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The determination of L-carnitine in several food samples

Knüttel-Gustavsen Seline *, Harmeyer Johein

Department of Physiology, School of Veterinary Medicine, Hanover, Germany

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Abstract

We designed this study to re-validate some methodological parameters of the radio-enzymatic assay, including phenomena of residual, non-carnitine radioactivity present in some assay mixtures of food samples. The second part of the paper presents L-carnitine concentrations (total-, free- and acyl-carnitine) in a wide range of food samples of animal and plant origin.

Due to an appropriate procedure of sample preparation and an elimination of influencing factors in the biological samples, more homogeneous and reliable results were obtained and smaller variances between the sample duplicates were obtained. For total and free carnitine, the inter-assay variances of the method were 6.5% and 7.2%, respectively. The lower limit of detection was 0.11 μmol carnitine/l of assay mixture.

The highest carnitine contents were found in muscle from kangaroo (6370 mg/kg DM (dry matter)) and horse (4230 mg/kg DM), followed by different cattle meats (3320–1870 mg/kg DM). The carnitine content in the tissues from pigs and fowls ranged from 135 to 830 mg/kg DM. The carnitine content in most dairy products ranged, approximately, between animal tissues and plant-derived food with concentrations ranging from 8 to 530 mg/kg DM.

The method was found to be easily and rapidly usable for the determination of L-carnitine in complex biological samples.

Keywords: L-Carnitine; Food samples; Muscle samples; Tissues; Radio-enzymatic assay; Deprotinization

1. Introduction

L-Carnitine (β-hydroxy-γ-trimethylaminobutyrate) is an essential constituent of mammalian tissues and is received by endogenous synthesis and from exogenous sources. The primary function of L-carnitine is to facilitate the transport of activated long chain fatty acids from the cytosol into the mitochondria, making them available for mitochondrial β-oxidation. L-carnitine is also important in modulation of the intramitochondrial CoA/acyl-CoA ratio, ketone body utilization and peroxisomal β-oxidation (Bremer, 1962; Fritz, 1963; Harmeyer & Schlumbohm, 1997; Idell-Wegner, Grotyohann, & Neely, 1982; Siliprandi, Sartorelli, & Di Lisa, 1989).

Under normal conditions, healthy humans can synthesize sufficient amounts of L-carnitine; therefore L-carnitine is not regarded as a true nutritional supplement (Vaz & Wanders, 2002). The carnitine content in m. pectoralis from human is 516 ± 48 mg/kg w/w (3.2 ± 0.3 μmol/g w/w) (Rudmann, Sewell, & Ansley, 1977). Surprisingly, the mean plasma carnitine concentration in vegetarians is only slightly lower than in carnivores (6.77 vs. 7.42 mg/l) (Lombard, Olson, Nelson, & Rebouche, 1989). However, subnormal L-carnitine concentrations have been reported in human blood, due to idiopathic defects in the L-carnitine synthesis or L-carnitine transport system (Di Donato, Cornelio, Balestrini, Bertagnolio, & Pluchetti, 1978; Rebouche & Engel, 1980, 1981; Scholte et al., 1990; Treem, Stanley, Finegold, Hale, & Coates, 1988). Additionally it can occur in dialysis patients (Bohmer, Bergrem, & Eiklid, 1978; Duran, Loof, Ketting, & Dorland, 1990), in patients during parenteral nutrition (Bowyer et al., 1986) or in patients as a consequence of

* Corresponding author. Present address: Norwegian Medicine Agency, Sven Oftedalsvei 8, 0950 Oslo, Norway. Tel.: +47 22897547.
E-mail address: seline.gustavsen@NoMA.no (K.-G. Seline).

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seriously impaired liver or kidney functions (Chalmers, Roe, Stacey, & Hoppel, 1984; Leschke et al., 1984; Nezu et al., 1999). Low concentrations of L-carnitine may reduce the rate of fatty acid oxidation and may result in muscle weakness, primarily in heart and skeletal muscle (Allen, Hansch, & Wu, 1982; Duran et al., 1990; Nezu et al., 1999; Roschinger et al., 2000; Scholte et al., 1990). During those pathologic conditions and certain physiological circumstances, such as pregnancy (Cederblad, Faahraeus, & Lindgren, 1986; Hahn, 1981; Kaise, 1997; Mitchell & Snyder, 1991; Schoderbeck et al., 1995) and during the new born period (Hahn, 1981; Rebouche & Engel, 1980; Scholte & De Jonge, 1987; Shortland, Walter, Fleming, Speidel, & Marlow, 1998), L-carnitine can be considered as an essential nutrient. Therefore, it is important to measure the carnitine status in these humans to determine whether or not they need an exogenous supplement of L-carnitine. Such a demand for carnitine could potentially be balanced with a well-advised assortment of food where the content of L-carnitine is known. L-carnitine contents in different types of food have been published (Götz, 1989; Demarquoy et al., 2004). The content varies greatly and the highest amounts of L-carnitine have been found in meats and dairy products, whereas fruit and vegetables contain negligible quantities. In order to measure the L-carnitine status in a wide range of biological materials, availability of a sensitive, reliable, relatively simple, cheap, and robust method for the assessment of the content of L-carnitine would be desirable.

