〈Original Article〉

Octacosanol ameliorates hyperlipidemia and oxidative stress in KKAy mice with type 2 diabetes

Koji Ohashi[1)], Hiroaki Ishikawa[2)] and Yoshiji Ohta[3)]

Summary We examined the effect of octacosanol (Oct) on hyperlipidemina and oxidative stress in type 2 diabetic KKAy mice. KKAy mice showed increases in body weight and serum glucose, insulin, triglyceride, total-cholesterol, and lipid peroxide (LPO) concentrations and hepatic triglyceride and cholesterol concentrations at 7 weeks of age and further increases in those levels at 12 weeks of age. In 12-week-old KKAy mice, hepatic LPO concentration increased and hepatic reduced glutathione concentration decreased. Dietary Oct administration (10 or 50 mg/kg body weight) for 5 weeks, starting at 7 weeks of age, did not affect obesity and the extent of diabetic status but attenuated the increased serum triglyceride and total-cholesterol concentrations and the increased serum and hepatic LPO concentrations. Oct (50 mg/kg body weight) attenuated the increased hepatic triglyceride and cholesterol concentrations and the decreased hepatic reduced glutathione concentrations. These results indicate that Oct ameliorates hyperlipidemina and oxidative stress in KKAy mice.

Key words: Octacosanol, Diabetes, hyperlipidemina, Oxidative stress, KKAy mice

1. Introduction

Policosanol is a natural mixture of long-chain primary aliphatic saturated alcohols that is isolated from sugarcane wax (*Saccharm officinarm* L.), wheat germ, rice bran or beeswax[1-3)]. Many studies reported by researchers in Cuba have shown that supplementation of policosanol isolated from sugarcane is effective in lowering increased serum or plasma total-cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations in hypercholesterolemic patients, monkeys, and rabbits[4-6)]. However, recent studies reported by researchers in other countries have demonstrated that supplementation of policosanol prepared form Cuba sugarcane or wheat germ is ineffective in lowering increased serum or plasma total-cholesterol and LDL-cholesterol concentrations in hypercholesterolemic patients and hamsters[7-10)]. In

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addition, it has been reported that supplementation of policosanol to patients with type 2 diabetes and hypercholesterolemia lowers not only plasma cholesterol and LDL-cholesterol concentrations but also plasma triglyceride and lipid peroxide (LPO) concentrations without affecting serum glucose and glycated hemoglobin levels and also raises the lag time of in vitro lipid peroxidation in isolated LDL and total plasma antioxidant activity[11], although there are reports showing no hypotriglyceridemic effect of supplemented policosanol in patients with type 2 diabetes and hypercholesterolemia[12, 13]. It is known that policosanol inhibits in vitro lipid peroxidation in liver microsomes and LDL prepared from rats treated with the compound[14]. It is also known that policosanol reduces the susceptibility of LDL-cholesterol isolated from humans administered with the compound to in vitro lipid peroxidation[15]. However, it has been shown that policosanol has no direct antioxidant activity[14, 16].

It is well known that dyslipidemia and oxidative stress as well as hyperglycemia, hyperinsulinemia, insulin resistance, and obesity occur in patients with type 2 diabetes[17-20]. It is also known that lipid is accumulated in the liver of patients with type 2 diabetes[21]. KKAy mice are widely used as an experimental model of human type 2 diabetes. KKAy mice develop type 2 diabetes spontaneously and are obese, hyperglycemic, hyperinsulinemic, and insulin resistant[22]. In addition, it is known that severe hypertriglyceridemia, mild hypercholesterolemia, and systemic oxidative stress occur in diabetic KKAy mice[23, 24]. It is also known that oxidative stress and accumulation of triglyceride and cholesterol occur in the liver of diabetic KKAy mice[25-27].

