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Comparison of the Plasma and Urinary Carnitine Profiles between Omnivore and Vegetarian Female College Students

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ABSTRACT

This study compared the effect of Korean vegetarian and omnivorous diets on plasma carnitine concentrations and urinary carnitine excretion. Twenty lactoovovegetarian and twenty omnivorous female college students consented to participate in this study. Daily nutritional intake and plasma and urinary nonesterified carnitine (NEC), acid-soluble acylcarnitine (ASAC), acid-insoluble acylcarnitine (AIAC), and total carnitine (TCNE) were determined. Daily protein, fat, retinol, vitamin B_2 and vitamin B_{12} intakes were significantly lower for vegetarians, however, fiber, carbohydrate, β-carotene, folic acid and vitamin C consumptions were much higher for vegetarians than omnivores. There were no differences in plasma NEC, ASAC, AIAC and TCNE concentrations between the two groups. Urinary carnitine excretion was lower in vegetarians, but only the differences in ASAC and TCNE excretions were statistically significant. These results suggest that the lower excretion of ASAC in vegetarians may be a reflection of their lipid metabolic state and that Korean vegetarian diets may accommodate lower carnitine intakes through efficient urinary conservation of carnitine. (*J Community Nutrition* 4(2) : 78~82, 2002)

KEY WORDS : carnitine · vegetarian · omnivore · food intake.

Introduction

Carnitine (β-hydroxy-γ-trimethylammonium butyrate) performs a critically important role in energy metabolism. Carnitine is synthesized predominantly in the liver and kidney of healthy adults. Carnitine functions primarily as a carrier molecule ; transporting long-chain fatty acids across the inner mitochondrial membrane, thereby facilitating β-oxidation (Bray et al. 1980). Humans obtain carnitine from either dietary sources or from de novo synthesis from the amino acids lysine and methionine, with the help of several vitamin and mineral cofactors. Under normal conditions, approximately 300 μmol carnitine per day is ingested from the diet (approximately 50mg) and 100 μmol of carnitine (approximately 20mg) is biosynthesized (Bremer 1983 ; Rebouche 1992).

Vegetarian diets have been associated with reduced risk for age-related degenerative disease including cancer and cardiovascular disease (Mill et al. 1994 ; Phillips et al. 1978). The health benefits of vegetarian diets are usually attributed to reduced saturated and total fat, reduced protein intake, altered amino acid composition, and increased dietary fiber. However, vegetarian diets may increase the risk of specific nutrient deficiencies. Mineral deficiency may result from vegetarian diets either because of decreased intake of a mineral or because of increased intake of phytate, oxalate, and fiber, which decrease mineral bioavailability (Freeland-Graves 1988). Since protein, vitamin C, vitamin B_6, and niacin are important for carnitine biosynthesis, deficiencies in these nutrients have been demonstrated to impair carnitine synthesis *in vivo* (Rebouche 1991 ; Borum 1983). In contrast to omnivores, vegetarians obtain very little carnitine from dietary sources and depend almost entirely on carnitine biosynthesis to meet their carnitine needs. It is therefore possible that vegetarians can develop carnitine deficiency (Siddiqui et al. 1980 ; Broquist et al. 1980).

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In this study, we examined the effect of Korean vegetarian diets on plasma carnitine concentrations and urinary carnitine excretion, as compared to normal omnivores.

Subjects and Methods

1. Subjects

The subjects in this study were 20 lactoovovegetarians and 20 omnivores aged between 18 to 27 years old. Vegetarians were included if they had followed a vegetarian diet that included milk, dairy products and eggs for at least 5 years.

2. Survey of dietary intake

Three-day records were used for dietary assessments (2 weekdays and 1 weekend day). All diets were assessed by the same investigator, using direct interviews for each subject with the aid of measuring instruments and books for eye measurement. Dietary analysis was performed using nutrient contents of Korean foods (Korean Nutrition Society 2000). For each subject, the average value of three days intake for a particular nutrient was used as the mean daily intake for the nutrient and compared with the Korean RDA. The dietary fiber content of the diets was calculated by using Hwang's list of foods (Hwang 1994).

3. Collection of blood and urine

Blood was drawn from each subject after 12 hour fasting in EDTA, centrifuged for 15 min. at 3,000rpm, and plasma separated and stored at −70℃ until assayed. Twenty-four hour urine samples from all subjects were collected into tared plastic containers. Toluene was used as a preservative for urine.