L-Carnitine analysis of biological materials is usually based on a radio-enzymatic assay reportedly Cederblad and Lindstedt (1972). Due to certain limitations (specificity, linearity and deproteinization), the original procedure has been repeatedly modified, e.g. with regard to different methodological features (Cederblad & Lindstedt, 1972; Christiansen & Bremer, 1978; Mc Garry & Foster, 1972, 1985; Parvin & Pande, 1977). Additionally, a number of other methods for determination of L-carnitine, based on various analytical principles, have been published. These include separation of D/L enantiomers of carnitine (De Witt et al., 1994; Hirota, Minato, Ishii, Nishimura, & Sato, 1994; Mardones, Ríos, Valcárcel, & Cicciarelli, 1999) or acylcarnitine esters by high-performance liquid chromatography (HPLC) (Vaz & Wanders, 2002; Zhu et al., 2000) and capillary electrophoresis (CE) (Deng et al., 2001; Heinig & Henion, 1999), modification of the eluted components and quantification by UV fluorescence or in combination with mass spectrometry and gas chromatography (GC) (Costa et al., 1997; Huang, Gage, Bieber, & Sweeley, 1991). Many of these methods do not appear to be sensitive enough to be used for samples with L-carnitine concentrations in the low μmol range, such as in blood plasma or vegetables. Additionally, expensive equipment is often necessary, precluding use in research or for routine measurements, e.g. in the field of human or animal nutrition.

The first part of this paper presents the re-evaluation of some methodological parameters of the radio-enzymatic assay, including phenomena of residual, non-carnitine radioactivity present in some assay mixtures. The second part of the paper presents L-carnitine concentrations in a wide range of food samples of animal and plant origin which were obtained with the current method.

2. Material and methods

2.1. Reagents and chemicals

L-Carnitine of analytical grade and L-carnitine acetyltransferase (CAT, EC.2.3.1.7.) from pigeon breast muscle in crystallized form and suspended in 2.9 M ammonium sulphate were purchased from Roche Diagnostic, Germany. The radiolabelled [^{3}H]-acetyl-CoA was obtained from Amersham Biosciences, Europe GmbH, Germany. HEPES (*N*-(2-hydroxyethyl)-piperazine-*N*-ethanesulphonic acid, 1 M, pH 7.4), EDTA (ethylenediaminetetraacetate, 12.5 M), and the anion-exchanger Dowex 1 × 8 (Cl^-, 400 mesh) were bought from Sigma–Aldrich, Germany. KOH (0.2 M), HCl (0.2 M) and NEM (*N*-ethylmaleimide) were from Merck, Germany. The Scintillastions-Coctail Ubisafe 1 was obtained from Zinsser Analytic GmbH, Germany. The Horse plasma which was used as an internal standard was supplied from the Horse Clinic of the Veterinary School in Hanover, Germany.

2.2. Dilution and storage of the chemicals

An L-carnitine stock solution (50 mmol/l) was prepared by dissolving 20 mg L-carnitine hydrochloride in 10 ml deionised water. An aliquot of 500 μl from this solution was diluted with 100 ml deionised water. CAT, with a specific activity of 80 U/mg was prepared by dissolving 20 mg of dried powder in 5.0 ml of deionised water. 240 μl of [^{3}H]-acetyl-CoA (1.85 MBq/ml) were mixed with 1.0 ml of an unlabelled 0.5 mM acetyl-CoA-trilithiumsalt-solution and diluted with 9.0 ml of deionised water. A NEM solution was prepared by (10 M *N*-ethylmaleimide in 0.1 M HEPES-buffer). One volume Dowex powder was treated with two lots of 1 M HCl, filtered and washed with water until the filtrate showed a neutral pH. For the radio-enzymatic assay, the semi dry Dowex was diluted 1:1 with deionised water.

The stock solutions and acetyl-CoA were stored at −20 °C; the NEM solution was prepared weekly and stored at +4 °C. The CAT, HEPES-buffer, EDTA and Dowex were stable at +4 °C for several months.

2.3. Instrumentation

A freeze-dryer from Alpha 2–4, Christ LDC-1 M (Martin Christ, Gefriertrocknungsanlagen GmbH, Germany) and a liquid scintillation counter Packard TriCarb® 460C (Packard Instruments, Germany) were used.

2.4. Sample preparation

Two aliquots were taken from all solid and liquid samples (Table 1) (see Fig. 1).

2.5. Principle of the radio-enzymatic assay

The method is based on the *in vitro* radioactive labelling of free L-carnitine (FC) in the sample by acetylation in the presence of carnitine acetyl transferase (CAT). It is essentially based on a modification of the method reported by Mc Garry and Foster (1972, 1985).

2.6. Assay procedure

2.6.1. General

All samples were analysed in duplicate so that the variation between samples in one assay could be detected. In addition, the samples were analysed with and without the addition of CAT to detect the "non-specific" signals (Section 2.7). In pilot experiments, HEPES was mixed with CAT to make the assay easier to operate. The reproducibility of L-carnitine was in this experiment approximately 100%.

Free carnitine (FC) (Section 2.6.1) was determined directly and the carnitine esters (CE) had to be hydrolysed

Table 1
Overview of analysed raw samples from animal and plant origins

Category	Products	Samples
Dairy products	Liquid (17)	(1) Buttermilk, (2) condensed milk, (4) creams, (3) curd cheese, (2) milk, (4), (1) whey
	Powder (4)	(1) Buttermilk, (1) skimmed milk, (2) yogurts
	Cheese (20)	Different products[a] from cow, goat and sheep
	Fatty (3)	(1) Butter, (1) margarine, (1) oil
Products from animal (muscle, visceral organ)	Cow (7)	(1) Beefsteak[a], (1) beef of the shoulder[a], (1) kidney, (1) liver[a], (1) ribs, (1) tartar, (1) tongue[a]
	Pig (4)	(1) Escalope, (1) liver[a],(1) minced meat, (1) spareribs
	Fowls (7)	(1) Broiler club, (1) duck filet, (1) liver, (1) pheasant breast, (1) pigeon breast[a],(1) quail breast, (1) turkey filet
	Kangaroo (1)	(1) m. glutaeus medius
	Horse (1)	(1) m. glutaeus medius[a]
Products from plants	Mushrooms (3)	(1) Chanterelle, (1) mushroom[a], (1) oyster[a]
	Vegetables (6)	(1) Avocado, (1) carrot, (1) cauliflower, (1) corn, (1) cucumber, (1) peas
	Fruits (4)	(1) Apple, (1) bananas, (1) guava, (1) orange

( ) number of analysed products/samples.
[a] Samples for the detection of so-called "non-specific" and "specific" carnitine-related signals.

