Effect of diet on plasma carnitine levels and urinary carnitine excretion in humans[1-3]

Gitten Cederblad, MD

ABSTRACT This investigation determines the effect of two isocaloric diet regimens on plasma carnitine and urinary carnitine excretion in man. Seven healthy men were served a high-carbohydrate, low-fat (C) or a low-carbohydrate, high-fat (F) diet for 2 wk, ie, one diet regimen for 4 d followed by a 3-d break and concluded with 4 d more on the other diet regimen. The two regimens contained the same amount of carnitine-rich food. Plasma free carnitine rose significantly from the initial value on F diet and was significantly higher from day 3 than C diet. Plasma acyl carnitine increased on both diets. Urinary excretion of carnitine increased only on F diet. Renal clearance of both free and acyl carnitine was significantly greater on F diet than on C diet. Results showed that composition of a diet with constant carnitine content influenced carnitine metabolism in man. *Am J Clin Nutr* 1987;45:725–9.

KEY WORDS Dietary composition, plasma carnitine, urinary carnitine excretion, normal healthy adults

Introduction

Carnitine is essential to the oxidation of long-chain fatty acids by mammalian tissue because it is required for entry of these substrates into the mitochondrial matrix, where β-oxidation takes place (1). Other functions for carnitine have been suggested although not yet confirmed. Several investigators have identified acyl carnitine derivatives of oxoanalogs from branched-chain amino acids in animal tissues (2, 3), and carnitine has been shown to facilitate removal of excess and potentially toxic acyl groups from mitochondrion and cell (4).

Carnitine is normally obtained from diet and from endogenous synthesis from lysine and methionine in human liver and kidney (5). Skeletal and cardiac muscle tissue have high carnitine concentrations and are therefore dependent on transport via plasma.

Factors such as sex, age (6, 7), nutritional status (8), fasting (9, 10), and disease (11–13) have been cited as influencing plasma carnitine levels in man. Recently there have been several reports about impact of diet on plasma carnitine levels in various species (14–16). Less has been published on plasma carnitine levels in man (8). In this study impact of diets on plasma and urinary carnitine levels in man is investigated.

Materials and methods

The studies were performed with seven apparently healthy male volunteers (**Table 1**). Informed consent was obtained from them all. During the 2 wk of the study, no regular medication (one day one subject took two 0.5 g acetylsalicylic acid tablets) or strenuous physical exercise were allowed. Two isocaloric diet regimens were used, one with a high-carbohydrate, low-fat content (C) and the other with a low-carbohydrate, high-fat content (F). After baseline blood samples were obtained, the diet regimens were maintained for 4 d, followed by a 3-d break, and then repeated for another 4 d. Four subjects started with C diet and three with F diet. The procedures of the study were in accord with the Helsinki Declaration (Tokyo, Japan, 1975).

Lunch and dinner were prepared before the start of the experiment and were frozen in individual servings. Breakfast (0730–0815) consisted of processed sour milk (skimmed or fat), cereals, various margarines, low- or high-fat cheese, orange marmelade, and coffee or tea. Lunch (1130–1300) consisted of pork fillet with mushroom sauce and rice. The sauce was prepared differently in the two

[1] From the Department of Clinical Nutrition, Sahlgren Hospital, University of Gothenburg, Gothenburg and the Department of Clinical Chemistry, Danderyd Hospital, Karolinska Institute, Danderyd, Sweden.

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[3] Address reprint requests to Dr Gitten Cederblad, Department of Clinical Chemistry, Danderyd Hospital, S-182 88 Danderyd, Sweden.

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TABLE 1
Characteristics of the seven subjects studied

			Weight			
			C diet		F diet	
	Age	Height	Before	After	Before	After
	yr	*cm*	*kg*			
Median value	33	184	69.1	68.9	68.9	68.3
Range	22–44	170–194	65.5–82.0	65.0–82.0	64.7–82.0	63.8–81.2

regimens as was the beverage (skimmed or high-fat milk). Dessert consisted of an apple. Tea or coffee, bread slices with margarine, and cheese and marmelade were served as a snack (1430–1530). Dinner (1730–1900) consisted of fillet of plaice, spinach, mashed potatoes, apple juice. All servings contained the same amount of the food items with the highest carnitine content and varied fat content, ie, fish and pork fillets, cheese and milk. All food items were weighed, and no other food intake was allowed except coffee and tea (unsweetened). Breakfast and lunch were served in the Department's experimental kitchen. Subjects ate snacks and dinner at work and at home. The composition of the regimens is given in **Table 2.** Blood samples were taken after overnight fasting on days 1, 3, and 5. Urine was collected daily.

