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Dietary Protein Level and Dietary Interaction Affect Quinolinic Acid Concentration in Rats

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Abstract: During tryptophan-niacin conversion, hepatic α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) [EC4.1.1.45] plays a key role in regulating NAD biosynthesis. ACMSD activity is greatly affected by many factors such as nutritional status and disease. The tryptophan catabolite quinolinic acid has been reported to be associated with the pathogenesis of various disorders and is a potential endogenous toxin. However the effects of dietary protein levels or dietary interaction between protein levels and fatty acid type to this process have not been investigated and are still unknown.

In this study, we examined whether dietary protein level, fatty acid type, namely saturated fatty acid and polyunsaturated fatty acid, and their interaction affect serum quinolinic acid concentration in rats. Male Sprague-Dawley rats (4-weeks old) were fed with 20% casein + 10% stearic acid diet (20C10S), 20% casein + 10% linoleic acid diet (20C10L), 40% casein + 10%stearic acid diet (40C10S), or 40% casein + 10% linoleic acid diet (40C10L) for 8 days, and serum quinolinic acid concentration and ACMSD activity were determined. Serum quinolinic acid concentration was significantly increased in the 40C10L group compared with other three groups. There was also the negative correlation between the sum of liver and kidney ACMSD activities, and serum quinolinic acid concentration per tryptophan intake ($r = 0.8209$, $p < 0.01$). Increased serum QA concentrations are probably due to a decreased ACMSD activity.

Key words: Tryptophan-niacin metabolism, tryptophan, protein, quinolinic acid, aminocarboxymuconate-semialdehyde decarboxylase

Introduction

Hepatic α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD; picolinic carboxylase) [EC4.1.1.45] plays a key role in regulating NAD biosynthesis from tryptophan, as shown in Figure 1. It has been reported that there is an inverse relationship between ACMSD activity and the production of NAD converted from tryptophan [1, 2].

The tryptophan catabolite quinolinic acid has been reported to be associated with the pathogenesis of certain neurodegenerative diseases, since it acts as an excitotoxic agonist of the N-methyl-D-aspartate receptor [3, 4]. Increase of quinolinic acid has been observed in human

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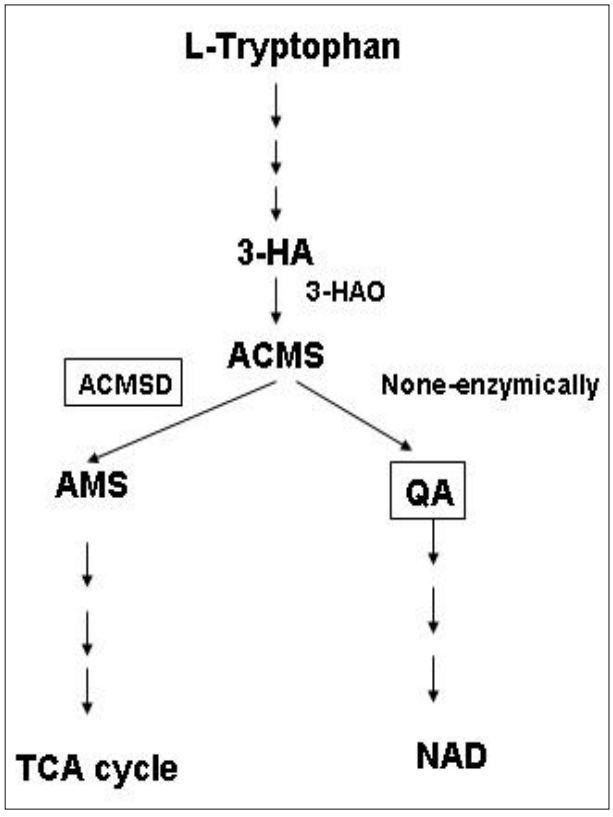

Figure 1: Schematic diagram of tryptophan-NAD metabolism. 3-HA, 3-Hydroxyanthranilate; ACMS, α-amino-β-carboxymuconate-ε-semialdehyde; AMS, α-aminomuconate-ε-semialdehyde; QA, quinolinic acid; TCA, tricarboxylic acid; NAD, nicotinamide adenine dinucleotide; 3-HAO, 3-hydroxyanthranilate 2,3-dioxygenase; ACMSD, α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase.

