



## CLINICAL RESEARCH ARTICLE

# Carnitine longitudinal pattern in preterm infants <1800 g body weight: a case–control study

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**BACKGROUND:** The aim of this study is to investigate free carnitine (C0) and total acylcarnitine levels (AC) in preterm infants with BW < 1800 g and the relationship with neonatal and perinatal factors.

**METHODS:** Ninety-three thousand two hundred and ninety-three newborns were screened between 2011 and 2013. Dried blood samples (DBS) were collected at 48–72 h, 14, and 30 days of age. We studied 92 consecutive preterm (BW < 1800 g) infants with low carnitine levels at 30 days of life (Group 1). As controls, we included the first 92 cases (BW < 1800 g) with normal carnitine levels (Group 2) and another 92 at or near-term newborns with BW > 1800 g (Group 3).

**RESULTS:** Compared to 48–72 h levels, C0 and AC decreased at 14 and 30 days of life in Groups 1 and 2 ( $p < 0.001$ ). In Group 2, the percentage of children with carnitine-free parenteral nutrition (PN) and BW < 1000 g was lower than in Group 1 ( $p < 0.001$ ). Only in Group 2 did C0 increase at 30 days ( $p < 0.001$ ). The multivariate regression analysis confirmed the influence of body weight and type of nutrition on C0 and AC.

**CONCLUSION:** Body weight and type of nutrition influenced the carnitine longitudinal pattern.

*Pediatric Research* (2019) 86:646–650; <https://doi.org/10.1038/s41390-019-0497-2>

## INTRODUCTION

L-carnitine plays a fundamental role as carrier for the acyl groups across the mitochondrial membranes for beta oxidation of fatty acids. These are activated in the cytosol into acylcarnitine (AC) by Acyl-CoA synthase and are then carried by specific AC transferases and translocases into the mitochondrial matrix where beta-oxidation occurs. In the neonatal period, adequate levels of carnitine are essential not only for adequate energy supply from the metabolism of fatty acids but also for neuroprotection. Many authors have argued that carnitine supplementation is recommended in children with low carnitine levels to prevent low energy production from fatty acid oxidation and reduce the risk of hypoglycemia, hypothermia, respiratory distress, and infection.<sup>1–4</sup> Jones et al. showed that ACs contribute to the overall energy status of the brain, increasing antioxidant activity, neurotransmitter synthesis, and function and modulating protein and gene expression.<sup>5</sup>

There are few and conflicting data on the normal ranges of free carnitine (C0) and AC concentrations especially in preterm infants and on the various perinatal factors (maturity, birth weight (BW), neonatal intensive care practices, parenteral nutrition (PN)) that may influence C0 and AC patterns. Some authors have reported a significant positive correlation between whole-blood carnitine levels and BW in preterm infants (<1800 g),<sup>6,7</sup> while others have emphasized the influence of gestational age.<sup>1,8–13</sup>

Chace et al. and Meyourg et al. found higher levels of blood total carnitine levels in preterm than in at-term infants, possibly due to reduced tissue mass and/or immature carnitine transporter of preterm newborns.<sup>1,10</sup>

Bernardini et al. reported that high carnitine levels found at birth in preterm infants may depend on higher carnitine placental transfer in the early stages of gestation.<sup>14</sup>

There are few longitudinal studies on the carnitine pattern in preterm newborns. Meybourg et al. and Mandour et al. reported a significant decrease in carnitine levels in preterm newborns during the first weeks of life.<sup>10,11</sup> In the study by Meybourg et al., the severity of prematurity negatively influenced carnitine levels at 2 weeks of life, possibly due to low tissue deposits and reduced capability of endogenous synthesis from the amino acid precursors, lysine and methionine, along with reduced carnitine exogenous supply.<sup>10</sup>