Octacosanol (Oct) [$CH_3(CH_2)_{26}CH_2OH$] is the major component of policosanol and occupies 60-70% of total aliphatic alcohols present in policosanol[1-3]. It is known that administration of Oct-containing diet (10 g/kg diet) to rats fed on a high-fat diet for 20 days decreases serum triglyceride concentration without changing serum cholesterol concentration and hepatic triglyceride and cholesterol concentrations[28]. We have reported that a single oral administration of Oct at a dose 50 or 100 mg/kg body weight (BW) decreases serum and hepatic triglyceride levels in normal rats and that a single oral administration of Oct (50 or 100 mg/kg BW) to rats intoxicated with carbon tetrachloride prevents liver cell damage by attenuating triglyceride accumulation and oxidative stress in the liver tissue[29, 30]. However, it is still unknown whether dietary Oct administration ameliorates hyperlipidemia and oxidative stress in experimental animals with type 2 diabetes.

Therefore, we examined whether dietary Oct administration ameliorates hyperlipidemia and oxidative stress without affecting obesity, hyperglycemia, and hyperinsulinemia in KKAy mice with type 2 diabetes.

2. Materials and Methods

1. Animals and Oct administration

Male KKAy mice aged 5 weeks were purchased from CLEA Japan Inc. (Tokyo, Japan). Age-matched male C57BL/6J (C57BL) mice were obtained from Nippon SLC Co. (Hamamatsu, Japan) and were used as the non-diabetic control. All mice were housed individually in cages under a daily controlled 12 h-light, 12-h dark lighting cycle at 23℃ and 50% humidity with free access to distilled water and Oriental MF chop diet (Oriental Yeast Co., Tokyo, Japan) for 3 weeks. Then KKAy mice were divided into 3 groups of 7 animals each. Two groups of them received Oriental MF powder diet containing Oct (Sigma Chemical Co., St. Louis, USA) for 5 weeks. The daily intake of Oct in one group was 10 mg/kg BW and that in the other group was 50 mg/kg BW. The remaining group of KKAy mice (n = 7) received the same amount of Oriental MF powder diet without Oct for the same period. Non-diabetic group of C57BL mice (n = 7) was maintained with free access to Oriental MF powder diet without Oct for the same period. The Oct-containing diet was prepared by mixing a certain amount of Oct with Oriental MF powder diet enough using a mortar. Food intake was recorded everyday. Based on the daily intake of the prepared Oct-containing diet, the amount of Oct added

to the prepared Oct-containing diet was changed weekly to adjust the daily intake of Oct to 10 or 50 mg/kg BW. During the first week of Oct administration, KKAy mice received 7.4 g of either the prepared diet containing Oct (50 or 250 mg/kg diet) or Oriental MF powder diet everyday. The consumption of administered diets was checked everyday. Body weight was recorded weekly until 12 weeks of age from 5 weeks of age. All animals received humane care in compliance with the guidelines of the Management of Laboratory Animals in Fujita Health University, Japan.

2. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed one week before the final day of Oct administration. KKAy mice with and without Oct administration and C57BL mice were orally administrated with 20% glucose solution at a dose of 2 g/kg BW after fasting for 18 h. Blood samples were immediately collected from the tail vein using a capillary tube coated heparin at 0, 30, 60 and 120 min after the start of glucose loading. Plasma was separated from the collected blood by centrifugation.

3. Sample preparation

KKAy and C57BL mice were sacrificed under anesthesia with pentobarbital (0.1 mg/g BW) at 8 weeks (i.e., at the starting day of Oct administration) or 12 weeks of age (i.e., at the ending day of Oct administration) after fasting for 8 h. Just before sacrifice, blood was collected by cardiac puncture and the collected blood was separated into serum by centrifugation. Immediately after sacrifice, each liver was perfused with ice-cold 0.9% NaCl from the portal vein to remove residual blood in the tissue and then removed from the body. The isolated liver was washed well in ice-cold 0.9% NaCl, clotted on a filter, and weighed. The isolated serum and livers were kept at -80℃ until use.