serum and cerebrospinal fluid and in animal models of severe hepatic injury [5, 6]. On the other hand, quinolinic acid is a cation chelator and inhibitor of gluconeogenesis [7]. Pawlak *et al* have shown that quinolinic acid can be a uremic toxin responsible for anemia associated with renal failure by reducing production of erythropoietin, which promotes erythrocyte formation [8]. The accumulation of a large amount of quinolinic acid within a disease state may be of functional significance. ACMSD seems to affect the production of quinolinic acid. We previously reported that the concentration of serum quinolinic acid of the 20% linoleic acid diet-fed rats increased two-fold compared to that of the fatty acid-free diet-fed rats [9]. However, we studied only two groups, namely the fatty acid-free group and the 20% linoleic acid group. The effects of other fatty acids beside linoleic acid, protein levels, and their interaction on the quinolinic acid concentration have not been investigated and are still unknown.

Hepatic ACMSD activity is greatly affected by many factors such as nutrients, hormones, and diseases [2, 10–13]. We presume that a factor that can greatly change hepatic ACMSD activity could also affect quinolinic acid concentration. Increase of ACMSD may play an important role in prevention of progression to disease according to reduction of the quinolinic acid toxicity. It is important to know the tryptophan-quinolinic acid conversion under the conditions of a disease associated with quinolinic acid toxicity from the viewpoint of nutrition and medicine.

In this study, we focused on the change of quinolinic acid concentration and examined whether protein level, fatty acid type, namely saturated fatty acid and polyunsaturated fatty acid, and their interaction affect the serum quinolinic acid concentration in rats, and studied the relationship between quinolinic acid concentration and ACMSD activity.

Materials and Methods

Chemicals. 3-Hydroxyanthranilic acid was obtained from Sigma Chemical Co. (St. Louis, MO,U.S.A.). Linoleic acid and stearic acid were purchased from Wako Pure Chemicals (Osaka, Japan). Their chemical form is free fatty acid, and is not in mono-, di-, or triglyceride form.

Animals. Rats were purchased from CLEA Japan (Tokyo, Japan) and housed in individual cages at 22 ± 1°C with a 12-hour light:dark cycle (lights on, 7:00–19:00 h). They were allowed free access to food and water until being sacrificed under Nembutal anesthesia at the end of the experiment.

Four-week-old male Sprague-Dawley rats were fed a commercial CE2 diet (CLEA Japan), which is adequate for normal growth. After this acclimatization period of 4 days, they were divided into 4 groups and fed 20% casein + 10% stearic acid (20C10S), 20% casein + 10% linoleic acid (20C10S), 40% casein + 10% stearic acid (40C10S), or 40% casein + 10%linoleic acid (40C10L) containing diets (Table I) for the next 8 days.

All rats were sacrificed between 9:00 and 11:00 h, and their livers were immediately perfused via the portal vein with ice-cold physiological saline solution (140 mmol/L NaCl) and excised. Blood was drawn by heart puncture under Nembutal anesthesia [0.13mL (pentobarbital sodium 6.5 mg)/100 g body weight]. Blood samples were immediately centrifuged at 3,000 rpm for 20 minutes, and serum was separated. And serum samples were stored at –30°C until analyzed. The liver samples were subjected to measurement of ACMSD activity. The care and treat-

Table I: Composition of diets

Ingredient	20C10S	20C10L	40C10S	40C10L
		g/kg diet		
Casein	200.00	200.00	400.00	400.00
DL-Methionine	3.00	3.00	3.00	3.00
Cornstarch	150.00	150.00	150.00	150.00
Sucrose	450.00	450.00	250.00	250.00
Mineral mixture*	35.00	35.00	35.00	35.00
Vitamin mixture*	10.00	10.00	10.00	10.00
Choline bitartrate	2.00	2.00	2.00	2.00
Cellulose powder	50.00	50.00	50.00	50.00
– Fatty acid				
Stearic acid	100.00	0.00	100.00	0.00
Linoleic acid	0.00	100.00	0.00	100.00

*AIN-76

20C10S: 20% Casein + 10% Stearic acid, 20C10L: 20% Casein + 10% Linoleic acid, 40C10S: 40% Casein + 10% Stearic acid, 40C10L: 40% Casein + 10% Linoleic acid.

