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Effect of dietary linoleic acid on the tryptophan–niacin metabolism in streptozotocin diabetic rats

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To make clear the mechanism of change of tryptophan–niacin metabolism in diabetic rats, we investigated the effect of dietary linoleic acid on the tryptophan–niacin metabolites and the activity of liver, α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD), a key enzyme of tryptophan–niacin metabolism, in streptozotocin diabetic rats. Moreover, we investigated the involvement of linoleic acid in the induction of hepatic ACMSD activity by streptozotocin diabetes. In diabetic rats, the sum of urinary excretion of nicotinamide, N^1-methylnicotinamide (MNA), N^1-methyl-2-pyridone-5-carboxamide (2-Py) and N^1-methyl-4-pyridone-3-carboxamide (4-Py) was higher in the fat free diet group than in the linoleic acid group, that was accompanied by the increase of tryptophan intake and reduction of body weight in the fat free diet group. In diabetic rats, hepatic ACMSD activity was higher in the fat free diet group than in the linoleic acid group. The results indicated that the induction of hepatic ACMSD activity by diabetes was not due to removal of the suppressive effect of the linoleic acid on the enzyme. In the diabetic + insulin group, hepatic ACMSD activity was significantly lower than in the diabetic group.

Key words: Aminocarboxymuconatesemialdehyde decarboxylase; Diabetes; Insulin; Linoleic acid; Nicotinamide; Rat; Tryptophan; Niacin.

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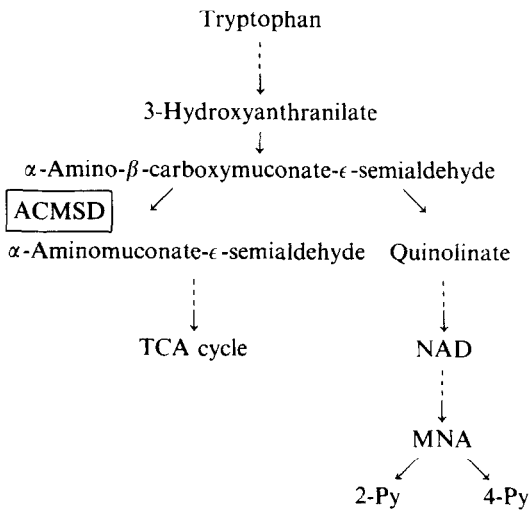
Introduction

Hepatic α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) (EC 4.1.1.45) seems to have a key role in regulating NAD biosynthesis from tryptophan. If ACMSD activity is high enough, most tryptophan is metabolized to enter the glutarate pathway. However if the activity is low, the amino acid is metabolized to enter the NAD pathway:



It has been reported that the level of liver ACMSD activity increased greatly in diabetic rats, and the urinary excretion of N^1-methylnicotinamide (MNA) was lower in diabetic rats

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Abbreviations: ACMSD; α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase, MNA; N^1-methylnicotinamide, 2-Py; N^1-methyl-2-pyridone-5-carboxamide, 4-Py; N^1-methyl-4-pyridone-3-carboxamide.

than in control rats when a large amount of tryptophan was loaded (Mehler *et al.*, 1958). Later, Ikeda *et al.* and Sanada *et al.* also found similar results (Ikeda *et al.*, 1965; Sanada *et al.*, 1980). On the other hand, Sanada *et al.* have previously reported that polyunsaturated fatty acids (PUFA) such as linoleic acid strongly suppressed hepatic ACMSD activity (Sanada, 1985). Later, we also found similar results (Egashira *et al.*, 1992, 1994). It has been known that lipogenesis was depressed and fatty acid oxidation was increased in diabetes. However, few investigations have been performed on the effect of dietary PUFA on the tryptophan–niacin metabolism in diabetic rats. In the present report, we investigated the effect of dietary linoleic acid on the tryptophan–niacin metabolites and its key enzyme, ACMSD activity in streptozotocin diabetic rats; and we studied the involvement of PUFA in the induction of hepatic ACMSD activity by diabetes; that is, we studied whether induction of ACMSD activity by diabetes was due to removal of the suppressive effect of linoleic acid on the enzyme. On the other hand, Mehler *et al.* (1958) reported that the liver ACMSD activity was normalized by the administration of insulin in diabetic rats. However, there is a contradictory report that insulin treatment was not able to normalize the ACMSD activity (Sanada *et al.*, 1980). Therefore, it is not clear whether induction of ACMSD activity by diabetes is caused directly or indirectly by lack of insulin. Thus, moreover we investigated the involvement of insulin in the induction of hepatic ACMSD activity by diabetes.