The difficulty in interpreting the literature is mainly due to the difference in the populations examined, in the experimental designs of the studies, and in the laboratory methodologies, as well as the lack of longitudinal studies. The implementation of screening programs for Inborn Errors of Metabolism (IEM), thanks to tandem mass spectrometry (MS/MS), has led to the assessment of the levels of C0, AC, and amino acids in dried blood samples (DBSs).<sup>15–17</sup> Many protocols have been developed for the routine repetition of specimens in preterm/very immature infants to evaluate the pattern of these metabolic parameters over time.<sup>15,17</sup>

The purpose of this study was to:

1. investigate C0 and AC levels longitudinally in a group of consecutive preterm infants with BW < 1800 g examined with uniform criteria in a single screening center subdivided according to the normal or abnormal C0 and AC levels shown during the screening protocol and compared to the full-term infants

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Received: 30 August 2018 Revised: 7 June 2019 Accepted: 12 June 2019

Published online: 10 July 2019

2. assess the influence of different neonatal and perinatal factors on the longitudinal profile of these metabolites
3. highlight possible neonatal prognostic factors in order to select patients for whom supplementation with carnitine could be recommended.

## PATIENTS AND METHODS

### Screening protocol

In our regional screening program, the expanded newborn screening (NBS) (IEM) using the same DBSs collected for endocrine diseases started in June 2011. DBSs are examined at a single laboratory and pathological results are sent to a single regional clinical center, which provides the recall procedures. The data transmission system is fully computerized. DBS handling from birth centers to the screening laboratory is managed via courier.

The demographic and clinical information recorded on the DBS case report form included gestational age, BW, time of sampling, duration of PN, blood transfusions, and neonatal diseases. For the purpose of this study, further specific clinical information—PN composition and duration, breastfeeding or formula, characteristics of neonatal intensive care—were obtained from each maternity clinic. DBS samples were obtained by a heel stick and not by the umbilical cord from all the infants and also from Neonatal Intensive Care Unit (NICU) infants. DBS samples were collected from all newborns at 48–72 h of life (in any case, the initial DBS was obtained before the start of PN and/or blood transfusion). In accordance with international guidelines,<sup>15,17</sup> additional specimens were routinely taken from preterm infants with BW < 1800 g on days 14 and 30. These newborns were recalled only if C0 or AC at 30 days of life were outside the normal cutoff value for at-term newborns. The normal cutoff for C0 and ACs are >4 μmol/L and >15.30 μmol/L, respectively.

C0 and AC levels in DBSs were measured using the MS/MS as described below. Parental consent was obtained for each subject and the protocol was approved by the local institutional review board.

The study was retrospective and longitudinal. Between October 2011 and December 2013, a total of 93,293 newborns were examined in our region, 2064 of who had a BW < 1800 g. Overall, 276 infants were included in this study. We evaluated C0 and AC levels in DBS obtained by a heel stick, taken after 48–72 h, 14, and 30 days of life in 92 consecutive infants with BW < 1800 g (Group 1) recalled for abnormal levels of C0 and/or AC at the end of the screening protocol.

A control infant was enrolled for each case in Group 1 (Group 2). This control infant was the first newborn with BW < 1800 g with normal levels of C0 and AC at the end of screening protocol (at 30 days of life) born in the same maternity clinic where the Group 1 case was born.

We also included 92 at- or near-term newborns with BW > 1800 g (Group 3) born in the same period from the same maternity clinic who underwent NBS at 48–72 h of life only. Extra samples were taken from Group 1 to evaluate the pattern of C0 and AC until the levels were normalized. Newborns with confirmed inherited metabolic diseases, with PN containing carnitine, neonates deceased after birth, and those who did not complete the protocol and/or with missing complete clinical data were excluded from the study.

