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Carnitine in milk: a survey of content, distribution and temporal variation

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Abstract

Acid-soluble free carnitine and short-chain acylcarnitines were released following a selective alkaline hydrolysis scheme and estimated with use of a coupled carnitine acetyl transferase–Ellman reaction. Liquid skim milk contained elevated total carnitine levels compared to whole milk, due both to the minor contribution of liposoluble long-chain acylcarnitines in milkfat and the higher solids-not-fat content. Data are also reported for a range of dried protein products derived from milk (1–64 mg/100 g), and the stability characteristics of endogenous carnitine are described. A study of a single lactating cow exhibited decreasing concentrations of total carnitine with time postpartum during transition from colostrum to mature milk (> 9 mg/100 g to ca. 3 mg/100 g). Regional influences on bovine milk carnitine levels within Australasia were shown to be insignificant, while carnitine content in milk powders and pooled herd milk were relatively constant across a production season. Bovine milk was compared against samples of caprine, ovine, equine, human, canine and feline milks. A survey of anhydrous infant formulas indicated diverse carnitine contents (6.9–30.1 mg/100 g) as a consequence of their complex and varied compositions and the common practice of supplementation.

1. Introduction

The physiological significance of L(−)-carnitine (3-hydroxy-4-*N*-trimethylaminobutyric acid), has been under intense investigation and is reviewed by various authors (Bieber, 1988; Borum, 1983; Bremer, 1983; Rebouche & Paulson, 1986; Rebouche & Seim, 1998). Its participation in facilitating transport of medium (C_{12}–C_{14}) and long-chain (C_{16}–C_{24}) fatty acyl–CoA into the mitochondria prior to β-oxidation, the initiation of ketogenesis and in the maintainance of thermogenesis are well documented. These and other suspected functions involve the substrate specific L-carnitine acyl transferases (Borum, 1983, 1991; Lowes & Rose, 1989; Marzo, Cardace, Monti, Muck, & Martelli, 1990).

Carnitine is available to both human and bovine species through de novo synthesis via a posttranslational modification of protein-bound lysine, requiring S-adenosyl methionine. The significance of exogenous dietary sources, notably meat and dairy products, to adult human health remains under investigation (Feller & Rudman, 1988; Kerner & Hoppel, 1998; Rebouche, 1988), however, absorption from food is both rapid and quantitative with tissue accretion and conservation well controlled. Uptake into the mammary gland has been separately postulated to occur through active transport into the milk gland (Snoswell & Linzell, 1975) and by passive diffusion into milk (La Count, Emmert, & Drackley, 1996), although the mechanism by which this transport process is regulated is currently unclear. Deficiency states are, however, recognised and may result from a range of congenital defects of intermediary metabolism, interaction with certain antagonistic therapeutics or a dietary inadequacy of either carnitine, a precursor or cofactor (Rebouche & Paulson, 1986). In infants, immature hepatic production of γ-butyrobetaine hydroxylase increases the importance of exogenous carnitine supply, even when plasma lysine and methionine levels are non limiting (Borum, 1991). Since carnitine levels are indicative of several inherited or aquired pathologies, the measurement of free and acylcarnitine has become a standard method for the investigation of children with certain metabolic disorders of intermediary

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metabolism and exogenous treatment with L-carnitine selectively advocated (Walter, 1996).

Human milk is reported to contain 0.5–1.5 mg/100 g carnitine (35–90 nmol/g), which while sufficient to maintain normal plasma levels, is significantly lower than bovine milk 2.5–4.3 mg/100 g (160–270 nmol/g) (Bindels & Harzer, 1984; Hamamoto, Shimoda, Matsuura, & Matsuura, 1988; Sandor, Pecsuvac, Kerner, & Alkonyi, 1982). It is therefore evident that milk-based formula should generally meet the needs of infant nutriture unless the carnitine concentration is altered during manufacture. Thus, certain formulas, either assembled from depleted raw materials or soy-based, despite an adequate lysine and methionine content, are potentially carnitine deficient and may require supplementation (Feller & Rudman, 1988).

