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EFFECT OF SIMVASTATIN (MK-733) ON PLASMA TRIACYLGLYCEROL LEVELS IN RATS

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Abstract—The effect of simvastatin (MK-733), an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, on plasma triacylglycerol (TG) levels was studied in rats. Dietary administration of MK-733 (0.055%, w/w) for 7 days significantly ($P < 0.05$) reduced plasma TG levels by 30.6% associated with a 44.3% significant ($P < 0.01$) reduction in very low density lipoprotein TG (VLDL-TG) as compared to those in the concurrent control rats. Clofibrate (0.08%, w/w) also significantly ($P < 0.05$) decreased plasma TG levels by 26.1%. MK-733 did not affect the triacylglycerol secretion rate (TGSR) during 0–1.5 hr after administration of Triton WR-1339, but reduced it by 33.9% during 1.5–3.0 hr. Clofibrate also decreased TGSR during 1.5–3.0 hr. MK-733 increased lipoprotein lipase (LPL) activity in epididymal adipose tissue and thigh muscle by 36.3 and 55.0% respectively. MK-733 significantly ($P < 0.05$) increased LPL activity in the post-heparin plasma by 21.5%, although it did not affect hepatic triacylglycerol lipase (H-TGL) activity. Clofibrate did not affect LPL activity in the tissues or LPL and H-TGL activities in the post-heparin plasma. It is considered that the mechanism of plasma TG-lowering effect of MK-733 is the removal of VLDL-TG by an increase in LPL activity in the tissues as well as a decrease in the TGSR.

Simvastatin (MK-733) [1], a chemical derivative of lovastatin (MK-803) [2], is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34), which is the rate-limiting enzyme in the cholesterol biosynthetic pathway [3]. The active open acid form of this compound, L-654,969, inhibits HMG-CoA reductase with an IC_{50} of 0.94×10^{-9} M, and is approximately twice as active as L-154,819, the active open acid form of MK-803 [1]. MK-733 has been reported to inhibit cholesterol synthesis from [^{14}C]acetate in different animal cell lines [4–6] and to reduce plasma cholesterol levels in experimental animals [4, 7, 8] and in humans [9, 10]. MK-733 reduced the intracellular concentration of cholesterol by inhibiting hepatic cholesterol synthesis and enhancing the removal of plasma low density lipoprotein (LDL) by up-regulating LDL receptors. In some clinical studies, MK-733 was found to reduce plasma triacylglycerol (TG) levels significantly and to increase high density lipoprotein (HDL) cholesterol in hyperlipidemic patients [11–14].

Recently, Yoshino *et al.* investigated the effects of pravastatin (CS-514) [15], another HMG-CoA reductase inhibitor, on TG kinetics in rats [16]. The data indicated that CS-514 suppressed very low density lipoprotein triacylglycerol (VLDL-TG) secretion in fed rats, whereas the drug did not affect it in fasted rats. They concluded that the observed reduction in VLDL-TG secretion was a cause for the TG-lowering effect of CS-514 in rats.

Theoretically, plasma TG concentration is regulated by TG secretion from the liver and/or intestine and TG removal by peripheral tissues. However, the effects of HMG-CoA reductase inhibitors on TG removal in rats have not been studied yet. Lipoprotein lipase (LPL, EC 3.1.1.34) and hepatic triacylglycerol lipase (H-TGL) play important roles in the catabolism of plasma lipoprotein TG. TG catabolism is initiated by the action of LPL, which is active at the endothelial surface of several tissues, mainly adipose tissue, skeletal muscle and heart [17]. The major function of LPL is the hydrolysis of TG in chylomicrons and very low density lipoprotein (VLDL) [17, 18]. Furthermore, it is recognized that some part of HDL particles is produced from surface components of chylomicrons and VLDL particles by LPL [19]. The role of H-TGL in lipoprotein metabolism has not been well defined yet. The preferred substrate for H-TGL is HDL (especially HDL_2) [20] and intermediate density lipoprotein (IDL) [21].

It has been reported that rodents are not suitable to evaluate the hypocholesterolemic effects of HMG-CoA reductase inhibitors [22, 23]. However, CS-514 has been clearly demonstrated to decrease plasma TG levels in rats [16]. Therefore, we used rats to evaluate the hypotriacylglycerolemic effect of MK-733. The present experiment was conducted in order to examine the TG-lowering effect of MK-733 and to elucidate the mechanism of action of the effect. Clofibrate was used as a reference hypotriacylglycerolemic drug.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley (Crj:CD) rats (5-weeks-old) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). The animals were housed in metal cages at an ambient temperature of

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23 ± 2° and at a humidity of 55 ± 15% in an air-controlled room which was lit artificially between 6:00 a.m. and 6:00 p.m. or between 6:00 p.m. and 6:00 a.m. The rats were fed a commercial chow pellet (CA-1, Clea Japan Inc., Tokyo, Japan) and were given water *ad lib.* for at least 1 week in order to acclimatize them.

