Effect of 4-(4'-Chlorobenzoyloxy)benzyl Nicotinate (KCD-232) on Cholesterol Metabolism in Rats Fed an Amino Acid Imbalance Diet

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The effect of KCD-232, a new hypolipidemic agent with the structure of 4-(4'-chlorobenzoyloxy)benzyl nicotinate, on cholesterol (Ch) metabolism was studied in rats fed a methionine-supplemented low casein (with threonine imbalance) diet.

A methionine-supplemented 10% casein (imbalance) diet caused an increase in serum Ch when compared to a 10% casein diet, while the supplement of methionine to a 20% casein diet caused no further increase. In rats fed the imbalance diet, KCD-232 reduced the serum Ch level. Measurement of Ch absorption using a dual-isotope serum ratio method showed neither an enhancement in the imbalance diet-fed rats nor a reduction in the drug-treated, imbalance diet-fed rats. However, radioactivities in the serum of orally given [^3H]Ch and intravenously given [^{14}C]Ch increased in the imbalance diet-fed rats and decreased in the KCD-232-treated rats. Hepatic sterol synthesis measured *in vivo* using [^3H] water was unchanged in the imbalance diet-fed rats and increased in the drug-treated rats.

These results suggest that the clearance of Ch from the circulation system, which may be impaired in rats fed the imbalance diet, is enhanced in the KCD-232-treated, imbalance diet-fed rats.

It is well known that dietary protein plays an important role in regulating the concentration of serum Ch.^{1,2)} A Ch-free, low fat, semi-purified diet containing casein, an animal protein, has been reported to develop equal hypercholesterolemia and atherosclerosis to a Ch-supplemented diet in rabbits.³⁾ The endogenous hypercholesterolemia can be enhanced by increasing the proportion of dietary casein in rabbits⁴⁾ and rats.⁵⁾ By contrast, soy protein, a vegetable protein, shows hypocholesterolemic properties in rabbits,^{1,2)} humans⁶⁾ and rats.⁷⁾ Purified whole egg protein has been reported to induce not only fatty liver but also an increase in serum LDL-Ch in rats fed a low protein diet containing the protein.⁸⁾ Feeding a diet containing low casein supplemented with cystine is known to develop fatty liver in rats, which is reduced if the diet is supplemented

with threonine.⁹⁾ There seem to exist similarities between the low whole egg protein and the sulfur-containing amino acid-supplemented low casein diets in regard to both the relative content of sulfur-containing amino acids and their effects on lipid metabolism. The specific effects of certain amino acids on serum Ch have been demonstrated; for instance, high glutamic acid intakes depressed serum Ch¹⁰⁾ while histidine caused hypercholesterolemia.¹¹⁾ KCD-232, 4-(4'-chlorobenzoyloxy)benzyl nicotinate, has been found to be a new hypolipidemic agent without any appreciable toxicity in the study of a series of compounds and has been shown to possess a hypocholesterolemic activity in rats.¹²⁾ The present work was undertaken to study the effect of KCD-232 on the Ch metabolism in rats fed a methionine-supplemented low casein (with threonine im-

Abbreviations: Ch, cholesterol; TG, triglyceride; PL, phospholipid; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

balance) diet.

MATERIALS AND METHODS

Chemicals. [4-¹⁴C]cholesterol (57 mCi/mmol) and [1 α ,2 α -³H]Ch (60 Ci/mmol) were obtained from The Radiochemical Centre, and [³H]water (100 mCi/ml) from New England Nuclear. KCD-232 was synthesized in this laboratory. Other chemicals were of the best grade commercially available.

