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L-CARNITINE CONCENTRATIONS IN MILK FROM MOTHERS ON DIFFERENT DIETS.

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INTRODUCTION

During most periods of mammalian life L-carnitine is nutritionally dispensable because it can be synthesized at sufficient rates from lysine and methionine (1). Only two conditions have been described so far where it becomes indispensable:

i) total parenteral nutrition lasting longer than 3 weeks (2) and
ii) early postnatal life (3).

A number of studies have contributed evidence that the role of milk L-carnitine is to provide the newborn with this coenzyme of fatty acid oxidation and to prevent deficient states during early postnatal life (4 - 7). Robles-Valdes and McGarry demonstrated that milk L-carnitine originating from the mother's L-carnitine synthesis reaches finally the hepatic L-carnitine pool of the newborn rat (8). We ourselves observed that the hepatic L-carnitine concentrations in the suckling newborn rat are dependent on the milk L-carnitine concentration (9).

Warshaw and Curry reported that soy-based formula-feeding to human newborns caused lower serum L-carnitine concentrations as compared to breast-feeding; interestingly, this was paralleled by lower serum ketone body concentrations indicating a reduced capacity of hepatic fatty acid oxidation (10).

Abbreviations: DTNB = 5,5' -Dithio- bis (-2-nitrobenzoic acid), HEPES = 4-(2-Hydroxyethyl)-piperazineethanesulfonic acid,
EDTA = Ethylene diamine tetraacetic acid,
CAT = Carnitine acetyltransferase (E.C. 2.3.1.7),
L-O-V = Lacto-Ovo-Vegetarians

From the metabolism of L-carnitine two modes may be anticipated by which nutrition might influence the L-carnitine concentration of mother's milk. First, variation of intake of precursor amino acids, i. e. lysine and/or methionine may be influential (11, 12). Second, different intakes of L-carnitine-containing foods, as for example meat or milk, might cause differences of milk L-carnitine concentrations.

As to our knowledge few studies have been published so far relating milk L-carnitine concentrations to dietary habits of the mothers we have initiated a survey on this topic. We report herein on the first results about the influence of a conventional as compared to a lacto-ovo-vegetarian diet.

MATERIAL AND METHODS

A. Milk Sampling

Milk specimen were obtained from 20 mothers in total, who gave their consent for the analyses planned and for giving a written qualitative protocol about the food consumed on the sampling day.

The donors were classified as "omnivorous" and "lacto-ovo-vegetarian" according to these written protocols and this conclusion was confirmed by interviewing them directly.

The donors were asked to express about 20 ml of milk from the second breast while nursing their baby. The milk was sampled during the initial time of nursing. Sampling time was 3 - 4 h after lunch before the evening meal.

The samples were stored immediately at -20°C until analysis.

Sampling started later than 50 days post partum. The lactation period was divided into 10 periods of 20 days each.

B. Analyses

Reagents: DTNB and Coenzyme A were from Boehringer Mannheim (Darmstadt, Germany). Acetyl-CoA was prepared as described by Stadtman (13). Carnitine acetyltransferase and palmitoyl-L-carnitine were from Sigma (Taufkirchen, Germany). The other chemicals were of reagent grade and obtained from Merck, Darmstadt.

Analyses: For the estimation of L-carnitine the method of Pearson et al (14) was used with some modifications.

Free L-carnitine: After thawing at 37°C, the samples were sonified with a Labsonic 1510 (B. Braun), setting at 100 Watt for 30 sec. An aliquot of the sonified milk was cooled on ice and deproteinized with perchloric acid (0.6 mol/l). After mixing the sample was centrifuged at 5° C for ten minutes at 27.500 x g. A fat-free aliquot of the supernatant was neutralized with K_3PO_4. Another aliquot of this supernatant was taken to estimate acid-soluble acylcarnitine esters (see below). The samples were put on ice for ten minutes. The precipitated $KClO_4$ was separated by centrifugation for 1 min at 11.000 x g.

An aliquot of the clear supernatant was analyzed for L-carnitine on a COBAS-BIO centrifugation analyzer (La Roche with the following components: HEPES 120 mmol/l, EDTA 30 mmol/l, acetyl-CoA 0.15 mmol/l, DTNB 0.125 mmol/l, CAT 1400 U/l. Correction of non-constant endpoint was done b extrapolating to start of reaction.