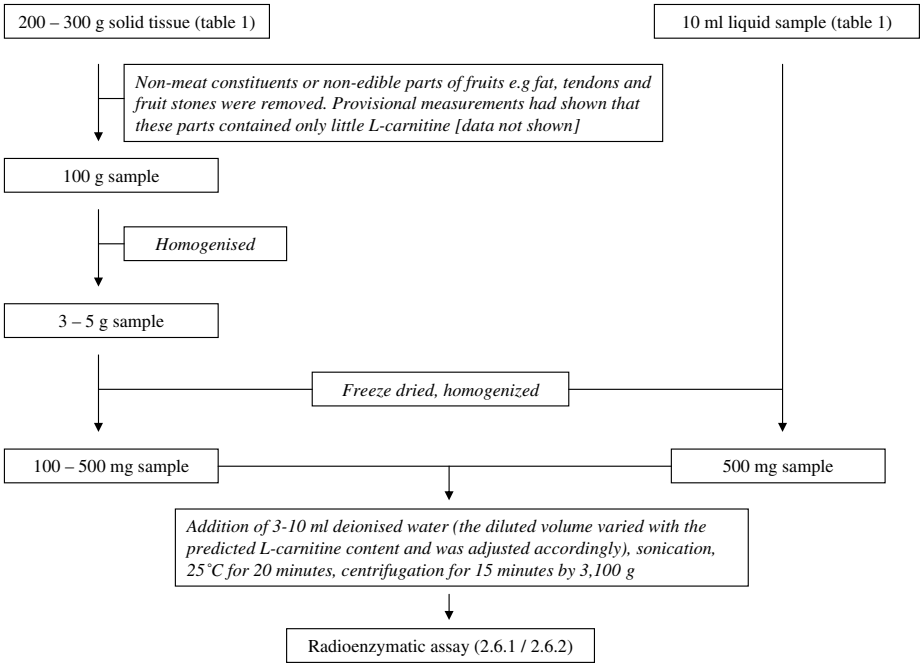

Fig. 1. Preparation of solid and liquid samples.

before they could be quantified by the assay. After hydrolysis was performed, the total carnitine (TC) (Section 2.6.2) content of the samples was obtained (CE + FC).

2.6.2. Assay of free carnitine

The assay procedure of the determination of free-carnitine and the different amounts of the used substances are shown in Fig. 2 and Table 2, respectively.

2.6.3. Assay of total carnitine

Four hundred microlitre of the supernatant extracts from the samples were pipetted into test tubes, treated with 1 ml KOH solution and incubated for 1 h in a water bath at 37 °C. Finally, the samples were neutralized with 1.0 ml HCl. All sample tubes were centrifuged at 3100*g* for 15 min. The samples were assayed according to the procedure of free L-carnitine (Section 2.6.1).

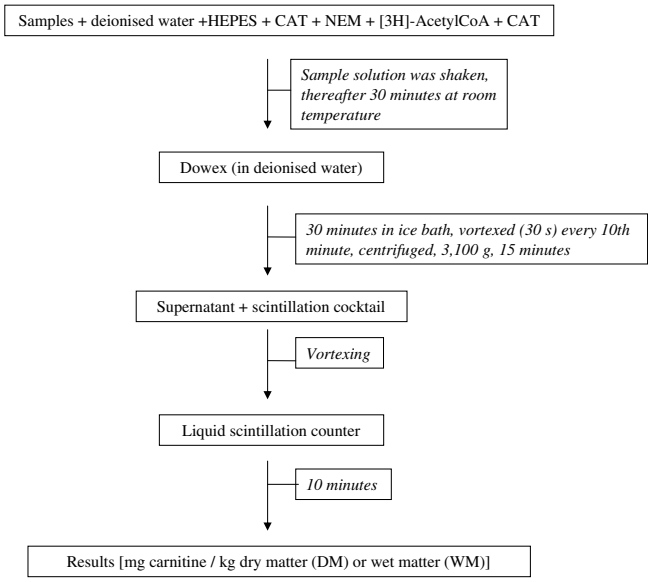

Fig. 2. An overview of the different steps in the radio-enzymatic assay.

2.7. Detection of "non-specific" carnitine-related radioactivity

In a number of food samples, the "non-specific" carnitine-related signals were determined and the effects of heating on the presence of these carnitine related signals were examined. Two subsamples from solid or liquid source materials (Table 1) were prepared according to Section 2.4. One sample (two test tubes) from each product was analysed untreated for free carnitine, according to Section 2.6.1. and one sample (two test tubes) was heated to 98 °C for 15 min and subsequently sonicated at 25 °C for 20 min. Fluid losses due to evaporation were replaced with deionised water. Thereafter, the samples were analysed for free carnitine according to Section 2.6.1. The "non-specific" signals are calculated from the percentage of the free carnitine in the samples with CAT and the free carnitine amount in the samples without CAT.

2.8. Statistical analyses

The statistical analyses were performed with Sigma Plot™ 9.0, Sigma Stat™ 1.0 (Jandel Scientific, Erkrath) and Exel 2000™ (Microsoft). All samples in one group were compared with samples in another group and the differences were assessed with ANOVA (dairy, plant, domestic ruminants, pork products: Dunn's test; fowl products: Student's *t*-test). Differences were considered significant at $p < 0.05$. Each sample was analysed in duplicate and the data were expressed either as means ± SD mg/kg of dry matter (DM) or wet weight (w/w).