Carnitine was determined by a radioenzymatic procedure (17) modified as described by Cederblad et al (18). Total carnitine was determined after alkaline hydrolysis and free carnitine was determined directly. Acyl carnitine was calculated as the difference between these two figures. The other substances were measured by routine methods.

For statistical analysis, paired "Student's" *t* test and Wilcoxon's nonparametric paired test, when indicated, were the methods used for comparing the two regimens.

Renal clearance (C) was calculated as C (mL/min) = $(U \cdot V)/P$ where U and P are the respective carnitine concentrations in urine and plasma and V is the urinary volume per unit of time. The mean values for carnitine (P) on days 3 and 5 and urinary carnitine excretion between these two samplings were used.

Results

Total plasma carnitine concentration and urinary carnitine excretion rose significantly from the initial values for subjects on F diet although not for subjects on C diet during the period of observation (**Table 3**). Both regimens produced an increase in the plasma acyl carnitine fraction. Accordingly, the plasma acyl carnitine:free carnitine ratio increased significantly ($p < 0.05$) from an initial value of 0.18 ± 0.09 (SD) to 0.30 ± 0.11 on day 5 with C diet and from 0.16 ± 0.09 to 0.26 ± 0.08 with F diet. The acyl carnitine:free carnitine ratio in urine did not change significantly from the first to the last 24-h collection periods (**Table 4**).

Comparisons between the two regimens showed that total and free carnitine levels in plasma were higher on F diet than on C diet by day 3 (Table 3). In urine, the levels of both the acyl and free carnitine fractions increased by day 2 on F diet (Table 4). The acyl carnitine: free carnitine ratio did not differ significantly in plasma or urine at the end of the observation period. The difference in urinary carnitine excretion appeared to be due to a 100% and 30% increase in the renal clearance of acyl and free carnitine, respectively, in subjects on F diet compared with excretion in subjects on C diet (**Table 5**). No changes occurred in the other serum values, ie, T3, cholesterol, triglycerides, and urate.

TABLE 2
Composition of experimental diets

	Energy	g (energy, %)		
	MJ	Protein	Fat	Carbohydrate
	kcal			
High-carbohydrate, low-fat diet (C)	10.04 (2400)	111 (19)	78 (30)	295 (51)
Low-carbohydrate, high-fat diet (F)	10.13 (2420)	101 (17)	140 (54)	169 (29)

TABLE 3
Plasma carnitine during isocaloric regimens with high-carbohydrate, low-fat (C) or low-carbohydrate, high-fat (F) content*

	Day						p	
	1		3		5		Day 1 vs 5	
Carnitine, μmol/L	C	F	C	F	C	F	C	F
Total	39.8 (3.4)	40.2 (3.6)	41.0 (4.1)	44.9† (4.1)	42.9 (3.5)	45.6† (4.0)	NS	< 0.05
Free	33.6 (2.6)	34.7 (3.8)	33.5 (2.3)	36.7† (2.3)	32.9 (3.5)	37.0† (2.2)	NS	NS
Acyl	6.1 (2.9)	5.5 (1.2)	7.8 (3.1)	8.2 (4.2)	10.0 (2.6)	9.6 (2.9)	< 0.05	< 0.05
Acyl:free ratio	0.18 (0.09)	0.16 (0.04)	0.23 (0.09)	0.23 (0.12)	0.30 (0.11)	0.26 (0.08)	< 0.05	< 0.05

* Mean values with SD in parentheses.
† 2 $p < 0.05$, C and F regimens compared.

Discussion

Intra- and interspecies differences in the levels of plasma carnitine are affected by diet (15, 19). In this study, F diet, but not C diet, caused an increase in the level of free carnitine in plasma and increased urinary excretion of both free and acyl carnitine. Plasma acyl carnitine increased on both regimens. Several mechanisms are consistent with this pattern. Firstly, carnitine content of F diet may have been higher. Even though carnitine content of diets was not analyzed, this does not seem likely but cannot be ruled out, as both diets contained the same high-carnitine food items. Extra fat was supplied as margarine, high-fat cheese, high-fat milk or cream instead of skim milk, etc. It is possible, at least theoretically, that absorption of dietary carnitine was increased by a high-fat content. Secondly, endogenous synthesis of carnitine could have increased as a result of the increased lipid load imposed by F diet. Thirdly, results may have been due to a redistribution of carnitine among various tissues. Plasma carnitine is known to be taken up by the liver during fasting, ie, a condition characterized by an increase in the rate of fatty acid oxidation (20).

