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Plasma Carnitine Profiles in Different Aged Normal Korean Women : Hypothesis of Possible Significance

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ABSTRACT

The present study was conducted to find out whether there are some differences in plasma carnitine levels among young-, middle-, and old-aged normal Korean women. Daily food intake, body fat content, plasma lipids and carnitine levels were measured in 153 samples from 44 young (20 - 24 years old), 49 middle-aged (30 - 49 years old), and 63 old (65 - 85 years old) normal volunteers. The differences in concentrations of nonesterified acylcarnitine and acid-soluble acylcarnitine were not statistically significant among them. However, acid-insoluble acylcarnitine (AIAC) level in plasma decreased with age. Moreover, total carnitine (TCNE) level in the young group was significantly higher than in old and middle-aged groups. Body fat content in the young group was significantly lower than in old and middle-aged groups. Plasma total cholesterol increased with age and triglycerides in the old group were significantly higher than in young and middle-aged groups. These results suggest that the higher levels of AIAC and TCNE in the young group may be a reflection of their lipid metabolic state, which is different from middle-aged and old groups.

KEY WORDS : body fat content · plasma lipids · carnitine · age.

INTRODUCTION

Carnitine (β-hydroxy-γ-trimethyammonium butyrate) is a natural constituent of higher organisms, particularly in cells of animal origin. It is readily available in a nonvegeterian diet and is also synthesized in normal healthy adults.[1] However, reports of systemic and conditional deficiency of carnitine continue to accumulate in the literature.[2-4] The best characterized function of carnitine is in transporting long chain fatty acids (the most common type in foods) across the mitochondrial membrane where they are oxidized with a resulting release in energy.[5] Carnitine also functions in the translocation of branched chain keto acids[6] and exit of excess acyl groups from inside of the mitochondrial matrix.[7] Without carnitine, most of the fatty acids that are eaten by human could not be metabolized for energy and thus an important energy source would be eliminated. This would result in less efficient utilization of fatty acids for energy and as a result a greater storage of fatty acids as adipose tissue.[8]

It has been traditionally believed that increased body fat content accompanies aging.[9] In middle and late adulthood all people experience a series of progressive changes in body composition. The lean body mass shrinks and the mass of adipose tissue expands. The contraction in lean body mass reflects atrophic processes in skeletal muscle, liver, kidney, spleen, skin, and bone.[10] These structural changes result in increased blood lipids and lipoprotein levels, unavoidable results of aging.

Therefore, it is interested to determine age-related variations in carnitine profiles. Since oriental diets contain less carnitine than in westernized countries[11] and because of the unique role of carnitine in the transport of fatty acids into mitochondria, it might be an implement of fat metabolic status.

The purpose of this study was to assess age-related differences in plasma carnitine profiles in normal Korean women and find out whether the carnitine levels might be correlated with body fat and plasma lipid profiles.

METHODS

1. Subjects

The subjects, 44 young (20 - 24 years old), 49 middle-aged (30 - 49 years old), and 63 old (65 - 85 years old) normal volunteers consented to participate in this study.

2. Physical assessment and blood collection

Height (m), weight (kg), and body fat content (%) were

taken at the time of blood sampling. BMI (body mass index) was calculated[12] by the weight/height^2 and fat content (%) was measured by the Bioelectrical Impedence Fatness Analyzer (Gil-Woo Trading Company). The blood was collected in heparinized tubes after 12 hours fast, and plasma was separated by centrifugation at 4℃ at 3, 000 rpm, prepared, frozen, and stored at −20℃ until analyzed.

3. Survey of dietary intake

The diet survey was conducted by 24-hour recall method for three consecutive days excluding weekends.[13][14] All diets were assessed by the same researcher for all three days for each individual. Direct interview for each subject was carried out with the aid of measuring instruments and books for eye measurement.[15] Intake of energy, carbohydrate, protein, and fat were calculated using nutrient contents of Korean foods.[16] For each subject, an average value of three days for a particular nutrient was used as the mean daily intake for the nutrient and compared with Korean RDA.