Total L-carnitine: An aliquot of the sonified milk sample was made 0.2 mol/l with KOH and the L-carnitine esters were hydrolized for 16 h at room temperature, to avoid too much browning products. The recovery of added palmitoyl-L-carnitine was 97 ± 0.5 % ($\bar{x}$ ± S.E.M., n = 5). The hydrolisates were cooled in a refrigerator for 15 min and then acidified with perchloric acid. The sample was spun in a refrigerated centrifuge for 10 min at 27.500 x g at 5° C. An aliquot of the slightly yellow, but clear supernatant was neutralized with K_3PO_4. After mixing and cooling, the precipitated $KClO_4$ was centrifuged down for 1 min at 11.000 x g. An aliquot of the supernatant was analyzed for L-carnitine as mentioned above.

Acid-soluble acylcarnitine esters (short- and medium chai acylcarnitine esters): An aliquot of the deproteinized samp (see above) was made alkaline by KOH (final concentration 0.2 mol/l) and the esters were hydrolized for 16 h at room temperature. The hydrolisates were neutralized with H_3PO_4. After mixing and cooling the samples were centrifuged and analyzed for L-carnitine as mentioned above.

The long-chain acylcarnitine esters were calculated as follows: [Total L-carnitine]-[short- and medium chain acylcarnitine esters + free L-carnitine] = long-chain acylcarnitine esters.

Cows and rat milk were deproteinized with $ZnSO_4$ and $Ba(OH)_2$. To estimate the acylcarnitine esters, the milk was first hydrolized by the addition of $Ba(OH)_2$ and then deproteinized by the addition of $ZnSO_4$ (5).

RESULTS

Milk sampling was started from the 50th day of lactation on.

Total milk L-carnitine concentrations did not change considerably with time of lactation in omnivorous mothers (Fig. 1). If there was a change there was a rise with lactation time in omnivorous and a fall in lacto-ovo-vegetarian mothers. At any time L-carnitine concentrations of the vegetarian donors' milk was lower than that of the omnivorous donors' milk. In 2 out of the 3 vegetarian mothers studied milk L-carnitine concentrations proved to be significantly lower as compared to omnivorous donors as judged by a sign test (15). In the third L-O-V-mother the number of observations was to small to ascertain significance.

There was a satisfying agreement of our results and values reported by earlier investigators (Tab. I).

Table II documents the averages of all total L-carnitine concentrations obtained from either omnivorous or lacto-ovo-vegetarian mothers irrespective of lactation time.

Fig. 2 and 3 show that the difference between the donor groups was caused more by the difference of free L-carnitine and short- and medium-chain acylcarnitine esters than by long-chain acylcarnitine esters.

Om 75-115
LOV 50-90
5 5

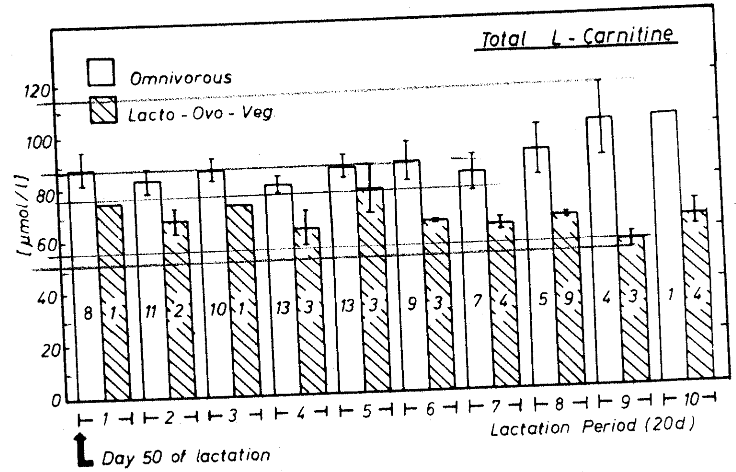

Fig. 1: Total L-carnitine concentrations in breast milk of "Omnivorous" and "Lacto-Ovo-Vegetarians". The columns show $\bar{x} \pm$ S.E.M. with the number of samples indicated in the column. Samples were taken between the 50th and 250th day of lactation.

TABLE I

HUMAN MILK L-CARNITINE AS REPORTED BY DIFFERENT INVESTIGATORS

The data relate to omnivorous or nutritionally not qualified donors. The range of lowest and highest averages, observed at different stages of lactation, are given.
n. d.: no data given.