### Mass spectrometry

All procedures for sample preparation and MS-MS analysis were performed by the NeoBase Non-derivatized Kit (PerkinElmer, USA) according to the manufacturer's protocol. A 3.2-μL-diameter dot per sample was taken from every DBS and punched out into a 96-well microtiter-plates by two punchers, one semi-manually and one automatically. Only one diskette was added for each well. The

first four wells contained a solution including internal standards, the next four contained low-level and high-level controls assayed in duplicate. The internal standard of carnitine was reconstituted with 1 mL of NeoBase extraction solution followed by its dilution to 1:110 to obtain the daily working solution of internal standards. In each well of the microtiter-plates, 100 μL of work solution were added containing an extraction solution and corresponding internal standards. The microtiter-plates were sealed and incubated at 45 °C in a forced air oven for 45 min with a shaking speed of 650 rpm. A total of 75 μL of the content of each well was transferred to a heat-resistant microtiter-plate (NUNC plate).

The analytic session was started with the injection of 30 μL per sample and evaluated the following day when a technical validation was also carried out with the transfer of results to the central server in order to manage the analytic data. Two TQD Acquity Waters triple quadrupole tandem mass spectrometers (one used as a backup) with an autosampler (2777C Sample Manager Waters) and a pump (Binary HPLC Pump 1524 Waters) were used for the analysis.

### Statistical analysis

Descriptive statistics were reported in terms of absolute frequencies and percentages for qualitative data. Pearson's chi-square test or Fisher's exact test, where appropriate, were applied to compare proportions, while Student's *t* test, was used to compare quantitative variables between groups.

Univariate and multivariate regression analyses of factors related to C0 and AC were also performed. The parameters considered for statistical analysis were: gestational age, BW, PN, sex, breastfeeding, and formula feeding.

All *p* values were double tailed and values <0.05 were considered statistically significant.

All the analyses were conducted using the STATA software.

## RESULTS

The demographic and clinical features of the patients are reported in Table 1.

The differences observed in Group 3 compared to Groups 1 and 2 depend on the study design. Compared to the infants in Group 2, those in Group 1 showed several significantly worse parameters of clinical outcome (antibiotic therapy rate, NICU admission rate, blood transfusion rate, duration of PN). In 72/92 infants in Group 1 and in 43/92 infants in Group 2 ( $\chi^2 = 117.37$ ;  $p < 0.001$ ) additional carnitine-free PN was carried out during the screening protocol. The rate of newborns that needed additional carnitine-free PN remained significantly greater at each step of the protocol in Group 1 than in Group 2 (78.3% vs 46.7% at 48–72 h; 83.7% vs 28.3% at 14 days; 58.7% vs 3.0% at 30 days;  $p < 0.001$ ).

Figures 1 and 2 show the C0 and AC levels in the infant groups examined at the various steps of the screening protocol.

At 48–72 h of life, C0 levels in Group 3 were significantly lower than those observed in Groups 1 and 2 ( $p < 0.05$ ); AC levels in Group 3 were only lower than in Group 2 ( $p < 0.05$ ).

At 14 and 30 days of life, in Groups 1 and 2, C0 and AC levels were significantly lower than those observed at 48–72 h of life ( $p < 0.001$ ). In Group 2, the C0 levels at 30 days increased compared to those observed at 14 days of life ( $p < 0.001$ ). In Group 1, the average time for the normalization of C0 levels was  $62 \pm 45$  days of life (range 28–286).

In both Groups 1 and 2, the body weight increased over time but always remained significantly lower in Group 1 than in Group 2 (Table 2). At 30 days of life, the percentage of infants still weighing <1000 g was significantly higher in Group 1 than in Group 2 [34 (37%) vs 3 (3%);  $p = 0.001$ ].

The univariate analysis showed the significant influence of body weight, gestational age, and duration of carnitine-free parenteral feeding on the C0 and AC levels in each group.

