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Radioisotopic determination of L-carnitine content in foods commonly eaten in Western countries

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Abstract

L-Carnitine is a vitamin-like nutrient essential for energy production and lipid metabolism in many organs and tissues such as skeletal muscle and heart. Even if L-carnitine can be synthesized, most of the carnitine present in human body is provided by food. Until now, no large study has been conducted where the content in L-carnitine of various foods was analyzed. The objective of this study was to determine the level of free L-carnitine present in food commonly consumed in Western countries. A radioisotopic assay was used to estimate L-carnitine content in raw and processed foods. From this study, it clearly appeared that meat products were the best sources for L-carnitine. Dairy products, seafood and fish are generally relatively low in carnitine whereas vegetables are mostly very low in carnitine. An omnivorous regimen allows to meet the general recommendation on L-carnitine intake. Vegetarian are clearly below recommendation and their carnitine homeostasis has to be carried out by a functional biosynthesis.

Keywords: Food; L-Carnitine; B-oxidation; Meat; Radioisotopic assay

1. Introduction

L-Carnitine plays a crucial role in fat metabolism directing fatty acids into the oxidative pathway. L-Carnitine is supplied to the body through endogenous biosynthesis and food intake. In mammals, L-carnitine is synthesized mainly in the liver and in some species in the testis and the kidney. This biosynthesis requires methionine and lysine as ultimate precursors and five enzymatic reactions (Vaz & Wanders, 2002). During this enzymatic pathway several cofactors are also needed such as vitamin C and iron, making this biosynthesis strongly influenced by the bioavailability of these elements. After its biosynthesis, L-carnitine is excreted out of the cell and transported by the blood stream to organs and tissues depending upon fatty acid oxidation for their energetic needs. Among these, muscle is certainly the main target for L-carnitine uptake. About 98% of the body L-carnitine is found in skeletal and heart muscles and the ratio between plasma and muscle carnitine concentration is around 1:50 (Siliprandi, Sartorelli, Ciman, & Di Lisa, 1989). None of these organs is able to synthesize L-carnitine, thus this molecule has to be imported from the blood stream using active mechanisms. Besides the biosynthetic origin, L-carnitine can also be provided by the food supply. Therefore, L-carnitine found in mammal tissues is always a mixture of biosynthetic and dietary origins. The relative importance of each source is quite difficult to precise since it depends on the age, the dietary habits, the availability of other elements, such as vitamin C or iron. Due to this dependence on micronutrients, L-carnitine is, sometimes, considered as a vitamin-like substance (Zurbriggen, 2000).

An adult body contains around 25 g of L-carnitine. Based on nutritional studies carried out in humans, it has been proposed that the daily need for L-carnitine is between 2 and 12 μmol/kg b.w./day (0.3 and 1.9 mg/kg/day) and can even be higher for special people such as athletes or pregnant women. L-Carnitine is an essential nutrient for infants since they are unable to synthesize carnitine due to an immature biosynthesis pathway (Galland et al., 1999).

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It has also been reported that carnitine biosynthesis is slow and it does not really keep up with rapid changes of the energetic metabolism (Bohles, 2000). Under normal dietary conditions, the total amount of L-carnitine brought by the food is absorbed. The absorption implies an active mechanism located in the proximal part of the small intestine. The levels of carnitine recovered in the plasma or the urine usually do not precisely reflect the level of reserves in organs such as liver, heart or muscle. The circulating level of carnitine remains stable. For male, carnitine concentration is around 59 μM whether for female the concentration is slightly lower: 52 μM. In children, this value is found between 35 and 45 μM (Galland, Le Borgne, Georges, & Demarquoy, 2001).

The primary function of L-carnitine is to permit the entry of esterified fatty acids into the mitochondrial matrix where β-oxidation occurs. The implication of carnitine at this point is critical as it has been shown that disorders in L-carnitine uptake in muscle or heart cells may lead to myopathy and heart diseases (Lahjouji, Mitchell, & Qureshi, 2001). Thus, because of its important role in fatty acid transport towards mitochondrial membrane, L-carnitine is a key element in fat metabolism.