Materials and Methods

Chemicals

3-Hydroxyanthranilic acid and N^1-methylnicotinamide chloride (MNA) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Linoleic acid was purchased from Nihon oil and fat (NOF) Co. (Tokyo, Japan). N^1-methyl-2-pyridone-5-carboxamide(2-Py) and N^1-methyl-4-pyridone-3-carboxamide(4-Py) were synthesized by the methods of Pullman and Colowick (1954) and Shibata *et al.* (1988), respectively.

Columns

ODS-II (4.6 × 250 mm, used for the measurement of MNA) were purchased from Shimadzu Co. (Kyoto, Japan). 7-ODS-(4.6 × 250 mm, used for measurement of nicotinamide, 2-py, and 4-py) were purchased from Chemco Scientific Co., Ltd (Osaka, Japan).

Animals and diets

Male rats of the Sprague Dawley (SD) strain, 7 weeks old were obtained from Clea Japan, Inc. The animals were housed in an air-conditioned room at 22 ± 1°C with 12 hr light and dark cycles. The rats were fed on a commercial diet (CE-2, obtained from Clea Japan Co.) for 1 week to accommodate them to the environment. Then, the rats were divided into two groups, a diabetic group and a normal group. One group was intraperitoneally injected with 80 mg streptozotocin/kg body weight as the solution (10 mg streptozotocin/ml buffer) which was dissolved in sterilized buffer (pH adjusted to 4.5 by the addition of 0.05 M citric acid), and the other was injected with sterilized 0.14 M NaCl solution (8 ml/kg body weight). On the 11th day, the urinary glucose was checked in each rat, using clinical test paper (Diastix, Sanyo Co., Ltd, Tokyo, Japan) and animals with glycosuria were used as diabetic rats for the present experiments. The rats were divided into the following six groups: normal + fat free (FF), normal + linoleic acid (L), diabetes + fat free (D–FF), diabetes + linoleic acid (D–L), diabetes + insulin + fat free (I–FF) and diabetes + insulin + linoleic acid (I–L). The animals were fed one of the experimental diets shown in Table 1 and water *ad libitum* for the following 13 days. We used six rats for each group, except for D–FF and I–L groups which included three and five rats, respectively.

To the I–FF and I–L groups, protamine zinc insulin (Takeda Chemical Industries, Ltd, Osaka, Japan) (2 units/head) was injected subcutaneously every 12 hr from the third to the 13th day of the start of administration of the experimental diets shown in Table 1. On the 11th day of the experimental diet, urine was collected in flasks containing 1 M HCl (1 ml/50 ml urine) for 24 hr. The urine was stored at −25°C until analysis for nicotinamide

Table 1. Composition of diets

Constituent (%)	FF*	L†
Casein	40.0	40.0
DL-Methionine	0.3	0.3
Corn starch	15.0	15.0
Sucrose	35.0	25.0
Mineral mixture (AIN-76)	3.5	3.5
Vitamin mixture (niacin free)‡	1.0	1.0
Choline bitartrate	0.2	0.2
Cellulose	5.0	5.0
Fatty acid	0.0	10.0

*Fat free.
†Linoleic acid.
‡The vitamin mixture (total: 1.00 g) contains (in mg) thiamine–HCl 0.6, riboflavin 0.6, pyridoxine–HCl 0.7, Ca-pantothenate 1.6, folic acid 0.2, biotin 0.02, vitamin B_{12} 0.001, vitamin A 0.22, vitamin E 5.0, vitamin D_3 0.0025, vitamin K_3 0.005 and sucrose 991.05.

and its metabolites. On the 13th day, the rats were killed under anesthesia with sodium pentobarbital (60 mg/kg body weight) 2 hr after the last insulin injection.

Enzyme assay

The fresh liver or kidney was added to three volumes of cold 0.14 M potassium chloride solution containing 0.02 M potassium phosphate buffer (pH 7.0) and homogenized with a Teflon homogenizer. The homogenate was centrifuged at 105,000 *g* for 60 min at 4°C. The supernatant was used as an enzyme source. The ACMSD activity was assayed as described in the previous paper (Sanada *et al.*, 1980). In the previous experiment, 100 μl of enzyme source was used, but 10–50 μl of enzyme source was used for the present experiments. The methods for measuring tryptophan oxygenase were performed as described previously (Knox *et al.*, 1970). Protein was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Measurement of others

Serum was analyzed for glucose using a "Glucose B Test wako" (Wako Pure Chemicals, Osaka, Japan). Urinary excretion of nicotinamide, MNA, 2-Py, and 4-Py was simultaneously measured by the method of Shibata *et al.* (1987, 1988).