3. Results

3.1. Validation of the method

A linear relationship existed over a full range up to 15 μmol L-carnitine/l with a correlation coefficient (r) of 0.9998. The variation coefficient of the radioactivity in the blank was 5.66% ($n = 50$). The carnitine content in a sample was significantly different from zero when the

Table 2
Schema of used substances in the assay (μl)

	Horse plasma	Blind	Totals	Standard	Samples	Samples
Sample	200	0	0	50–300[a]	50–550[a]	50–550[a]
Deionised water	Ad 550	Ad 550	Ad 550	Ad 550	Ad 550	Ad 550
HEPES/CAT	100	100	100	100	100	0
HEPES	0	0	0	0	0	100
EDTA	100	100	100	100	100	100
NEM	200	200	200	200	200	200
^{3}[H]-Acetyl-CoA	50	50	50	50	50	50
DOWEX®	600	600	0	600	600	600
Deionised water	0	0	600	0	0	0
Supernatant	500	500	500	500	500	500
Scintillation-cocktail	4000	4000	4000	4000	4000	4000

[a] Depending of the predicted L-carnitine concentration in the samples or standards 2.5, 5.0, 10.0 and 15 μmol/l, the total was included to detect the grade of radioactivity of the [^{3}H]-acetyl-CoA.

radioactivity in the sample minus the blank was larger than twice the mean standard deviation of the blanks. The lower limit of detection was calculated from the variance of the carnitine free blank and was found to be 0.11 μmol carnitine/l assay mixture. The precision of the inter-assay variance was calculated from horse plasma samples, which were routinely analysed during every assay. The variance in 65 measurements was, for free carnitine 7.2% and, for total carnitine, 6.5%. The intra-assay variance was detected in muscle samples from cattle and plasma samples from horse and ranged from 3.2% to 7.6% (Table 2). Accuracy was >99%, as assessed by the analytical recovery of the stock solution ($n = 50$). In complex biological samples, the accuracy decreases with the amount of disturbing factors (e.g. enzymes, proteins and fat) (Table 3).

3.2. Detection of "non-specific" carnitine-related radioactivity

The amount of "non-specific" signals ranged from 27% to 70% of the free carnitine amount with CAT. After a heating procedure of 98 °C for 15 min, no "non-specific" signals were any longer detectable (Table 4).

3.3. Food samples

3.3.1. General

The results of the L-carnitine measurements in different food samples from animal and plant origins varied significantly between the different species. Low carnitine contents were found in vegetables and fruit, while higher carnitine concentrations were found in animal products.

3.3.2. Meat products

The L-carnitine concentrations in meat products are presented in Table 5. The highest total carnitine content was found in the muscle from kangaroo with 6370 mg/kg DM, followed by muscles from horse (4230 mg/kg DM), cow (mean ± SD; 1950 ± 432 mg/kg DM) and pig (mean ± SD; 613 ± 214 mg/kg DM). The lowest carnitine content was found in fowls (mean ± SD; 380 ± 224 mg/kg DM) and visceral organs (mean ± SD; 127 ± 26 mg/kg DM). The free carnitine concentrations were of the same order as the total carnitine concentrations.

Table 3
Intra-assay variance in different samples from cattle and plasma from horse

Sample	N	$X \pm$ SD (mg/kg DM)	Coefficient of variation
Beefsteak	6	2302 ± 86	3.7
Plasma	6	12.2 ± 0.39	3.2
Beef of the shoulder	5	1906 ± 121	6.4
Plasma	5	3.5 ± 0.13	3.7
Tongue	3	1131 ± 72	7.6
Plasma	3	12.1 ± 0.46	3.8

DM: dry matter.

Table 4
Measurement of the amount of non-specific signals in different samples during the determination of free carnitine

Sample	Treatment (°C)	With CAT (mg/kg DM)	Without CAT (mg/kg DM)	Non-specific signals (%)
Cow liver	25	185	77	42
	98	110	2.0	2
Pig liver	34	156	95	61
	98	63	2.0	3
Pigeon (breast)	25	548	320	58
	98	244	5.0	2
Mushroom	25	304	120	39
	98	196	1.0	1.0
Oyster mushroom	25	530	144	27
	98	383	0	0
Gorgonzola	25	27	10	39
	98	17	1.0	3.0
Gouda	25	25	15	60
	98	9	0.65	7
Guava	25	244	76	31
	98	1.0	0	0
Banana	25	15	10	70
	98	3.0	3.0	0

DM: dry matter; WW: wet weight; TC: total carnitine; FC: free carnitine; CE: carnitine ester.

3.3.3. Dairy products

The L-carnitine concentrations in dairy products varied with the different products, consistently with fat content and animal origin. The results are expressed in dry matter so their L-carnitine contents could be compared with other food products.

The results of carnitine measurements in different dairy products are summarized in Tables 6–8.

The total carnitine content in liquid dairy products ranged from 68 to 428 mg/kg DM (mean ± SD; 249 ± 118 mg/kg DM). The free carnitine content ranged from 36 to 232 mg/kg DM (mean ± SD; 152 ± 64 mg/kg DM) which represented approximately 63 ± 14% of the total carnitine pool in the liquid dairy products.

Yoghourt, cheese curd and milk are based on the same type of milk and their amounts of total carnitine are shown in Fig. 3.

The total carnitine content in cheese products ranged from 14 to 149 mg/kg DM (mean ± SD; 54 ± 41 mg/kg DM), the free carnitine content ranged from 7 to 102 mg/kg DM (mean ± SD; 32 ± 28 mg/kg DM) which represented approximately 59 ± 21% of the total carnitine content. Fat contained almost no carnitine. The total carnitine concentration ranged from 0 to 11 mg/kg DM, the free carnitine concentration ranged from 0 to 8 mg/kg DM.