Despite its relevance to human health, there is currently limited information regarding the carnitine content and distribution in milk, its derivative products and infant formulas (Bosi & Refrigeri, 1983; Erfle, Fisher, & Sauer, 1970; Hamamoto et al., 1988; Indyk & Woollard, 1995; Ohtani, Higashi, & Matsuda, 1985; Roos, de Vrese, Schulte-Coerne, & Barth, 1992; Sandor et al., 1982; Woollard, Indyk, & Woollard, 1997). It is, however, accepted that a reliable estimate of total carnitine in milk is achieved from the sum of the acid-soluble free and short-chain acylcarnitines, with the acid-insoluble long-chain esters contributing less than 3–4% (Hamamoto et al., 1988; La Count, Drackley, & Weigel, 1995; Roos, et al.). In a recent series of bovine studies involving carnitine supplementation (via the rumen, abomasum or diet) increased milk carnitine levels were reported, while milk yields and composition were unaffected (La Count et al., 1995; La Count, Emmert, & Drackley, 1996; La Count, Ruppert, & Drackley, 1996).

Radioenzymatic techniques have traditionally supported clinical studies of carnitine metabolism and have been successful in determining free carnitine and acylcarnitines classes (Borum, 1990; Cederblad & Lindstedt, 1972; De Sousa, English, Stacey, & Chalmers, 1990; McGarry & Foster, 1976; Pace, Wannemacher, & Neufeld, 1978; Rossle, Kohse, Franz, & Furst, 1985), although a microbiological assay based on the growth of *Torulopsis bovina* has also been recently reported (Baker et al., 1992). More recently, selective HPLC (Arakawa, Ha, & Otsuka, 1989; Kamimori, Hamashima, & Konishi, 1994; Longo, Bruno, Curti, Mancinelli, & Miotto, 1996; Matsumoto, Ichitani, Ogasawara, Yuki, & Imai, 1994; Minkler & Hoppel, 1993; Schmidt-Sommerfeld, Zhang, Bobrowski, & Penn, 1995; Takeyama, Takagi, Adachi, & Tanaka, 1986; Van Kempen & Odle, 1992) and capillary electrophoresis (Kiessig & Vogt, 1997) techniques have facilitated the identification of individual esters in metabolic pathologies. Available techniques have been comprehensively reviewed previously (Lowes & Rose, 1989; Marzo & Curti, 1997; Marzo, Cardace, Monti, Muck, & Martelli, 1990).

Validation of an enzymatic procedure coupled to the colourimetric Ellman reaction has previously been reported for application to milk in both manual and automated formats (Indyk & Woollard, 1995) and has recently been adapted for flow injection analysis (Ferreira, Macedo, & Ferreira, 1997). The enzymatic technique, when utilised subsequent to a selective ester hydrolysis scheme, has proven reliability for the estimation of both free and total (acid-soluble) carnitine in milk and infant formula (Woollard et al., 1997). While this approach does not discriminate individual acylcarnitines, the dominance in biological fluids of both free and short chain acylcarnitines (primarily acetylcarnitine), renders the method appropriate for a survey of carnitine distribution in milk and its derivatives.

This study reports data obtained for fluid milks, milk powders, milk protein fractions and a range of bovine, goat and soya-based infant formulas. In addition, the extensive pasture grazing and synchronised calving practises in New Zealand facilitates an authentic study of temporal variability of endogenous carnitine. We therefore communicate the lactational and seasonal carnitine trends in pooled bovine milk and of an individual lactating animal. The technique was also applied to a comparison of several mammalian species milks.

2. Experimental

Apparatus and reagents have been described previously for the enzymatic estimation of free and total acid-soluble acylcarnitines utilising an automated centrifugal analyser (Woollard et al., 1997). In brief, a preliminary removal of protein and fat by coprecipitation with perchloric acid is followed by mild saponification to release acid-soluble acylcarnitines, which may be estimated subsequent to subtraction of free carnitine determined in a concurrent non-hydrolysed extract. The extracts (20 μL), are subjected to an automated, coupled carnitine acetyl transferase–Ellman reaction forming the nitrophenolate anion chromophore (405 nm), with a Cobas Fara II centrifugal instrument.

Samples were analysed for fat, protein and total solids by reference procedures (Rose Gottlieb, Kjeldahl and oven drying respectively) as described by AOAC and IDF standards.

2.1. Sample collection

Market fluid and powdered skim and whole milks were sourced from several Australasian retail and production sites and were representative of predominantly Friesian–Jersey cross supply herds. Two New Zealand production sites were selected and skim and wholemilk powders sampled at monthly intervals across an entire season.

Raw milk was collected from a single 4-year-old Jersey–Friesian cross (3rd calving) between days 1 and 36 post partum. Caprine, ovine and single donor equine milks were obtained from pasture-grazed animals. Pooled domestic cat (*n*: 5) and dog milks were collected from animals fed commercial pet food and pooled human (*n*: 5) milk was collected from mothers at between 1 and 5 months post partum. Liquid milks were held frozen until required for analysis. Following thawing, liquid milks were tempered at 37°C for 15 min prior to sampling.