**Table 1.** Neonatal demographics and care characteristics in the three groups examined

Characteristics	Group 1	Group 2	Group 3
M/F	44/48	41/51	51/41
Caucasians, <i>n</i> (%)	73 (79.3)	73 (79.3)	79 (85.9)
BW, g; mean ± SD	967.4 ± 375.2 <sup>§</sup>	1392.6 ± 324.3	3027.7 ± 561.3*
GA, weeks; mean ± SD <sup>c</sup>	27.6 ± 2.9 <sup>§</sup>	31.6 ± 2.8	38.3 ± 2.2*
SGA, <i>n</i> (%)	11 (12.1)	15 (16.3)	5 (5.4)
Twins, <i>n</i> (%)	35 (38.0) <sup>§</sup>	29 (31.5)	8 (8.8)*
Antibiotics, <i>n</i> (%)	86 (93.5) <sup>§</sup>	66 (71.7)	16 (17.4)*
NICU, <i>n</i> (%)	90 (97.8)**	80 (87.0)	16 (17.4)*
Jaundice, <i>n</i> (%)	41 (44.6)	40 (43.5)	8 (8.7)*
Blood transfusion, <i>n</i> (%)	70 (76.1) <sup>§</sup>	11 (12.0)	0
Duration of PN, days; mean ± SD	21.0 ± 11.8 <sup>§</sup>	10.6 ± 5.1	0

F female, BW birth weight, GA gestational age, M male, NICU Neonatal Intensive Care Unit, PN parenteral nutrition, SGA small for gestational age \**p* < 0.0001 vs Groups 1 and 2; <sup>§</sup>*p* < 0.0001 vs Group 2; \*\**p* < 0.01 vs Group 2

**Table 2.** Weight of infants in Groups 1 and 2 examined at the various steps of the screening protocol

	Birth weight (g)	Weight at 14 days (g)	Weight at 30 days (g)
Group 1	967 ± 375 <sup>§</sup>	1038 ± 397 <sup>§</sup>	1289 ± 478 <sup>§</sup>
Group 2	1393 ± 324*	1528 ± 370	1942 ± 473

Data are expressed as mean ± SD  
\**p* < 0.0001 vs 14 and 30 days; <sup>§</sup>*p* < 0.0001 vs Group 2

Table 3 shows the percentage of infants with a different type of nutrition in each group at each stage of the study protocol.

Figures 3 and 4 show the C0 and AC levels of breastfed vs formula-fed children at each stage of the study protocol, excluding the patients on carnitine-free PN.

Overall, C0 and AC levels decreased from 48–72 h to 30 days of life. C0 and AC levels were lower in breastfed than in formula-fed children at 14 days (*p* < 0.05) and at 30 days of life (*p* < 0.001).

The multivariate regression analysis confirmed the association between carnitine levels (C0 and AC) and body weight at 48–72 h of life and at 30 days of life (*p* < 0.001), carnitine-free PN at 14 (*p* < 0.01) and 30 days of life (*p* < 0.001), and formula feeding at 30 days of life (*p* < 0.05). The relationship between gestational age and carnitine levels was not confirmed by multivariate regression analysis.

## DISCUSSION

To the best of our knowledge, this is the first longitudinal study that has evaluated a large sample of preterm infants with BW < 1800 g examined with uniform criteria at a single screening center, who were recalled on the basis of a specific protocol of screening and compared with infants with BW < 1800 g not recalled and at-or near-term infants with BW > 1800 g.

There are few and conflicting data on the effects of gestational age, BW, and weight trend on C0 and AC concentrations from birth to the first weeks of life in preterm infants.

Our results seem to confirm that BW and weight trend are the principal factors that influence carnitine levels in the first month of life in preterm infants.

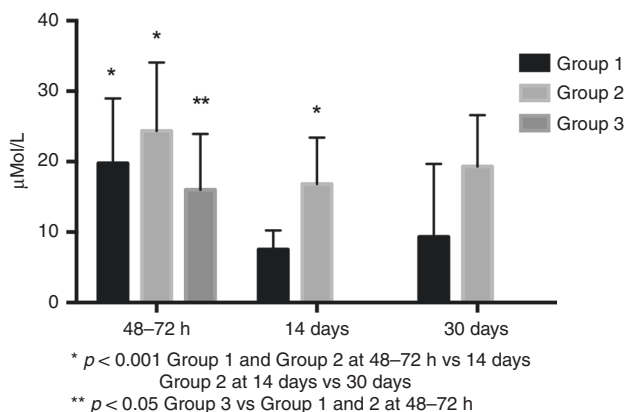
The role of BW in influencing the C0 levels in the first days of life has been reported in several studies, albeit with conflicting results.<sup>6,11</sup> However, the weight trend was not taken into consideration in the few longitudinal studies reported in the literature.<sup>10,11</sup>