The amounts of tryptophan in 20% and 40% casein diet were 2200 and 4400 mg/kg diet, respectively. The niacin content of each diet was 30 mg/kg diet.

ment of the rats were carried according to the guideline prescribed in Faculty of Horticulture, Chiba University and the National Institutes of Health Guide for the care and use of laboratory animals [14].

Quantification of quinolinic acid. Quinolinic acid in serum was quantified by an electron capture negative chemical ionization gas chromatography/mass spectrometry assay as previously described [15].

Assay for ACMSD activity. Livers or kidneys were homogenized in 3 volumes of ice-cold buffer, 50 mmol/L potassium phosphate buffer (pH 7.0) containing 140 mmol/L potassium chloride, 5 mmol/L 2-mercaptoethanol, 1 mmol/L dithiothreitol, 1 mmol/L EDTA-2Na and 1 mmol/L phenylmethanesulfonyl fluoride, with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 105,000 × g for 1 hour at 4°C. Activity of ACMSD in the cytosolic fraction was determined by measuring the decrease in absorbance of ACMS at 360 nm, as described previously [16, 17]. In brief, the pre-assay mixture (total volume 2.8 mL) consisted of 24 μM 3-hydroxyanthranilic acid (3HA), and 10–20 m-units/mL 3HA dioxygenase solution prepared from rat liver in 71 mM Tris-acetate buffer solution, pH 8.0 was incubated at 25°C, with monitoring of the increase in absorbance at 360 nm due to the formation of α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) from 3-HA. After the reaction was completed within approximately 2 minutes, 200 μL of cytosolic fraction was added, and the decrease in absorbance at 360 nm was followed at 15-second intervals. The rate of the decrease in absorbance caused by ACMSD was calculated by subtracting that of the control reaction mixture without ACMSD from that decreased above. For the calculation of activity, a molar absorption coefficient of 4.5×10^4 for ACMS was used under these conditions [16, 17]. ACMSD is known as the only enzyme that reacts with ACMS [18]. The ACMSD activity was expressed as μmol ACMS disappearance per hour per g of wet weight tissue or per whole tissue.

Statistical analysis. Data are shown as mean ± SEM. The effects of fatty acid types and protein levels were analyzed by two-way ANOVA. Tukey's multiple-range test was used for comparisons among groups. A difference with $p < 0.05$ was considered significant.

Results

Initial body weights were similar in all groups (Table II). Food intakes were significantly lower ($p < 0.01$) in the linoleic acid-fed groups (20C10L, 40C10L) than in the stearic acid-fed groups (20C10S, 40C10S). Food efficiencies were significantly higher ($p < 0.01$) in the linoleic acid-fed groups (20C10L, 40C10L) than in the stearic acid-fed groups (20C10S, 40C10S). Fatty acid type affected food intake and food efficiency.

Serum quinolinic acid concentration of 40C10L was significantly higher than other groups (Figure 2A). There were interactions between protein levels and fatty acid type on serum quinolinic acid concentrations. Serum quinolinic acid concentrations per milligram of tryptophan intake were higher in rats fed diets with linoleic acid than those in the rats fed diets with stearic acid (Figure 2B). Protein levels and fatty acid type affected serum quinolinic acid concentration.

Table II: Body weight gain, food intake, and liver and kidney weight in rats[1]

	20C10S	20C10L	40C10S	40C10L	*P-Value*		
					P[2]	F[3]	P×F[4]
Initial weight, g	130 ± 2	130 ± 1	130 ± 2	130 ± 1	NS[5]	NS	NS
Body weight gain, g/8 days	62.4 ± 2.4	65.4 ± 1.9	61.6 ± 2.2	68.8 ± 1.7	NS	< 0.05	NS
Food efficiency[6]	0.41 ± 0.01[c]	0.54 ± 0.01[b]	0.41 ± 0.01[c]	0.58 ± 0.01[a]	< 0.05	< 0.01	NS
Food intake, g/day	19.1 ± 0.5[a]	15.2 ± 0.3[b]	18.3 ± 0.5[a]	14.8 ± 0.2[b]	NS	< 0.01	NS
Liver weight, g	9.73 ± 0.28	10.5 ± 0.1	9.65 ± 0.30	10.1 ± 0.3	NS	< 0.05	NS
Kidney weight, g	1.81 ± 0.10[ab]	1.59 ± 0.03[b]	1.91 ± 0.05[a]	1.85 ± 0.04[a]	< 0.01	< 0.05	NS