Drugs. MK-733 (Lot No. L-644, 128-000U123, purity 99.3%) was prepared by Merck Sharp & Dohme Research Laboratories (MSDRL, Rahway, NJ, U.S.A.). Clofibrate (Lot No. F031301 and H010201, purity of each 100.0%) was obtained from Shizuoka Caffeine Industries Co. (Shizuoka, Japan).

Chemicals. Glycerol tri[1-^{14}C]oleate (114 mCi/mmol) and [^{3}H]oleic acid (5.0 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). Triton WR-1339 (Tyloxapol), heparin sodium (porcine intestinal mucosa, 181 units/mg), glycerol trioleate, bovine serum albumin (essentially fatty acid free) and fluorescamine were obtained from Sigma (St. Louis, MO, U.S.A.). All other chemicals used were standard commercial high purity materials.

Drug administration. Each drug was administered to rats in the diet (CA-1, Clea Japan Inc.). Either MK-733 at a concentration of 0.055% (w/w) or clofibrate at a concentration of 0.08% (w/w) was incorporated into the diet. Diet containing each drug was stored at −20° until used. Food intake was measured daily in order to estimate the dosage during the experimental period.

Plasma lipid levels. Six-week-old rats were divided into approximately equal initial body weight groups. Rats were fed diets containing either MK-733 or clofibrate for 7 days. Body weight was measured on the initial and final days of the experiment. In the morning of day 8, non-fasting blood samples were collected from an abdominal artery of each rat under ether anesthesia. An aliquot of fresh plasma was provided for analysis of plasma and lipoprotein lipids. At necropsy, the liver was removed and weighed.

Triacylglycerol secretion rate (TGSR). TGSR was determined by the Triton method according to the method of Steiner *et al.* [24]. Triton WR-1339 has been reported to block the removal of circulating TG completely [25]. TGSR was determined in the late dark on day 7, since TGSR during the dark phase was higher than during the light phase in our exploratory experiment (unpublished data). Triton WR-1339 was dissolved in physiological saline (300 mg/mL) and injected into the tail vein (600 mg/kg) of conscious rats in the late dark ($D_{10} \sim D_{11}$, the average-point was $D_{10.5}$). Blood samples (0.4 mL) were collected from the tail vein before and 1.5 hr after the injection of Triton WR-1339. At 3 hr after the injection, the rats were anesthetized with ether and blood was taken from an abdominal artery. Chylomicron and VLDL in plasma were fractionated with an ultracentrifuge (L8-80M, Beckman Instruments Inc., Fullerton, CA, U.S.A.) using an angle rotor (Type 40.3, Beckman) for the determination of TG concentrations in each fraction. Lipid and protein concentrations in the secreted VLDL were determined.

Tissue LPL. Epididymal adipose tissue and thigh muscle were obtained from the intact rats in the late dark for determination of tissue LPL activity. Each tissue was removed and immediately immersed in liquid nitrogen. The frozen tissue was stored at −80° until used.

Post-heparin plasma LPL and H-TGL. Post-heparin blood samples were collected from an abdominal artery exactly 5 min after injection of heparin at a dose of 2000 units/kg into the tail vein in the late dark on day 7 for the determination of LPL and H-TGL activity. Post-heparin plasma (PHP) was prepared by centrifugation at 2800 rpm at 4° for 10 min. PHP was frozen with liquid nitrogen and stored at −80° until used.

Lipoprotein fractionation. Plasma lipoprotein fractions were separated according to Havel *et al.* [26]. The chylomicron ($d < 0.96$) fraction was separated by centrifugation at 26,000 *g* and 10° for 30 min as described by Hatch and Lees [27]. After removal of the chylomicron fraction, lipoprotein fractions were separated into VLDL ($0.96 < d < 1.006$), LDL ($1.006 < d < 1.063$) and HDL ($d > 1.063$) by ultracentrifugation as described previously [8].

Determination of lipid concentrations. Plasma TG, total cholesterol (TC), phospholipid (PL) and free fatty acid (FFA) levels were measured with an autoanalyzer (Centrifichem, Encore, Baker Instruments Co., Allentown, PA, U.S.A.), using Determiner TG-S555, Determiner TC555, Determiner PL (Kyowa Medex Co., Tokyo, Japan) and Iatro-MA701 NEFA (Iatron, Tokyo, Japan) respectively. TG concentrations in each plasma lipoprotein fraction were measured manually by the enzymatic method as described above and were calculated as described previously [8].

Plasma glucose and serum insulin. Concentrations of plasma glucose and serum insulin were determined in the late dark on day 7 using an enzymatic kit (Glucose/Hexokinase, Baker Instruments Co.) and an enzyme immunoassay kit (Insulotec Mochida, Mochida Pharmaceutical Co., Tokyo, Japan) respectively.

Preparation of the antiserum against H-TGL. H-TGL was partially purified from rat PHP using heparin-agarose (Bethesda Research Laboratories Life Technologies Inc., MA, U.S.A.) column chromatography, and antiserum against H-TGL was prepared in rabbits as described by Murase and Uchimura [28]. Antiserum against H-TGL was stored at −80° for use. Antiserum completely inhibited H-TGL activity, but it did not affect LPL activity.