Author	Ref.	Milk L-carnitine concentration Total	Free
		µmol/l	
Schmidt-Sommerfeld et al.	(16)	39 - 63	n. d.
Sandor et al.	(17)	35 - 70	n. d.
Warshaw and Curry	(10)	70 - 110	n. d.
Donzelli et al.	(18)	n. d.	32
This study		81 - 103	20 - 44

TABLE II

TOTAL L-CARNITINE CONCENTRATIONS IN MILK OF OMNIVOROUS OR LACTO-OVO-VEGETARIAN DONORS

Averages ($\pm$ S.E.M.) of all results obtained irrespective of lactation. N = number of observations.

Donors	N	Total L-Carnitine Concentration
		µmol/l
Omnivorous	81	86.5 $\pm$ 1.9
Lacto-ovo-vegetarian	18	65.4 $\pm$ 1.6

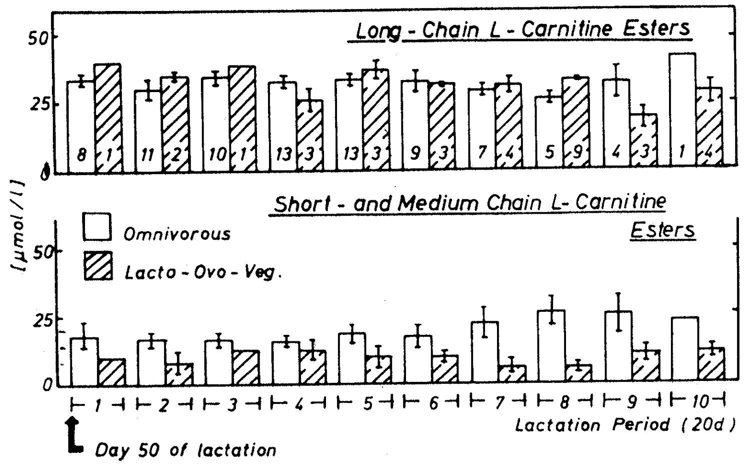

Fig. 3: Long chain acylcarnitine esters and short- and medium-chain acylcarnitine esters in breast milk of "Omnivorous" and "Lacto-Ovo-Vegetarians". For further explanation see Fig. 1.

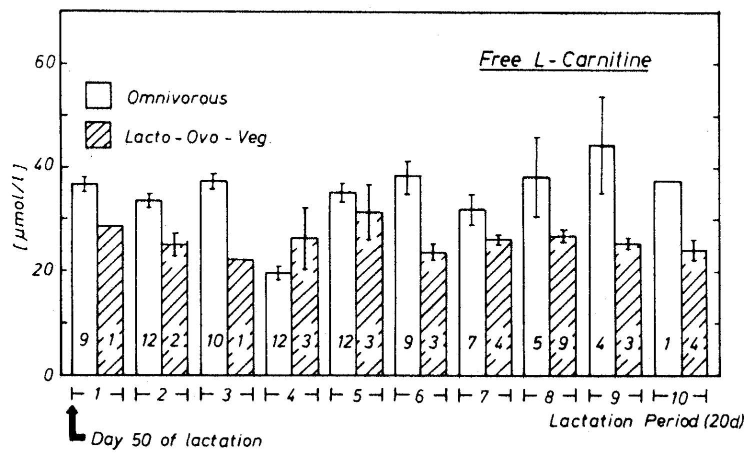

Fig. 2: Free L-carnitine concentrations in breast milk of "Omnivorous" and "Lacto-Ovo-Vegetarians". For further explanation see Fig. 1.

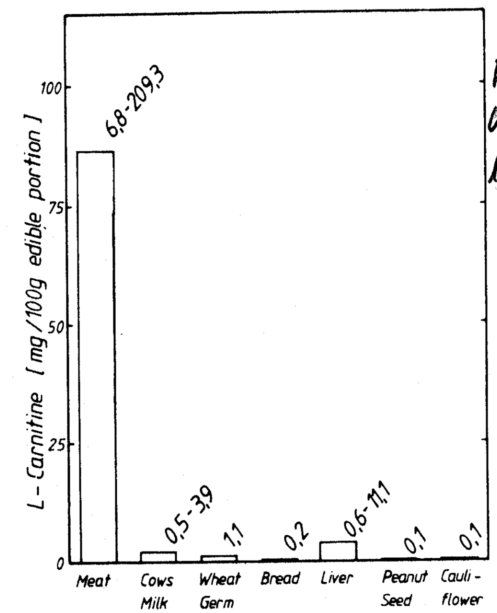

Fig. 4: Total L-carnitine concentrations in various food items. Columns show the averages. Numbers indicate the range of L-carnitine contents calculated from (23).