Table 5
The L-carnitine content in different products from animals

Samples, $n = 2$	% DM	TC (mg/kg DM)	FC (mg/kg DM)	TC (mg/kg WW)	FC (mg/kg WW)	CE (%)
Horse (m. glutaeus medius)	28	4230	3010	1170	832	29
Kangaroo steak	26	6370	5970	1660	1550	6
Beef of the shoulder (cow)	32	1390	1020	452	330	27
Kidney (cow)	22	310	66	67.6	14.4	79
Liver (cow)	30	156	110	46.8	33.0	29
Ribs (cow)	28	2260	1800	635	506	20
Beef steak (cow)	28	2320	1360	661	386	42
Tartar (cow)	27	1830	1230	488	329	33
Tongue	24	1890	1320	446	311	30
Escalope (pig)	24	830	778	224	213	6
Liver (pig)	34	107	63.5	36.4	21.6	41
Spareribs (pig)	36	402	329	144	118	18
Minced meat (pig)	34	608	388	209	133	36
Broiler club	23	344	336	80.5	78.7	2
Duck filet	39	732	340	288	133	54
Liver (broiler)	28	119	62	33.3	17.4	48
Pheasant (breast)	24	135	104	32.3	25.0	23
Pigeon 1 (m. pectoralis)	30	528	244	161	74.3	54
Quail breast	57	291	122	166	70	58
Turkey filet	25	514	449	130	113	13

DM: dry matter; WW: wet weight; TC: total carnitine; FC: free carnitine; CE: carnitine ester.

Table 6
The L-carnitine concentrations in different "liquid" dairy products

Samples; $n = 2$	% Fat	DM %	TC (mg/kg DM)	TC (mg/kg WW)	FC (mg/kg DM)	FC (mg/kg WW)	CE (%)
Liquids							
Milk	1.5	15.0	340	34.9	175	18.0	48
Milk	3.5	27.0	330	41.8	152	19.3	54
Buttermilk	0.6	6.7	385	34.4	224	20.0	42
Cream	30.0	83.3	86.0	31.3	35.6	13.0	59
Café cream	12.0	12.0	166	33.2	89.8	17.9	46
Condensed milk	4.0	4.0	396	97.2	234	57.8	40
Condensed milk	10.0	10.0	214	75.5	193	68.0	10
Yogurt	0.3	3.0	399	38.9	210	24.1	38
Yogurt	2.6	10.0	142	36.3	103	26.4	27
Yogurt	3.5	25.0	278	38.4	166	23.0	40
Yogurt	10.0	52.6	151	28.2	103	19.2	32
Crème fraiche	30.0	78.9	68.0	26.0	42.0	16.0	38
Sour cream	10.0	52.6	197	38.1	87.5	16.9	56
Whey	0.2	3.3	355	22.0	230	14.0	35
Curd cheese	20.0	20.0	266	28.3	199	21.2	25
Curd cheese	40.0	40.0	299	30.3	211	21.4	29
Curd cheese	<10	12.0	225	27.9	159	19.8	29
Powder							
Buttermilk	–	97	428	415	232	225	46
Yogurt	1.5	96	149	143	134	129	10
Yogurt	3.5	97	126	122	104	101	17
Skimmed milk	1.5	96	400	384	200	190	50

DM: dry matter; WW: wet weight; TC: total carnitine; FC: free carnitine; CE: carnitine ester.

3.3.4. Vegetables, fruit and mushrooms

Compared to dairy- and animal products, the L-carnitine concentrations in products of plant origin were very low, except those of mushrooms (Table 9). The L-carnitine concentrations in mushrooms were comparable with the L-carnitine contents in pigs. The total carnitine concentration in mushrooms ranged from 133 to 530 mg/kg DM (mean ± SD; 320 ± 199 mg/kg DM). The free carnitine concentration ranged from 73 to 383 mg/kg DM (mean ± SD; 218 ± 156 mg/kg DM), which represented 65 ± 8% of the total carnitine content. The total carnitine content in fruits and vegetables ranged from 2.94 to

Table 7
The L-carnitine concentrations in different cheese products

Samples; $n = 2$	% Fat	DM %	TC (mg/kg DM)	TC (mg/kg WW)	FC (mg/kg DM)	FC (mg/kg WW)	CE (%)
Babybel[a]	45	40	15.9	6.37	7.24	2.91	54
Brie[a]	60	46	58.5	26.7	43.4	19.8	26
Butter cheese[a]	45	61	14.0	8.48	9.90	5.5	35
Camembert[a]	55	33	55.0	18.2	37.1	12.3	32
Classic blue[a]	70	61	18.2	11.1	12.0	7.30	34
Cream cheese[a]	80	36	43.0	15.5	21.9	7.9	49
Edamer[a]	40	54	28.0	15.0	9.38	5.09	66
Feta cheese (cow)	40	42	86.3	34.6	68.5	28.9	21
Feta cheese (sheep)	48	44	149	65.0	89.5	39.0	40
Feta cheese (sheep/cow)	45	49	96.8	47.0	53.5	26.0	45
Cream cheese with herbs[a]	52	32	35.1	11.2	15.7	5.0	55
Gorgonzola[a]	48	51	20.3	10.4	16.8	8.59	17
Gouda, young[a]	48	55	28	14.3	9.35	6.84	35
Gouda, old[a]	48	73	26	20.4	5.16	2.82	82
Harzercheese[a]	2	54	31.0	17.0	28.0	15.0	12
Kochkäse[a]	20	26	116	30.6	41.1	10.8	65
Mozzarella (buffalo)	45	33	55.0	18.0	21.0	7.0	61
Norwegian goat cheese	31	93	137	127	102	95	25
Roquefort (sheep)	52	58	18.8	10.8	18.2	10.2	3
Snøfrisk (goat cheese)	83	30	56.2	16.8	32.2	9.61	43

DM: dry matter; TC: total carnitine; FC: free carnitine; CE: carnitine ester.
[a] Products from cow.

44.5 mg/kg DM. The free carnitine content ranged from 1.00 to 25.5 mg/kg DM, which represented 13% to 79% of the total carnitine content.