Determination of LPL and H-TGL in PHP. LPL activity was assayed by the method of Yamada *et al.* with some modifications [29]. The composition of substrate mixture was as follows: 2 μCi of glycerol tri[1-^{14}C]oleate, 0.133 g of cold trioleate, 0.9 mL of 4% bovine serum albumin solution (pH 8.6), 0.9 mL of 1% Triton X-100 and 10.2 mL of Tris–HCl buffer (pH 8.6). The substrate mixture was sonicated on ice for 3 min with a sonifier (Sonifier 185, Branson Sonic Power Co., Danbury, CT, U.S.A.). Post-heparin plasma was incubated with an equal volume of H-TGL antiserum for 60 min at 4° and then diluted ten times with 4% BSA in 0.2 M Tris–

HCl buffer (pH 7.4). After centrifugation, the supernatant was used for the determination of LPL activity; 0.1 mL of the supernatant was incubated with 0.2 mL of the substrate mixture, 0.025 mL of pooled rat serum as an activator for LPL, and 0.175 mL of 4% BSA in 0.2 M Tris–HCl buffer (pH 7.4). After incubation at 37° for 30 min, the reaction was terminated by the addition of 2 mL of 1.5 M H_2SO_4/isopropyl alcohol (1:40, v/v). Approximately 8,000–10,000 dpm of [^{3}H]oleate as an internal standard was added to the reaction mixture for calculating recovery. Extraction of [^{14}C]oleate formed was performed according to the procedure of Schots *et al.* [30] and elsewhere [28, 29]. The radioactivity was counted with a liquid scintillation counter (TRI-CARB 2000CA, Packard Instrument Co., IL, U.S.A.). Total lipase activity was measured using non-immune rabbit serum instead of anti-H-TGL serum and other procedures were the same as those in the LPL assay. H-TGL activity was calculated by subtracting LPL activity from total lipase activity. LPL and H-TGL activities are expressed as micromoles of free fatty acid formed per milliliter per hour.

Determination of tissue LPL. Each tissue was thawed and weighed exactly. One hundred milligrams of adipose tissue or 300 mg of thigh muscle was minced and incubated with 2 mL of 95% O_2–5% CO_2 gas-aerated Krebs–Ringer bicarbonate (pH 7.4) containing 5 units/mL heparin and 4% (w/w) BSA at 25° for 15 min. After centrifugation, 0.25 mL of supernatant was added to the incubation mixture containing 0.2 mL of substrate mixture, 0.025 mL of rat serum and 0.05 mL of 4% BSA in 0.2 M Tris–HCl buffer (pH 7.4). The assay was carried out as described above (the LPL assay for PHP). LPL activity is expressed as micromoles of free fatty acid formed per gram per hour.

Determination of protein. Protein concentration in the secreted VLDL was determined using fluorescamine as described by Sims and Carnegie [31] to avoid the effect of Triton WR-1339.

Data analysis. Data from these studies were statistically analyzed using one-way analysis of variance (ANOVA) and then the Newman–Keuls test [32]. Values are expressed as means ± SD.

RESULTS

Food intake, body weight and liver weight. There was no significant difference in the initial and final body weights between the control and drug-treated groups (data not shown). Daily food intakes were 25.9 ± 2.6, 25.9 ± 2.0 and 25.5 ± 1.6 g/day in the control, MK-733-treated and clofibrate-treated groups respectively. Daily food intake was not affected by dietary administration of MK-733 or clofibrate. The estimated doses of MK-733 and clofibrate, which were calculated from food intakes and average body weights on the initial and last days of the experiment, were 64 ± 5 and 93 ± 8 mg/kg respectively.

As shown in Table 1, MK-733 did not affect the liver to body weight ratio, whereas clofibrate significantly increased this ratio by 11.9% compared to that in the control group.

Plasma lipid levels. Plasma lipid levels on day 8 are summarized in Fig. 1. MK-733 reduced plasma TG significantly from 111 ± 30 to 77 ± 29 mg/dL (30.6%). MK-733 reduced plasma TC (14.8%) and PL (17.8%) significantly, and increased FFA (38.9%). Clofibrate decreased TG, TC and PL levels significantly by 26.1, 25.1 and 20.0% respectively. FFA levels were not affected by clofibrate. Reduction percentages of plasma TG by MK-733 and clofibrate in this experiment and those in all the following experiments were almost comparable (data not shown).

TG concentrations in lipoprotein fraction. As shown in Fig. 2, most plasma TG existed in the chylomicron and VLDL fractions. MK-733 reduced VLDL-TG levels significantly by 44.3% as compared with that of the control group. Chylomicron-TG, LDL-TG and HDL-TG levels were not affected by MK-733. Clofibrate reduced VLDL-TG levels significantly by 27.5%. Chylomicron-TG, LDL-TG and HDL-TG were not affected by clofibrate.