[1] Values are means ± SEM, n = 5; Means in a row with different superscript letters differ, $p < 0.05$. (For example, food efficiency of 20C10S (alphabet c) was significantly lower than those of 20C10L (alphabet b) and 40C10L (alphabet a). However, food efficiency of 20C10S (alphabet c) was not significantly different from that of 40C10S (alphabet c). Alphabet order shows the order of a high value.

[2] Significant main effect of protein level.

[3] Significant main effect of fat.

[4] Significant protein × fat interaction.

[5] NS, not significant, $p > 0.05$.

[6] Food efficiency = weight gain/food intake.

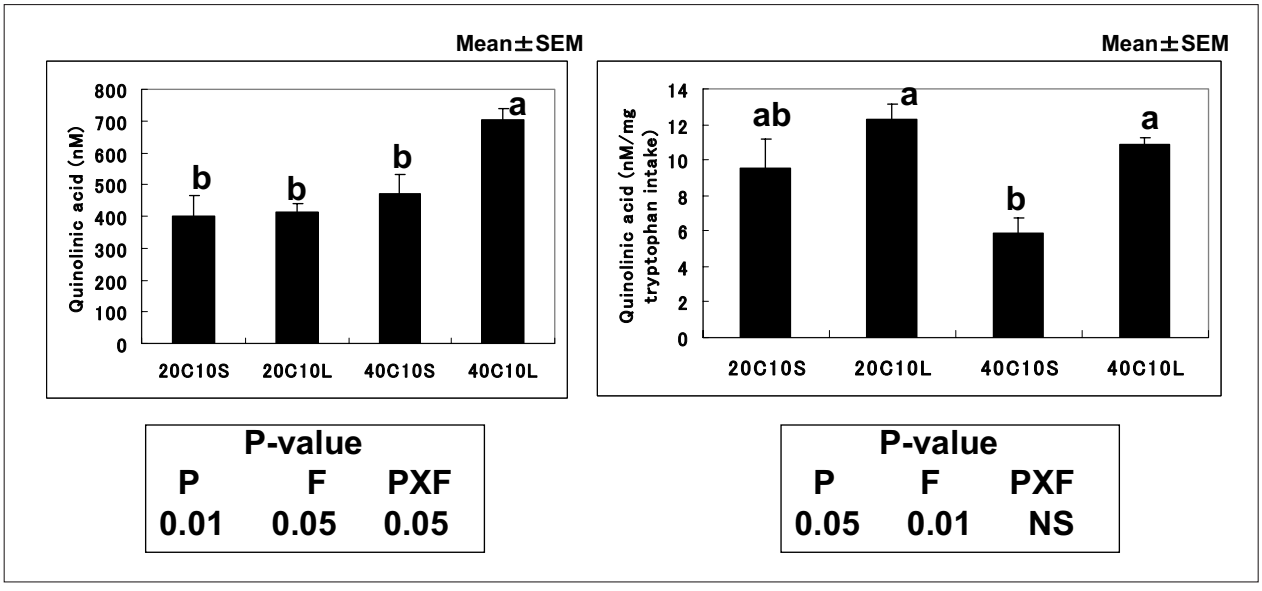

Figure 2: Effects of dietary protein level and the fatty acid type on quinolinic acid concentration. Values are means ± SEM, n = 5. Different superscript letters indicate significant difference, $p < 0.05$. 20C10S: 20% Casein + 10% Stearic acid, 20C10L: 20% Casein + 10% Linoleic acid, 40C10S: 40% Casein + 10% Stearic acid, 40C10L: 40% Casein + 10% Linoleic acid.

Hepatic ACMSD activities of the rats fed linoleic acid (20C10L, 40C10L) were significantly much lower ($p < 0.01$) than those of the stearic acid-fed groups (20C10S, 40C10S) (Figure 3A,B). In rats fed with stearic acid groups, hepatic ACMSD activities of the rats fed with 40% casein (40C10S) were significantly higher ($p < 0.01$) than those of the 20% casein-fed groups (20C10S). There were interactions between protein levels and fatty acid type on liver ACMSD activity (Figure 3A,B). Kidney ACMSD activity was lower in rats fed diets with 20% casein than in those fed diets with 40% casein, regardless of the type of fatty acid (Figure 3C,D). Protein level affected kidney ACMSD activity (Figure 3C,D).