Various protein and infant formula raw ingredients were sourced from a range of suppliers. A selection of carnitine supplemented and non-supplemented infant formulas of widely diverse composition were sourced both locally and internationally (New Zealand, Australia, UK, Japan, USA, Denmark, Germany, The Netherlands, China, Thailand, Ireland, France, Indonesia, Venezuela and Switzerland).

3. Results

Acid-soluble free and short-chain acylcarnitine content of retail milks of varying fat composition are compiled in Table 1, indicating an inverse correlation with milkfat content.

A survey of skim (< 1% fat) and whole milk (25–30% fat) powders produced in Australia and New Zealand was conducted to identify any regional influence on carnitine expression in bovine milk. Temporal influences were also investigated by determining free and total carnitine levels for both skim and whole milk powders produced at monthly intervals from a single region across a southern hemisphere season (August–May). These data are summarised in Table 2.

Neither geographical nor seasonal variation have a measurable influence on either total carnitine levels or free:acylcarnitine ratio.

Changes in the concentration of free and total carnitine during early bovine lactation were investigated in the milk of an individual animal. The data are presented in Table 3 and illustrated graphically in Fig. 1.

Species milks were compared and the data presented in Table 4.

Human milk is notable for its low carnitine content relative to other species, while the significantly higher content in ewe's milk may partially reflect a high total solids contribution.

The stability of endogenous carnitine in both fluid and powdered infant formulas held under storage was

Table 1
Carnitine in liquid retail milks (mg/100 g)[a]

Milk	Fat (%)	Protein (%)	Total carnitine[b]	Free carnitine	Free:total
Full cream	5.4	3.3	3.65 (226.3)[c]	2.01 (124.5)	0.55
Standard	3.3	3.3	3.78 (234.4)	2.04 (126.6)	0.54
Reduced fat	1.6	4.0	4.18 (259.2)	2.38 (147.7)	0.57
Trim	0.5	4.1	4.33 (268.5)	2.29 (142.3)	0.53
Super trim	0.1	4.4	4.64 (287.7)	2.51 (155.4)	0.54
UHT reduced fat	2.1	4.2	4.03 (249.9)	2.29 (142.4)	0.57

[a] Mean of triplicates.
[b] Free + short-chain acylcarnitines.
[c] nmol/g in parentheses (1 nmol/g 0.1612 mg/litre liquid milk).

Table 2
(a) Regional survey of carnitine in Australasian milk powders (mg/100 g)[a]; (b) seasonal survey of carnitine in Australasian milk powders[b]

Region	Product	Total carnitine[c]	Free carnitine	Free:total
a				
A[d]	SMP[e]	29.5	16.3	0.55
B	SMP	30.4	15.8	0.52
C	SMP	28.5	16.0	0.56
D	SMP	28.8	15.5	0.54
E	SMP	27.6	15.4	0.56
A	WMP[e]	23.8	12.0	0.53
B	WMP	24.7	13.5	0.52
C	WMP	24.6	11.9	0.53
D	WMP	24.0	12.7	0.54
E	WMP	22.9	12.0	0.53
b.				
B	SMP	29.4 (25.0–31.8)	16.2 (13.2–18.1)	0.55
B	WMP	24.7 (22.7–26.4)	13.3 (12.0–14.4)	0.54

[a] Mean of three random samples from each region.
[b] August 1996–May 1997; samples taken at monthly intervals; mean (seasonal range).
[c] Free + short-chain acylcarnitines.
[d] New Zealand regions (A, Northland; B, Waikato; C, Taranaki; D, Southland); E, Victoria, Australia.
[e] SMP, skim milk powder; WMP, whole milk powder.