Animals and diets. Male Wistar rats (CLEA Japan Inc., Tokyo) were used throughout the experiments. They were maintained on a stock pellet (CE-2, CLEA Japan) in an air-conditioned room with a 6 a.m. to 6 p.m. light cycle until use. The animals were then divided into groups with equal body weights and fed the experimental diets. The composition of the basal diets expressed in percentage weight is shown in Table I. In the initial experiment shown in Fig. 1, 0.3 or 0.6% DL-methionine (Met) was added to the 10 or 20% casein diet at the expense of sucrose. In other experiments, to the 10% casein diet were added 0.4% Met, 0.4% Met plus 0.48% L-threonine (Thr), and 0.4% Met plus 0.3% KCD-232 at the expense of sucrose as shown in each table or figure. The feeding periods are also noted in each table and figure. Where indicated, KCD-232 was suspended in 0.5% carboxymethyl cellulose and orally given to the animals by gastric intubation (100 mg/5 ml/kg body weight) once a day during the experimental periods. Under these conditions, the rats in the drug-untreated groups received carboxymethyl cellulose alone.

Lipid analyses. The rats were sacrificed by decapitation, and blood was collected in a glass tube and left to clot at room temperature. The liver was quickly removed, washed with cold 0.9% NaCl solution, blotted on filter paper and weighed. The serum (1 ml), obtained by centrifugation, and the liver (0.5 g) were homogenized with 20 vol. of chloroform-methanol (2:1, v/v) and total lipids were

extracted according to the procedure of Folch *et al.*¹³⁾ After aliquots of the chloroform phase had been dried under nitrogen, Ch,¹⁴⁾ TG¹⁵⁾ and PL¹⁶⁾ were determined by colorimetric methods. In the case of determining the liver Ch content, the extracted lipids (ca. 2 mg) were hydrolyzed with 3 ml of 6% ethanolic KOH for 1 hr at 45°C. At the end of saponification, 3 ml of distilled water was added and the sterols were extracted with 5 ml of hexane. Aliquots of the hexane layer were evaporated to dryness and the Ch content was determined.¹⁴⁾

Lipoprotein separation and cholesterol determination. The serum lipoproteins were separated into HDL and VLDL+LDL fractions by a slightly modified version of the precipitation method described by Burstein *et al.*¹⁷⁾ and Lopes-Virella *et al.*¹⁸⁾ To 0.2 ml of serum was added 2 ml of a solution containing 0.02% sodium phosphotungstate and 50 mM MgCl₂. After standing for 20 min, centrifugation was performed for 15 min at 800 $\times g$. The precipitate was dissolved in 2 M NaCl, diluted with distilled water and used for determining the Ch content of VLDL+LDL. The supernatant was used for the Ch determination of HDL. The total Ch contents of unfractionated whole serum (S-Ch), HDL (HDL-Ch) and VLDL+LDL [(VLDL+LDL)-Ch] were determined by an enzymatic method using a Determiner TC "555" kit (Kyowa Hakko Kogyo Co., Ltd., Tokyo).

Determination of cholesterol absorption. Cholesterol absorption was measured by the dual-isotope ratio method described by Zilversmit and Hughes.¹⁹⁾ The intravenous dose of [4-¹⁴C]Ch was prepared by dissolving in 25 μ l of 95% ethanol. Colloidal Ch was obtained by adding 0.5 ml of 0.9% NaCl. The resulting mixture was injected intravenously within 1 hr of its preparation, each rat receiving 0.762 μ Ci of [¹⁴C]Ch per 0.5 ml. The oral dose of [1 α ,2 α -³H]Ch was prepared as follows: [³H]Ch (3.424 μ Ci) was dissolved with 6 mg of carrier Ch in 156 mg of triolein (Tokyo Kasei Kogyo Co., Ltd., Tokyo), the oil phase of which was then suspended by sonication in water containing 7.5 mg of sodium taurocholate (Sigma Chemical Co.) and the final volume was adjusted with water to 2 ml. Each rat therefore received 6 mg of Ch plus 3.424 μ Ci of [³H]Ch per 2 ml.