Plasma glucose and serum insulin levels. Plasma glucose and serum insulin levels were 138 ± 7 mg/dL and 45.0 ± 12.5 μunits/mL, respectively, in the control rats. Plasma glucose levels were 139 ± 10 and 133 ± 11 mg/dL in the MK-733-treated and clofibrate-treated groups respectively. Serum insulin levels were 49.1 ± 16.3 and 47.5 ± 15.6 μunits/mL in the two groups respectively. MK-733 and clofibrate did not affect plasma glucose and serum insulin levels.

TGSR. The effects of MK-733 on TGSR were examined in the late dark period, since TGSR in the late dark was higher than that in the other phase in normal rats (data not shown). When Triton WR-1339 was injected intravenously in normal rats, the plasma TG concentrations were increased linearly for at least 3 hr after the injection (data not shown). Under these conditions, MK-733 and clofibrate did not affect TGSR during 0–1.5 hr, but both drugs decreased it significantly during 1.5–3.0 hr by 33.9 and 30.6% respectively (Fig. 3). Plasma was fractionated into chylomicrons and VLDL. Both drugs significantly reduced VLDL-TG levels without affecting chylomicron-TG levels (Fig. 4). The VLDL-lipid and VLDL-protein concentration are shown in Table 2. The concentrations of all lipid classes in VLDL and VLDL-protein were affected significantly by MK-733. Clofibrate also reduced VLDL-TG and VLDL-protein. The ratio of FC/TG, PL/TG and protein/TG in the MK-733-treated group was similar to that in the control group, but the ratio of EC/TG was increased by MK-733, whereas clofibrate did not alter the ratios of any lipid classes/TG or protein/TG in the VLDL.

Tissue LPL. LPL activity in epididymal adipose tissue was higher than that in thigh muscle. MK-733 significantly increased LPL activity in adipose tissue and muscle by 36.3 and 55.0%, respectively, as compared to those in the control rats (Fig. 5). Clofibrate did not affect LPL activity in adipose tissue and muscle.

H-TGL and LPL activities in the PHP. The functional LPL activity is often estimated in intact animals by TG lipolytic activity in PHP. It is well known that LPL and H-TGL are released when

Table 1. Effects of MK-733 and clofibrate on the liver to body weight ratio in rats

Treatment	N	Liver weight (g)	Liver to body weight ratio (%)
Control	18	12.2 ± 0.7	4.87 ± 0.21
MK-733	8	12.0 ± 0.8	4.80 ± 0.24
Clofibrate	10	13.7 ± 0.8*	5.45 ± 0.29*

Rats in the control group were fed a normal diet. Either MK-733 (0.055%, w/w) or clofibrate (0.08%, w/w) was administered in the diet of the drug-treated animals. Rats were fed each diet for 7 days. Values represent the mean ± SD in each group; N indicates the number of animals.

* Significantly different from the values in the control group as determined by the Newman–Keuls test, $P < 0.01$.

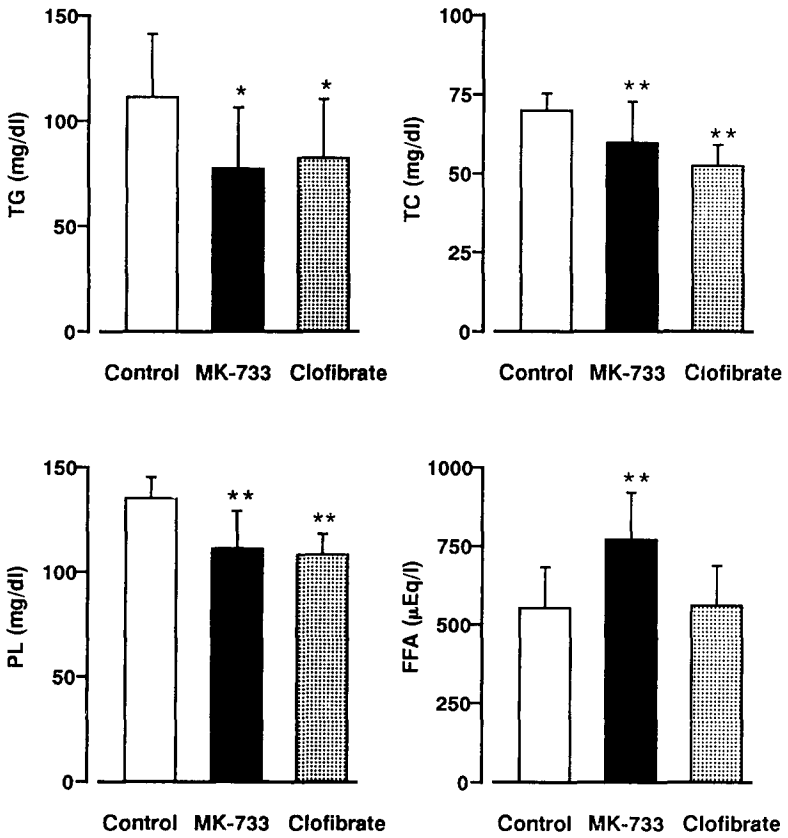

Fig. 1. Effects of MK-733 and clofibrate on plasma lipid levels. Either MK-733 (N = 8) or clofibrate (N = 10) was administered in the diet for 7 days. Rats in the control group (N = 18) were fed a normal diet. Plasma triacylglycerol (TG), total cholesterol (TC), phospholipid (PL) and free fatty acid (FFA) levels were determined on day 8 as described in Materials and Methods. Values represent the mean ± SD in each group. Key: significantly different from the value in the control group as determined by the Newman–Keuls test: (*) $P < 0.05$, and (**) $P < 0.01$.

heparin is injected in animals. As shown in Fig. 6, MK-733 significantly increased LPL activity by 21.5% as compared to that in the control rats, but it did not affect H-TGL activity. On the other hand, clofibrate did not affect LPL or H-TGL activity.