4. Analysis of creatinine and carnitine

Urinary creatinine was determined by the Jaffe's method (Henry 1967). Carnitine was assayed using a modified radioisotopic method of Cederblad and Lindstedt (Cederblad et al. 1972 ; Sachan et al. 1984). Acid-insoluble acylcarnitine (AIAC) was precipitated with perchloric acid and centrifugation, leaving the short-chain acid-soluble acylcarnitine (ASAC) and the nonesterified free carnitines (NEC) in the supernatant. An aliquot of the supernatant was assayed to determine the NEC and another aliquot hydrolyzed with 0.5ml/L KOH to assay all acid-soluble acylcarnitine (ASAC + NEC). ASAC was calculated as the difference between the NEC and the total acid-soluble carnitines. The pellets containing the AIAC were drained, washed, and hydrolyzed in 0.5mol/L KOH for 60 min. in a hot water bath at 60?. In each case, carnitine was assayed using carnitine acetyltransferase (Sigma Chemical Co., St. Louis, Mo, USA) to esterify the carnitine to a [^{14}C] acetate from [1-^{14}C] acetyl CoA (Amersham, Little Chalfont, Buckinghamshire, England). Radioactivies of samples were determined in a Beckman LS-3801 liquid scintillation counter (Beckman Instruments, Palo Alto, CA).

5. Statistical analysis

All values are expressed as group mean ± SD. Significance of differences were determined using a paired t-test. Comparisons with a $p < 0.05$ were considered significantly different.

Results and Discussion

1. Anthropometry and nutrient intake

Anthropometric parameters of subjects are shown in Table 1. The average age of the vegetarians was younger than that of omnivores ; however, the other parameters such as height weight, body fat and lean body mass (LBM) were similar between the two groups. BMIs of both vegetarians and omniveres were in the normal range (20 − 25kg/m^2), but lower than average for the same age of Koreans according to the National Nutrition Survey Report (Ministry of Health and Welfare 2001).

The mean daily intake of energy, total fat, carbohydrate, protein, and other nutrients are shown in Table 2. The average energy intake was 1770 ± 200kcal for vegetarians, and 1682 ± 166kcal for omnivores, which was not significantly different. Energy intake was 88.5% of the Korean RDA in vegetarians, and 84.1% in omnivores.

Energy intake of neither group met the RDA (Korean Nutrition Society 2000). It has been suggested that many college women suffer from malnutrition due to the fear of

Table 1. Anthropometric measurements of subjects

Parameters	Vegetarian (n = 20)	Omnivores (n = 20)
Age (years)	19.8 ± 1.9[1)*]	21.8 ± 1.6
Height (cm)	159.7 ± 6.7	161.5 ± 5.3
Weight (kg)	51.7 ± 5.7	52.3 ± 6.7
Body fat (%)	20.1 ± 2.5	20.8 ± 3.5
LBM (kg)[2)]	40.9 ± 3.8	40.3 ± 5.6
BMI (kg/m^2)[3)]	20.2 ± 1.5	19.9 ± 2.3

1) Mean ± SD, * : p<0.01 2) Lean body mass
3) Body mass index

Table 2. Daily intake of energy and nutrients

Parameters	Vegetarians (n = 20)	Omnivores (n = 20)	RDA[2)]
Energy (kcal)	1770 ± 200[1)]	1682 ± 166	2000
Protein (g)	55.5 ± 8.3*	65.8 ± 19.1	55
% energy	12.6 ± 0.7***	16.3 ± 4.1	
Fat (g)	43.7 ± 6.9*	49.8 ± 7.5	
% energy	20.9 ± 3.8**	24.8 ± 3.2	
Cabohydrate (g)	293.2 ± 45.2***	236.2 ± 30.7	
% energy	66.4 ± 3.8***	58.8 ± 4.5	
Protein/ Carbohydrate	0.1 ± 0.02***	0.2 ± 0.09	
Dietary fiber (g)	31.3 ± 6.9***	21.1 ± 9.6	
Insoluble	15.6 ± 3.1	11.3 ± 3.6	
Soluble	15.7 ± 4.3	9.9 ± 6.3	
Vitamin A (μg RE)	593.7 ± 73.9	501.7 ± 220.4	700
Retinol (μg)	64.0 ± 38.2**	111.7 ± 62.9	
β-Carotene (μg)	3028.2 ± 429.2*	2247.2 ± 1216.8	
Vitamin B_1 (mg)	1.2 ± 0.2	1.1 ± 0.2	1.0
Vitamin B_2 (mg)	0.8 ± 0.2*	1.1 ± 0.3	1.2
Niacin (mg NE)	11.4 ± 2.2	13.5 ± 4.0	13
Vitamin B_6 (mg)	1.1 ± 0.3	1.1 ± 0.3	1.4
Pantothenic acid (mg)	1.5 ± 0.5	1.2 ± 0.6	
Vitamin B_{12} (μg)	0.8 ± 0.4***	2.7 ± 1.9	
Folic acid (μg)	176.6 ± 56.7***	99.1 ± 26.2	250
Vitamin C (mg)	67.4 ± 16.9*	54.3 ± 21.4	70
Vitamin E (mg)	10.7 ± 3.0	10.2 ± 4.1	10