4. Discussion

4.1. General

Since its discovery in 1905 by Gulewitsch and Krimberg (1905), a number of different methods for the quantification of L-carnitine have been published (Cederblad & Lindstedt, 1972; Costa et al., 1997; Christiansen & Bremer, 1978; Deng et al., 2001; De Witt et al., 1994; Heinig & Henion, 1999; Hirota et al., 1994; Huang et al., 1991; Mardones et al., 1999; Mc Garry & Foster, 1972, 1985; Parvin & Pande, 1977; Zhu et al., 2000; Vaz et al., 2002). Most of these methods were designed for the analysis of carnitine in plasma and urine. Additionally, different methods of sample preparation and for the detection and quantification of carnitine in food samples have been described in the literature (Erfle, Sauer, & Fischer, 1974; Götz, 1989; LaCount, Drackley, & Weigel, 1995; Leibetseder, 1995; Panter & Mudd, 1969; Rudmann et al., 1977; Snoswell & Linzell, 1975). However, a specific, reliable and sensitive method for the quantitative determination of L-carnitine and carnitine esters in foods of plant and animal origins was essential to provide the best results in our study. Therefore, due to its high sensitivity (20–2000 pmol), the present investigation was based on the method of Mc Garry and Foster (1972, 1985). An appropriate procedure of sample preparation and a modification of the assay (non-specific carnitine signals) accomplished the elimination of influencing factors in biological samples. Due to these changes, more homogeneous and reliable results were obtained, which showed smaller variances between the sample duplicates. The method was found to be easily usable and rapid for the determination of L-carnitine in our work.

Table 8
The L-carnitine concentrations in different fats

Samples; $n = 2$	% DM	TC (mg/kg DM)	FC (mg/kg DM)	CE (%)
Butter	97	11.0	8.0	11
Margarine	92	0.58	0.04	93
Sunflower oil	100	0	0	0

DM: dry matter; TC: total carnitine; FC: free carnitine; CE: carnitine ester.

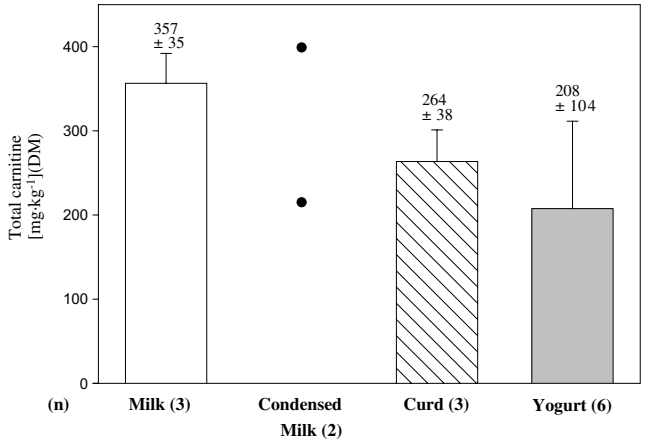

Fig. 3. The content of total carnitine in different products from cow milk.

Table 9
The L-carnitine content in mushrooms, vegetables and fruits

Samples, $n = 2$	% DM	TC (mg/kg DM)	FC (mg/kg DM)	TC (mg/kg WW)	FC (mg/kg WW)	CE (%)
Chanterelle	10	133	73.5	13.2	7.3	45
Mushroom	9	298	196	26.2	17.2	34
Oyster mushroom	9	530	383	49.8	36.0	28
Apple	15	2.94	2.31	0.45	0.35	21
Banana	25	3.98	2.99	0.99	0.75	25
Guava	20	8.2	0.9	1.64	0.18	89
Orange	4	3.59	2.25	0.14	0.09	37
Avocado	25	17.2	11.6	4.3	2.89	81
Carrot	11	37.3	25.1	3.99	2.69	33
Cauliflower	11	32.6	21.3	3.58	2.34	35
Corn	24	6.82	3.26	1.63	0.78	52
Cucumber	4	44.5	25.5	1.85	1.06	42
Peas	24	5.93	2.35	1.39	0.55	60

DM: dry matter; WW: wet weight; TC: total carnitine; FC: free carnitine; CE: carnitine ester.

4.2. "Non-specific carnitine signals"

According to the concept of the radio-enzymatic assay from Mc Garry and Foster (1972, 1985), radioactive labelled acetyl carnitine should only be formed in the assay mixture in the presence of carnitine acetyltransferase (CAT). In some biological samples, acetyl carnitine is formed, despite no exogenous CAT being added. This is because biological material contains enzymes, proteins and other substances which can interfere with the selected assay and form false carnitine radioactivities ("non-specific" carnitine signals). Additionally, biological samples can contain endogenous CAT which can start the reaction before the exogenous CAT is added and therefore develop "specific" carnitine signals. These signals do not falsify the measured carnitine results, but could be detected in the samples without the addition of exogenous CAT. In a number of pilot experiments, some radioactivity of unknown origin was detected in assay mixtures in the absence of CAT. This radioactivity could obviously not be removed by the anion-exchange resin, thus leading to a significantly higher radioactivity in the assay solution than that found in the blank solutions. It was impossible to distinguish whether the obtained radioactivity was due to "specific" or "non-specific" carnitine-related signals. So if correct results should be obtained the interfering substances in the assay had to be removed.

Some authors have reported the usage of perchloric acid to remove proteins from the sample mixtures (Alhomida, Duhaiman, Al-Jafari, & Junaid, 1995; Cederblad, Carlin, Constantin-Teodosiu, Harper, & Hultman, 1990; Mc Garry & Foster, 1985; Pearson, Tubbs, & Chase, 1974; Tegelaers, Pickkers, & Seelen, 1989; Wan & Hubbard, 1998). In our investigations we elaborated the amounts of "non-specific" and "specific" carnitine-related signals and the effect of heating on these signals. The so-called "specific" carnitine-related signals were not detected during our investigations but, in some samples, the amount of "non specific" carnitine signals ranged from 27% to 70%. After the heating procedure (Section 2.7), no "non-specific" signals were any longer detectable (Table 4). It seems that the "non-specific" signals were thermolabile and disappeared after heating of the samples. Consequently, deproteinization by heating is not only a quicker way of preparing samples than is deproteinization with perchloric acid or other agents; but it also avoids further dilution of the samples, which improves the precision and the sensitivity. Further on, a neutralization step at the end of the procedure is not necessary.