The intravenous isotope dose was injected into the tail vein of the rats. This was immediately followed by the oral isotope dose which was given by stomach tube to the rats which were all in the fed state. Three days after the doses, the animals were decapitated and blood was collected. To 0.8 ml of serum was added 2 ml of 15% ethanolic KOH. The mixture was saponified at 85°C for 2 hr and the nonsaponifiable fraction was extracted three times each with 4 ml of petroleum ether into a counting vial. After the solvent had been dried, radioactivity was counted with a liquid scintillation spectrometer (Packard Tri-Carb model C2425) after adding 10 ml of toluene scintillator. Cholesterol absorption was calculated with a ³H/¹⁴C ratio

TABLE I. COMPOSITION OF EXPERIMENTAL DIETS (%)

Ingredient	10% Casein	20% Casein
Casein ^a	10.0	20.0
Sucrose ^a	81.0	71.0
Corn oil ^b	1.0	
Mineral mixture ^c	5.0	
Vitamin mixture ^c	1.0	
Cellulose powder ^a	2.0	

^a Oriental Yeast Co., Ltd., Tokyo.

^b Yuro Yakuhin Co., Ltd., Tokyo.

^c Mineral mixture 1 and vitamin mixture 1 arranged by Oriental Yeast Co., Ltd. were used.

in the nonsaponifiable fraction and administration doses.¹⁹⁾

Hepatic sterol and fatty acid syntheses in vivo using [³H]water. The rats were deprived of their diets at 9 a.m. on the 21st day of feeding. [³H]water (2 mCi/0.5 ml isotonic NaCl solution/rat) was intraperitoneally injected 90 min prior to their sacrifice which was conducted by decapitation from 1:30 p.m. The animals were not given any further water during this 90 min period. Blood was collected in a glass tube and left to clot at room temperature. The liver was quickly removed, washed with cold 0.9% NaCl solution, blotted on filter paper and weighed. The minced liver weighing about 1 g was placed in a screw-capped tube. After adding 1 ml of water and 2 ml of 15% ethanolic KOH, the mixture was saponified at 85°C for 5~6 hr until totally dissolved. The nonsaponifiable lipids were then extracted three times each with 4 ml of hexane. Carry-over [³H]water was eliminated by adding anhydrous sodium sulfate as a dehydrating agent to the pooled hexane and the mixture was filtered to remove the sodium sulfate. The filtrate was pooled in a glass tube, dried under nitrogen and dissolved in 1 ml of acetone-ethanol (1:1, v/v). Subsequently, the digitonin-precipitable sterols were removed, isolated and washed as described by De Matteis.²⁰⁾ The digitonide was dissolved in 1 ml of methanol, transferred quantitatively to a scintillation vial and counted after adding the toluene scintillator.

The residue remaining after the extraction of sterols was acidified to pH 2 with concentrated HCl and the total fatty acids synthesized were extracted three times each with 4 ml of petroleum ether. The pooled extracts were dried under nitrogen, dissolved in 2 ml of chloroform and washed twice with water. The chloroform layer was quantitatively transferred to a scintillation vial, dried and counted as stated above.

Fifty μ l of serum was taken into a scintillation vial and its radioactivity counted after adding 10 ml of Insta Gel (Packard Instrument Co., Inc.). The specific radioactivity of the serum water at the time each rat was killed was calculated by considering the water content of the serum to be 93%, and the μ moles of water incorporated into the sterols²¹⁾ and total fatty acids²²⁾ was calculated by the specific radioactivity.

Statistical method. The statistical analysis was carried out using Student's *t* test for paired data, and *p* value of <0.05 was considered significant.

RESULTS

The initial study was attempted to examine the effect of a Met supplement to a 10% casein or 20% casein diet on the serum Ch level (Fig. 1). The serum Ch level was significantly increased by adding 0.3% Met to the 10% casein

FIG. 1. Effect of the Methionine Supplement to Casein Diets on the Serum Cholesterol Levels of Rats.