DISCUSSION

In the present study, dietary administration of MK-733 (0.055%, w/w) for 7 days significantly reduced plasma TG (30.6%), TC (14.8%) and PL (17.8%) levels and increased FFA (38.9%) levels as compared to those in the control rats. HMG-CoA reductase inhibitors have been reported to show a clear species specificity in hypocholesterolemic effect and not to reduce plasma TC in rats [22, 23]. These mechanisms have not been defined clearly thus far, but an increase of hepatic HMG-CoA reductase

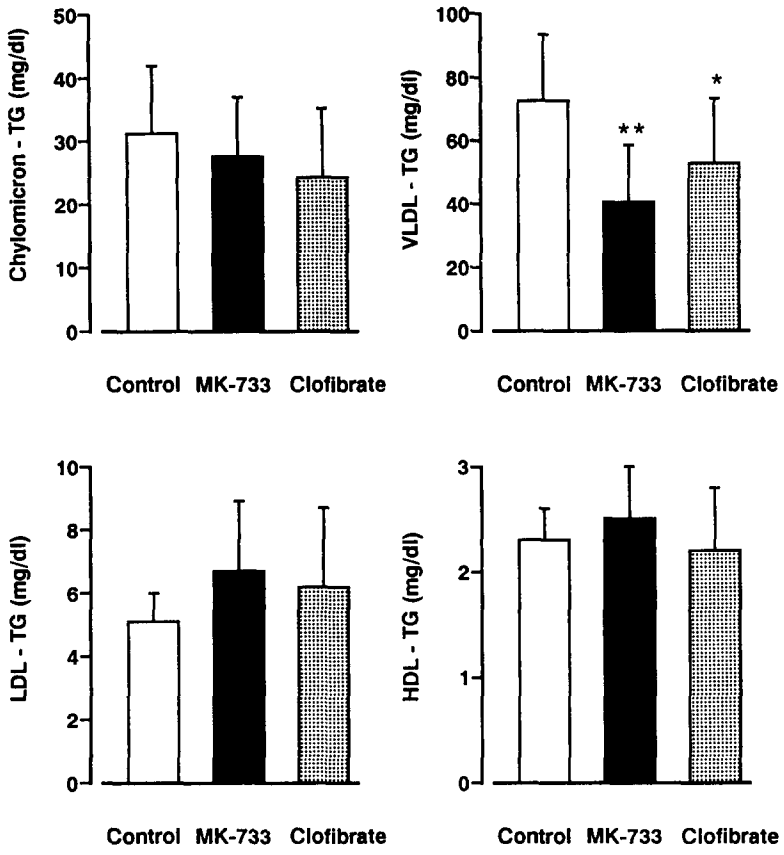

Fig. 2. Effects of MK-733 and clofibrate on plasma lipoprotein triacylglycerol levels. Plasma lipoprotein triacylglycerol levels were determined on day 8. Each lipoprotein fraction was prepared by ultracentrifugation as described in Materials and Methods. The numbers of animals used in the control, MK-733-treated and clofibrate-treated groups were 18, 8 and 9 respectively. Values represent the mean ± SD in each group. Key: significantly different from the value in the control group as determined by the Newman–Keuls test: (*) $P < 0.05$, and (**) $P < 0.01$.

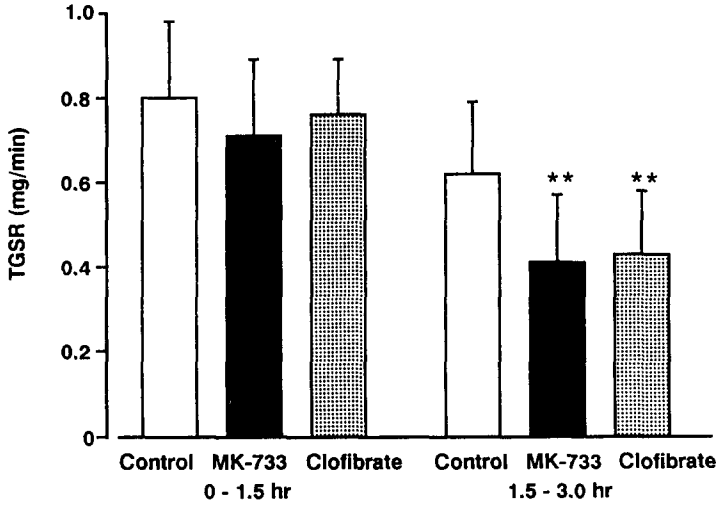

Fig. 3. Effects of MK-733 and clofibrate on triacylglycerol secretion rate (TGSR). Triton WR-1339 at a dose of 600 mg/kg was injected intravenously and TGSR was determined during the indicated times as described in Materials and Methods. The number of animals used in each group was 12. TGSR is expressed as milligrams of triacylglycerol secreted per minute. Values represent the mean ± SD in each group. Key: significantly different from the value in the control group as determined by the Newman–Keuls test: (**) $P < 0.01$.