1) Mean ± SD
2) Recommended Dietary allowances for Koreans (7th reversion, 2000) * : p<0.05, ** : p<0.01, *** : p<0.001

Table 3. Plasma carnitine levels in the subjects

Parameters	Vegetarians (n = 20)	Omnivores (n = 20)
NEC[2)] (nmol/ml)	53.38 ± 10.45[1)]	61.82 ± 12.90
ASAC[3)] (nmol/ml)	78.32 ± 19.96	72.84 ± 17.26
AIAC[4)] (nmol/ml)	1.39 ± 0.67	1.38 ± 0.61
TCNE[5)] (nmol/ml)	133.08 ± 28.73	136.04 ± 27.81

1) Mean ± SD 2) Nonesterified acylcarnitine
3) Acid soluble acylcarnitine 4) Acid insoluble acylcarnitine
5) Total carnitine

obesity (Moon et al. 1992 ; Kang et al. 1995 ; Kim et al. 1996). Recently the social view of beauty has emphasized excessive thinness (Ryu 1997 ; Won 1995), resulting in decreased food intake to give the appearance of fitness (Sung 1997). The Nutritional Survey for Korean College Women (Lee et al. 1998 ; Lee et al. 1998) also reported that female college students consume less energy than the Korean RDA.

Protein intake of vegetarians (55.5 ± 8.3g/d) was significantly lower than that of omnivores (65.8 ± 19.1g/d) ; likewise, fat intake of vegetarians was lower than omnivores ; (43.7 ± 6.9g/d) and (49.8 ± 7.5g/d), respectively. The omnivores consumed 109.7% of the RDA for protein, but vegetarians did not meet protein requirements (92.6% of the RDA for protein). The mean fat intake of both groups was higher than in the Korean Nutritional Survey Report of 1998 (Ministry of Health and Welfare 2001). However, the mean carbohydrate intake in vegetarians (293.2 ± 45.2g) was significantly higher than that of omnivores (236.2 ± 30.7g).

When the intake of an energy-providing nutrient was expressed as a percentage of total calories, vegetarians had a significantly higher percentage of energy from carbohydrate than omnivores (66.4 ± 3.8% vs. 58.8 ± 4.5%, respectively) and lower percentages from fat (20.9 ± 3.8% vs. 24.8 ± 3.2%, respectively) and protein (12.6 ± 0.7 vs. 16.3 ± 4.1, respectively). The ratio of percentage energy from carbohydrate : fat : protein was 66.5 : 20.9 : 12.6, respectively for vegetarians ; and 58.8 : 24.8 : 16.4, respectively for omnivores.

2. Blood and urinary carnitine levels

Plasma carnitine data is summarized in Table 3. There were no significant differences in NEC, AIAC, ASAC, or TCNE concentrations in plasma between groups. When the fatty acid oxidation was accelerated, the acylcarnitine concentration in the plasma increase in general (Cha et al. 1998 ; Cha et al. 1999). However, this phenomenon was not observed in this study.

Urinary carnitine data is summarized in Fig. 1. There were no significant differences between the two groups in NEC or AIAC. Vegetarians, however, had a significantly lower ASAC and TCNE than omnivores. Urinary excretion of carnitine has been shown to reflect carnitine status and changes usually parallel those in blood and dietary carnitine intake (Maebashi et al. 1976). However, reduced carnitine excretion can be a sensitive indicator of carnitine conservation, independent from plasma carnitine concentrations. For example, choline supplementation in humans can decrease carnitine excretion to less than one-fourth of that seen in non-supplemented persons, with little or no effect on plasma concentrations (Daily et al. 1995).