At birth, our preterm infants with a lower BW (Group 1 and 2) had higher levels of C0 compared to the full-term infants with BW > 1800 g. This finding, which has been previously reported by other authors, may be related to reduced tissue mass and/or fetal reduced carnitine tissue uptake and the higher carnitine placental transfer that takes place in the early stages of gestation.<sup>1,8,10,14</sup>

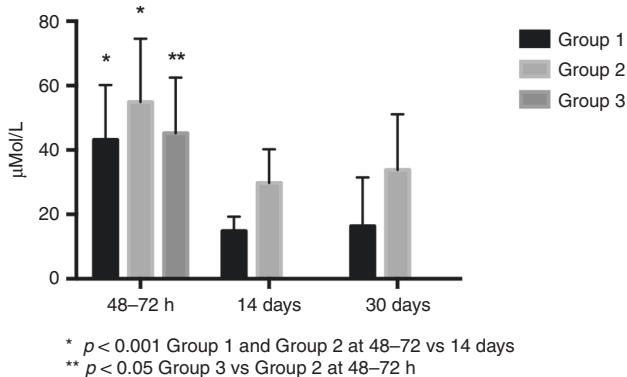
At 14 and 30 days of life, C0 and AC levels of all our infants with BW < 1800 g were significantly lower than those observed at 48–72 h of life. However, the C0 levels at 30 days increased only in Group 2 compared to those observed at 14 days of age.

In a longitudinal study, Meyburg et al. reported that gestational age influenced whole-blood ACs in preterm newborns. In their cohort, during the first weeks of life, the severity of prematurity played a key role in determining the carnitine longitudinal pattern.<sup>10</sup> However, the influence of gestational age was not confirmed by our data. These results are probably related to the different characteristics of the two cohorts, since Meyburg et al. also evaluated infants at <28 weeks of gestational age.

We believe that, in our infants, the higher levels of C0 at 30 days of life in Group 2 compared to Group 1 are probably still linked, at



**Fig. 1** Whole-blood free carnitine (C0) values (mean ± SD) in infants examined at the various steps of the screening protocol

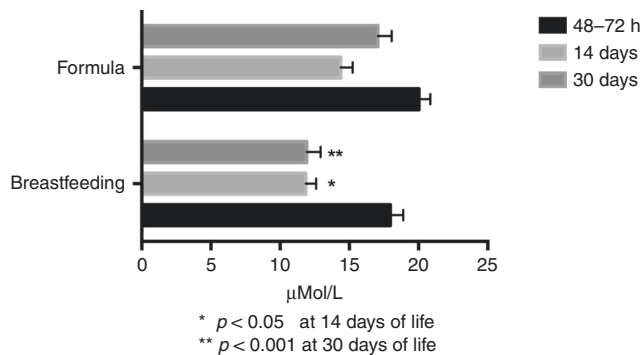


**Fig. 2** Whole-blood acylcarnitine (AC) values (mean ± SD) in infants examined at the various steps of the screening protocol

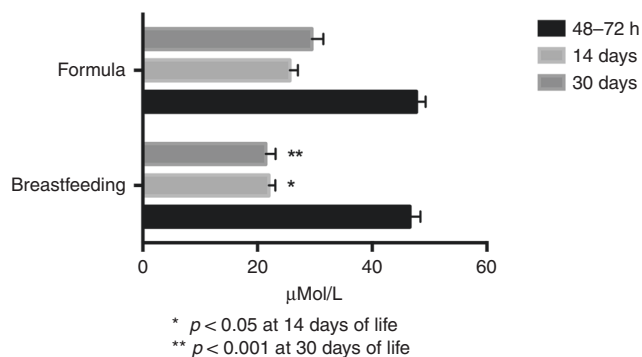
**Table 3.** Types of nutrition examined at the various steps of the screening protocol