4. Plasma lipid parameters

Plasma total cholesterol was analyzed with a commercial kit (Youngdong Pharmaceutical Co., Korea). High density lipoprotein (HDL)-cholesterol was analyzed with the same method, following the precipitation of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) with dextran sulfate-Mg^{++} (Kyotto Pharmaceutical Co., Japan). LDL-cholesterol was calculated by substrating the combined VLDL and HDL, which were assayed as mentioned above after precipitation of LDL-cholesterol using an assayed Quantolip commercial kit (Imuno AG, Wein, Austria). Triglyceride in plasma was assayed using a commercial kit (Youngdong pharmaceutical Co., Korea).

5. Analysis of carnitine

Carnitine was assayed using a modified radioisotopic method of Cederblad and Lindstedt.[17][18] Acid-insoluble (long chain) acyl carnitine (AIAC) was precipitated with perchloric acid and centrifugation leaving the short-chain acid-soluble acyl carnitine (ASAC) and the nonesterified free carnitine (NEC) in the supernatant. An aliquot of the supernatant was assayed to determine the NEC and another aliquot hydrolyzed with 0.5 mol/L KOH to assay all acid-soluble carnitine (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 0.5 mol/L KOH for 60 min in a hot water bath st 60℃. In each case, carnitine was assayed by using carnitine acetyltransferase (Sigma Chemical Co., St. Louis, Mo, USA) to esterify the carnitine to a [^{14}C]acetylcarnitine from [1-^{14}C]acetyl CoA (Amersham, Little chalfont, Buckinghamshire England). Radioactivity of samples was determined in a Beckman LS-3801 liquid scintillation counter (Beckman Instruments, Palo Aalto, CA).

6. Data analysis

All data was expressed as mean±SEM and differences among group means were determined by analysis of variance and Tukey test using GraphPad Version 2.0 (GrapPad San Diego, CA, USA). Results were considered significant at $p<0.05$.

RESULT AND DISCUSSION

The average age of young, middle-aged, and old groups was 22.67, 39.27, and 75.7 years old, respectively (Table 1). Weight and height averages of the old group were 47. 94 kg and 146.3 cm, significantly lower than those of young and middle-aged groups. It has been reported that atrophic processes in skeletal muscle, liver, kindney, spleen, skin, and born are unavoidable results of aging.[2] There were no statistically significant difference in their daily nutritional intake (Table 2) and there were significant correlations among groups (Table 6). we conducted

Table 1. Anthropometric parameters of the subjects

Variable	Groups		
	Young (n=44)	Middle-aged (n=50)	Old (n=60)
Age	22.67±0.14$^{a1)2)}$	39.27±1.17^{b}	75.74±1.49^{c}
Weight	50.13±1.59^{ab}	55.6 ±1.37^{b}	47.94±2.12^{c}
Height	160.27±1.87^{a}	157.9 ±1.37^{a}	146.25±1.44^{c}
BMI[3]	18.66±0.90^{a}	22.6 ±0.53^{b}	22.46±0.87^{b}
MUAC[4]	23.3 ±0.52^{a}	27.7 ±0.6^{b}	23.95±0.49^{a}

1) Mean±SEM
2) Differnet superscripts in the same row indicate significant differences ($p<0.05$) between groups by Tukey test
3) Body mass index
4) Mid-upper-arm-circumference

Table 2. Daily nutrient intake of the subjects

Variable	Groups		
	Young (n=44)	Middle-aged (n=50)	Old (n=60)
Energy (Kcal)	1766±454[1)]	1945.63±82	1872.99±140.75
Protein (g)	81.3±47.8	75.48±5.3	51.39±5.4
Lipid (g)	55.02±21	30.45±5.2	20.96±2.82
Carbohydrate (g)	232±87	334.58±15.3	367.20±27.04
Fiber (g)	10.5±7.52	8.54±0.5	5.70±0.54

1) Mean±SEM

this experiment by using a bioelectric impedance fatness analysis that can detect not only subcutanous fat, but also abdominal fat. Previous studies of middle school students [19] and middle-aged women[20] showed that BMI positively correlated with their body fat content. The present study showed that BMI of middle-aged and old groups positively correlated with their body fat (%), but not in the young group. However, the BMI of young and middle-aged groups were positively correlated with their LBM, but not in the old group (Table 6). These results indicated that LBM shrinks and the mass of adipose tissue expands as people are getting old.