Deficiencies in L-carnitine may occur in several conditions, it could be due to an impaired biosynthesis such as what happened in vitamin C deficiency or iron deficiency or due to an impaired intracellular import of L-carnitine. Whatever the origin is, the results are usually the same, i.e., muscle atrophy, neurological disorders and heart diseases. These symptoms are linked to the main effect of carnitine on fatty acid metabolism. Besides this main effect, L-carnitine also plays a role in the peroxisomal oxidation of fatty acids (Wanders et al., 2001).

These data show the importance of L-carnitine in providing energy to the human body but, until now no complete studies on L-carnitine levels in food have been published. In this paper, L-carnitine levels in various food including meat, dairy products but also, fruits and vegetables are described. Taken together, these data allow to have a picture of daily intake in L-carnitine.

2. Materials and methods

2.1. Chemicals

Chemicals or drugs were purchased from Sigma (Sigma, France) and were of analytical grade. [^{3}H]acetyl-coenzyme A was obtained from Amersham (UK).

2.2. Sample preparation

Samples of meat products were either purchased or provided by local butchers. Other raw foods were collected from retail outlets. Processed foods were obtained "out of the plate", these foods were previously purchased in supermarkets. Foods were kept at 4 °C until carnitine determination. The delay between sample collection and L-carnitine determination was always less than 6 h. Soft samples (meat, vegetables and fruits) were minced, washed three times in an ice-cold homogenization buffer, briefly dried and weighted. The composition of this buffer was: Tris–HCl 100 mM, sucrose 25 mM, EDTA 1 mM, pH 7.5 (TSE buffer). They were homogenized in this buffer with six strokes of a loose-fitting Teflon pestle at 200 rpm in an ice-cold Elvehjem potter. Generally, 100 mg of sample were dissolved in 0.7 ml of buffer. If carnitine concentration determination appeared out of range other dilution ratios were used. The homogenate was centrifuged at 13,000g for 30 min at 4 °C. Hard samples such as seeds or nuts were first powdered using a pestle and a mortar. They were subsequently taken in TSE buffer (1 ml/100 mg) and treated as above. The resulting supernatant was subsequently centrifuged at 100,000g for 1 h at 4 °C. The final supernatant was immediately used for carnitine amount determination.

2.3. Carnitine determination in tissues

The amount of L-carnitine present in food was estimated as described by McGarry and Foster (McGarry & Foster, 1976) modified by Galland (Galland, Le Borgne, Guyonnet, Clouet, & Demarquoy, 1998). Briefly, 100 μl (and up to 300 μl) of sample was mixed with 300 μl of a medium containing 20 mM of Tris–HCl (pH 7.3), 2 mM of sodium tetrathionate and 25 nCi of [methyl-^{3}H]acetylCoA. One unit of L-carnitine acetyltransferase and the fraction were added. After 15 min of incubation at 37 °C, 600 μl of Dowex resin (1 × 8, 200–400 mesh) were added to the incubation mixture and vigorously shaken. The resin was used to remove the unused acetylCoA. The tubes were incubated at 4 °C for 10 min and centrifuged at 4000g for 2 min. The supernatant was mixed with scintillation solution and counted in a scintillation counter. L-Carnitine content was expressed in μmol or mg per 100 g of fresh food for solids and per 100 ml for liquids. L-Carnitine determination was done in triplicate on each sample. Only samples giving comparable values were used (more than 95%). Others were redone. Based on previous experiments done in our lab or in others, the recovery has been estimated more than 85%.

2.4. Statistical analysis

Each foodstuff was analyzed at least three times in three separate experiments.

3. Results

3.1. L-Carnitine in meat products

To assess the presence of L-carnitine in meat products, L-carnitine amount was estimated in various commonly eaten meat products (Table 1). These included beef, pork, lamb, veal, etc. As a general rule, these data confirmed the presence of L-carnitine, in relatively high concentration, in basically each meat product tested. Among those, veal muscle appeared as the best source for carnitine. In general, muscles appeared as good sources of carnitine. Liver carnitine levels were also generally high except for the veal. Levels of carnitine in raw meat products were comprised between 40 and 540 μmol/100 g (i.e., 6.5 and 87.5 mg/100 g, respectively). Processed meats were comparatively low in carnitine.