Table 3
Carnitine content of colostrum, transitional and mature milk from a single lactating cow (mg/100 g)[a]

Day	Fat (%)	Protein (%)	Total solids (%)	Total carnitine[b]	Free carnitine	Free:total
1	6.4	6.4	20.1	8.61 (534.4)[c]	1.22 (75.6)	0.14
2	6.1	5.1	18.5	9.28 (575.4)	2.01 (124.6)	0.22
3	5.7	4.3	17.9	7.17 (444.5)	3.12 (193.4)	0.44
4	5.0	4.1	16.8	5.53 (342.9)	2.88 (178.6)	0.52
5	4.6	4.3	15.2	5.28 (327.4)	2.64 (163.7)	0.50
6	4.9	4.2	14.3	4.66 (288.9)	2.37 (147.0)	0.52
7	5.2	4.2	14.9	3.95 (244.9)	1.86 (115.3)	0.47
8	4.9	3.9	14.5	3.82 (236.8)	1.98 (122.8)	0.52
15	4.8	3.7	13.8	2.92 (181.0)	1.44 (89.3)	0.49
22	4.8	3.4	14.1	2.89 (179.2)	1.32 (81.8)	0.46
29	4.5	3.5	13.9	2.91 (180.4)	1.34 (83.1)	0.46
36	4.6	3.4	13.3	2.99 (185.4)	1.40 (86.8)	0.47

[a] Mean of duplicates.
[b] Free + short-chain acylcamitines.
[c] nmol/g in parentheses.

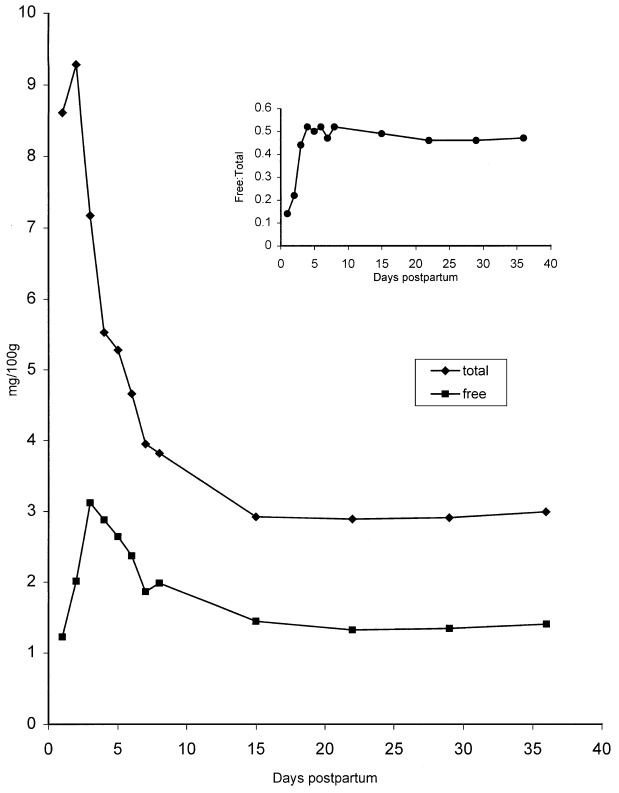

Fig. 1. Carnitine in milk of individual lactating cow.

Table 5
Storage stability of carnitine (total) in infant formula

Sample	Trial (Ambient)	Time (months)			
		0	12	24	36
IF powder[a]	1	10.3	10.4	11.0	10.1
	2	11.2	10.8	10.9	10.6
	3	12.0	11.5	11.5	11.8
	4	10.1	9.9	10.9	9.8
	Mean	10.9	10.7	11.1	10.6
IF powder[a]	Trial (37°C)	0	6	12	18
	1	10.3	11.1	11.7	10.8
	2	11.2	11.2	10.4	10.9
	3	12.0	12.1	11.0	11.9
	4	10.1	9.5	8.8	9.7
	Mean	10.9	11.0	10.5	10.8
UHT[b]	Trial (Ambient)	0	6	12	18
	1	19.2	18.3	18.3	20.1
	2	21.0	19.7	17.9	18.9
	3	20.6	20.3	21.0	19.3
	Mean	20.3	19.4	19.1	19.4
UHT[b]	Trial (37°C)	0	6	12	18
	1	19.2	20.0	19.3	19.1
	2	21.0	22.1	20.3	21.6
	3	20.6	19.7	19.8	20.3
	Mean	20.3	20.6	19.8	20.3

[a] Non-carnitine supplemented infant formula powder (mg/100 g).
[b] Non-carnitine supplemented ready-to-feed infant formula (mg/litre).