The relationship between ACMSD activity in the liver and serum quinolinic acid concentration per milligram of tryptophan intake was highly negatively correlated as shown in Figure 4A. The correlation coefficient value was 0.8065 ($p < 0.01$, $n = 20$). There was also the negative cor-

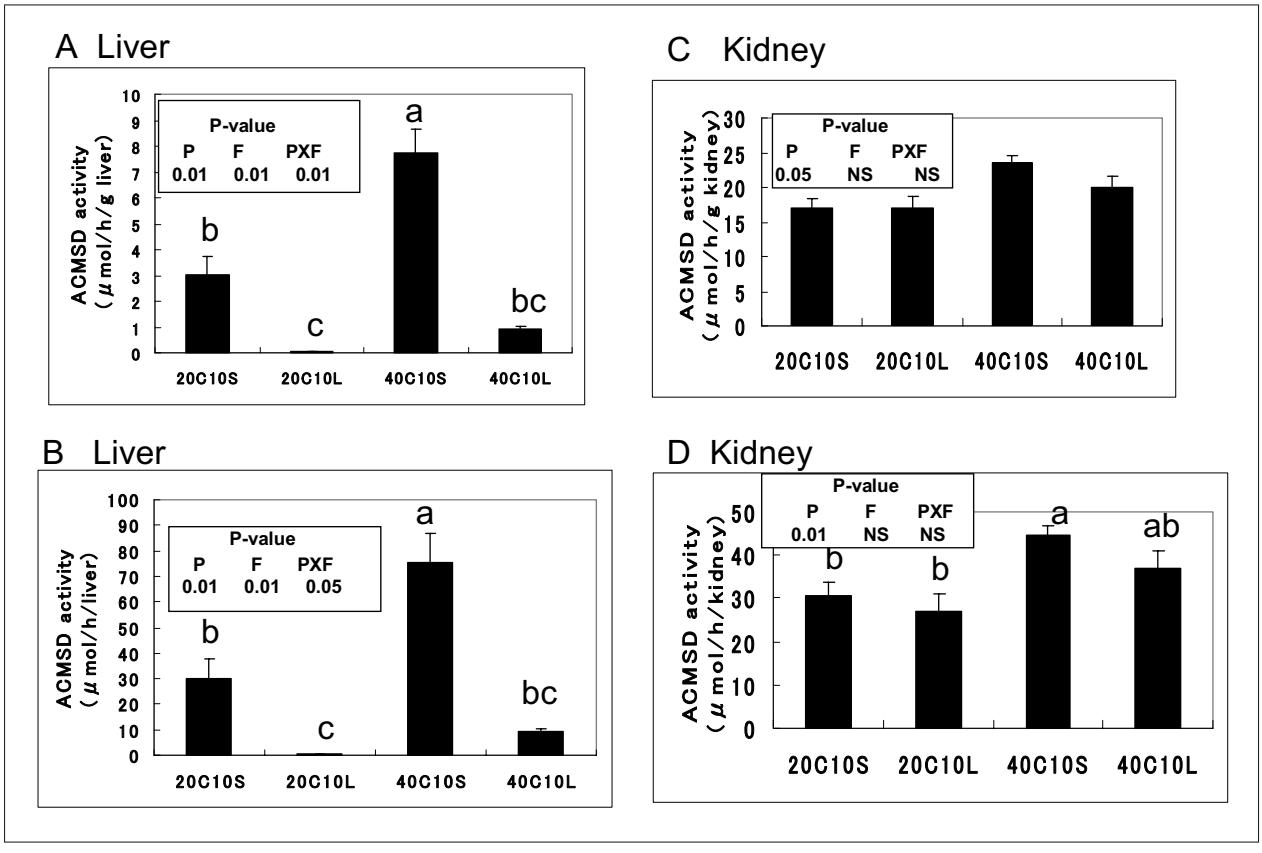

Figure 3: Effects of dietary protein level and the fatty acid type on α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) activity of rats. Values are means ± SEM, n = 5. Different superscript letters indicate significant difference, $p < 0.05$. 20C10S: 20% Casein + 10% Stearic acid, 20C10L: 20% Casein + 10% Linoleic acid, 40C10S: 40% Casein + 10% Stearic acid, 40C10L: 40% Casein + 10% Linoleic acid.