Statistical analysis

Data were analyzed by a two way analysis of variance, which was followed by an inspection of difference by Student's *t*-test.

Results

Changes in body weight gain and the glucose levels in blood

Table 2 shows the body weight gain and food intake of the experimental animals. The body weight gain was lower in the diabetic (D–FF, D–L) groups than in the non-diabetic (FF, L) and diabetic + insulin (I–FF, I–L) groups, although the food intake was the highest in the diabetic (D–FF, D–L) groups. The body weight gain was higher in the D–L group than in the D–FF group, although the food intake was lower in the D–L group than in the D–F group. The body weight gain was higher in the I–L group than in the I–FF group, although the food intake was lower in the I–L group than in the I–FF group. The blood glucose concentrations of rats in the diabetic groups (D–FF, D–L) and the diabetic + insulin groups (I–FF, I–L) were 3.8–4.8 and 0.2–0.3-fold of normal rats, respectively. There is no significant difference between the fat free and linoleic acid diet group (data are not shown).

Enzyme activities

The ACMSD activity and tryptophan oxygenase activity are shown in Table 3. The activities of hepatic ACMSD and tryptophan oxygenase were increased in the diabetic (D–FF, D–L) groups. These results are consistent with previous reports (Mehler *et al.*, 1958; Sanada *et al.*, 1980), and these activities were decreased by the injection of insulin. In all groups fed linoleic acid (L, D–L, I–L), hepatic ACMSD activity was suppressed, but the activity of tryptophan oxygenase was not changed by feeding the linoleic acid diets. The kidney ACMSD activity was increased slightly in the diabetic groups, but the enzyme activity of the insulin groups was at the same level as for the non-diabetic groups.

Urinary excretion of nicotinamide and its metabolites

The urinary excretion of nicotinamide, MNA, 2-Py and 4-Py in rats for the last 24 hr is shown in Table 4. In the normal groups, the urinary excretion of the sum of nicotinamide, MNA, 2-Py and 4-Py was higher in the L group than in the FF group. These results are consistent with a previous report (Shibata and Onodera, 1992). In the diabetic groups (D–FF, D–L), the urinary excretion of 2-Py and 4-Py was not significantly different between the D–FF group and D–L group. The urinary excretion of MNA was higher in the D–FF group than in the D–L group. The sum of urinary excretion of nicotinamide, MNA, 2-Py and 4-Py was higher in the D–FF group than in the D–L group. In the insulin injection groups, the urinary excretion of 4-Py was higher in the I–L group than in the I–FF group. The total urinary excretion of nicotinamide, MNA, 2-Py and 4-Py tended to be higher in the I–L group than in the I–FF group.

Discussion

It has been reported that the administration of pyrazinamide, a strong inhibitor of ACMSD, caused an increase in the urinary excretion of nicotinamide and such metabolites as MNA, 2-Py and 4-Py (Shibata, 1990). This result indicates that ACMSD is critical in tryptophan–niacin conversion.

In diabetic rats, a marked increase was observed in the activity of ACMSD. However, the mechanism for this phenomenon has still not been completely elucidated, so we studied whether induction of ACMSD activity by diabetes was due to removal of the suppressive

Table 2. Body weight gain and food intake of the experimental animals during 13 days