4. Assays of serum and liver components

Serum insulin was assayed using a commercial ELISA kit for mouse insulin assay (Mercodia, Uppsala, Sweden). Serum or plasma glucose and serum triglyceride and total-cholesterol were assayed using commercial kits, Glucose CII-Text Wako, Triglyceride E-Test Wako, and Cholesterol E-Test Wako (Wako Pure Chemistry Industries Ltd., Osaka, Japan), respectively. Serum LPO was fluorometrically measured by the thiobarbituric acid method of Yagi[31]. The concentration of LPO is expressed as the amount of malondialdehyde (MDA) equivalents. In order to assay liver triglyceride and cholesterol, approximately 0.2 g of each liver tissue was homogenized and then extracted in a mixture of chloroform and methanol (2 : 1 v/v) according to the Folch method[32]. The extract was dried at 45℃ under nitrogen gas stream and then the extracted lipid was dissolved in a 5% solution of defatted bovine serum albumin (Sigma Chemical Co., St. Louis, USA). Triglyceride and cholesterol in the solution were assayed using the same commercial kits as used for the assays of serum triglyceride and total cholesterol. For the assays of LPO and reduced glutathione (GSH), each liver tissue was homogenized in 9 volumes of ice-cold 0.05 M Tris-HCl buffer (pH 7.4) containing 0.1 mM ethylenediaminetetraacetic acid. LPO in the liver homogenate was colormetrically determined by the thiobarbituric acid method of Ohkawa et al.[33]. The concentration of LPO is expressed as the amount of MDA equivalents. GSH in the liver homogenate was determined by the method of Sedlak and Lindsay[34] using Ellman's reagent and GSH as a standard.

5. Statistical analysis

All results obtained are expressed as the mean ± standard error (S.E.). The statistical analyses of the results were performed using a computerized Excel statistical package. Each mean value was compared by one-way analysis of variance (ANOVA) and Bonferroni/Dunn for multiple comparisons. The significance level was set at $P < 0.05$.

3. Results

As shown in Fig. 1, the amount of diet consumed (expressed as g/week/animal) in KKAy mice without Oct administration was significantly larger than that in normal C57BL mice between 5 weeks and 12 weeks

of age. Administration of Oct (10 or 50 mg/kg BW) to KKAy mice for 5 weeks, starting at 7 weeks of age, had no significant effect on the increase in food intake (Fig. 1).

Body weight in KKAy mice without Oct administration was significantly heavier than that in normal C57BL mice between 5 weeks and 12 weeks of age (Fig. 2). Administration of Oct (10 or 50 mg/kg BW) to KKAy mice for 5 weeks had no significant effect on the increase in body weight (Fig. 2).

Serum glucose and insulin concentrations in untreated KKAy mice were significantly higher than those in C57BL mice at the starting and ending points of Oct administration (Fig. 3). Administration of Oct (10 or 50 mg/kg BW) to KKAy mice did not attenuate the increased serum glucose and insulin concentrations (Fig. 3). When OGTT was performed in KKAy mice with and without Oct administration and C57BL mice, plasma glucose concentrations in KKAy mice with and without Oct administration were significantly higher than that in C57BL mice at each time point (Fig. 4). There was no significant difference in increased plasma glucose concentration between KKAy mice administered with and without Oct (10 or 50 mg/kg BW) at each time point (Fig. 4).

KKAy mice without Oct administration had higher serum triglyceride concentration than C57BL mice at the starting and ending time points of Oct administration, although the increase in serum triglyceride concentration tended to be larger at the starting time point than at the ending time point of Oct administration (Fig. 5A). The increased serum triglyceride concentration in KKAy mice was significantly attenuated by administration of Oct at a dose of 10 or 50 mg/kg BW and the attenuating effect of Oct tended to be larger at a dose of 50 mg/kg than at a dose of 10

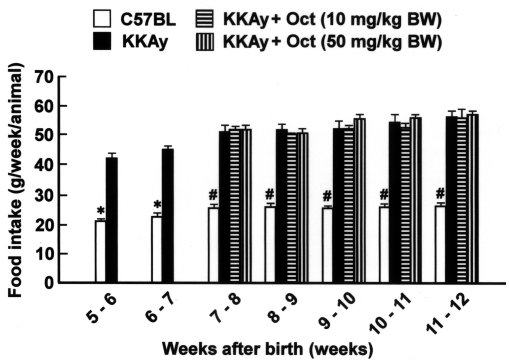

Fig. 1 Changes in food intake in KKAy mice administered with and without Oct and C57BL mice. Administration of Oct (10 or 50 mg/kg BW) to KKAy mice was conducted until 12 weeks of age from 7 weeks of age. Each vale is a mean ± S.E. (n = 7). *, significantly different from untreated KKAy mice at $P < 0.05$; #, significantly different from KKA administered with and without Oct (10 or 50 mg/kg) at $P < 0.05$.