Generally, it has been considered that analysis of blood lipid fractions is the first method of diagosis of coronary heart disease and atherosclerosis. It has been reported that blood total cholesterol, total lipids, triglycerides, and LDL-cholesterol concentrations increase with age.[21] In our study, blood HDL- and LDL-cholesterol concentrations among the groups were not statistically different. However, total cholesterol (TC) and Triglyceride (TG) levels in blood gradually increased with age, as TC and TG concentrations in the old group showed significantly higher values than in the of young group. There were no correlations between blood lipids and anthropometric parameters in this study, except the positive correlation between blood TG and BMI on middle-aged group (Table 6).

Carnitine is an essential factor for the oxidation of fatty acids. It is endogenously synthesized in humans, but seems to act as an essential nutrient under special circumstances that either increase the need for carnitine or decrease its synthesis.[22] For example, in rats maintained at 2℃ there was an increase in body carnitine that was apparently related to increased fat oxidation to meet increased thermogenic needs.[23] Carnitine is readily available in nonvegeterian diets and is also synthesized in normal healthy adults.[3] However, reports of systemic and conditional deficiency of carnitine continue to accumulate in the literature.[4-6] Especially, patients with Alzheimer's disease had consistantly lower (25-40%) carnitine concentrations in their brain tissues when compared with control subjects.[24] Carnitine is of particular interest as an antiaging compound because, when chronically administered to aged rats, it has been shown to reduce age-related cognitive, [25][26] mophological, and neurochemical[27] changes through its cholinomimetic activities.[28] It has been generally accepted that endogenous carnitine biosynthesis is enough to meet carnitine pools for normal persons, but exogenous carnitine supplements would be helpful for people who are either unhealthy (i.e. those with cancer or dementia) or who over exert themselves (i.e. athletes). Dodson *et al.*[5]

Table 3. Body fat of the subjects

Variable	Groups		
	Young (n=44)	Middle-aged (n=50)	Old (n=60)
Body fat (%)	22.2 ±2.5[a1)2)]	28.4±0.8[b]	28.31±1.25[b]
Fat weight (kg)	10.06±1.7[a]	15.5±0.7[b]	13.14±1.06[ab]
Lean body mass (kg)	30.6 ±2.5[a]	38.5±1.0[b]	33.26±2.14[a]
Total water (%)	32.42±1.0[a]	28.6±0.6[b]	54.8±0.94[c]

1) Mean±SEM
2) Differnet superscripts in the same row indicate significant differences ($p<0.05$) between groups by Tukey test

Table 4. Plasma lipids and lipoprotein concentration in subjects

Variable	Groups		
	Young (n=44)	Middle-aged (n=50)	Old (n=60)
T-chol[3)]	108.72±8.4[a1)2)]	156.67±19.8[ab]	195.40±15.6[b]
TG[4)]	49.56±11.5[a]	88.67±8.79[a]	161.25±14.5[b]
HDL-C[5)]	51.33±13.9	65.67±4.27	40.67±3.24
LDL-C[6)]	108.27±28	90.28±8.6	118.67±5.30

1) Mean±SEM
2) Differnet superscripts in the same row indicate significant differences ($p<0.05$) between groups by Tukey test
3) Total cholesterol 4) Triglycerides
5) HDL-cholesterol 6) LDL-cholesterol

Table 5. Plasma carnitine concentration in the subjects

Variable	Groups		
	Young (n=44)	Middle-aged (n=50)	Old (n=60)
NEC[3)] (μmol/L)	43.93±2.87[1)2)]	39.80±2.06	39.70±3.35
ASAC[4)] (μmol/L)	18.39±2.86	10.70±2.47	12.73±2.11
AIAC[5)] (μmol/L)	7.56±0.52[a]	3.03±0.63[b]	1.39±0.16[b]
TCNE[6)] (μmol/L)	66.54±3.68[a]	52.62±3.4[b]	51.95±3.36[b]

1) Mean±SEM
2) Differnet superscripts in the same row indicate significant differences ($p<0.05$) between groups by Tukey test
3) Nonesterified acylcarnitine 4) Acid-soluble acylcarnitine
5) Acid-insoluble acylcarnitine 6) Total carnitine

