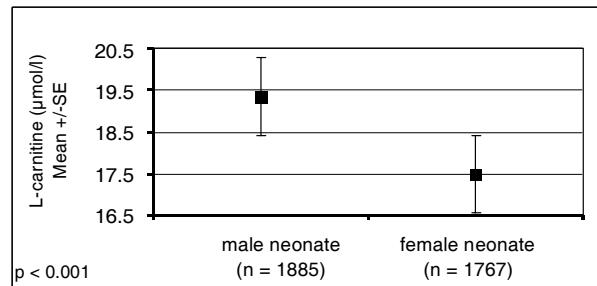


Fig. 2. L-carnitine level in neonates and sex

Neonates with so-called "respiratory distress syndromes" (RDS) were compared to those without RDS. Starting from 34/0 SSW the occurrence of the RDS can be regarded as rare event. The analysis therefore referred to all newborn children which were born between 23/0 and 33/6 SSW. No significant difference of the L-carnitine levels in the blood of neonates with RDS could be seen as compared to those without RDS (21.9 $\mu\text{mol/l}$, $n = 113$ versus 21.0 $\mu\text{mol/l}$, $n = 162$; $p = 0.54$; see Figure 3).

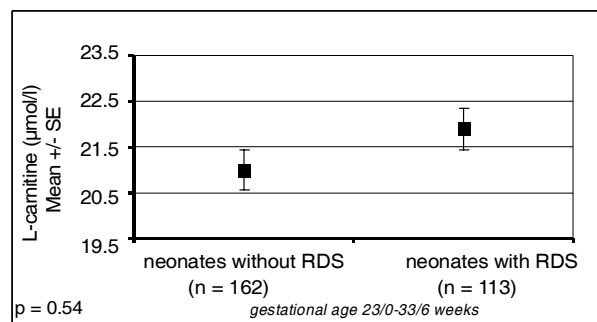


Fig. 3. L-carnitine level in neonates with and without RDS

In the group of neonates whose mothers smoke, the L-carnitine levels at third day post delivery was elevated compared to the group of neonates whose mothers were non-smokers (18.3 $\mu\text{mol/l}$, $n = 456$ versus 17.2 $\mu\text{mol/l}$, $n = 1925$; $p < 0.01$).

In order to test the hypothesis that L-carnitine levels in the blood of neonates correlates with birth weight, term neonates with 38/0 weeks GA to 40/6 weeks GA were examined (see results further above: within this period there is no dependence of the L-carnitine levels to GA!). In order to minimize the bias possible by macrosome neonates of diabetic mothers or neonates with growth retardation, only children with a birth weight between the 5th and 95th percentiles were included in the analysis.

When dividing the examined neonates into four groups (group 1: 2500-2999 g; group 2: 3000-3499 g; group 3: 3500-3999 g and group of 4: 4000-4350 g), the average L-carnitine levels between neonates of group 2, 3 and 4 show no significant difference (group 2: 17.3 $\mu\text{mol/l}$, $n = 886$; Group 3: 17.5 $\mu\text{mol/l}$, $n = 635$; Group 4: 17.9 $\mu\text{mol/l}$, $n = 108$; $p_{2,4} \geq 0.5$; see Figure 4). Only the L-carnitine levels of neonates with a birth weight between 2500 g and 2999 g are marginally signi-

ificantly higher than that of the neonates of group 2 (group 1: 18.7 $\mu\text{mol/l}$, $n = 302$; $p_1 = 0.04$; see Figure 4).

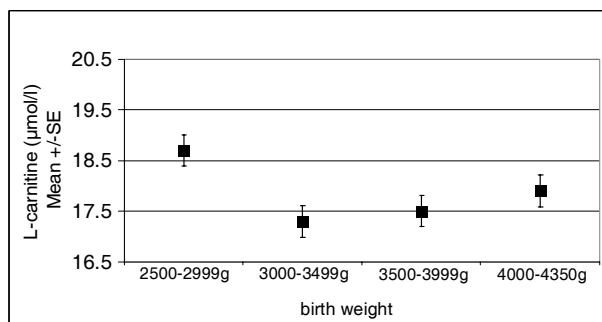


Fig. 4. L-carnitine level in neonates and birth weight

In our study neonates of mothers with gestational diabetes show a tendency to higher L-carnitine levels in full blood than the newborn children of metabolically healthy women (18.9 $\mu\text{mol/l}$, $n = 120$ versus 17.3 $\mu\text{mol/l}$, $n = 2266$; $p = 0.03$; see Figure 4).

When the appropriate parameters for the specific analysis (e.g. data for the metabolic situation of the mother) were not available, these cases were excluded so the total number of the examined neonates in the specific analyses does not correspond to the total number of all neonates in this study.