Rats weighing 190 g in all groups were fed a 10% casein (10C), 0.3% methionine-supplemented 10% casein (10CM), 20% casein (20C) or 0.6% methionine-supplemented 20% casein (20CM) diet *ad libitum*. They were deprived of these diets at 9 a.m. on the 28th day and decapitated from 1 p.m. Each value and vertical bar represent the mean of eight rats and the standard error, respectively. **p* < 0.01 to the 10CM group.

diet (groups 10C vs. 10CM), while the supplement of 0.6% Met to the 20% casein diet induced no further increase in the serum Ch level (groups 20C vs. 20CM). The Ch level of rats fed the 20% casein diet tended to be higher (*p* < 0.1) than that of rats fed the 10% casein diet (groups 10C vs. 20C). From these results, the enhancing effect of Met on the serum Ch level was verified in the case of the low casein diet. Hence the Met-supplemented low casein (with Thr imbalance) diet was subsequently used.

In the next experiment, the amount of Met supplemented to the 10% casein diet was increased from 0.3 to 0.4%, and the feeding period was curtailed from 4 weeks to 2 weeks. Under these conditions, the effects of Thr as well as KCD-232 on the serum and liver lipids were estimated because of the fact that Thr could prevent fatty liver induced by feeding a low casein diet supplemented with a sulfur-containing amino acid (cystine) in rats.⁹⁾ The results are shown in Table II. Despite the shortening of feeding period, the serum Ch level was significantly elevated by the supplement of 0.4% Met to the 10% casein diet (groups 1 vs. 2). Threonine failed to reduce the serum Ch level at a content of 0.48% (groups 2 vs. 3), whereas KCD-232 significantly suppressed it at a content of 0.3% (groups 2 vs. 4). No significant changes were observed in the

TABLE II. EFFECTS OF THREONINE AND KCD-232 ON HYPERCHOLESTEROLEMIA OF RATS INDUCED BY A METHIONINE SUPPLEMENT TO A LOW CASEIN DIET

Group	Treatment	Initial body wt (g)	Final body wt (g)	Relative liver wt (g/100 g bwt)	Serum lipid levels			Liver lipid levels		
					Ch	TG (mg/dl serum)	PL	Ch	TG (mg/g wet liver)	PL
1	10% casein (C)	241 ± 6	267 ± 7	3.15 ± 0.05	87 ± 6 ^a	125 ± 12	154 ± 8	2.0 ± 0.1	12.0 ± 1.5	32.2 ± 0.6
2	C + 0.4% Met (CM)	240 ± 6	274 ± 7	3.07 ± 0.05	102 ± 3	102 ± 14	160 ± 8	2.3 ± 0.3	21.5 ± 4.2	30.3 ± 1.7
3	CM + 0.48% Thr (CMT)	240 ± 6	281 ± 7	3.38 ± 0.31	104 ± 4	115 ± 22	168 ± 7	2.1 ± 0.1	16.8 ± 3.2	29.6 ± 0.3
4	CM + 0.3% KCD-232 (CMK)	240 ± 6	281 ± 7	3.16 ± 0.05	67 ± 4 ^b	65 ± 10 ^a	100 ± 6 ^b	2.0 ± 0.1	5.9 ± 0.9 ^a	37.4 ± 1.5 ^a

Rats were fed experimental diets for 14 days *ad libitum*. On the 14th day, the animals were deprived of their diets at 9 a.m. but allowed free access to water until sacrifice which was performed by decapitation from 1 p.m. Each value represents the mean of six rats ± S.E.M. ^a*p* < 0.05 and ^b*p* < 0.001 to Group 2, respectively.