Table 1
Carnitine level in meat products

	Carnitine (μmol/100 g)	Carnitine (mg/100 g)
Beef		
Beef (steak)	401	65.0
Ground Beef	540	87.5
Beef (tenderloin)	485	78.6
Beef (T bone)	520	84.2
Beef (loin)	399	64.6
Chicken, duck and turkey		
Chicken liver (uncooked)	427	69.2
Chicken liver (cooked)	580	94.0
Chicken meat (wo skin)	64	10.4
Chicken wing meat (wo skin)	62	10.0
Duck (steaklet)	165	26.7
Duck (gizzard)	9	1.5
Turkey meat	131	21.2
Turkey skin	95	15.4
Lamb and pork		
Lamb chop	250	40.5
Pork leg	109	17.7
Pork shoulder	130	21.1
Pork (believed ham)	330	53.5
Pork (muscle)	130	21.1
Pork (cooked plug)	80	13.0
Pork (white ham)	207	33.5
Veal		
Veal (shoulder)	483	78.2
Veal (sirloin)	820	132.8
Veal (liver)	40	6.5
Processed meat		
Beef Sausage (merguez)	409	66.3
Beef Chorizo	54	8.8
Pork sausage	44	7.1
Andouillette	18	2.9

Unless indicated free L-carnitine level was determined on raw food.

3.2. L-Carnitine in dairy products

The concentration of L-carnitine was estimated in several dairy products (Table 2). In manufactured milk (1/2 skimmed) the amount of L-carnitine was found to be 26 μmol/100 ml (4.2 mg/100 ml). Due to the process, a

Table 2
Carnitine level in fish and seafood

	Carnitine (μmol/100 g)	Carnitine (mg/100 g)
Anchovy (cnd in oil)	11	1.8
Shrimp	4	0.7
Cod (Atlantic)	11	1.8
Hake (boiled)	18	2.9
Whiting (boiled)	13	2.1
Mussels (cooked)	16	2.6
Salmon (cooked)	36	5.8
Smoked salmon	6	1.0
Surimi	7	1.1
Tuna (white cnd in water)	9	1.5

Unless indicated free L-carnitine level was determined on raw food.

Table 3
Carnitine level in milk and dairy products

	Carnitine (μmol/100 g or μmol/100 ml)	Carnitine (mg/100 g or mg/100 ml)
Milk and butter		
Milk dry whole	62	10.0
Milk 2% fat	18	2.9
Milk 4% fat	14	2.3
Butter	8	1.3
Cream (low fat)	11	1.8
Cream (whipping)	8	1.3
Cream (fluid)	11	1.8
Cheese		
Boursin™	0	0
Camembert (AOC)	89	14.4
Comté (AOC)	75	12.2
Feta	11	1.8
Goat cheese	98	15.3
Gruyere	40	6.5
Mozzarella	2	0.3
Munster	122	19.8
Parmesan	4	0.7
Reblochon	27	4.4
Saint Môret	77	12.5
Yogurt		
Yogurt 0% fat	77	12.5
Yogurt regular	75	12.2
Petit suisse	8	1.3
Fromage blanc	11	1.8
Egg		
White	2	0.3
Yolk	5	0.8

Unless indicated free L-carnitine level was determined on raw food.

higher level of L-carnitine was recovered in dry milk. Even if L-carnitine is a hydrophilic molecule, a significant amount of carnitine was found in liquid cream, probably due to the presence of water coming from the milk. On the other hand butter was very low in L-carnitine.

3.3. L-Carnitine in fish and seafood

L-Carnitine concentration in fish and various marine products was determined (Table 3). Clearly, the average amount of L-carnitine present in fish is closer to dairy products than to meat products. Among all tested fishes, salmon appeared as the best marine source for carnitine.