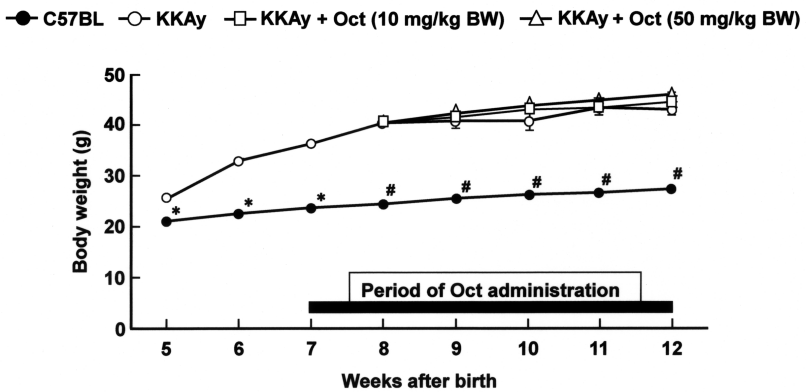

Fig. 2 Changes in body weight in KKAy mice administered with and without Oct and C57BL mice. Oct was administered to KKAy mice at a dose of 10 or 50 mg/kg BW. Each vale is a mean ± S.E. (n = 7). *, significantly different from untreated KKAy mice at $P < 0.05$; #, significantly different from KKA administered with and without Oct (10 or 50 mg/kg) at $P < 0.05$.

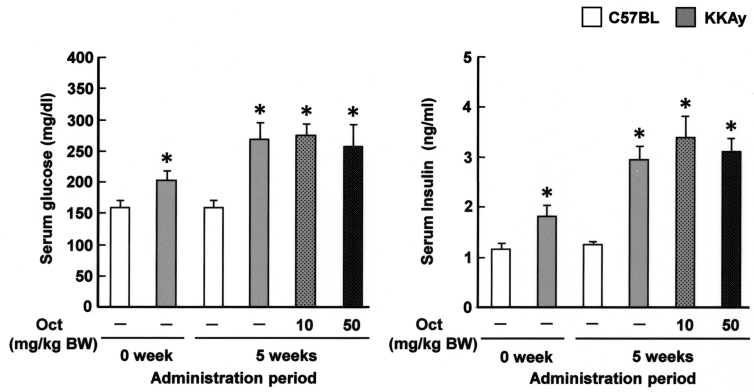

Fig. 3 Changes in serum glucose and insulin concentrations in KKAy mice administered with and without Oct and C57BL mice. Administration of Oct (10 or 50 mg/kg BW) to KKAy mice was started at 8 weeks of age. All mice were sacrificed after fasting for 8 h and then serum was isolated. Each vale is a mean ± S.E. (n = 7). *, significantly different from the corresponding C57BL mice at $P < 0.05$.

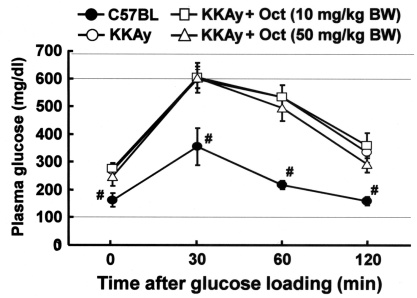

Fig. 4 Changes in plasma glucose concentration in KKAy mice administered with and without Oct and C57BL mice in OGTT. A solution of glucose (2 g/kg BW) was orally administered to KKAy mice with and without Oct administration and C57BL mice after fasting for 18 h. Each vale is a mean ± S.E. (n = 7). #, significantly different from KKA administered with and without Oct (10 or 50 mg/kg BW) at $P < 0.05$.