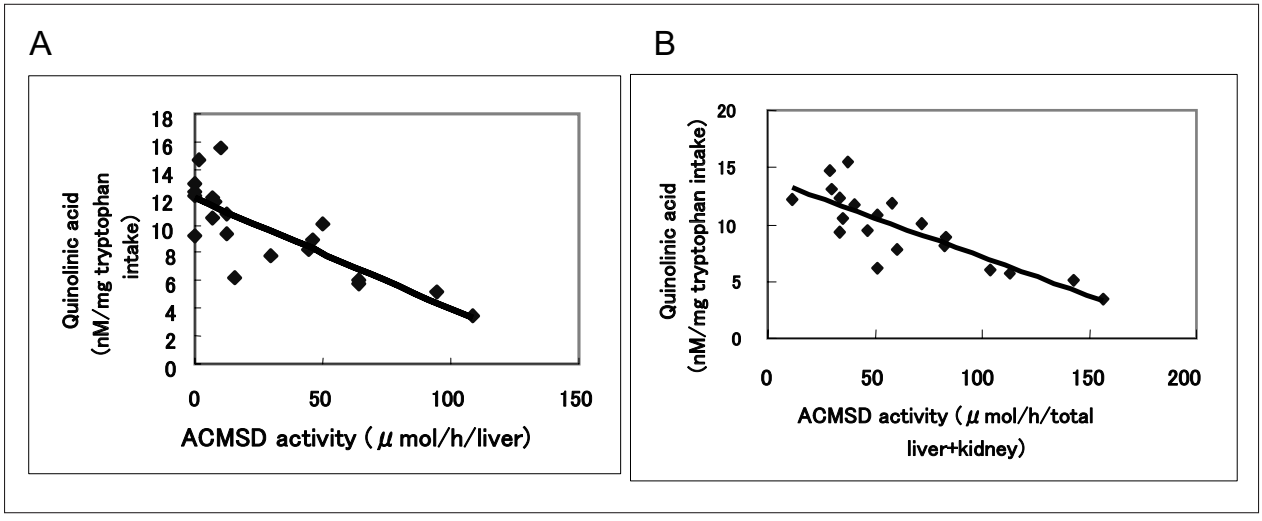

Figure 4: Relationship between the α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) activity and the serum quinolinic acid levels per milligram tryptophan intake. n = 20.

relation between the sum of ACMSD activities in the liver and kidney, and serum quinolinic acid concentration per milligram of tryptophan intake ($r = 0.8209$, $p < 0.01$, $n = 20$).

Discussion

In this study, we have shown a dietary interaction between dietary protein level and fatty acid type affecting serum quinolinic acid concentration, accompanied with changing ACMSD activity. Although food intakes in the linoleic acid-fed groups were significantly lower than in the stearic acid-fed groups, the body weight gain of the linoleic acid-fed groups were significantly higher than the stearic acid-fed groups. It was reported that longer-chain saturated fatty acids such as stearic acid are less readily, or less completely, absorbed than the more highly unsaturated fatty acids are [19]. Our previous study showed that apparent absorption of linoleic acid (free-form, not triglyceride) and stearic acid (free-form) are 94.3% and 67.9%, respectively [20]. Difference of absorption of each diet would affect the body weight gain.

In this experiment, the 40C10L group increased serum quinolinic acid concentration (Figure 2A). This phenomenon is due to a suppression of liver ACMSD activity and the increase of tryptophan intake. Liver ACMSD activity of 20C10L group is also low, however tryptophan intake is low compared with 40% casein groups. So, serum quinolinic acid concentration in 20C10L would be lower than that in 40C10L. Quinolinic acid concentration per tryptophan intake in linoleic acid groups tended to be higher than those of stearic acid groups. Polyunsaturated fatty acid would significantly affect quinolinic acid per tryptophan intake compared with saturated fatty acid. Liver and kidney ACMSD activities are affected by dietary protein levels. We previously reported that mRNA expression of rat hepatic ACMSD is increased by a high protein diet [21] and is decrease by a polyunsaturated fatty acid diet [9]. In this experiment, the transcription level of ACMSD would be modulated by dietary protein and polyunsaturated fatty acid. Shin et al reported that mouse hepatic ACMSD gene expression was found to be under the control of both hepatocyte nuclear factor 4 α and peroxisome-proliferator activated receptor-α [22]. It would be necessary to follow the pathway with further investigations to determine whether dietary polyunsaturated fatty acids affect these transcription factors and whether these transcription factors affect quinolinic acid production.

