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Effect of Long-Term Feeding of Taurine in Hereditary Hyperglycemic Obese Mice

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1) Feeding of 5% taurine diet for 14 weeks caused marked reduction of the body weight increase in young mice of a hereditary hyperglycemic obese strain (KK). Exchange of the diet for control accelerated gain of the body weight in these animals for the subsequent 6 weeks.

2) Adult KK mice fed 5% taurine diet for 10 weeks demonstrated a slight but significant decrease of the initial body weight, mainly due to a reduction of the size of the abdominal fat pads, whereas such effects of long-term feeding of taurine were not observed in adult lean (BALB/C) and experimentally obese (GTG) mice.

3) Suppression of development of obesity was also produced in young KK mice by adding taurine to tap water in 1.0 and 0.5% for 20 weeks. The abdominal fat pads were decreased markedly in size at the high dose of taurine, as compared to those of controls. There was no difference in blood sugar and liver cholesterol between the treated and untreated groups. The long-term feeding of taurine did not cause any change in the fatty acid composition of lipid in the abdominal fat pads, but this treatment served to liver lipid pattern of composition similar to that from BALB/C mice.

4) Whole body macroautoradiography demonstrated that administered taurine-S^{35} was accumulated largely in the liver, gall bladder, gastrointestinal walls, kidney and spleen. There was no significant difference in distribution pattern of taurine-S^{35} between KK and BALB/C mice.

As a physiological function taurine conjugates with bile acids in the livers of many animals.[2)] This conjugation mechanism appears to connect taurine to lipid metabolism, contributing to the intestinal absorption of lipid by affecting the processes of lipolysis,[3)] micelle formation,[4)] and re-esterification of fatty acids within intestinal mucosal cells.[5)] It is also suggested that bile salt formation causes an increase in the transfer of cholic acid from blood to liver,[6)] and results in the elimination of body cholesterol by promoting its conversion to bile acids.[7)]

KK mice are known to be a hereditary hyperglycemic obese strain which has been established originally by Kondo, *et al.*[8)] as inbred mice from a Japanese dealer's stock. Adult mice of this strain have such characteristics as sluggishness, moderate obesity, polyphagia and polyuria. Nakamura[9)] has studied in detail the morphological and histological background

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of this strain. Tsuchida[10] has pointed out several metabolic disorders of lipid and carbohydrate hereditarily present in KK mice.

The present study deals with the effect of long-term feeding of taurine on development of obesity in these mice.

Materials and Methods

Animals——Female mice were used in this study. KK mice which had been derived initially from Dr. Kondo[11] and kept in this laboratory were used as hereditary obese. BALB/C strain also kept in this laboratory was used as normal control. ICR mice were obtained from CLEA,[12] in which strain experimental obesity was induced by an intraperitoneal injection of 20 mg per mouse of aurothioglucose (Solganal B4, Schering). These mice were referred later as GTG mice. All the animals were housed 2—3 numbers in plastic cages with sterile wood shavings and allowed to stand in a thermostatically controlled room throughout the entire periods of the experiments.

Diet and Drinking Water——For the feeding experiments, taurine was mixed uniformly with a commercial animal chow (CLEA CE-2) in 5%. This was designated later as 5% taurine diet. The unmodified diet was given to control animals. For the drinking experiment, taurine was dissolved in tap water in 1.0 and 0.5%, respectively. These solutions were prepared daily and given freely to animals which were maintained on another commercial diet (Oriental CMF).

Procedure I——The same numbers of newly weaned KK and BALB/C mice (16 numbers each) were used together at the age of 4 weeks. The half of each group were given 5% taurine diet for the initial 14 weeks and control diet for the subsequent 6 weeks. Another half were fed consistently control diet for 20 weeks. Body weight was measured twice a week, and simultaneously food and water intakes were recorded.

Procedure II——Twenty KK, sixteen GTG and twenty BALB/C mice at the same age of 15 weeks were used in this experiment. They were divided into two groups each and given 5% taurine diet and control diet for 10 weeks, respectively. Gain of body weight and consumption of food and water were recorded regularly as mentioned above. At the end of the experimental period, all animals were sacrificed by cervical dislocation and the organs were weighed.