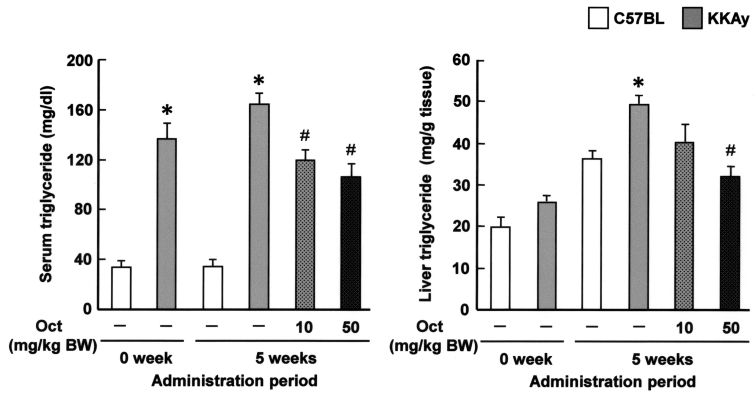

Fig. 5 Changes in serum and hepatic triglyceride concentrations in KKAy mice administered with and without Oct and C57BL mice. Administration of Oct (10 or 50 mg/kg BW) to KKAy mice was started at 8 weeks of age. All mice were sacrificed after fasting for 8 h and then serum and livers were isolated. Each vale is a mean ± S.E. (n = 7). *, significantly different from the corresponding C57BL mice at $P < 0.05$; #, significantly different from KKAy mice without Oct administration at $P < 0.05$.

mg/kg (Fig. 5A). Hepatic triglyceride concentration in untreated KKAy mice was significantly higher than that in C57BL mice at the ending timepoint of Oct administration, although untreated KKAy mice tended to have higher hepatic triglyceride concentration than C57BL mice at the starting time point of Oct administration (Fig. 5B). The increased hepatic triglyceride concentration in KKAy mice was significantly attenuated by Oct administered at a dose of 50 mg/kg BW, although the increased hepatic triglyceride concentration tended to be attenuated by Oct administered at a dose of 10 mg/kg BW (Fig. 5B). The hepatic triglyceride concentration in KKAy mice administered with Oct (50 mg/kg BW) was not significantly different from that in C57Bl mice (Fig. 5B).

Serum total-cholesterol concentration in untreated KKAy mice was significantly higher than that in C57BL mice at the starting and ending time points of Oct administration, although the increased total-cholesterol concentration in untreated KKAy mice was significantly higher at the ending time point than at the starting time point of Oct administration (Fig. 6A). The

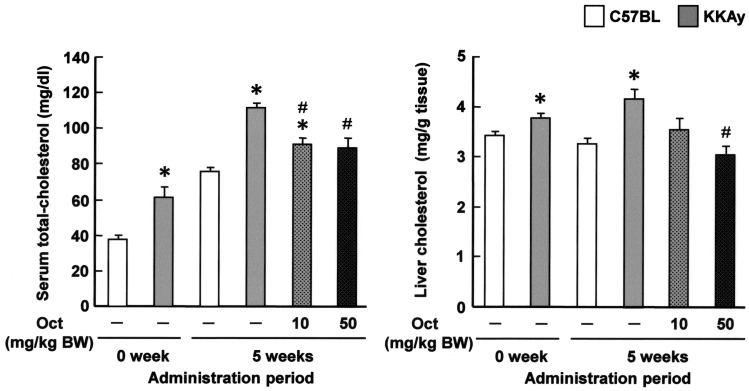

Fig. 6 Changes in serum total-cholesterol concentration and hepatic cholesterol concentration in KKAy mice administered with and without Oct and C57BL mice. Administration of Oct (10 or 50 mg/kg BW) to KKAy mice was started at 8 weeks of age. All mice were sacrificed after fasting for 8 h and then serum and livers were isolated. Each vale is a mean ± S.E. (n = 7). *, significantly different from the corresponding C57BL mice at $P < 0.05$; #, significantly different from KKAy mice without Oct administration at $P < 0.05$.