Vegetarian diets have been shown to cause greater reduction in the carnitine concentrations of urine than plasma. Lombard et al. (1989) compared to nonvegetarian, lactoovo-vegetarian, and strict vegetarian adult women and found pla-

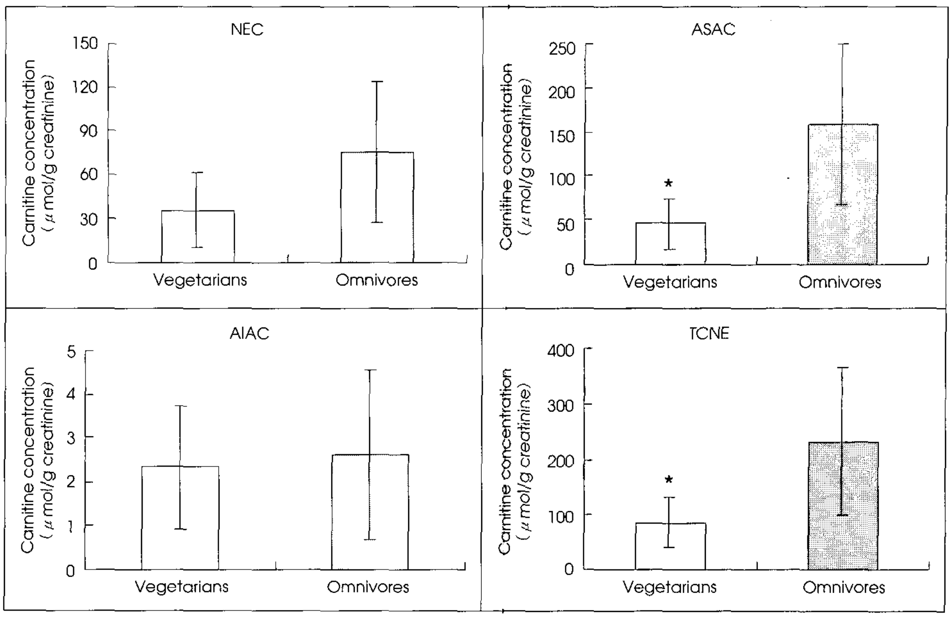

Fig. 1. Urinary carnitine levels in the subjects. The error bars show the standard deviations of the means for vegetarians and omnivores. Bars that have asterisks are significantly different ($p < 0.05$) between two groups by paired t-test. NEC : Nonesterified acylcarnitine ; ASAC : Acid-soluble acylcarnitine, AIAC : Acid-insoluble acylcarnitine ; TCNE : Total carnitine.

sma concentrations to be 43.3 ± 5.5, 36.1 ± 7.7, and 36.6 ± 8.2 nmol/ml, respectively ; and 24-hour urinary excretion was 4.66 ± 2.31, 1.55 ± 0.50, and 1.08 ± 0.31 μmol/kg/d, respectively. Those results indicated that carnitine homeostasis may be regulated to a large extent by changes in the renal threshold for carnitine. This study shows that people who habitually consume diets low in carnitine (vegetarian diets) have somewhat lower plasma carnitine concentrations and markedly lower urinary carnitine excretion than do subjects consuming a normal diet (omnivorous diet). However, plasma carnitine levels remained within the normal range in for both groups. It appears, therefore, that carnitine biosynthesis in conjunction with renal conservation is effective in maintaining carnitine status when there is low carnitine intake.

Other dietary factors, related to carnitine biosynthesis, may also influence plasma carnitine excretion. Cederblad (1987) reported that a high-fat, low-carbohydrate diet increased plasma carnitine concentrations and urinary carnitine excretion, whereas the reverse was true for a low-fat, high-carbohydrate diet. Because vegetarian diets are high in carbohydrate and low in fat, compared to omnivorous diets, our results may be attributable to, at least in part, the differences in the energy composition of the diets. Furthermore, Mitchell (1991) reported that dietary carnitine intake was positively related to food intake of zinc, protein, iron, and fat. Urinary carnitine output, however, was only related to dietary carnitine and protein. Therefore, lower carnitine excretion should be expected in vegetarians, because their diets are low in protein.

On the basis of the results of this study, we suggest that the higher levels of urinary acylcarnitine in omnivores may be a reflection of their lipid metabolic state. Korean vegetarian diets increase the renal conservation of carnitine.

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