In this experiment, we did not measure the level of quinolinic acid in urine. Fukuwatari *et al* reported that phthalate ester increased urinary excretion of quinolinic acid in rat [23]. In our preliminary experiment, phthalate ester increased serum quinolinic acid, too. Urinary excretion of quinolinic acid might reflect serum quinolinic acid concentration or quinolinic acid production.

Under normal conditions, most of the tryptophan is known to be converted to kinurenine and subsequently to NAD and acetyl CoA in the liver through the kinurenine pathway [24]. During the metabolism of tryptophan, liver ACMSD seems to be able to directly affect the production of quinolinic acid. Quinolinic acid is non-enzymically generated from ACMS in the absence of ACMSD activity. Serum quinolinic concentration would be affected by ACMSD activity, tryptophan intake, and body weight gain.

In this study, we showed that dietary interaction affected serum quinolinic acid concentration. This phenomenon is partly attributable to the ACMSD activity. Relationships between ACMSD activity in the liver or in the liver and kidney, and serum quinolinic acid concentration per tryptophan intake, were highly negative correlated. This study provides the information that a factor which can change hepatic ACMSD activity could also affect quinolinic acid concentration.

Quinolinic acid possesses a suppressive effect on erythroid colony and lymphocyte blast formation [25]. Pawlak *et al* reported that the inadequate erythropoietin production in uremic patients was partially attributable to the inhibitory effect of quinolinate on the erythropoietin production [8]. This information may be helpful to reduce quinolinic acid toxicity by diet or under disease conditions associated with quinolinic acid toxicity.

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References

1. Mehler, A.H., Yano, K. and May, E.L.(1964) Nicotinic acid biosynthesis: Control by an enzyme that competes with a spontaneous reaction. Science 145, 817.
2. Sanada, H. and Miyazaki, M. (1980) Regulation of tryptophan-niacin metabolism by hormones. J. Nutr. Sci. Vitaminol. 26, 617.
3. Schwarcz, R., Whetsell, W.O. Jr. and Mangano, R.M. (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. Science 219, 316.
4. Perkins, M.N. and Stone, T.W. (1983) Pharmacology and regional variations of quinolinic acid-evoked excitations in the