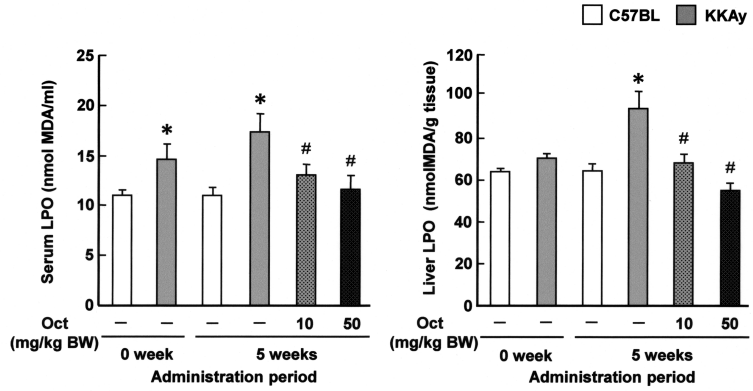

Fig. 7 Changes in serum and hepatic LPO concentrations in KKAy mice administered with and without Oct and C57BL mice. Administration of Oct (10 or 50 mg/kg BW) to KKAy mice was started at 8 weeks of age. All mice were sacrificed after fasting for 8 h and then serum and livers were isolated. Each vale is a mean ± S.E. (n = 7). *, significantly different from the corresponding C57BL mice at $P < 0.05$; #, significantly different from KKAy mice without Oct administration at $P < 0.05$.

increased serum total-cholesterol concentration in KKAy mice was significantly attenuated by Oct administered at a dose of 10 or 50 mg/kg BW and the attenuating effects of Oct at both doses were nearly equal (Fig. 6A). KKAy mice without Oct administration had significantly higher hepatic cholesterol concentration than C57BL mice at the starting and ending time points of Oct administration, although the increased cholesterol concentration tended to be larger at the ending time point than at the starting time point of Oct administration (Fig. 6B). The increased hepatic cholesterol concentration in KKAy mice was significantly attenuated by Oct administered at a dose of 50 mg/kg, although Oct administered at a dose of 10 mg/kg tended to attenuate the increased hepatic cholesterol concentration (Fig. 6B). The hepatic cholesterol concentration in KKAy mice administered with Oct (50 mg/kg BW) was not significantly different from the hepatic cholesterol concentration in C57BL mice (Fig 6B).

KKAy mice without Oct administration had significantly higher serum LPO concentration than C57BL mice at the starting and ending time points of Oct administration, although the increased LPO concentration tended to be higher at the ending time point than at the starting time point of Oct administration (Fig. 7A). The increased serum LPO concentration was significantly attenuated by Oct administered at a dose of 10 or 50 mg/kg BW, although the attenuating effect of Oct tended to be larger at a dose of 50 mg/kg BW than at a dose of 10 mg/kg BW (Fig. 7A). In addition, the serum LPO concentration in KKAy mice administered with Oct (10 or 50 mg/kg BW) was not significantly different from that in C57BL mice (Fig. 7A). There was no significant difference in hepatic LPO concentration between untreated KKAy mice and C57BL mice at the starting time point of Oct administration but untreated KKAy mice had significantly higher hepatic LPO concentration than C57Bl mice at the ending time point of Oct administration (Fig. 7B). The increased hepatic LPO concentration in KKAy mice was significantly attenuated by Oct administered at a dose of 10 or 50 mg/kg BW and the attenuating effect of Oct tended to be larger at a dose of 50 mg/kg BW than at a dose of 10 mg/kg (Fig. 7B). In addition, there was no significant difference in hepatic LPO concentration between KKAy mice administered with Oct (10 or 50 mg/kg BW) and C57BL mice (Fig. 7B).

As shown in Fig. 8, there was no significant difference in hepatic GSH concentration between untreated KKAy mice and C57BL mice at the starting time point of Oct administration but untreated KKAy mice had significantly lower hepatic GSH concentration than C57BL mice at the ending time point of Oct administration. The decreased hepatic GSH concentration in KKAy mice was significantly recovered by Oct administered at a dose of 50 mg/kg BW, but not 10 mg/kg BW, to the level of C57BL mice (Fig. 8).

4. Discussion

Castaño et al.[11] have reported that supplementation

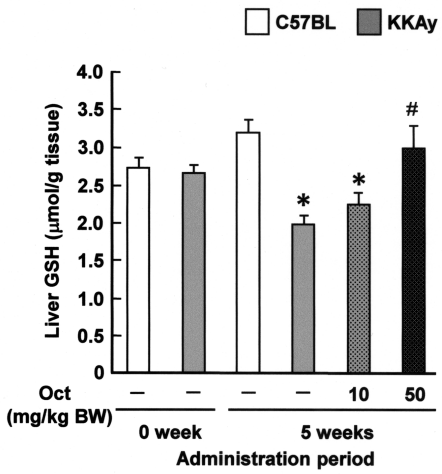

Fig. 8 Changes in hepatic GSH concentration in KKAy mice administered with and without Oct and C57BL mice. Administration of Oct (10 or 50 mg/kg BW) to KKAy mice was started at 8 weeks of age. All mice were sacrificed after fasting for 8 h and then livers were isolated. Each vale is a mean ± S.E. (n = 7). *, significantly different from the corresponding C57BL mice at $P < 0.05$; #, significantly different from KKAy mice without Oct administration at $P < 0.05$.