TABLE 4
Urinary excretion of carnitine during isocaloric regimens with high-carbohydrate low-fat (C) or low-carbohydrate, high-fat (F) content*

	Days								p	
	1–2		2–3		3–4		4–5		Days 1–2 vs 4–5	
Carnitine, μmol/24 h	C	F	C	F	C	F	C	F	C	F
Total	364 (117)	498 (176)	349 (105)	610† (206)	440 (175)	692† (166)	478 (170)	714† (208)	NS	< 0.05
Free	139 (63)	215 (87)	143 (78)	253† (104)	178 (101)	284† (103)	205 (107)	283† (99)	NS	NS
Acyl	226 (61)	283 (94)	206 (72)	357† (108)	261 (76)	408† (81)	272 (71)	431† (119)	NS	< 0.05
Acyl:free ratio	1.91 (0.84)	1.56 (0.63)					1.40 (0.38)	1.63 (0.48)	NS	NS

* Mean values with SD in parentheses.
† 2 $p < 0.05$, C and F regimens compared.

TABLE 5
Renal carnitine clearance during isocaloric regimens with high-carbohydrate, low-fat (C) or low-carbohydrate, high-fat (F) content

	mL/min*		p†
Carnitine	C	F	C vs F
Free	4.0 (2.0)	5.3 (1.7)	< 0.01
Acyl	21.0 (6.4)	40.0 (26.5)	< 0.05

* Mean values with SD in parentheses; calculated from plasma values for days 3 and 5 and urine carnitine excretion for the last 2 days.

† Wilcoxon's paired test.

Or, there may have been a combination of the last two options.

The feeding of a high-fat diet to rats for 2 days resulted in serum free carnitine levels half as great as the value recorded after a high-carbohydrate diet, but no difference was found in acyl carnitine values (16). Liver and muscle carnitine concentrations were measured in another study in which rats were fed a high-fat diet for 30 days (21). The muscle carnitine concentration declined on the high-fat diet but liver carnitine increased. When the diet of a nonhuman primate, *Macaca artoides,* was switched from a high-carbohydrate, low-fat diet to a low-carbohydrate, high-fat diet for 90 d, mean level of total serum carnitine declined from 64 to 43 μmol/L after 6 d. However, mean values increased to 58 μmol/L, approaching the baseline level, between the 2nd and 3rd wk (22). These patterns in experimental animals may reflect increased tissue demand for carnitine, presumably mainly by liver, in order to oxidize the fatty acids in high-fat diets and possibly even to compensate for a concomitant increase in urinary loss of carnitine. In rat, this demand appears to be met at the expense of muscle carnitine in addition to a possible increase in endogenous synthesis capacity. In monkey, serum carnitine level was restored after 2–3 wk by some unknown mechanism after an initial drop.

In this study, the two regimens were supplied in an experiment of brief duration. However, there was no initial drop in plasma carnitine levels on F diet as was found in experimental animals. On the contrary, both the plasma carnitine level and urinary carnitine excretion increased. The carnitine concentration in human muscle tissue is approximately five times higher than in rat whereas plasma levels are of the same order of magnitude (19). Thus, humans have a much larger carnitine *buffer* capacity, a circumstance which could be one explanation for the absence of any initial drop in plasma carnitine. This is still consistent with the hypothesis of an increased flow of carnitine and acyl groups through carnitine as a response to a high-fat diet.

Both diets increased plasma acyl carnitine levels compared with the initial levels. In spite of this, renal clearance of free and acyl carnitine was of the same order of magnitude in subjects on C diet and in normal subjects on an ordinary diet, ie, 4 and 25 mL/min for free and acyl carnitine, respectively, (23) in contrast to the 100% increase of acyl carnitine clearance found in subjects on F diet. The reason for this difference is not clear.

To summarize, this study showed that diet can affect plasma carnitine levels and excretion of urinary carnitine in man. The mechanism(s) responsible for the increase in plasma and urinary carnitine levels in subjects on the low-carbohydrate, high-fat diet, ie, increase in carnitine synthesis capacity, tissue redistribution, or both, could not be determined.

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