Table 6. Correlation coefficients in young, middle-aged, and old groups

		BMI[1)]	Body fat (%)	TG (mg/dl)
Body fat (%)	Young	0.117		-0.190
	Middle-aged	0.389**	-	-0.370
	Old	0.523*		-0.370
LBM[2)] (kg)	Young	0.687***	0.208	0.431
	Middle-aged	0.465***	-0.115	0.001
	Old	0.271	0.147	0.156
TG[3)] (mg/dl)	Young	0.249	-0.190	
	Middle-aged	0.318*	0.210	-
	Old	-0.255	-0.370	
NEC[4)] (μmol/L)	Young	0.069	-0.102	0.469*
	Middle-aged	0.227	0.314*	0.299*
	Old	-0.199	-0.148	-0.165

Pearson correlation coefficients ($p<0.05$, **$p<0.01$, ***$p<0.001$)
1) Body mass index 2) Lean body mass
3) Triglycerides 4) Nonesterified acylcarnitine

reported that all fractions of carnitine were significantly decreased in female cancer subjects, which can be partially attributed to the increased renal clearance of ASAC and AIAC and decreased tubular reabsorption of carnitine. The present study evaluated the effects of age on blood carnitine fractions (Table 5).

There were no differences in nonesterified acylcarnitine (NEC) and acid-soluble acylcarnitine (ASAC), but acid-insoluble acylcarnitine was significantly lowered with age. Over all, blood total carnitine (TCNE) in the young group showed significantly higher values than those of both middle-aged and old groups. It is known that renal handling of carnitine influences blood carnitine level.[29] More than 90% of all filterated carnitine is reabsorbed by the kidney under phsiological conditions.[30] We did not study whether renal handling of carnitine has an effect on age-dependence in plasma carnitine on this study. However, some previous reports with other populations clarified the effects of age-related variations and the sex-related differences on serum carnitine profiles. They reported that serum carnitine is regulated by sexual hormones,[11] as androgens and estrogens influence the serum carnitine levels in rats.[31] It has also been show that plasma levels of acylcarnitine are sharply elevated under physiological conditions of accelerated fatty acid oxidation.[32][33]

Therefore, our results showing higher levels of AIAC and TCNE in the young group than in the old group might imply that there are some metabolic differences in maintaining plasma carnitine concentrations among different-aged groups. Also, there was found to exist statistically significant correlations between carnitine concentrations, body compositions, and blood lipid profiles. Alteration of carnitine concentrations with age might have led to the increased accumulation of lipid in old age because of its role in fatty acid oxidation. Our group has clarified that exogenouly added L-carnitine has an inhibitory role on the differentiation of the 3T3-L1 cells (unpublished). However, to clarify this assumption in vivo, further research is needed on the short term or long term carnitine supplementation studies with Korean subjects of different age groups.