Procedure III——Thirty KK mice 6 weeks old were divided into three groups and let to drink 1.0 and 0.5% taurine solutions and usual tap water for 20 weeks, respectively. Increase of body weight and changes of food and water intake were observed throughout the entire period. At the end of 20 weeks all mice were sacrificed by bleeding from the vein of the thigh under ether-anesthesia. Blood was collected for determinations of cell counts, hemoglobin value and blood sugar. Organs were removed and weighed. The ovarian fat pads and livers were extracted with the mixture of chloroform-methanol (2:1) for the determination of total lipid content. Fatty acid composition of lipid was determined by gaschromatography using diethylene glycol succinate columes.[13] Liver cholesterol was determined by the method of Rosental, *et al.*[14]

Whole Body Autoradiography——Adult mice of both KK and BALB/C strains were given intravenously 5 μCi of taurine-S^{35} (RCC) and sacrificed by ether-anesthesia 3 hours later. Whole body was placed in a holder and immersed in dry ice-acetone. The subsequent procedures for preparation of whole body macroautoradiogram was followed by the method of Aoyagi.[15] Autoradiogram was scanned by a Macrodensitometer (Joyce-Loeble, Mark III. C. S. and relative concentration of the taurine-S^{35} distributed in various organs and tissues were determined.

Result

Feeding Experiments

Fig. 1 shows the growth curves for mice of both KK and BALB/C strains in one feeding experiment starting from the age of 4 weeks old. Average intakes of food and water were also given in the figure.

When control diet was made freely available, KK mice were increased considerably in body weight up to 18 weeks of age and then reached to a plateau (32—33 g). In contrast

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the animals fed 5% taurine diet demonstrated a significant depression of the body weight increase from the initial 4 weeks of the treatment. The difference of body weight became larger between the treated and untreated groups in the subsequent period. Although

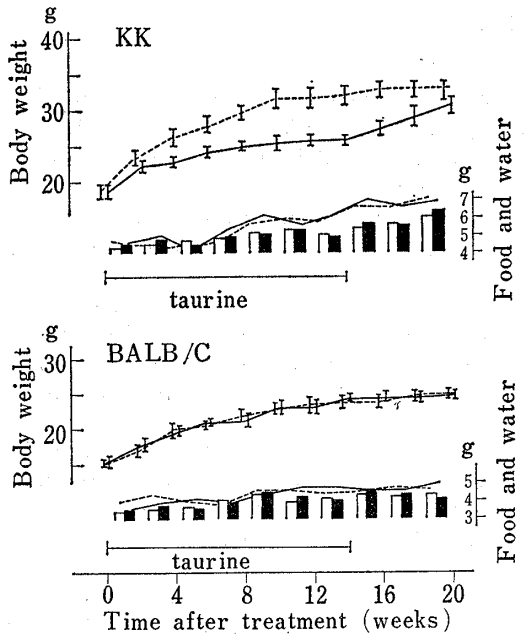

Fig. 1. Effect of Long-Term Feeding of Taurine on Body Weight in Young Mice

body weight and water: treated (——), untreated (------), diet: treated (▓▓), untreated (▭), S.E (⊥)

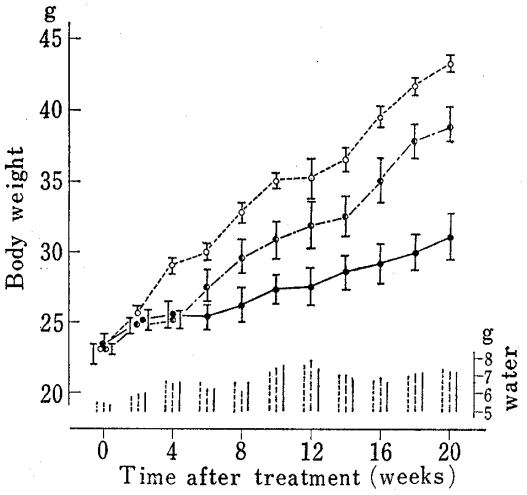

Fig. 2. Effect of Long-Term Drinking of Taurine Aqueous Solutions in KK Mice

1.0% treated (——), 0.5% treated (----), untreated (------), S.E. (⊥)

TABLE I. Body Weight and Organ Size in Adult Female Mice Fed 5%-Taurine Diet for 10 Weeks