Table 4
Comparison of carnitine content of species milks (mg/100 g)

Species	Total carnitine[a]	Free carnitine	Free:total
Cow	2.92 (181.0)[b]	1.65 (102.3)	0.57
Goat	3.15 (195.3)	1.70 (105.4)	0.54
Sheep	10.04 (623.1)	4.86 (301.3)	0.48
Human	0.90 (55.6)	0.51 (31.7)	0.57
Horse	3.64 (225.9)	1.77 (109.5)	0.48
Dog	4.03 (249.7)	2.37 (147.1)	0.59
Cat	5.52 (342.6)	3.37 (209.2)	0.61

[a] Free + short-chained acylcarnitines.
[b] nmol/g in parentheses.

investigated and the results presented in Table 5. Carnitine was found to be retained with no measurable loss over 36 months under ambient conditions and 18 months at 37°C. In addition, two intralaboratory control infant formulas were used over the duration of these trials and both demonstrated stability of the measurand under ambient storage between 1993 and 1997 (mean: 12.8 mg/100 g, range: 12.2–13.7, *n*: 16; mean: 26.2 mg/100 g, range: 24.9–27.3, *n*: 26).

There are a wide range of milk protein products commercially available for usage in many food processing applications. Many of these are based on whey protein concentrates and/or isolates, although total milk protein products are also available. A survey of such commodities for total endogenous carnitine is presented in Table 6.

The above also includes a low protein mineral concentrate powder (described as a milk calcium salt), which was low in carnitine. A range of casein and caseinates were also tested and no measurable carnitine was detected in these products. Spray dried whey produced without ultrafiltration was found to contain the highest levels of carnitine.

A wide range of infant formulas ($n = 179$) were sourced internationally and surveyed for total carnitine and the data presented in Table 7.

Apart from the large number of individual formulas sampled, the above survey included values for the NIST SRM 1846 which currently has no declaration for carnitine.

4. Discussion

The sum of free and short-chain acylcarnitines present in commercial milks is consistent with literature data for total carnitine, inclusive of acid-insoluble long-chain acylcarnitines (reported range 2.1–5.8 mg/100 g, equivalent to 130–360 nmol/g, Bosi & Refrigeri, 1983; Erfle et al.,

Table 6
Survey of total carnitine in protein products (mg/100 g)

Product	Protein (%)	Source	Carnitine	
			Mean (*n*)	Range
Whey protein concentrate	55–80	Cheese whey	16.8 (9)	7.4–36.0
Whey protein concentrate	78–82	Rennet casein whey	18.1 (6)	12.9–24.9
Whey protein concentrate	78–82	Lactic acid casein whey	30.2 (6)	24.1–37.1
Whey protein concentrate	78–82	Sulphuric acid casein whey	27.2 (4)	24.1–29.9
Whey protein isolate	89–92	Cheese whey	9.9 (5)	7.7–11.2
Whey protein isolate	89–92	Skimmed milk	10.5 (5)	9.3–11.4
Whey powder	14–15	Rennet casein whey	41.3 (9)	38.1–44.1
Whey powder	14–15	Cheese whey	63.9 (2)	63.6–64.1
Mineral concentrate powder	6–9	Lactic acid casein whey	3.2 (7)	1.1–5.3
Milk protein concentrate	55–80	Skimmed milk	23.5 (6)	20.1–26.7

Table 7
Survey of total carnitine in infant formulas (mg/100 g)

Type	Carnitine	
	Mean (*n*)	Range
Milk-based[a]	17.9 (85)	6.9–30.1
Soya-based[b]	14.7 (24)	7.8–20.0
Lactose-hydrolysed[c]	13.2 (9)	10.1–16.9
Protein-hydrolysed[d]	15.8 (3)	14.7–17.0
Follow-on[e]	22.0 (48)	11.1–29.1
Health formula[f]	13.9 (11)	8.1–25.2
NIST SRM 1846[g]	16.3 (10)	14.8–18.7

[a] Whey/total milk protein (12–14%), bovine/goat, partially/totally oil-filled.
[b] Soya protein (12–14%), carnitine supplemented.
[c] Milk protein (12–15%), lactose-free, dextrins and carnitine supplemented.
[d] Carnitine supplemented.
[e] Follow-on formulas, 6–12 months/1 + years, protein (11–24%).
[f] Children's flavoured health formulation, milk protein (13–18%), carbohydrate > 50%.
[g] Dry-blended SRM, no declaration for carnitine.

1970; Hamamoto et al., 1988; La Count et al., 1995; La Count, Emmert, & Drackley, 1996; Roos et al., 1992; Sandor et al., 1982; Woollard et al., 1997) and is confirmed to be a reliable estimate of total carnitine content. The inverse relationship with fat content demonstrates the association of the acid-soluble carnitines with the milk serum phase and while long-chain esters were not quantitated in the method utilised presently, they have been shown previously to be concentrated appreciably in milkfat (Hamamoto et al., 1988). The data also confirms that non-esterified carnitine represents the major individual component of the carnitine pool (50–60%) in bovine milk.