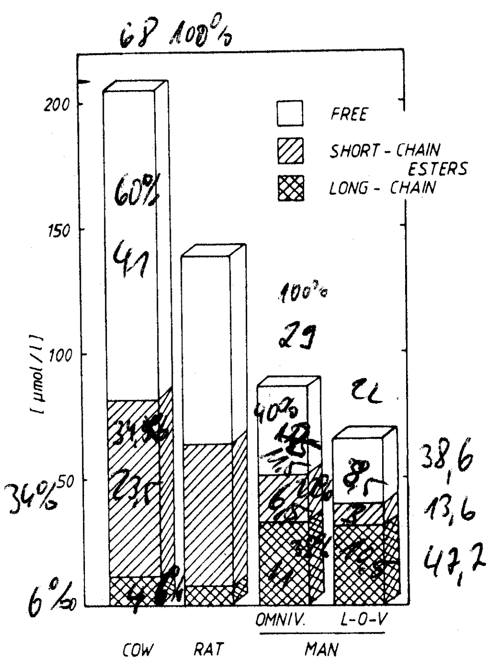

Fig. 5: Free and esterified L-carnitine concentrations in mature milk of various species. The time of sampling for cow, rat and man were the 100th - 110th day, 15th day and 50th - 250th day of lactation, respectively.

DISCUSSION

We report herein a reduced milk L-carnitine concentration in lacto-ovo-vegetarian mothers at later stages of lactation. This reduction concerns mainly the free L-carnitine fraction.

There is no convincing evidence that deficiency of the precursor amino acids lysine and/or methionine might contribute to this difference. A somewhat lower intake of these amino acids by vegetarians is possible because they consume relatively more vegetable protein than the average population in western society (19). However, studies in humans (20) and rats (11,21) have shown that a severe lysine deficiency is a prerequisite to cause lowering of tissue L-carnitine concentrations. Moreover, a low-lysine diet did not cause lower milk L-carnitine concentrations in rats (22). All these findings suggest an alternative explanation for our results.

Fig. 4 shows the outstanding nutritional role of meat containing 50 times more L-carnitine (by weight) than the next important food which is cow's milk (23). This makes it very probable that the differences of meat intake, that is to say of L-carnitine itself, determine the different milk L-carnitine concentrations observed in our study. Therefore, we consider it worthwile to study milk L-carnitine concentrations in vegans, who do not even consume dairy products.

Another aspect of carnitine physiology might be worth of mentioning here. The physiological significance and particular metabolic fate of free vs. esterified L-carnitine are not fully understood so far. A surprising observation in this study was the relatively high proportion of long-chain acylcarnitine esters in human milk (Fig. 5). It amounted to 43 % of total milk L-carnitine as compared to 6 % and 5 % in cows and rats, respectively. When lactating rats consume a fat-rich diet (16 weight % corn oil) this percentage rose to 26 % (24).

Thus there seems to be a positive correlation between dietary fat and milk long-chain acylcarnitine esters. It is tempting to speculate that the high proportion of milk long-chain acylcarnitine esters is caused by a high influx of preformed dietary fatty acids into the mammary gland, suppressing fatty acid synthesis de novo (25). When, on the other hand, the mammary gland relies on its own fatty acid biosynthesis, as in the rat and cow (26), the substrate pool of acyl-CoA for the carnitine-acyl-transferase(s) is dominated by short- and medium-chain fatty acids.

It remains to be established whether the high concentration of long-chain acylcarnitine esters is of relevance for the intestinal absorption of L-carnitine by the newborn.

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Discussion

AKESSON: Is there any relation between blood carnitine levels and those of the milk of lactating mothers?

BARTH: Yes, there are. In the milk we found carnitine levels of about 80 umol/l and in adult plasma the levels are at about 40 - 50 umol/l, so there has an active transport to be assumed.

AKESSON: Could you demonstrate the plasma levels of carnitine also among the vegetarian mothers?

BARTH: No, we did not look for that.

HARZER: Is there anything known about the biological activity of the different carnitine forms, I mean are the long chain carnitines probably more active, do they have a higher transport capacity?

BARTH: No, nothing is known about up to now, besides an abstract from the last FEBS meeting regarding the distribution of the different carnitines in human milk. But nothing is known about the metabolic significance of that.

BLANC: Is there any influence of carnitine in milk for instance during storage even in the mammary gland on the fatty acids, I mean could fatty acid be attacked by carnitine?

BARTH: If I understand your question right I am not aware of any data where the metabolism of fatty acids in the mammary gland has been studied under the influence of carnitine. I do not think that this is of great relevance because if anything happens in the mammary gland then fatty acids are synthesized and not oxidized.