Organ		KK mice $n=10$		G.T.G. mice $n=8$		BALB/C mice $n=10$	
		Treated mg±S.E.	Untreated mg±S.E.	Treated mg±S.E.	Untreated mg±S.E.	Treated mg±S.E.	Untreated mg±S.E.
Body[a] weight	initial	36.9±0.62	34.6±0.58	55.4±4.4	54.2±4.5	24.3±0.23	24.1±0.43
	final	33.5±0.77	37.4±0.70	58.5±4.2	58.5±4.2	25.5±0.42	24.4±0.32
Heart		120±3.1 (0.3)	122±3.8 (0.3)	178±18 (0.3)	169±15 (0.3)	116±3.1 (0.5)	114±3.5 (0.5)
Lung		155±3.9 (0.4)	190±7.1 (0.5)	194±8.9 (0.3)	200±10.1 (0.3)	147±4.1 (0.6)	154±2.8 (0.6)
Liver		1295±45 (3.9)	1382±31 (3.7)	2250±218 (3.8)	2120±198 (3.7)	1112±48 (4.4)	1108±19 (4.5)
Pancreas		179±14.1 (0.5)	208±14.0 (0.6)	309±31.8 (0.5)	295±20.2 (0.5)	190±15.4 (0.7)	187±13.5 (0.8)
Spleen		97±3.7[b] (0.3)	130±14.0 (0.3)	163±15.9 (0.3)	170±14.4 (0.3)	109±3.3 (0.4)	103±2.6 (0.4)
Abdominal fat		3148±154[c] (9.4)	4834±276 (12.9)	8250±1230 (14.1)	8035±1300 (13.9)	354±29.0 (1.4)	341±25.0 (1.4)
Kidney		294±8.2[b] (0.9)	345±10.8 (0.9)	481±23.8 (0.8)	470±31.0 (0.8)	307±7.5 (1.2)	297±4.5 (1.2)
Carcass[a]		22.3±0.52[c] (66.5)	23.9±0.48 (64.0)	35.8±2.17 (61.1)	36.0±3.00 (62.2)	17.5±0.30 (68.8)	16.9±0.19 (69.1)

a) unit in g b) $p<0.05$ c) $p<0.01$
Numbers in parenthesis indicate percent of final body weight.

sluggishness and fat-deposition in the body were clearly obvious in the controls older than 10 weeks, none of these features appeared in the animals given daily taurine. However, by exchanging the diet at the 14 weeks of the treatment, they showed a gradually accelerated increase of body weight and finally gained body weight at the same level to controls. On the other hand, the growth curves for BALB/C mice were not changed by long-term feeding of 5% taurine diet. The shape of the curves for these mice were fairly comparable to those for the treated group of KK mice.

There was no significant difference in daily consumption of food and water between the treated and the untreated group of both strains throughout the entire period. As compared with BALB/C mice, KK strain took largely diet and water with increasing age. Therefore average daily intake of taurine for KK mice was calculated to be 260 mg per mouse against 190 mg for the BALB/C strain.

Another feeding experiment was performed on adult mice of the three types,; KK, BALB/C and G.T.G mice. The data were summarized in Table I.

These adult mice older than 15 weeks were in a static phase of growth, so that a meaningful rise of body weight was not observed in the control group of each strain during the tested period. Feeding of 5% taurine diet caused a slight but significant decrease of body weight only in KK mice, while the controls of other two strains showed rather a slight increase of body weight. KK and G.T.G mice demonstrated together markedly large size of abdominal fat pads. Feeding of taurine caused a definite reduction of depot-fat only in KK mice, but not in experimental obese mice. The other organs of the treated KK mice, except the liver and lung, were relatively smaller in the size than those of controls. There was no difference in the organ-size between the treated and the untreated group of the other two types after the long-term feeding of taurine.

Drinking Experiment

Fig. 2 shows the growth curves for KK mice drunk daily the tap water containing taurine. The animals fed ad libitum the control diet different from the above show demonstrated a 190% increase of body weight from the initial week to the end of the treatment. This rate of growth was larger than that found in the control animals of the feeding experiment, probably due to the difference in calorie-intake from the diet. However, daily injestion of taurine in these mice also caused suppression of development of obesity as shown in Fig. 2.

With the dose level of taurine the growth of KK mice was depressed significantly from the 4 weeks of the treatment, and final weight gain of both 1.0 and 0.5% groups were approximately 30 and 10% below control weight. No appetite was changed in the treated mice by long-term drinking of taurine. Average daily injestion of taurine was estimated to be 66 and 33 mg per mouse for the high and low dose group, respectively.

Of the mice given the high dose of taurine almost all the organs, especially the abdominal fat pads, were decreased significantly in the size, while hematological values were not different from those of controls (Table II).