Literature cited

1) Bieber LL. Carnitine. *Ann Rev Biochem* 57 : 261-283, 1988
2) *Winter SC, Simon M, Zorn EH, Szabo-Aczel S, Vance WH, O'Hara T,* Higashi L. Relative carnitine insufficiency in children with Type I diabetes mellitus. *AJDC* 143 : 1337-1339, 1989
3) Dodson WL, Sachan DS, Krauss S, Hanna W. Alteration of serum and urinary carnitine profiles in cancer patients : Hypothesis of possible significance. *J Am College Nutr* 8 : 133-142, 1989
4) Olson AL, Nelson SE, Rebouche CJ. Low carnitine intake and altered lipid metabolism in infants. *Am J Clin Nutr* 49 : 624-628, 1989
5) Fritz I. Action of carnitine on long-chain fatty acid oxidation by liver. *Am J Physiol* 197 : 297-304, 1959
6) May M, Aftring R, Buse M. Mechanisms of stimulation of branched chain fatty acid oxidation in liver by carnitine. *J Biol Chem* 255 : 8394-8397, 1980
7) Hochachka P, Neely J, Driedzic W. Integration of lipid utilization with Krebs cycle activity in muscle. *Fed Proc* 36 : 2009-2014, 1977
8) Rebouche CJ, Paulson BJ. Carnitine metabolism and function in humans. *Ann Rev Nutr* 6 : 41-46, 1986
9) Beauchene RE, Bales CW, Bragg CS, Hawkins ST, Mason RL. Effect of age of initiation of feed restriction on growth, body composition, and longevity of rats. *J Gerontology* 41(1) : 13-19, 1986
10) Rudman D. Growth hormone, body composition, and aging. *J Am Geriatr Soc* 33 : 800-807, 1985
11) Takiyama N, Matsumoto K. Age and sex-related differences of serum carnitine in a Japanese population. *J Am College Nutr* 17(1) : 71-74, 1998
12) Lohman TG. Anthropometry and body composition. In Lohman TG, Foche AF, Martrorell R. (eds.), "Anthoropometric standarisation reference manual" Champaign, III : Human Kinetics Books, 1988
13) Lee H, McGuire V, Boyd NF. A review of the methods used by studies of dietary measurement. *J Clinical Epidemiology* 42 : 269-279, 1989
14) Block G. A review of validation of dietary assessment methods. *Am J Epidemiology* 115 : 492-505, 1982
15) The society of Korean Food and Technology, Food Research Center. The amounts of food and meal by eye measurement for surveying dietary intake, 1988
16) Recommended dietary allowances for Koreans, 6th revision, the Korean Nutrition Society, Seoul, 1995
17) Cederblad G, Lindstedt S. A method for determination of carnitine in picomole range. *Clin Chim Acta* 37 : 335-343, 1972
18) Sachan DS, Rhew TH, Ruark RA. Ameliorating effects of carnitine and its precursors on alcohol-induced fatty liver. *Am J Clin Nutr* 39 : 738-744, 1984
19) Kim HS, Lee YN, Mo SM, Choi HM. Assessment of obesity through anthropometric assessment in junior high school boys and girl. *Korean J Lipidology* 4(1) : 41-49, 1994
20) Cha YS, Kim IS, Joo EJ. Comparison of body fat metabolism in middle-aged women depending upon swimming practice. *Korean J Nutr* 28(5) : 397-405, 1995
21) Austin MA. Atherosclerosis reviews. (Gotto AM, Paoletti R eds) 22 pp. 65-69, Raven Press, LTD, NY, 1993
22) Feller AG, Rudman D. Role of carnitine in human nutrition. *J Nutr* 118 : 541-548, 1990
23) Tsai AC, Ramsos DA, Leveille GA. Determination of carnitine turnover in choline-deficient and cold exposed rats. *J Nutr* 105 : 301-307, 1974
24) Rajesh NK, Sami IH. Carnitine acetyltransferase activity in the human brain and its microvessels is decreased in Alzheimer's disease. *Ann Neurol* 32 : 583-586, 1992
25) Spagnoli A, Lucca U and Menasece G. Long-term acetyl-L-carnitine

treatment in Alzheimer's disease. *Neurology* 41 : 1726-1732, 1991

26) Castorina M, Ferraris L. Acetyl-L-carnitine affects aged brain receptorial system in rodents. *Life Science* 54(17) : 1205-1214, 1994

27) Angelucci L, Ramacci MT. Biological Psychiatry. Development in Psychiatry. pp.1140, Elsevier Science Publishing, New York, 1986

28) Sterri SH, Fonnum F. Acetyl-CoA synthesizing enzymes in cholinergic nerve terminals. *J Neurochem* 35 : 249-254, 1980

29) Cederblad G, Fahrseus L, Lindgren K. Plasma carnitine and renal carnitine clearance during pregnancy. *Am J Clin Nutr* 44 : 379-383, 1986

30) Rebouche CJ, Mack DL. Sodium gradient-stimulated transport of L-carnitine into renal brush border membrane vesicles : Kinetics specificcity and regulation by dietary carnitine. *Arch Biochem Biophys* 235 : 395-402, 1984

31) Borum PR. Regulation of the carnitine concentration in plasma In Frenkel RA, McGarry JD (eds) : "Carnitine Biosynthesis, Metabolism and Function", New York Academic Press, pp.115-126, 1980

32) Cha YS, Li B, Sachan DS, Whelan J. Dietary docosahexaenoic acid decreases plasma triglycerides with mixed effects on indices of β-oxidation. *Korean J Nutr* 30(9) : 1067-1072, 1997

33) Osmundsen H, Bremer J, Pedersen JI. Metabolic aspects of peroxisomal β-oxidation. *Biochim Biophys Acta* 1085 : 141-158, 1991