Discussion

L-carnitine levels in the blood of neonates correlates closely with L-carnitine levels in the maternal blood [8-11]. This correlation remains up to the seventh day post delivery both for preterm as well as term neonates, even if according to an investigation of Meyburg et al., the concentration of free L-carnitine levels in the blood of preterm neonates during this period continuously drops [13, 14]. That is, L-carnitine levels in the blood of neonates is also a parameter for L-carnitine levels in the blood of the mother at the time of delivery.

There is a clear dependence of L-carnitine levels and gestational age at birth. The L-carnitine levels seem to reach a minimum around 40/0 weeks GA. The levels then rise again when the pregnancy comes above 40 weeks GA. Whether the significantly lower L-carnitine levels of term neonates opposed to preterm neonates can be explained by the fact that the mother's L-carnitine levels diminish throughout the pregnancy cannot be proven so far [3, 15]. These results correspond however to the results of smaller studies by other authors [13, 16].

Whether there is a correlation between sex and L-carnitine levels was examined in a Japanese study comparing 296 male and 258 female inhabitants of Japan age 0 to 65 years. On the basis of this investigation it was shown that levels of free L-carnitine correlate with the sex. The authors assumed that estrogen has an influence on the lower free L-carnitine levels of the female population. A significant negative correlation of L-carnitine levels with estrogen has been shown, which is not found for testosterone in men [17]. Our investigations indirectly confirm a higher L-carnitine level in the blood of male fetus compared to female fetus (plus approx. 11%) already intrauterine. However the sex specific L-carnitine levels cannot be explained completely with the theories

published so far (dependency on the estrogen levels and nutrition).

Already in the 90's animal models proved that L-carnitine promotes the synthesis of surfactant (and lung maturity) [3]. A combination therapy with betamethasone and L-carnitine is said to promote the lung maturation more effectively than the betamethasone therapy alone [3]. This thesis is supported by newer studies, which analysed the concentration of free L-carnitine in premature newborn with RDS compared to the concentration of free L-carnitine in premature newborn without RDS [18, 19]. At the third day post delivery the free L-carnitine levels in the group with RDS were significantly lower than the free L-carnitine levels in the control group [19]. Our results cannot confirm these findings. The blood L-carnitine levels of preterm neonates with RDS is minimally (not significantly!) higher compared to preterm neonates without RDS.

Possibly our data would question the dependency of surfactant on the availability of L-carnitine. On the other hand, lack of surfactant could be, among other factors, the expression of a disturbance in the "novel organic cation transporter 2 (OCTN-2/"carnitine transporters"). Further studies need to be done for clarification.

Numerous investigations report about a strict positive correlation between birth weight and L-carnitine level in neonates [e.g. 14, 20]. Other authors describe a negative correlation between birth weight and L-carnitine levels [15]. Own investigations with animal models showed that birth weight of the piglets does not differ when the sow is supplemented with L-carnitine compared to controls. In the first weeks of life, more weight gain was seen in piglets whose mothers were supplemented [12, 21]. According to the study presented here there is no correlation of L-carnitine levels with birth weight in human neonates. The nearly significant higher L-carnitine levels in neonates with a rather low birth weight (2500-2999 g) could be explained first by a bias through a number of children, whose birth date was wrongly computed and who were born with normal birth weight but were preterm – and therefore having higher levels of L-carnitine. Thus several neonates with < 38/0 weeks GA could falsify the values of the group 1.

On the contrary to the expected lower levels of free L-carnitine in neonates of mothers with gestational diabetes [22] the neonates showed a tendency of higher L-carnitine levels when their mothers had the disease in our study. Whether an under-supply with L-carnitine is associated with pregnancy-induced metabolic diseases such as gestational diabetes, still remains unclear seeing the results of this study and must be evaluated by further investigations. Recent references show both: disturbed L-carnitine metabolism may and also may not play a role in the development of gestational diabetes [23].

In an explanation already on the 18. 10. 2005 the German Association of Gynecologists draws the attention to "the meaning of L-carnitine in pregnancy and lactation". Without establishing sufficient broad studies, which can clarify the open questions concerning the meaning of L-carnitine for mother and child, an evidence-based recommendation concerning L-carnitine supplementation is not possible.

This analysis, which represents the largest in its area, is to contribute in placing the discussion about the effects of L-carnitine in pregnancy on mother, fetus and the neonate on

a more solid scientific basis. Still further studies are needed to enlighten the situation concerning the remarked controversial results.

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Gregor Seliger

Martin Luther University Halle-Wittenberg

Department of Obstetrics

Ernst-Grube-Strasse 40, D-06097 Halle (Saale), Germany

e-mail: gregor.seliger@medizin.uni-halle.de