There was no difference in the blood sugar and liver cholesterol levels among the treated and untreated groups (Table III).

Fatty acid composition of lipid in the fat pads and livers from the animals of each group was presented in Table IV, togather with the data from ad libitum fed controls of BALB/C strain at the same age.

The total lipid content of the fat pads was higher in KK mice than in BALB/C mice. A relatively lower proportion of short chain fatty acids was noted in the lipid from the fat pads of KK mice than in that of BALB/C mice. The fraction of an unsaturated fatty acid which was corresponding with linolic acid in chromatographical behavior was significantly high in comparison with KK mice. The long-term drinking of taurine did not modified the pattern of fatty acid composition of lipid in the fat pads of KK mice, despite of a marked reduction in the tissue size. There were some differences in fatty acid composition of liver

Table II. Organ Size and Blood Cell Count in Taurine-Drunk KK Mice

Organ and blood cell	Unit	1.0% Taurine	0.5% Taurine	Untreated
Heart	mg	127±8.4[a] (0.4)	146±7.5 (0.4)	106±4.1 (0.4)
Lung	mg	200±3.7 (0.6)	196±5.7 (0.5)	205±6.7 (0.5)
Liver	mg	1400±87[a] (4.4)	1800±180 (4.7)	2000±110 (4.5)
Pancreas	mg	288±22.1 (0.9)	321±16.6 (0.8)	304±15.7(0.7)
Spleen	mg	95±9.8[a] (0.3)	125±8.1 (0.3)	132±3.9 (0.3)
Abdom. fat	mg	2800±700[a](8.8)	4200±950 (11.0)	5800±300 (13.0)
Kidney	mg	339±14.0[a](1.1)	382±14.3[b](1.0)	417±9.2 (0.9)
Carcass	g	21.0±0.92[a](65.4)	24.9±1.81(65.3)	27.9±0.41(62.4)
Final body weight	g	32.1±1.25[a]	38.1±1.44[a]	44.8±0.82
WBC[c]	10^3/mm^3	6.5±0.64	5.9±0.88	5.6±0.48
RBC[d]	10^6/mm^3	10.5±0.85	11.5±0.66	11.4±0.96
Hb[e]	g/dl	14.6±0.66	14.5±0.63	14.2±0.65

a) $p<0.01$ *b*) $p<0.05$ *c*) white blood cell *d*) red blood cell *e*) hemoglobin
Numbers in parenthesis indicate percent of final body weight.

Table III. Blood Sugar and Liver Cholesterol Levels in Taurine-Treated and Untreated KK Mice

Group	Blood sugar (mg %)	Liver cholesterol (mg per g of wet wt.)	
		Total	Free
1.0% taurine (4)	154±5.6	2.06±0.282	0.69±0.055
0.5% taurine (4)	155±9.9	1.96±0.253	0.58±0.100
Control diet (5)	156±6.9	2.09±0.142	0.66±0.097

number of determinations in parenthesis

lipid between the two strains of mice. A fraction of a fatty acid (20:4) largely present in the livers of BALB/C mice was not found in those of KK mice. This compound agreed chromatographically with the authentic sample of arachidonic acid. Stearic acid (18) was markedly low but oleic acid (18:1) was significantly high in comparison with KK mice, as compared with those in BALB/C mice. The long-term drinking of taurine in KK mice tend to modify the compositions of these fatty acids in the liver lipid and to show a pattern similar to those found in BALB/C mice, although arachidonic acid fraction remained still absent.

Distribution of Administered Taurine-S^{35} in Mice

As shown in Table V, administered taurine-S^{35} was accumulated largely in the liver, gall bladder, gastro-intestinal walls, kidney and spleen. Urinary excretion appeared to be very rapid. Localization of a relatively large amount of taurine-S^{35} was observed in several glands and mucosal tissues. Incorporation of radioactivity into the brain, spinal cord, heart and muscle which were rich in endogenous taurine[2] was extremely slow. There was no difference in the distribution pattern of taurine-S^{35} between the two strains. Relative concentration of radioactivity in respective organs and tissues was also in the same order of magnitude. Radioactivity was not found wholly in the abdominal fat pads of KK mice, but partly on the capsule of the tissue.

Discussion

The present study demonstrates that the body weight increase of hereditary hyperglycemic obese mice is depressed considerably by long-term injestion of taurine, mainly due to inhibition of excess fat deposition in the body.