			Diabetes		Diabetes + insulin		ANOVA		
	FF	L	D–FF*	D–L†	I–FF‡	I–L§	D¶	FA‖	FA × D**
Initial weight (g)	279 ± 3^{a}	279 ± 2^{a}	181 ± 21^{b}	181 ± 18^{b}	181 ± 18^{b}	181 ± 17^{b}	—	—	—
Final weight (g)	353 ± 6^{a}	357 ± 4^{a}	169 ± 18^{b}	212 ± 27^{b}	284 ± 14^{c}	305 ± 10^{c}	$P < 0.01$	NS	NS
Weight gain (g)	73.2 ± 3.5^{a}	77.5 ± 3.1^{a}	-8.67 ± 6.44^{d}	31.3 ± 10.8^{b}	103 ± 7^{c}	119 ± 9^{c}	$P < 0.01$	NS	$P < 0.01$
Food intake (g/day)	19.2 ± 3.3^{ab}	18.2 ± 1.2^{a}	41.7 ± 2.3^{d}	32.1 ± 2.4^{c}	30.9 ± 1.5^{c}	23.9 ± 1.7^{b}	$P < 0.01$	NS	$P < 0.05$
Liver weight (g)	16.1 ± 0.6^{ad}	14.9 ± 0.5^{a}	9.69 ± 1.33^{b}	10.2 ± 0.6^{b}	22.8 ± 1.4^{c}	16.9 ± 0.5^{d}	$P < 0.01$	$P < 0.01$	NS
Kidney weight (g)	2.77 ± 0.11^{a}	2.72 ± 0.06^{a}	3.71 ± 0.42^{b}	3.25 ± 0.36^{c}	3.19 ± 0.09^{b}	2.92 ± 0.14^{ab}	$P < 0.01$	NS	NS

*Diabetic + fat free (FF).
†Diabetic + linoleic acid (L).
‡Diabetic + insulin + FF.
§Diabetic + insulin + L.
¶Diabetic.
‖Fatty acid.
**Diabetic × fatty acid.
Values are means ± SEM.
The statistical significance of differences among values was analyzed by two-way ANOVA, using fatty acid (fat-free and linoleic acid) and diabetes (normal, diabetes and diabetes + insulin) as factors.
Values without a common superscript letter are significantly different by Student's t-test ($P < 0.05$).
NS: not significant.

Table 3. ACMSD and tryptophan oxygenase activities in rats

			Diabetes		Diabetes + insulin		ANOVA		
	FF	L	D–FF	D–L	I–FF	I–L	D	FA	FA × D
Liver:									
ACMSD activity									
(μmol/hr/g liver)	7.36 ± 1.65^{a}	0.41 ± 0.12^{b}	91.5 ± 3.5^{c}	41.8 ± 7.2^{d}	18.4 ± 1.3^{e}	5.86 ± 1.42^{a}	$P < 0.01$	$P < 0.01$	$P < 0.01$
(μmol/hr/g protein)	59.3 ± 14.4^{a}	2.69 ± 0.78^{b}	569 ± 40^{c}	300 ± 70^{d}	147 ± 9^{e}	44.1 ± 10.7^{a}	$P < 0.01$	$P < 0.01$	$P < 0.01$
Tryptophan oxygenase activity									
(μmol/hr/g liver)	0.62 ± 0.16^{a}	0.85 ± 0.17^{a}	7.40 ± 0.92^{b}	4.29 ± 0.68^{c}	0.99 ± 0.13^{a}	1.29 ± 0.34^{a}	$P < 0.01$	NS	$P < 0.01$
(μmol/hr/g protein)	3.98 ± 1.08^{a}	5.57 ± 1.11^{a}	46.9 ± 9.3^{b}	34.0 ± 7.1^{b}	8.37 ± 1.86^{a}	9.60 ± 2.66^{a}	$P < 0.01$	NS	NS
Kidney:									
ACMSD activity									
(μmol/hr/g kidney)	23.7 ± 1.4^{a}	24.5 ± 1.3^{a}	41.1 ± 8.3^{b}	30.2 ± 1.0^{bc}	24.7 ± 2.6^{acd}	18.4 ± 1.1^{d}	$P < 0.01$	NS	$P < 0.05$
(μmol/hr/g protein)	187 ± 15^{a}	236 ± 12^{b}	442 ± 62^{c}	376 ± 28^{c}	240 ± 22^{ab}	188 ± 11^{a}	$P < 0.01$	NS	$P < 0.05$

Values are means ± SEM.
The statistical significance of differences among values was analyzed by two-way ANOVA, using fatty acid (fat-free and linoleic acid) and diabetes (normal, diabetes and diabetes + insulin) as factors.
Values without a common superscript letter are significantly different by Student's t-test ($P < 0.05$).
NS: not significant.