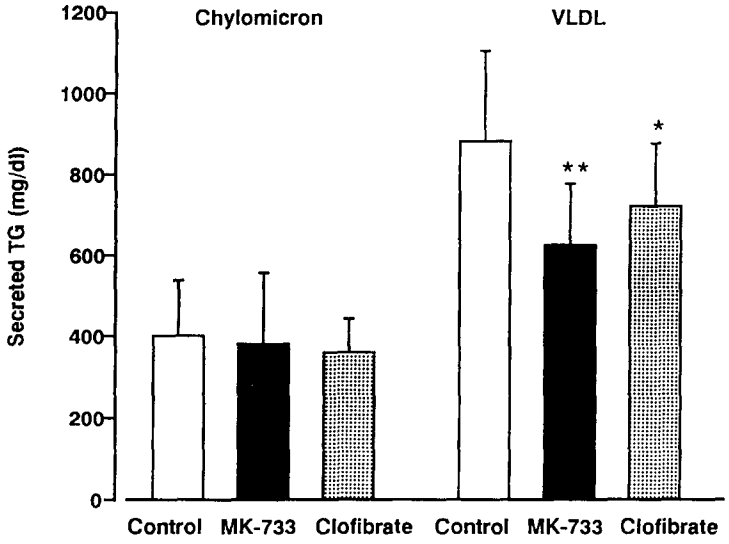

Fig. 4. Effects of MK-733 and clofibrate on the secreted triacylglycerol (TG) in chylomicrons and very low density lipoprotein after the injection of Triton WR-1339. Blood samples were collected 3 hr after the injection of Triton WR-1339. Plasma chylomicron-TG and very low density lipoprotein (VLDL)-TG were determined as described in Materials and Methods. The number of animals used in each group was 12. Values represent the mean ± SD in each group. Key: significantly different from the value in each control group as determined by the Newman–Keuls test: (*) $P < 0.05$, and (**) $P < 0.01$.

Table 2. Effects of MK-733 and clofibrate on the composition of VLDL

	VLDL-lipid and VLDL-protein concentrations (mg/dL)				
Group	FC	EC	PL	TG	Protein
Control	41 ± 7	34 ± 11	121 ± 21	881 ± 224	106 ± 13
	(4.7)	(3.9)	(13.7)	(100.0)	(12.0)
MK-733	29 ± 6*	55 ± 12*	88 ± 13*	624 ± 153*	83 ± 7*
	(4.7)	(8.8)	(14.1)	(100.0)	(13.3)
Clofibrate	36 ± 10	32 ± 7	109 ± 24	721 ± 155†	95 ± 14†
	(5.0)	(4.4)	(15.1)	(100.0)	(13.2)

VLDL-lipids and VLDL-protein were determined as described in Materials and Methods. The weight ratio of VLDL-lipids and VLDL-protein relative to VLDL-TG is shown in parentheses. Abbreviations: FC, free cholesterol; EC, esterified cholesterol; PL, phospholipid; and TG, triacylglycerol. Twelve animals per group were used. Values represent the mean ± SD in each group.

*, † Significantly different from the values in each control group as determined by the Newman–Keuls test. (*) $P < 0.01$, and (†) $P < 0.05$.

activity and a reduction of bile acid excretion were observed in rats treated with an HMG-CoA reductase inhibitor [22]. HMG-CoA reductase inhibitors at relatively higher doses were shown to decrease plasma TC levels in rats, but not consistently [MK-803, data on file, MSDRL (unpublished data) and fluvastatin (XU 62-320)*].

We have already reported that MK-733 inhibited HMG-CoA reductase, resulting in a reduction of cholesterol synthesis, but that it did not affect the synthesis of TG in primary cultured rat hepatocytes [5] and the human hepatoma cell line, Hep G2 cells [6]. Therefore, inhibition of hepatic TG synthesis was not thought to be a cause of reduction in plasma TG by MK-733.

MK-733 significantly reduced plasma TG levels and increased FFA levels. A significant reduction in VLDL-TG (44.3%) was observed in the MK-733 group as compared to that in the control group. Plasma TG levels are thought to be regulated by TG secretion by the liver and/or intestine and TG removal by peripheral tissue. Therefore, TGSR was measured *in vivo* by the method using Triton WR-1339 [24]. When Triton WR-1339 was injected

* Kathawala FG, Scallen T, Engstrom RG, Weinstein DB, Schuster H, Stabler R, Kratunis J, Wareing JR, Jewell WF, Wilder L and Wattanasin S, XU 62-320, an HMG-CoA reductase inhibitor, more potent than compactin and lovastatin. In: *Eighth International Symposium on Atherosclerosis* Rome, 9–13 October 1988, p. 445.