Since colostrum and early transitional milk is withdrawn from the commercial collection pool, the apparent independence of carnitine expression from season reflects consistent levels at which carnitine is transported from bovine liver to the mammary gland. This time course may well signify the particular physiological requirements of the calf for exogenous carnitine, despite its removal after several days, as is common in commercial dairy husbandary. A similar stability in late lactation milk carnitine concentration has previously been reported in a northern hemisphere study (Roos et al., 1992).

Declining concentrations of total carnitine with time postpartum were observed for a single lactating animal, with early colostrum containing greater than 9 mg/100 g (550 nmol/g), decreasing to relatively stable levels in transitional and mature milk of ca. 3 mg/100 g (200 nmol/g) after 5–7 days. The absolute levels and temporal trend are similar to those reported in previous studies in bovine milks, with acylcarnitine levels in mature milk ca. 35% of that in colostrum (Erfle et al., 1970; Roos et al., 1992). The proportion of non-acylated to acylated carnitine was also observed to change during this transition, reaching an equilibrium free:total ratio of ca. 0.49 in mature milk. The decline in total carnitine over the lactation period can be attributed principally to the acylcarnitines, since an increase in free carnitine secretion evident in early colostrum was followed by a return to initial levels. Similar observations have been noted in other lactational studies of bovine milk (Erfle, Sauer, & Fisher, 1974; Roos et al., 1992), human milk (Bindels & Harzer, 1984; Ohtani et al., 1985; Sandor et al., 1982) and rat milk (Robles-Valdez, McGarry, & Foster, 1976), although the rate of decline in total carnitine and the free:acylcarnitine ratios were species dependent. Apart from the influence of species differences between bovine and human, long-chain acylcarnitines are reported to be relatively invariant over lactation, with time of milk collection and number of lactations also significant determinants of carnitine secretion in milk (Roos et al., 1992).

Pooled, mature human milk has been confirmed as containing significantly lower carnitine relative to bovine milk, with levels comparable to previous studies (range: 0.5–1.5 mg/100 g, equivalent to 35–90 nmol/g, Bindels & Harzer, 1984; Ohtani et al., 1985; Sandor et al., 1982). Caprine milk is equivalent in carnitine content to bovine milk, with values concordant to that reported previously (Sandor et al., 1982). We have reported in this study data for ovine, equine, canine and feline milk, for which there appears to be an absence of earlier literature. A noteworthy observation was that the proportion of total carnitine represented by the free form is remarkably independent of species (range: 0.48–0.61). The significantly higher carnitine content of ovine milk is only partially rationalised based on a high total solids contribution, since both feline and canine milks have greater total solids content (Rosenthal, 1991). That the high levels of carnitine in milk of the sheep and cat reflect the specific demands of the animal neonate for utilisation of fatty acids may be assumed, although specific species requirements for carnitine are currently unknown.

The present study has confirmed the stability of endogenous milk carnitine under storage at ambient and 37°C. Similar observations were reported previously for milk and infant formulas even at significantly higher temperature (Hamamoto et al., 1988).

The distribution of carnitine in protein products suggests an association with the whey protein fraction. Thus, casein products are essentially devoid of carnitine, while whey powders prepared directly from liquid whey contain high levels. In contrast, whey protein concentrates (and isolates) are generally prepared by incorporating additional ultrafiltration and demineralisation processes, which may reduce carnitine in the final product.

Infant formulas were found to contain a range of total carnitine consistent with diversity of composition, although different formula types were reasonably consistent in overall range. Formulas made from low carnitine raw ingredients (caseinate, soya) were supplemented to meet nutritional requirements and where indicated, carnitine levels were found to comply with label declaration. The levels estimated in this study are equivalent to those reported in other studies (Bindels & Harzer, 1984; Ferreira et al., 1997; Hamamoto et al., 1988; Ohtani et al., 1985; Sandor et al., 1982) and confirm that the exogenous carnitine upon which the non breast fed infant is dependent, is present in formulas in amounts adequate to meet requirements.

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References

Arakawa, N., Ha, T. Y., & Otsuka, M. (1989). An improved high-performance liquid chromatographic assay for the determination of free and esterified carnitine in animal tissues. *Journal of Nutritional Science and Vitaminology, 35*, 475–479.