rat central nervous system, J. Pharmacol. Exp. Ther. 226, 551.
5. Record, C.O., Buxton, B., Chase, R.A., Curzon, G., Murray-Lyon, I.M. and Williams, R. (1976) Plasma and brain amino acids in fulminant hepatic failure and their relationship to hepatic encephalopathy. Eur. J. Clin. Invest. 6, 387.
6. Moroni, F., Lombardi, G., Carla, V., Pellegrini, D., Carassale, G.L. and Cortesini, C. (1986) Content of quinolinic acid and of other tryptophan metabolites increases in brain regions of rats used as experimental models of hepatic encephalopathy. J. Neurochem. 46, 869.
7. McDaniel, H.G., Reddy, W.J. and Boshell, B.R. (1972) The mechanism of inhibition of phosphoenolpyruvate carboxylase by quinolinic acid acid. Biochim. Biophys. Acta 276, 543.
8. Pawlak, D., Koda, M., Pawlak, S., Wolczynski, S. and Buczko, W. (2003) Contribution of quinolinic acid in the development of anemia in renal insufficiency. Am. J. Physiol. Renal Physiol. 284, F693.
9. Egashira, Y., Murotani, G., Tanabe, A., Saito, K., Uehara, K., Morise, A., Sato, M. and Sanada, H. (2004) Differential effects of dietary fatty acids on rat liver alpha-amino-beta-carboxymuconate-epsilon-semialdehyde decarboxylase (ACMSD) activity and gene expression. Biochim. Biophys. Acta 1686, 118.
10. Egashira, Y., Nakazawa, A., Ohta, T., Shibata, K. and Sanada, H. (1995) Effect of dietary linoleic acid on the tryptophan-niacin metabolism in streptozotocin diabetic rats. Comp. Biochem. Physiol. 111A, 539.
11. Egashira, Y., Komine, T., Ohta, T., Shibata, K. and Sanada, H. (1997) Change of tryptophan-niacin metabolism in D-galactosamine-induced liver injury in rat. J. Nutr. Sci. Vitaminol. 43, 233.
12. Egashira, Y., Isagawa, A., Komine, T., Yamada, E., Ohta, T., Shibata, K. and Sanada, H.(1999) Tryptophan-niacin metabolism in liver cirrhosis rat caused by carbon tetrachloride. J. Nutr. Sci. Vitaminol. 45, 459.
13. Sanada, H. and Miyazaki, M. (1984) Effect of high-protein diet on liver α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase in rats. J. Nutr. Sci. Vitaminol. 30, 113.
14. National Research Council (1985) Guide for the Care and Use of Laboratory Animals. Publication no. 85–23. National Institutes of Health, Washington, D.C.
15. Saito, K., Fujigaki, S., Heyes, M.P., Shibata, K., Takemura, M., Fujii, H., Wada, H., Noma, A. and Seishima, M. (2000) Mechanism of increases in L-kynurenine and quinolinic acid in renal insufficiency. Am. J. Physiol. Renal Physiol. 279, F565.
16. Egashira, Y., Kouhashi, H., Ohta, T. and Sanada, H. (1996) Purification and properties of α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD), key enzyme of niacin synthesis from tryptophan, from hog kidney. J. Nutr. Sci. Vitaminol. 42, 173.
17. Ichiyama, A., Nakamura, S., Kawai, H., Honjo, T., Nishizuka, Y., Hayaishi, O. and Senoh, S. (1965) Studies on the metabolism of the benzene ring of tryptophan in mammalian tissue: II enzymic formation of α-aminomuconic acid from 3-hydroxyanthranilic acid. J. Biol. Chem. 240, 740.
18. Mehler, A.H. (1956) Formation of picolinic and quinolinic acids following enzymatic oxidation of 3-hydroxyanthranilic acid. J. Biol. Chem. 218, 241.
19. Hofmann, A.F. and Borgstrom, B. (1963) Hydrolysis of long-chain monoglycerides in micellar solution by pancreatic lipase. Biochim. Biophys. Acta. 70, 317.
20. Egashira, Y., Yamamiya, Y. and Sanada, H. (1992) Effects of various dietary fatty acids on α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase activity in rat liver. Biosci. Biotechnol. Biochem. 56, 2015.
21. Tanabe, A., Egashira, Y., Fukuoka, S., Shibata, K. and Sanada, H. (2002) Expression of rat hepatic 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase is affected by a high protein and by streptozotocin-induced diabetes. J. Nutr.132, 1153.
22. Shin, M., Kim, I., Inoue, Y., Kimura, S. and Gonzalez, F.J.(2006) Regulation of mouse hepatic alpha-amino-beta-carboxymuconate-epsilon-semialdehyde decarboxylase, a key enzyme in the tryptophan-NAD pathway, by hepatocyte nuclear factor 4alpha and peroxisome proliferator-activated receptor alpha. Mol. Pharmacol. 70, 1281.
23. Fukuwatari, T., Suzuki, Y., Sugimoto, E. and Shibata, K. (2002) Elucidation of the toxic mechanism of the plasticizers, phthalic acid esters, putative endocrine disrupters: effects of dietary di(2-ethylhexyl)phthalate on the metabolism of tryptophan to niacin in rats. Biosci. Biotechnol. Biochem. 66, 705.
24. Bender, D.A. (1982) Biochemistry of tryptophan in health and disease. Mol. Aspects Med. 6, 101.
25. Kawashima, Y., Sanaka, T., Sugino, N., Takahashi, M. and Mizoguchi, H. (1987) Suppressive effect of quinolinic acid and hippuric acid on bone marrow erythroid growth and lymphocyte blast formation in uremia. Adv. Exp. Med. Biol. 223, 69.

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