TABLE III. CHOLESTEROL ABSORPTION IN RATS FED A METHIONINE-SUPPLEMENTED LOW CASEIN DIET AND THE EFFECT OF KCD-232

Group	Treatment	Initial body wt (g)	Final body wt (g)	Relative liver wt (g/100 g bwt)	Cholesterol levels		Serum ³ H-Ch (PO) (dpm/ml)	Serum ¹⁴ C-Ch (IV) (dpm/ml)	Absorbed cholesterol (%)
					Serum (mg/dl)	Liver (mg/g)			
1	10% Casein (C) & CMC	295 ± 9	302 ± 11	3.71 ± 0.24	109 ± 3 ^b	2.9 ± 0.3	14591 ± 834 ^a	8396 ± 379 ^a	38.8 ± 2.3
2	C + 0.4% Met (CM) & CMC	299 ± 5	310 ± 10	3.75 ± 0.10	144 ± 9	2.7 ± 0.1	18185 ± 1160	11079 ± 847	37.0 ± 3.2
3	CM & KCD-232 (100 mg/kg/day)	298 ± 5	319 ± 8	3.99 ± 0.14	61 ± 7 ^c	2.5 ± 0.1	7582 ± 380 ^c	4393 ± 429 ^c	39.0 ± 2.0

Rats were fed experimental diets for 17 days *ad libitum*. On the 14th day, 0.762 μCi of [4-¹⁴C]cholesterol was injected into the tail vein followed immediately by 3.424 μCi of [1α, 2α-³H]cholesterol dose which was given by stomach tube. The experimental diets were then resumed *ad libitum*. The final administration of carboxymethyl cellulose (CMC) or KCD-232 was orally performed at 9 a.m. on the 17th day. The rats were allowed free access to the diets and water until sacrifice which was conducted by decapitation from 1 p.m. Each value represents the mean of four rats ± S.E.M. ^a*p* < 0.05, ^b*p* < 0.01 and ^c*p* < 0.001 to Group 2, respectively.

FIG. 2. Cholesterol Distribution in Serum Lipoproteins of Rats Fed a Methionine-supplemented Low Casein Diet and the Effect of KCD-232.

Rats were given a 10% casein (C) diet, C diet supplemented with 0.4% methionine (CM) or CM diet with oral administration of KCD-232 at a dose of 100 mg/kg of body weight once a day (CMK) for 14 days. The final oral administration of KCD-232 was performed at 9 a.m., at which time the animals were deprived of their diets but allowed free access to water until sacrifice. They were decapitated from 1 p.m. The initial body weights of the C, CM and CMK groups were 217 ± 7 , 216 ± 8 and 218 ± 7 g (the mean of six rats $\pm$ S.E.M.), respectively. A, total cholesterol content of unfractionated whole serum (S-Ch). B, HDL-Ch (□) and (VLDL+LDL)-Ch (▨). Each value and vertical bar represent the mean of six rats and the standard error, respectively. * $p < 0.05$ and ** $p < 0.01$ to the CM group.

serum TG and PL levels by the Met supplement. Threonine had no significant effects on the serum TG and PL levels but KCD-232 significantly decreased both lipid levels. The liver TG content showed a tendency ($p < 0.1$) to be increased by the Met supplement. Threonine slightly prevented, although not significantly, this tendency to increase, and KCD-232 strongly reduced it. The liver PL content was not changed by the Met supplement and Thr had no further effect on it, while KCD-232 significantly increased it. The liver Ch contents were almost constant among the four groups. The growth of the rats and their relative liver weight were not affected by Met, Thr and KCD-232. As illustrated in Fig. 2, to estimate the Ch distribution among serum lipoproteins in the rats, the lipoproteins were separated into HDL and VLDL+LDL fractions by the precipitation method and the Ch content of each fraction was determined. The total Ch content of unfractionated whole serum (S-Ch) of the rats fed the Met-supplemented 10% casein (CM) diet signifi-

FIG. 3. Synthetic Rates of Hepatic Sterols and Fatty Acids in Rats Fed a Methionine-supplemented Low Casein Diet and the Effect of KCD-232.