Table 4. Urinary excretion of nicotinamide and its metabolites in experimental animals

			Diabetes		Diabetes + insulin		ANOVA		
	FF	L	D–FF	D–L	I–FF	I–L	D	FA	FA × D
Tryptophan intake (mg/day)	123 ± 21^{ab}	117 ± 8^{a}	267 ± 15^{e}	194 ± 22^{cd}	197 ± 9^{c}	153 ± 11^{bd}	$P < 0.01$	$P < 0.05$	NS
Nicotinamide									
(nmol/daily urine)	285 ± 33^{a}	457 ± 38^{b}	269 ± 75^{ac}	192 ± 61^{ac}	162 ± 16^{c}	158 ± 12^{c}	$P < 0.01$	NS	$P < 0.05$
(nmol/mg tryptophan intake)	3.34 ± 1.00^{ab}	4.20 ± 0.36^{a}	1.04 ± 0.32^{bcd}	1.00 ± 0.31^{bcd}	0.81 ± 0.06^{c}	1.04 ± 0.08^{d}	$P < 0.01$	NS	NS
MNA									
(nmol/daily urine)	248 ± 35^{ac}	3150 ± 1340^{abc}	2470 ± 305^{b}	624 ± 169^{a}	419 ± 125^{ac}	165 ± 24^{c}	$P < 0.05$	NS	$P < 0.01$
(nmol/mg tryptophan intake)	3.01 ± 1.02^{acd}	28.5 ± 11.1^{ab}	9.23 ± 1.48^{b}	3.15 ± 0.86^{ac}	2.09 ± 0.59^{cd}	1.07 ± 0.12^{d}	$P < 0.05$	NS	$P < 0.05$
2-Py									
(nmol/daily urine)	214 ± 29^{a}	480 ± 56^{b}	432 ± 159^{a}	209 ± 393^{ac}	108 ± 6^{d}	129 ± 11^{cd}	$P < 0.01$	NS	$P < 0.01$
(nmol/mg tryptophan intake)	2.51 ± 0.68^{ac}	4.40 ± 0.49^{c}	1.68 ± 0.67^{ab}	1.11 ± 0.21^{ab}	0.55 ± 0.03^{d}	0.85 ± 0.07^{b}	$P < 0.01$	$P < 0.05$	$P < 0.05$
4-Py									
(nmol/daily urine)	2818 ± 391^{a}	3060 ± 758^{ab}	1007 ± 402^{bcd}	1259 ± 272^{bd}	574 ± 119^{c}	1066 ± 154^{d}	$P < 0.01$	NS	NS
(nmol/mg tryptophan intake)	33.3 ± 10.3^{ab}	28.3 ± 7.2^{a}	3.91 ± 1.69^{bcd}	6.57 ± 1.32^{c}	2.96 ± 0.64^{d}	6.93 ± 0.76^{c}	$P < 0.01$	NS	NS
Nicotinamide + MNA + 2 − Py + 4 − Py									
(nmol/daily urine)	3565 ± 480^{ac}	7148 ± 711^{b}	4178 ± 593^{a}	2285 ± 423^{cd}	1263 ± 157^{d}	1517 ± 195^{d}	$P < 0.01$	$P < 0.01$	$P < 0.01$
(nmol/mg tryptophan intake)	42.1 ± 13.0^{ab}	65.4 ± 5.9^{a}	16.0 ± 3.0^{bd}	12.0 ± 2.0^{bd}	6.42 ± 0.79^{c}	9.89 ± 0.95^{d}	$P < 0.01$	$P < 0.05$	NS

Values are means ± SEM.

The statistical significance of differences among values was analyzed by two-way ANOVA, using fatty acid (fat-free and linoleic acid) and diabetes (normal, diabetes and diabetes + insulin) as factors.

Values without a common superscript letter are significantly different by Student's t-test ($P < 0.05$).

NS: not significant.

effect of the linoleic acid on the enzyme. The activity of liver ACMSD was increased remarkably in the D–FF group. In addition, hepatic ACMSD activity was higher in the D–FF group than in the FF group. These results suggest that linoleic acid does not participate in the induction of liver ACMSD activity by diabetes, because, if the induction of ACMSD activity by diabetes is due to removal of the suppressive effect of linoleic acid on the enzyme, the activity of liver ACMSD should be the same as for the levels in the FF and D–FF and D–L groups. However, feeding the linoleic acid diet suppressed hepatic ACMSD activity in diabetic rats. Therefore, these results are considered to indicate that the mechanism of suppression by dietary linoleic acid and the induction by diabetes may be independent.