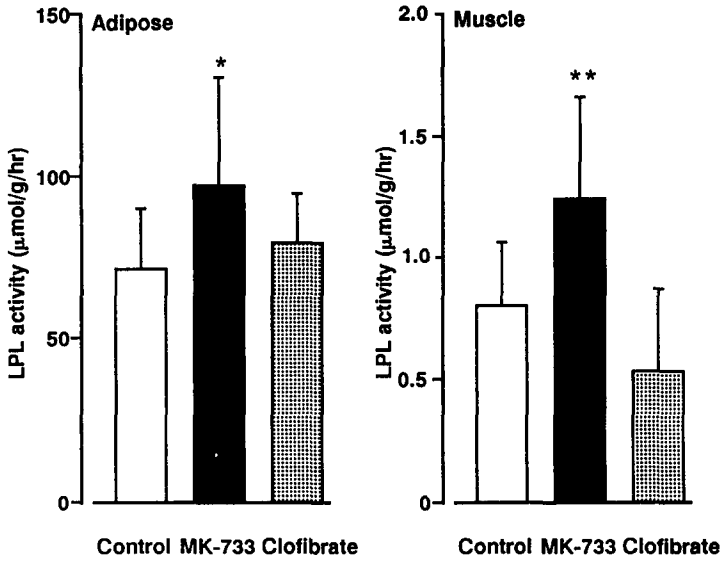

Fig. 5. Effects of MK-733 and clofibrate on lipoprotein lipase activity in the epididymal adipose tissue and thigh muscle. The epididymal adipose tissue and thigh muscle were obtained from the rats in each group and lipoprotein lipase (LPL) activity was determined as described in Materials and Methods. Twelve rats per group were used. LPL activity is expressed as micromoles of free fatty acid formed per gram per hour. Values represent the mean ± SD in each group: Key: significantly different from the value in the control group as determined by the Newman–Keuls test: (*) $P < 0.05$, and (**) $P < 0.01$.

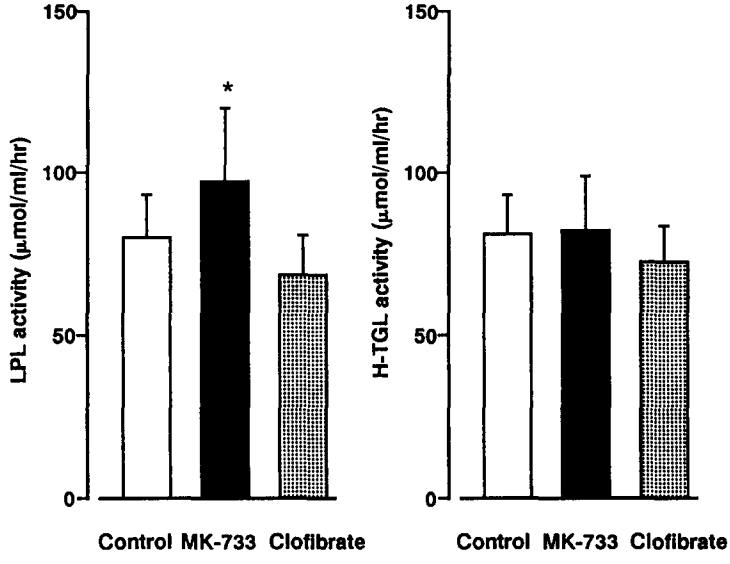

Fig. 6. Effects of MK-733 and clofibrate on lipoprotein lipase and hepatic triacylglycerol lipase activities in the post-heparin plasma. Post-heparin plasma (PHP) was obtained from the rats injected with heparin in each group and lipoprotein lipase (LPL) and hepatic triglyceride lipase (H-TGL) activities were determined as described in Materials and Methods. Ten rats per group were used. LPL and H-TGL activities are expressed as micromoles of free fatty acid formed per milliliter per hour. Values represent the mean ± SD in each group. Key: significantly different from the value in the control group as determined by the Newman–Keuls test: (*) $P < 0.05$.

intravenously in rats, the concentration of plasma TG was increased linearly for at least 3 hr as reported [25]. MK-733 did not affect TGSR during 0–1.5 hr after the administration of Triton WR-1339, but reduced it during 1.5–3 hr. MK-733 increased plasma FFA levels in the present experiment. We previously observed that MK-733 did not affect FFA synthesis in primary cultured rat hepatocytes and Hep G2