Table 4
Carnitine levels in selected fruits, vegetables, cereals and seeds

	Carnitine (μmol/100 g)	Carnitine (mg/100 g)
Fruits		
Apple (wo skin)	1	0.2
Apricot	3	0.5
Banana (wo skin)	1	0.2
Blackcurrant	0	0
Kiwi (wo skin)	1	0.2
Mango (wo skin)	5	0.8
Pear (wo skin)	2	0.3
Vegetables and vegetables products		
Asparagus	0	0
Avocado (wo skin)	50	8.1
Carrot	2	0.3
Cucumber peeled	0	0
French been	0	0
Lentil	13	2.1
Mushroom	3	0.5
Olive	3	0.5
Onion	4	0.7
Palmtree	1	0.2
Pea	35	5.7
Potato (boiled)	0	0
Potato	15	2.4
Spinach	0	0
Sweet pepper	2	0.3
Sweet potato	7	1.1
Tomato	0	0
Zucchini squash	7	1.1
Spices and herbs		
Chive	29	4.7
Garlic	8	1.3
Ginger root	1	0.2
Mint	5	0.8
Sage	15	2.4
Nut and seed products		
Peanut	1	0.2
Manioc	0	0
Hazel	1	0.2
Pinion	13	2.2
Raisin	5	0.8
Taro	11	1.8
Cereal grains and pasta		
Rice (white boiled)	0	0
Noodle (boiled)	0	0

Unless indicated free L-carnitine level was determined on raw food. For nuts and seeds shells were removed if uneaten.

3.4. L-Carnitine in vegetables, fruits, cereals and derived products

It can be concluded from the data presented in Table 4 that all vegetables and fruits are low in carnitine except avocado and to a lesser extend green peas, lentil and several mixed herbs such as chive and sage. Bread is also low in L-carnitine.

4. Discussion

This study allowed to draw a figure of L-carnitine availability in many commonly consumed foods in Western countries. From the data presented in this paper, it clearly appeared that the major source of free L-carnitine in the food is meat (Fig. 1) and especially muscle-derived foodstuff. Similar data were reported in the past and reviewed by Borum (Borum, 1983). This finding is in agreement with previous findings showing that between 95% and 98% of the carnitine in the body is recovered in muscle (reviewed by Galland: Galland et al., 2001). However, variations exist in this group, the lower level of carnitine was found in veal liver (40 μmol/100 g or 6.5 mg/100 g), this relatively low amount may be related to the immaturity of the carnitine biosynthesis in the liver of young animals or human (Arenas, Rubio, Martin, & Campos, 1998; Galland et al., 1999).

The presence of L-carnitine in milk was extensively studied by Woollard (Woollard, Indyk, & Woollard,

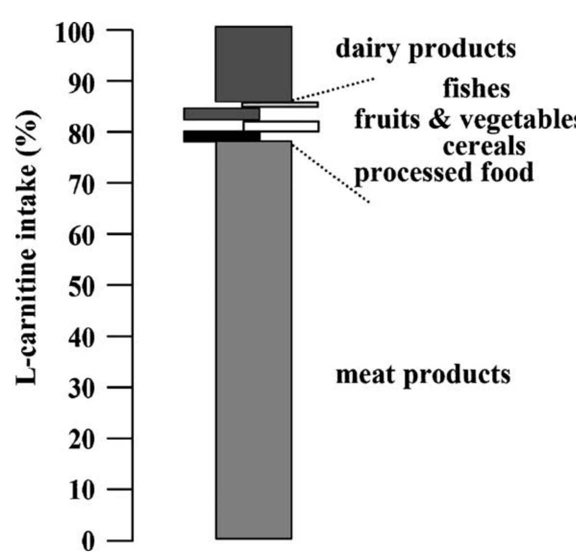

Fig. 1. Schematic representation of the origin of free L-carnitine from foods. This representation is based on a regular food intake, including meat and dairy products. It is expressed in percentage of the total intake.