There are some contradictory reports that the liver ACMSD activity was normalized by the administration of insulin or not. In the present experiment, the liver ACMSD activity was suppressed by the administration of insulin and approached the normal ACMSD activity. A marked increase of liver ACMSD activity by diabetes was due to the lack of insulin. Therefore, linoleic acid does not participate in the induction of liver ACMSD activity by diabetes. Even in insulin treated rats, liver ACMSD activity was suppressed by dietary linoleic acid. Accordingly, these results are considered to indicate that the suppression by dietary linoleic acid and by insulin may be independent. After insulin treatment, tryptophan oxygenase was normalized, and blood glucose concentration was low, but liver ACMSD activity of insulin treatment groups was not normalized completely. Thus, this seems to be influenced by some unknown mechanism.

Mehler *et al.* (1958) and Sanada *et al.* (1980) reported that there was a marked reduction of urinary MNA in a normal diet containing 6.4% soybean oil in diabetic rats compared with normal rats. In the present experiment with the 10% linoleic acid diets (L and D–L groups), when the normal group was compared with the diabetic group, the urinary excretion of nicotinamide, MNA, 2-Py, 4-Py was lower in the D–L group than in the L group, and hepatic ACMSD activity was higher in the D–L group than in the L group. However, when the D–FF group was compared with the D–L group, the daily urinary excretion of nicotinamide + MNA + 2-Py + 4-Py was 1.8 times higher in the D–FF group than in the D–L group, although hepatic ACMSD activity was 2.2 times higher in the D–FF group than in the D–L group. In diabetic rats, the reduced amount of urinary nicotinamide + MNA + 2-Py + 4-Py in the D–L group was considered to be attributable to the elevated retention of niacin and its derivatives, and to the reduction of liver tryptophan oxygenase activity, the first limiting enzyme on tryptophan–niacin metabolism. The above-mentioned phenomena were explained as follows; although the body weight of D–L group increased and that of the D–FF group decreased, the food intake and tryptophan intake were 1.3–1.4 times higher in the D–FF group than in the D–L group. In diabetic rats, the D–L group could use dietary linoleic acid as the energy source, but the D–FF group ate a fat-free diet, so the D–FF group may degrade their body protein for gluconeogenesis to produce free amino acids, including free tryptophan. Tryptophan is a substrate of tryptophan–niacin metabolism, so urinary excretion of nicotinamide and its metabolites may increase.

On the other hand, urinary excretion of nicotinamide/(nicotinamide + MNA + 2-Py + 4-Py) of the D–FF group and that of the D–L group was approximately equal (D–FF, 0.06; D–L, 0.08). 2-Py/(nicotinamide + MNA + 2-Py + 4-Py) was also equal (D–FF, 0.10; D–L, 0.09), while MNA/(nicotinamide + MNA + 2-Py + 4-Py) in the D–FF group and the D–L group was 0.59 and 0.27, respectively. 4-Py/(nicotinamide + MNA + 2-Py + 4-Py) of D–FF group was 0.24 and that of the D–L group was 0.55. Since Shibata (1991) reported that 4-Py-forming MNA oxidase was higher in the 5% corn oil group than in the oil free group, the reduced urinary MNA and increased urinary 4-Py in the D–L group may be attributable to the elevated 4-Py-forming MNA oxidase, with the conversion enzyme of MNA to 4-Py.

In comparison with diabetic groups and insulin-treated diabetic groups, although liver ACMSD activity was suppressed by insulin, the urinary excretion of nicotinamide + MNA + 2-Py + 4-Py was lower in the insulin-treated diabetic groups than in the diabetic groups. These phenomena may be explained by the difference of potency for the retention of niacin. Shibata and Murata (1984, 1986) reported the niacin concentration in various organs to be almost constant, except for liver and blood. Sanada *et al.* (1980) reported that the total concentration of niacin in the liver of diabetic, insulin-treated diabetic and non-diabetic rats was almost constant (Sanada *et al.*, 1980). These reports indicated that niacin concentration in the tissue did not change; so, the retention of niacin and its derivatives in the whole body in the insulin-treated diabetic group was supposed to be increased without a change of niacin concentration in tissue since its growth rate was greater in the insulin-treated groups than in the diabetic groups, even though the food intake was lower in the insulin-treated diabetic groups.

Therefore, the reduced amount of urinary nicotinamide and its metabolites in insulin-treated diabetic groups was considered to be attributable to the elevated retention of niacin and its derivatives.

The mechanism of these phenomena is still not completely clarified. It depends largely upon future multilateral studies.

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