cells [5, 6]. The reason why MK-733 increased plasma FFA levels in rats has not been made clear yet, but the increased triacylglycerol metabolism by LPL in peripheral tissues might increase plasma FFA levels. The observed increase in plasma FFA may have affected VLDL secretion or minimized the apparent decrease, since fatty acids increase formation and secretion of VLDL by the liver [33, 34]. Yoshino *et al.* [16] reported that CS-514 reduces the TGSR in rats. They measured TGSR in non-fasted rats and concluded that a reduction in TGSR was a cause for the TG-lowering effect of CS-514 in non-fasted rats. Khan *et al.* [35] reported that dietary administration of MK-803 (0.1%, w/w) for 1 week severely depresses the secretion of VLDL lipids by the isolated perfused rat liver. They suggested that cholesterol is an obligate component of the VLDL required for its secretion. Furthermore, secretions of VLDL-TG, -PL, -FC and -EC were all decreased, but the percent decrease in TG was greater than that in FC and EC, resulting in secretion of a VLDL particle enriched in sterol relative to TG. In our experiments, an increase in the ratio of EC/TG in VLDL was observed after treatment with MK-733. Clofibrate reduced TGSR significantly by 30.6% during 1.5–3.0 hr, but it did not affect the lipid composition of VLDL. Therefore, the mechanism of suppression of TGSR by clofibrate was thought to be different from that of HMG-CoA reductase inhibitors. It is considered that the reduction of TGSR by MK-733 is one of the mechanisms of TG-lowering effects in rats.

Recently, Khan *et al.* [36] demonstrated that the secretion of VLDL was reduced in rats fed MK-803 (0.1%, w/w) as compared to that in control rats using the Triton WR-1339 model. They reported that MK-803 decreases the secretion of VLDL *in vivo* by a reduction in the putative cholesterol pool, but not by a suppression of triacylglycerol synthesis. Furthermore, they speculated that the reduction of VLDL output by the liver did not result from an increased fatty acid oxidation and subsequently reduced fatty acid available for esterification and secretion. Mechanisms of action of HMG-CoA reductase inhibitors on hepatic VLDL secretion have not been clearly elucidated yet, but a reduction in the putative cholesterol pool by MK-733 may be related to a reduction in hepatic VLDL secretion.

MK-733 significantly increased LPL activity in epididymal adipose tissue and thigh muscle by 36.3 and 55.0% respectively. Fielding *et al.* [37] reported that there are two kinds of LPL in rats. One, present in the heart, has a high affinity for chylomicron-TG; the other, most of the enzyme found in adipose tissue, has a considerably lower affinity for this substrate. In the postprandial state, which increases plasma TG concentrations, uptake into adipose tissue will increase. On the other hand, in the heart, uptake will remain constant since this enzyme is already saturated [38]. This mechanism would ensure a constant rate of TG fatty acid uptake into heart muscle, regardless of nutritional state, and an effective channeling of excess TG for storage in adipose tissue. In addition to passive control, LPL activity is reported to be actively regulated. Adipose tissue of fed animals has markedly higher LPL activity than that of fasted ones, and the reverse is true of heart and muscle [39]. We also confirmed the reciprocal LPL activity as reported in the adipose tissue and muscle during diurnal variation in rats (data not shown). These changes in enzyme activity are presumed to be under hormonal control [17]. LPL activity in the adipose tissue and muscle was increased in rats treated with MK-733, but MK-733 did not affect plasma glucose and serum insulin concentrations. LPL in peripheral tissue has been reported to hydrolyze both chylomicron-TG and VLDL-TG [17, 18]. In the present study, MK-733 decreased VLDL-TG, but did not affect chylomicron-TG. Further studies are needed to elucidate the mechanisms of action of MK-733 on LPL activity. We are planning to investigate the mechanisms of action using rat adipose tissue *in vitro*. It is considered that the plasma TG-lowering effect of MK-733 may be related to an observed increase of LPL activity in the adipose tissue and muscle as well as a decrease in TGSR.

PHP lipolytic activity has been shown to consist of LPL and H-TGL. The heparin-releasable fraction of tissue LPL is regarded as the physiologically active pool of the enzyme. LPL activity in the PHP was also increased by MK-733, but H-TGL activity was not affected. This increased LPL activity may reduce plasma TG levels, accompanied by a reduction in VLDL-TG. It is recognized that HDL_2 particles are formed following the LPL-catalyzed hydrolysis of VLDL-TG when apoproteins and surface phospholipids released from VLDL fuse with pre-existing HDL_3 particles [19]. The larger, less dense HDL_2 is the large part of the total HDL cholesterol while the smaller HDL_3 particles vary only within narrow limits. The formation of HDL (HDL_2) depends on the activity of LPL and it is promoted under conditions of high VLDL turnover [40]. Therefore, an increase in LPL may be related to a reduction in plasma TG and a slight increase in HDL cholesterol, which were observed in hyperlipidemic patients.

Dietary administration of clofibrate (0.08%, w/w) for 7 days increased the liver to body weight ratio as compared with that of the control group. The results were in agreement with those of Kähönen *et al.* [41]. It is well known that clofibrate induces marked proliferation of hepatic peroxisomes and increases the activity of peroxisomal β-oxidation in association with hepatomegaly [42, 43]. MK-733 did not affect the liver to body weight ratio. Therefore, the mechanism of action of MK-733 was clearly different from that of clofibrate.

From the present results, it is considered that the plasma TG-lowering effect of MK-733 is due to an increase of LPL activity in the adipose tissue and muscle as well as a decrease in the TGSR.

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