To analyse biological samples without deproteinization is only feasible if it has been established that the samples do not contain significant amounts of interfering factors or endogenous CAT. This can be done by the measurement of all samples with and without the addition of exogenous CAT. If "non-specific" carnitine signals are found, deproteinization through heating accomplishes a clearance of the samples from those signals.

4.3. Food samples

The bioavailability of dietary carnitine from food is 54% to 87% (Evans & Fornasini, 2003; Rebouche & Chenard, 1991). The intestinal absorption depends on both the carnitine content and the composition of the food (Rebouche & Chenard, 1991). In a balanced diet, humans ingest about 10–100 mg of carnitine (Mitchell, 1978), with the most carnitine coming from tissues of animals. In our investigations, the highest L-carnitine content was found in the muscle from kangaroo (6370 mg/kg DM) and horse (4230 mg/kg DM), followed by beef steak and ribs from cow (2320 and 2260 mg/kg DM). The lowest L-carnitine content was found in muscles from pigs (830–402 mg/kg DM) and fowls (732–135 mg/kg DM). The visceral organs contained only 10–30% of the L-carnitine content of the associated species; e.g., kidney from cow contained 310 mg/kg DM compared to beef filet which contained 1390 mg/kg DM. No explicit results of the L-carnitine content in animal tissues can be found in the literature. Götz (1989) for example, detected in beef steak 23% more carnitine and in turkey filet 13% less carnitine than was found in

our investigations. However, the distribution of carnitine between the different species was similar. Foster and Harris (1992) measured the carnitine content in the middle gluteal muscle from horse to be 4385 ± 677 mg/kg DM, which is approximately the same as values obtained in our study (4230 mg/kg DM). Similar results were obtained from ribs from cow (our study 2260 mg/kg DM vs. 2188 mg/kg DM) (Erfle, Sauer, & Fischer, 1970). In contrast, the carnitine content in the broiler was, in our study, 344 mg/kg DM compared to 295 mg/kg DM as described in the literature (Leibetseder, 1995). The partly different carnitine contents could be generated due to differences in the samples (tissues, age, gender and water content), in the sample preparations and due to different methods of L-carnitine determination. To investigate the interferences between different species, further studies with more samples from each species under different conditions are necessary.

The amount of free carnitine varied in pork and cow from 64% to 94% and from 58% to 80%, respectively. Consequently, free carnitine is not a stable value and the total carnitine shows a more definite degree of accuracy than does free-carnitine. The measured free-carnitine amounts in food samples from Demarquoy et al. (2004) were higher than the free carnitine amounts measured in our investigations, e.g. beef: 731 ± 98.5 vs. 407 ± 89.9 mg/kg carnitine wet weight (w/w), Pork: 200 ± 19.6 vs. 155 ± 51.1 mg/kg carnitine w/w; chicken: 104 vs. 79 mg/kg carnitine w/w. A comparison between the free-carnitine contents of Demarquoy et al. (2004) and our free- and total-carnitine amounts is difficult to perform even though the same radio-enzymatic method was used. In our investigation we distinguish between free and total carnitine. In addition to the reasons for different carnitine contents mentioned above, Demarquoy and his colleges used a different sample preparation. It is possible that, due to this procedure, not only free-carnitine but additionally some total carnitine was measured.

The measured amount of L-carnitine in our dairy products is located between the amounts of L-carnitine in plant and animal tissues. The mean L-carnitine concentration of liquid dairy products from cow was 259 ± 118 mg/kg DM and was lower than the carnitine content in muscle tissues from cow and pigs and is comparable with the carnitine content in fowls. The carnitine contents in yogurts (208 ± 104 mg/kg DM) and in cheese curd (264 ± 38 mg/kg DM) were statistically significantly lower than those in milk (357 ± 35 mg/kg DM). This could be due to different samples, analytical methods, sample preparations, and preprocessing procedures. The milk had been pasteurized, homogenized, separated into different fat categories and maybe even freeze-dried (milk powder). Afterwards, dairy products are mixed with powdered milk, microbial cultures and essences, though, in some of these procedures (development towards butter, cheese), carnitine in the milk could be decomposed.

Morover, the L-carnitine contents in milk varied even though the samples had been through the same manufactory processes. The L-carnitine contents in milk from one species can be influenced by different lactation durations and numbers of lactations. Directly *post partum*, cows show an increased carnitine content in the milk (Roos, Devrese, Schult-Coerne, & Barth, 1992). During early lactation, dairy cows secrete as much as 80.6 mg carnitine/l of milk (w/w) and, after 8 weeks, the values decrease to approximately one-half (Erfle et al., 1974; Penn, Dolderer, & Schmidt-Sommerfeld, 1987). The concentration of L-carnitine in cow milk is negative correlated with the milk capacity. Milk from five couples of twin cows showed, in the morning, a carnitine concentration of 32.4 ± 0.8 mg/l (w/w) (approximately 0.16 mg per milking) and, in the evening, 36.4 ± 0.76 mg/l (w/w) (approximately 0.33 mg per milking) (Roos et al., 1992). Simultaneously, the first lactation gives a higher carnitine amount per day than do later lactations (Roos et al., 1992). These variations in the cow milk could also be found in the literature (Table 10), but an additional source of error here was the different analytical methods. In contrast, human milk shows minor fluctuations in the lactation period (Sandor, Pecsuvac, Kerner, & Alkonyi, 1982).

The mean L-carnitine concentration in the milk from five couples of twin cows after 40 lactation weeks was 29.5 ± 0.4 mg/kg (w/w) (Roos et al., 1992), corresponding to our obtained data (milk 1.5%, fat 34.9 mg/kg (w/w) and milk 3.5%, fat 41.8 mg/kg (w/w)).