Baker, H., DeAngelis, B., Baker, E. R., Reddi, A. S., Khalil, M., & Frank, O. (1992). Routine microbiological assay for carnitine activity in biological fluids and tissues. *Food Chemistry, 43*, 141–146.

Bieber, L. L. (1988). Carnitine. *Annual Reviews of Biochemistry, 57*, 261–283.

Bindels, Von J. G., & Harzer, G. (1984). Carnitine in Muttermilch und Sauglingsmilchfertignahrungen. *Sozialpadiatrie., 6* (8), 431–433.

Borum, P. R. (1983). Carnitine. *Annual Reviews of Nutrition, 3*, 233–259.

Borum, P. R. (1990). Carnitine: determination of total carnitine using a radioenzymatic assay. *Journal of Nutritional Biochemistry, 1*, 111–114.

Borum, P. R. (1991). Carnitine. In L. Machlin (Ed.), *Handbook of vitamins* (2nd ed., pp. 557–563). New York: Marcel Dekker.

Bosi, G., & Refrigeri, M. S. (1983). Determinazione Gascromatografica della Carnitina nel Latte. *Rassegna Chimica, 35*, 421–426.

Bremer, J. (1983). Carnitine metabolism and function. *Physiol Rev., 63*, 1420–1468.

Cederblad, G., & Lindstedt, S. (1972). A Method for the determination of carnitine in the picomole range. *Clinica Chimica Acta, 37*, 235–243.

De Sousa, C., English, N. R., Stacey, T. E., & Chalmers, R. A. (1990). Measurement of L-carnitine and acylcarnitines in body fluids and tissues in children and in adults. *Clinica Chimica Acta, 187*, 317–328.

Erfle, J. D., Fisher, L. J., & Sauer, F. (1970). Carnitine and acetylcarnitine in the milk of normal and ketotic cows. *Journal of Dairy Science, 53*, 486–489.

Erfle, J. D., Sauer, F., & Fisher, L. J. (1974). Interrelationships between milk carnitine and blood and milk components and tissue carnitine in normal and ketotic cows. *Journal of Dairy Science, 57*, 671–676.

Feller, A.G., & Rudman, D. (1988). Role of carnitine in human nutrition. *Journal of Nutrition, 118*, 541–547.

Ferreira, I. M. P. L. V. O., Macedo, M. N., & Ferreira, M. A. (1997). Enzymatic flow injection determination of free L-carnitine in infant formulae. *Analyst, 122*, 1539–1541.

Hamamoto, M., Shimoda, K., Matsuura, N., & Matsuura, H. (1988). Determination of free-, short-chain acyl and long-chain acyl carnitines in milk and milk products. *Journal of the Japanese Society of Nutrition and Food Science, 41*, 397–404.

Indyk, H. E., & Woollard, D. C. (1995). Enzymatic determination of free carnitine in milk and infant formula. *J. AOAC International, 78*, 69–74.

Kamimori, H., Hamashima, Y., & Konishi, M. (1994). Determination of carnitine and saturated-acyl group carnitines in human urine by high-performance liquid chromatography with fluorescence detection. *Analytical Biochemistry, 218*, 417–424.

Kerner, J., & Hoppel, C. (1998). Genetic disorders of carnitine metabolism and their nutritional management. *Annual Reviews of Nutrition, 18*, 179–206.

Kiessig, S., & Vogt, C. (1997). Separation of carnitine and acylcarnitines by capillary electrophoresis. *Journal of Chromatography A., 781*, 475–479.

La Count, D. W., Drackley, J. K., & Weigel, D. J. (1995). Responses of dairy cows during early lactation to ruminal or abomasal administration of L-carnitine. *Journal of Dairy Science, 78*, 1824–1836.

La Count, D. W., Ruppert, L. D., & Drackley, J. K. (1996). Ruminal degradation and dose response of dairy cows to dietary L-carnitine. *Journal of Dairy Science, 79*, 260–269.

La Count, D. W., Emmert, L. S., & Drackley, J. K. (1996). Dose response of dairy cows to abomasal administration of four amounts of L-carnitine. *Journal of Dairy Science, 79*, 591–602.

Longo, A., Bruno, G., Curti, S., Mancinelli, A., & Miotto, G. (1996). Determination of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine in human plasma by HPLC after pre-column derivatization with 1-aminoanthracene. *Journal of Chromatography B., 686*, 129–139.

Lowes, S., & Rose, S. (1989). Analytical procedures for determining acylcarnitines in biological fluids. *Trends in Analytical Chemistry, 8* (5), 184–188.