Rats weighing 204 g in all groups were fed a 10% casein (C) diet, C diet supplemented with 0.4% methionine (CM) or CM diet supplemented with 0.3% KCD-232 (CMK) *ad libitum* and deprived of their diets at 9 a.m. on the 21st day. [^3H]Water (2 mCi/rat) was intraperitoneally injected 90 min prior to sacrifice which was conducted by decapitation from 1:30 p.m. The animals were not given any further water during this period of 90 min. The [^3H]water incorporation into hepatic digitonin-precipitable sterols (A) and total fatty acids (B) was determined as described in MATERIALS AND METHODS. Each value and vertical bar represent the mean of six rats and the standard error. * $p < 0.05$ and ** $p < 0.01$ to the CM group.

cantly increased by 57% in comparison with that of the rats fed the 10% casein (C) diet. This rise was due to an increase in both HDL-Ch and (VLDL+LDL)-Ch by 49 and 67%, respectively, although the latter was an increasing tendency ($p < 0.1$). KCD-232 significantly reduced the S-Ch level of CM rats by significantly decreasing both HDL-Ch and (VLDL+LDL)-Ch (CM vs. CMK).

The next group of studies was conducted to deduce the possible mechanism(s) involved in the amino acid imbalance-induced hypercholesterolemia and the action of KCD-232 on it. Table III shows Ch absorption from the intestine evaluated by a dual-isotope serum ratio method.¹⁹⁾ Neither an enhancement in Ch absorption in rats fed the imbalance diet (groups 1 vs. 2) nor a reduction in rats treated with KCD-232 (groups 2 vs. 3) was observed. However, the radioactivities in the serum of ^3H derived from an oral dose of [$1\alpha,2\alpha\text{-}^3\text{H}$]Ch and ^{14}C derived from an intravenous dose of [$4\text{-}^{14}\text{C}$]Ch significantly stagnated in the imbalance diet-fed rats (groups 1 vs. 2) and significantly diminished in the drug-treated rats (groups 2 vs. 3). Figure 3 shows the

synthetic rates of hepatic sterols and fatty acids *in vivo*. [^3H]water was used for measuring the sterol and fatty acid synthetic rates, since the incorporation of ^3H from [^3H]water has been reported to provide reliable estimates for the absolute rates of sterol²¹⁾ and fatty acid²²⁾ syntheses *in vivo*. The results are expressed as both $\mu\text{moles of H}_2\text{O incorporated/hr/g}$ of wet liver and $\mu\text{moles of H}_2\text{O incorporated/hr/whole liver/100 g}$ of body weight. The supplement of Met to the low casein diet had no significant effect on the sterol synthetic rate (C vs. CM), while the drug addition to the CM diet showed a marked increase in it (CM vs. CMK). On the other hand, the fatty acid synthetic rate was highly enhanced by the Met supplement (C vs. CM) and KCD-232 significantly reduced it (CM vs. CMK). The serum Ch levels of the C, CM and CMK groups were 90 ± 6 , 114 ± 7 and $80 \pm 7 \text{ mg/dl}$ of serum (the mean of six rats $\pm \text{SEM}$, $p < 0.05$ for C vs. CM and $p < 0.01$ for CM vs. CMK), respectively. The liver TG levels of the C, CM and CMK groups were 17.2 ± 1.7 , 49.3 ± 12.1 and $5.5 \pm 0.8 \text{ mg/g}$ of liver (the mean of six rats $\pm \text{SEM}$, $p < 0.05$ for C vs. CM and CM vs. CMK).