Table IV. Fatty Acid Composition of Total Lipid Extracted from Adipose Tissue and Liver

(A) Abdominal Adipose Tissue

Group	Total lipid (%/wet wt.)	Fatty acid composition (%)					
		14	16	18	18:1	18:2	18:3
KK							
1.0% Taurine	87.9±3.77	0.5±0.05	15.8±0.80	2.8±0.23	35.8±0.98	39.7±0.51	3.0±0.27
0.5% Taurine	83.6±3.77	0.4±0.01	14.6±0.24	2.7±0.12	35.8±1.35	41.2±0.89	3.0±0.24
Control diet	89.5±2.31	0.4±0.02	15.0±0.60	3.3±0.25	37.0±1.11	39.4±1.19	2.7±0.26
BALB/C							
Control diet	79.0±2.33	1.0±0.01	19.0±0.48	3.9±0.22	37.0±1.54	33.6±1.23	2.7±0.20

(B) Liver

Group	Total lipid (%/wet wt.)	Faffy acid composition (%)					
		16	16:1	18	18:1	18:2	18:3
KK							
1.0% Taurine	3.6±0.83	27.8±1.41	2.9±0.42	12.1±2.52	28.1±3.77	28.7±1.45	
0.5% Taurine	3.1±0.53	26.7±1.73	2.3±0.22	13.1±1.17	25.8±1.30	32.0±1.24	
Control diet	3.6±0.29	25.3±0.79	3.5±0.28	8.6±0.85	34.8±2.01	27.8±1.56	
BALB/C							
Control diet	6.0±0.33	24.9±0.78	1.1±0.14	15.3±1.21	16.9±2.17	24.0±0.83	17.5±0.92

Average value ± S.E. was determined from four experiments except for the control group of KK mice (five determination).

Table V. Autoradiographic Density of Taurine-S^{35} in Mice 3 Hours after Intravenous Administration

Organs	KK mice	BALB/C mice
Blood	20	14— 15
Liver	130—197	127—159
Heart muscle	51	21— 46
Lung	26	32
Gall bladder	157	149
Thymus	20	33
Submaxilary gland	45	45
Stomach	120	114—138
Small intestine	65—127	86— 99
Large intestine	59	51
Epidermis	19— 42	28— 36
Tongue	56	62
Brain	4— 10	11— 15
Spinal cord	7	6
Bone marrow	34	48— 51
Spleen	77	63—101
Kidney	91	134
Adrenal	43	38
Muscle	16— 18	15
Lacrimal gland	56	62
Abdominal fat	7— 9	
Urinary bladder	125	408

Based on the density of back ground (0).
Scanned by a Microdenstiometer Mark III C.S. (Joyce–Loeble, slit width: 3.8 mm, objective lens:X10, differential control:5)

Mayer, *et al.*[16] have described that in obesity more than 90% of body weight gain are assigned to excess deposition of fat, independent of changes of other biological factors, *e.g.*, increases in the content of water, protein or ash in the body. It is well-known in general that the injection of thyroid hormone, the injection of fat mobilizing substances and starvation cause a weight-loss in mice. However, no information has been available on such an effect of taurine on obesity as mentioned in this paper.

It is observed in whole body macroautoradiography that radioactivity of administered taurine-S^{35} is localized greatly in the liver and gall bladder of mice. The previous authors have described that administered taurine is readily available for the formation of bile salts in the livers of animals.[6b,17] Autoradiogram also shows that taurine-S^{35} is concentrated highly in the walls of the gastro-intestines. It is suggested in the literature that intestinal absorption of such substances as calcium[18] and salicylate[19] may be accelerated by the presence of taurine. As an explanation for the result of this study a possibility cannot be excluded that long-term feeding of taurine would contribute to suppression of excess fat deposition in the hereditary obese mice by improvement of the intestinal absorption of nutrients, especially of lipid. Neverthless, the finding that experimental obesity mainly due to polyphagia is not modified by feeding of taurine is likely to suggest alternatively that injected taurine would serve direct influence on lipid and carbohydrate metabolism in other portions, *e.g.*, the liver than the intestines of KK mice.

Long-term feeding of taurine produced no adverse effect on the growth of BALB/C mice. There is also no evidence of any toxical effect of taurine in animals including human in the literature.[20] Histological examinations on various organs and tissues from the KK mice fed 5% taurine diet are now in progress.

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