1999), a few years ago. Basically, in this study, comparable values for L-carnitine concentration in milks were found. L-Carnitine content for several cheeses and dairy products was added. While there was a concentration of 14–18 μmol of carnitine per 100 ml of milk (2.3–2.9 mg/100 ml), L-carnitine was subsequently recovered in higher concentration in cheese and all dairy products, except butter, made from exclusively the fat part of milk.

In fish, L-carnitine level is basically comparable to that of dairy products. The best source was found to be the salmon. Shrimp, on the other hand, appeared in this study as one of the lowest source of L-carnitine.

Exception made from avocado, lentil and green pea, vegetables and fruits are low in free L-carnitine. Some of them even appeared as totally free in L-carnitine. Mixed herbs are also usually rich in L-carnitine especially sage and chive. However, the quantitative importance of these herbs on the total food intake is very low and under regular food intake habits these herbs probably do not participate in a major way in L-carnitine supply.

The average intake of free L-carnitine per day and per capita was determined. It was clear that this intake would be depending on age and food preferences. Free L-carnitine intake was estimated in a standard meal and in vegetarians. Based on food intake information provided by the French Food Agency (Afssa) and the Inca study (for more information: see www.afssa.fr), a regular diet provides around 472 μmol (76.5 mg) of carnitine per day, 77.8% of these 472 μmol are provided by meat products and 14.4% by dairy products and eggs. Based on the actual range of recommended carnitine intake (i.e., from 2 to 12 μmol/kg/day or from 22.7 to 136.1 mg/day for a 70 kg human being), a standard diet provides enough carnitine, 3.4 times the lower level or 96% of the average recommendation (Fig. 2).

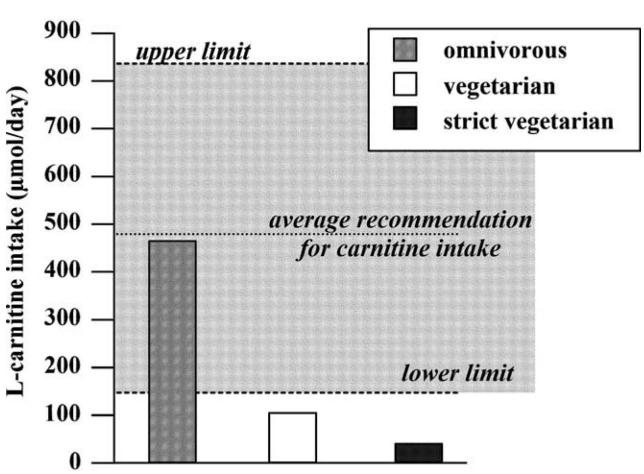

Fig. 2. Estimated L-carnitine intake according to dietary habitudes. The dashed line represents the average recommended carnitine intake. The gray box the upper and lower limits for carnitine intake. Histograms showed estimated daily free L-carnitine intake in omnivorous (gray histogram), vegetarian (white histogram) and strict vegetarian (black histogram).

With a vegetarian diet, the total amount of ingested carnitine is significantly reduced and found to be 100.4 μmol/day (16.3 mg/day). As shown by Fig. 2, this value is slightly under the lower limit of the recommendation while it is one-fifth of the average recommendation for carnitine intake. A strict vegetarian diet, i.e., without any animal derived food will provide only 22.5 μmol (3.6 mg) of carnitine per day. This value represents only 4.6% of the lowest recommendation for carnitine intake. This indicates that vegetarians rely almost entirely on carnitine biosynthesis to meet their carnitine needs. This also suggests that a vegetarian diet implies an adequate supply in vitamin C and iron, two major cofactors in carnitine biosynthesis as it has been already suggested by Krahenbuhl (Krahenbuhl, 2000).

5. Conclusions

For healthy people receiving a regular diet, it seems that there is a good coverage of carnitine needs. Most of the L-carnitine intake is related to meat consumption. For vegetarians, the L-carnitine intake cannot take charge of the coverage of carnitine needs. In this case, efficient biosynthetic pathway is necessary to ensure carnitine homeostasis that implies that all substrates and cofactors required in this pathway are made available, especially vitamins C and B6 and iron.

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