DISCUSSION

The initial study showed that increasing dietary casein tended to increase the serum Ch levels of rats (Fig. 1). This result is in good agreement with the study of Terpstra *et al.*⁴⁾ who found a gradual increase in serum Ch concentrations of rabbits receiving 10, 20 and 40% casein diets. The supplement of Met to the 10% casein diet caused a further increase in the serum Ch of rats, whereas that of Met to the 20% casein diet showed no further increase in serum Ch (Fig. 1). The latter finding is similar to that of Hamilton and Carroll²³⁾ who have reported that a Met supplement to a 27% casein diet low in fat failed to induce any further increase in the serum Ch concentrations of rabbits. The former finding shows that an amino acid imbalance diet (with Thr imbalance) induces endogenous hypercholes-

terolemia, in addition to fatty liver^{9,24)} in rats. Fatty liver induced by such an amino acid imbalance is known to be prevented by Thr.⁹⁾ Threonine, however, failed to reduce the hypercholesterolemia developed by the imbalance diet low in fat at a dietary content of 0.48% and showed little effect on accumulated liver TG (Table II). The reason for the failure of Thr is not clear at present, but it may be related to the low content of dietary fat (1% corn oil) and/or an inadequate amount of Thr supplement. The elevation in serum Ch of the imbalance diet-fed rats is shown to be due to those in both HDL-Ch and (VLDL+LDL)-Ch (Fig. 2). KCD-232 reduced the serum Ch level of the imbalance diet-fed rats by decreasing both HDL-Ch and (VLDL+LDL)-Ch.

It seems unlikely that the elevation in the serum Ch of the imbalance diet-fed rats is due to either an enhanced Ch absorption from the intestine or an enhanced Ch synthesis in the liver. The drug also had neither a suppressive effect on Ch absorption nor an inhibitory effect on hepatic Ch synthesis (Table III, Fig. 3). Hepatic sterol synthesis, far from decreasing, is increased by KCD-232 treatment despite its ability to inhibit sterol synthesis due to a KCD-232 metabolite, 4-(4'-chlorobenzyl-oxy)benzoic acid.²⁵⁾ On the contrary, radioactivities in the serum of ^3H derived from an oral dose of [^3H]Ch, and ^{14}C derived from an intravenous dose of [^{14}C]Ch were significantly higher in rats fed the Met-supplemented 10% casein diet than those fed the 10% casein diet. Conversely, KCD-232 decreased the radioactivities of [^3H]Ch and [^{14}C]Ch present in the serum. The serum Ch is excreted partly as sterols and partly as bile acids; in rats, the conversion of Ch to bile acids is much the more important of these two pathways.²⁶⁾ It is therefore suggested that the Ch clearance from the circulation, which may have been impaired in the imbalance diet-fed rats, was enhanced in the KCD-232-treated, imbalance diet-fed rats, presumably by enhancing the bile acid formation in the liver.

Fatty liver induced in rats fed the Met-supplemented 10% casein diet was reduced by

KCD-232 treatment. Hepatic lipogenesis, above all the synthesis of TG, from [^{14}C] acetate has been reported to increase in liver slices of rats fed a Met-supplemented low casein diet.²⁴⁾ The current study, which shows a significant increase in the *in vivo* synthesis of hepatic fatty acid in rats fed the Met-supplemented 10% casein diet (Fig. 3), is consistent with the previous work.²⁴⁾ The amino acid imbalance-induced enhancement in hepatic fatty acid synthesis was prevented by KCD-232. This drug has been reported to inhibit hepatic fatty acid synthesis in rats which is attributable to its metabolite 4-(4'-chlorobenzoyloxy)benzoyl-coenzyme A,²⁵⁾ like natural fatty acyl-coenzyme A thioesters.^{27,28)} KCD-232 is therefore considered to reduce both the serum TG and development of the amino acid imbalance-induced fatty liver by inhibiting TG synthesis *via* a suppression of the enhanced fatty acid synthesis in the liver. KCD-232 often reduced the serum Ch level of the Met-supplemented 10% casein diet-fed rats below that of the 10% casein diet-fed rats (Tables II and III). The hypotriglyceridemic activity of KCD-232 may partly explain this phenomenon; the Ch moiety as well as TG moiety of serum lipoproteins can be reduced by the strong hypotriglyceridemic activity. Further studies are needed to clarify this aspect.

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