The L-carnitine contents vary greatly between different species. Penn et al. (1987) determined the carnitine content in milks from sheep, goat and cow. In their study, the L-carnitine content in sheep milk was found to be 152 mg/ml (w/w), goat milk 22 mg/ml (w/w), cow milk 27 mg/ml (w/w) and horse milk 12.1 mg/ml (w/w). These results show that sheep milk has a relatively high amount of carnitine compared to other species. The significant species differences in the L-carnitine amount depend, not on the different dry matter amount, but also may be related to differences in the rate of the maternal carnitine biosynthesis, the maternal carnitine body storage and the maternal carnitine plasma concentrations. These species differences in the amounts of L-carnitine are also seen for cheese products. Feta cheese from sheep contains 149 mg carnitine/kg DM, in contrast to feta cheese from sheep and cow which contains 97 mg carnitine/kg DM and feta cheese from cow which contains 86 mg carnitine/kg DM. The sheep cheese contains approximately 50% more carnitine than does cow cheese. Cheese contains less L-carnitine than does milk because, during the cheese manufacturing, the milk is

Table 10
L-Carnitine contents in milk samples measured with different methods

Cow	mg/kg[a]	Method	Reference
Milk	19.6	Enzymatic	Erfle et al. (1970)
Milk	58.3	Radio-enzymatic	LaCount et al. (1995)
Milk	29.5	Enzymatic	Roos et al. (1992)
Milk	34.1	Spectrophometrically	Götz (1989)

[a] mg/kg ~ mg/l wet weight.

curdled and strained and the liquid products, e.g. whey or milk plasma, are removed from the cheese products. Because of the hydrophilic characteristics of L-carnitine, the liquid by-products from the cheese development contain more L-carnitine than does the solid sample (whey: 355 mg/kg DM vs. edamer: 28 mg/kg DM). Naturally, cheese, where a lot of whey is subtracted, contains less carnitine (e.g. edamer 28 mg/kg DM) than does cheese where less whey is subtracted (e.g. camembert 55 mg/kg DM). Not only does the subtraction of whey affect the L-carnitine content in cheese, but also the maturation process. When cheese is stored, the dry matter increases because of the evaporation of water and the carnitine concentration increases in the solid sample (old gouda 28 mg/kg DM vs. new gouda 26 mg/kg DM). Götz (1989) detected minor or zero amounts of L-carnitine in tissues from cheese products, which could be due to the use of a less sensitive spectrophotometric method (usage of 5.5′-dithiobis-2.2′-nitrobenzoic acid) for the determination of L-carnitine in his investigations. For example, the content of L-carnitine could not be detected in Gorgonzola and Gouda. The Norwegian goat cheese is a whey product and contains 137 mg carnitine/kg DM. Because of different manufactory processes, the Norwegian goat cheese can not be compared to other cheeses. But our results cast doubt on the results from Penn et al. (1987) reporting that goat milk contains less carnitine than does cow milk. Further measurements are necessary to draw a proper conclusion.

The amount of free-carnitine showed large variations in dairy products (18–97% of the total carnitine amount). Again, our determined free-carnitine concentrations were lower than those from Demarquoy et al. (2004) due to the reasons stated above.

Fruit and vegetables contained less than 4 mg carnitine per wet weight (<50 mg/kg DM). These results are among the lowest measured carnitine concentrations in our study. There are few data on different L-carnitine contents in the tissues from plant and mushrooms in the literature. Panter and Mudd (1969) found 48 mg carnitine/kg DM in avocado which is three times more than our measurements showed (17.2 mg/kg DM). A possible explanation is probably the different pre-processing of the samples. Panter and Mudd (1969) used a methanol–water mixture for the carnitine extraction instead of the extraction with water in our investigations. In contrast, cauliflower showed approximately half of our measured carnitine concentration (14 mg/kg (DM) vs. 33 mg/kg (DM)).

Plants and mushrooms are classified in two different kingdoms. The carnitine content in mushrooms is higher than those in tissues from plants and lower than those in muscles from animals. In the oyster, a concentration of 530 mg carnitine/kg of dry matter was detected. This is equivalent to the amount of carnitine found in pig muscles (830–402 mg/kg DM). Currently it is not known whether mushrooms need carnitine for fat oxidation. Further investigations are necessary for a more detailed understanding of this area.

The free-carnitine content in tissues from plants and mushrooms showed a variation similar to dairy products. Demarquoy et al. (2004) detected between 2.6 and 5.7 times more free-carnitine than in our investigations which could be due to the reasons stated above.

5. Conclusions

The method was found to be easily and rapidly usable for the determination of L-carnitine in complex biological samples. The fraction of L-carnitine varied greatly between the different food products; tissues from animals contained a much larger amount of L-carnitine than did tissues from plants. Under normal conditions healthy humans can synthesize sufficient amounts of L-carnitine; therefore, L-carnitine is not regarded as a true nutritional supplement (Vaz & Wanders, 2002). However, low carnitine plasma concentrations are present in infants fed with carnitine-free soy formulas (Novak, Wiser, Buch, & Hahn, 1979), in individuals receiving long-term, total parenteral nutrition (Hahn, Seccombe, & Towell, 1982), and possibly in adults undergoing physical stress (Volek et al., 2002), pregnancy (Cederblad et al., 1986; Hahn, 1981; Kaise, 1997; Mitchell & Snyder, 1991; Schoderbeck et al., 1995) and work/fitness (Foster & Harris, 1992; Harmeyer & Schlumbohm, 1997). In these humans, primary and secondary carnitine deficiencies can occur (Bowyer et al., 1986; Duran et al., 1990; Leschke et al., 1984; Nezu et al., 1999; Roschinger et al., 2000; Scholte & De Jonge, 1987). The knowledge about L-carnitine concentrations in different biological samples is helpful when humans are suffering from carnitine deficiency and an exogenous supplementation is needed.

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