Marzo, A., Cardace, G., Monti, N., Muck, S., & Martelli, E. A. (1990). Chromatographic and non-chromatographic assay of L-carnitine family components. *Journal of Chromatography, Biomedical Applications, 527*, 247–258.

Marzo, A., & Curti, S. (1997). L-carnitine moiety assay: an up to date reappraisal covering the commonest methods for various applications. *J. Chromatogr. B, 702*, 1–20.

Matsumoto, K., Ichitani, Y., Ogasawara, N., Yuki, H., & Imai, K. (1994). Precolumn fluorescence derivatisation of carnitine and acylcarnitine with 4-(2-aminoethylamino)-7-nitro-2,1,3-benzoxadiazole prior to high-performance liquid chromatography. *Journal of Chromatography A, 678*, 241–247.

McGarry, J. D., & Foster, D. W. (1976). An improved and simplified radioisotopic assay for the determination of free and esterified carnitine. *Journal of Lipid Research, 17*, 277–281.

Minkler, P. E., & Hoppel, C. L. (1993). Quantification of carnitine and specific acylcarnitines by high performance liquid chromatography: application to normal human urine and urine from patients with methylmalonic aciduria, isovaleric acidemia or medium-chain acyl-CoA dehydrogenase deficiency. *Journal of Chromatography, Biomedical Applications, 613*, 203–221.

Ohtani, Y., Higashi, A., & Matsuda, I. (1985). Carnitine concentration of formulas. *Journal of Pediatric Gastroenterology and Nutrition, 4*, 845–846.

Pace, J. A., Wannemacher, R. W., & Neufeld, H. A. (1978). Improved radiochemical assay for carnitine and its derivatives in plasma and tissue extracts. *Clin. Chem., 24*, 32–35.

Rebouche, C. J. (1988). Carnitine metabolism and human nutrition. *J. Appl. Nutr., 40*, 99–111.

Rebouche, C. J., & Paulson, D. J. (1986). Carnitine metabolism and function in humans. *Annual Reviews of Nutrition, 6*, 41–66.

Rebouche, C. J., & Seim, H. (1998). Carnitine metabolism and its regulation in microorganisms and mammals. *Annual Reviews of Nutrition, 18*, 39–61.

Robles-Valdez, C., McGarry, J. D., & Foster, D. W. (1976). Maternal fetal carnitine relationships and neonatal ketosis in the rat. *Journal of Biological Chemistry, 251*, 6007–6012.

Roos, von N., de Vrese, M., Schulte-Coerne, H., & Barth, C. A. (1992). L-Carnitin in Milch von Monozygoten Zwillingskuhen. *Kieler Milchwirtschaftliche Forschungsberichte, 44* (4), 363–370.

Rosenthal, I. (1991). Production of milk. In I. Rosenthal (Ed.), *Milk and dairy products* (Table 8, p. 60). New York: Balaban Publishers, VCH.

Rossle, C., Kohse, K. P., Franz, H.-E., & Furst, P. (1985). An improved method for the determination of free and esterified carnitine. *Clinica Chimica Acta, 149*, 263–268.

Sandor, A., Pecsuvac, K., Kerner, J., & Alkonyi, I. (1982). On carnitine content of the human breast milk. *Pediatric Research, 16*, 89–91.

Schmidt-Sommerfeld, E., Zhang, L., Bobrowski, P. J., & Penn, D. (1995). Quantitation of short- and medium-chain acylcarnitines in plasma by radioisotopic exchange/high-performance liquid chromatography. *Analytical Biochemistry, 231*, 27–33.

Snoswell, A. M., & Linzell, J. L. (1975). Carnitine secretion into milk of ruminants. *J. Dairy Res., 42*, 371–380.

Takeyama, N., Takagi, D., Adachi, K., & Tanaka, T. (1986). Measurement of free and esterified carnitine in tissue extracts by high-performance liquid chromatography. *Analytical Biochemistry, 158*, 346–354.

Van Kempen, T. A. T. G., & Odle, J. (1992). Quantitation of carnitine esters by high-performance liquid chromatography. effect of medium-chain triglycerides on the plasma carnitine ester profile. *Journal of Chromatography, Biomedical Applications, 584*, 157–165.

Walter, J. H. (1996). *L-carnitine*. *Archives of Disease in Childhood*, *74*, 475–478.

Woollard, D. C., Indyk, H. E., & Woollard, G. A. (1997). Enzymatic determination of carnitine in milk and infant formula. *Food Chemistry, 59* (3), 325–332.