	Group 1			Group 2			Group 3
	48–72 h	14 days	30 days	48–72 h	14 day	30 days	48–72 h
Breastfeeding	25	33	33	22	43	27	41
Formula	17	31	45	61	55	72	59
Carnitine-free PN	58	36	22	17	2	1	0

Data are expressed in percentage



**Fig. 3** Whole-blood free carnitine (C0) values (mean ± SD) of breastfed vs formula-fed children at the various steps of the screening protocol



**Fig. 4** Whole-blood acylcarnitine (AC) values (mean ± SD) of breastfed vs formula-fed children at the various steps of the screening protocol

least in part, to a weight factor. In fact, the better weight increase in subjects of Group 2 probably led to a greater amount of carnitine tissue deposits. Parenteral feeding is another factor that seems to have a significant influence on the carnitine pattern in the first month of life. In fact, almost all infants in Groups 1 and 2 required intensive neonatal care and were unable to carry out enteral nutrition. In these infants, particularly those in Group 1, the tissue fat deposits were very small. Parenteral feeding favors adequate caloric intake with solutions that are rich in essential fatty acids and lipid emulsions but that are carnitine-free. Moreover, PN provides sufficient amino acid precursors for carnitine biosynthesis (lysine and methionine); however, in these children carnitine would not be sufficiently produced owing to ineffective enzymatic systems.<sup>2</sup>

It is possible that Group 1 children failed to restore C0 levels at 30 days of life due to their worse clinical conditions, which led to a

prolonged carnitine-free PN and slowed their weight gain. On the other hand, Group 2 children showed more favorable clinical features at birth, increased their body weight more rapidly, and therefore were able to start enteral feeding sooner, which probably increased the carnitine intake.

Note that most formula milk is enriched with carnitine, while human milk fortifiers are not.<sup>17</sup>

Our results are thus in agreement with the recent data by Bene et al. who reported higher C0 levels in formula-fed than breastfed preterm infants.<sup>18</sup>

In conclusion, infants with BW < 1800 g represent a heterogeneous population in terms of neonatal features and perinatal and postnatal care. There are few and conflicting data in the literature, in particular in relation to longitudinal studies, on the C0 and AC pattern at the beginning of life and the possible influence of different perinatal factors on their profile. Some studies have highlighted the positive effects on growth and metabolic parameters in very low BW infants who underwent carnitine-supplemented PN. These studies, however, were only carried out on few cases and with a short follow-up.<sup>2,4</sup> In the study by Clark et al., l-carnitine supplementation is associated with higher carnitine levels but is not associated with improved short-term hospital outcomes in preterm infants.<sup>19</sup> As well as establishing a basis for determining normative data in the population of infants with BW < 1800 g, our data could be very useful to select subjects for whom a supplementation with carnitine should be recommended. Our results do not enable us to identify a critical weight and thus to select a particular at-risk group in the infant population in order to recommend carnitine supplementation. A body weight of 1000 g is considered by neonatologists to be a very significant prognostic factor for an infant's outcome, and our results highlight the significantly higher percentage in Group 1 compared to Group 2 of newborns who were not able to reach a weight of 1000 g at 30 days. Prospective long-term follow-up studies are also needed to evaluate the potential benefits on psycho-intellectual development in these subjects compared to non-supplemented subjects.

**AUTHOR CONTRIBUTIONS**

A.C., F.B. and B.R. conceived the study. B.R., I.B. and R.O. collected the data. F.R. performed the biochemical analyses. F.B., A.C. and B.R. drafted the article, and all authors contributed substantially to its revision. A.C., F.B., R.R. and F.R. made substantial contributions to conception and design and, analysis and interpretation of data. F.B., B.R. and A.C. were responsible for drafting the article and/or revising it critically for important intellectual content. G.F., A.C. and A.P. gave final approval of the version to be published.

**ADDITIONAL INFORMATION**

**Competing interests:** The authors declare no competing interests.

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