of policosanol to patients with type 2 diabetes and hypercholesterolemia lowers plasma cholesterol, LDL-cholesterol, triglyceride, and lipid peroxide concentrations without affecting serum glucose and glycated hemoglobin levels. The present study has clearly shown that dietary administration of Oct at a dose of 10 or 50 mg/kg BW for 5 weeks can ameliorate hypertriglyceridemia, hypercholesterolemia, and oxidative stress in type 2 diabetic KKAy mice without affecting diabetic status associated with obesity, hyperglycemia, and hyperinsulinemia. Thus, these results obtained in the present study were well consistent with the results reported by Castaño et al.[11]. Therefore, the lowering effect of supplemented policosanol on plasma cholesterol, LDL-cholesterol, triglyceride, and lipid peroxide concentrations in patients with type 2 diabetes and hypercholesterolemia in the report of Castaño et al.[11] may be, at least in part, due to Oct present in the supplemented policosanol.

In the present study, KKAy mice showed clear diabetes at 7 weeks of age, i.e., at the starting time point of Oct administration, and advanced diabetes at 12 weeks of age, i.e., at the ending time point of Oct administration, judging from the changes in body weight, serum glucose and insulin levels and from OGTT. Oct administered at a dose of 10 or 50 mg/kg BW for 5 weeks to KKAy mice had no effect on the increases in body weight, serum glucose and insulin concentrations and glucose intolerance. Thus, Oct was found to be ineffective in ameliorating diabetic status itself in KKAy mice.

It is known that severe hypertriglyceridemia, mild hypercholesterolemia, and systemic oxidative stress occur in diabetic kKAy mice[23, 24]. It is also known that diabetic KKAy mice possess accumulated triglyceride and cholesterol and oxidative stress associated with an enhancement of lipid peroxidation and GSH depletion due to an increase in reactive oxygen species production in the liver[25-27]. In the present study, serum triglyceride, total-cholesterol, and LPO concentrations were significantly higher in untreated diabetic KKAy mice than in non-diabetic C57BL mice at the starting and ending time points of Oct administration. Untreated diabetic KKAy mice showed significant increases in hepatic triglyceride and LPO concentrations at the ending time point of Oct administration and a significant increase in hepatic cholesterol concentration at the starting and ending time points of Oct administration as compared with C57BL mice. Furthermore, untreated diabetic KKAy mice had significantly lower hepatic GSH concentration than non-diabetic C57BL mice at the ending time point of Oct administration. Thus, increases in serum triglyceride, total-cholesterol, and LPO concentrations and hepatic triglyceride, cholesterol, and LPO concentrations and a decrease in hepatic GSH concentration were found in diabetic KKAy mice. These results indicate that hyperlipidemia and oxidative stress are enhanced with the development of diabetes in KKAy mice. These results also suggest that the changes in triglyceride, cholesterol, LPO, and GSH levels in the liver tissue of diabetic KKAy mice could contribute to systemically observed hperlipidemia and oxidative stress at the advanced stage of diabetes.

The increases in serum triglyceride, total-cholesterol, and LPO concentrations in diabetic KKAy mice were significantly attenuated by dietary administration of Oct at a dose of 10 or 50 mg/kg BW for 5 weeks. These attenuating effects of Oct tended to be larger at a dose of 50 mg/kg BW than at a dose of 10 mg/kg BW. However, Oct administered at a dose of 50 mg/kg BW could not attenuate the increased serum triglyceride and total-cholesterol concentrations completely, while the same dose of Oct could attenuate the increased serum LPO concentration completely. Oral administration of Oct at a dose of 50 mg/kg BW significantly attenuated the increases in hepatic triglyceride, cholesterol, and LPO concentrations in diabetic KKAy mice to those level of non-diabetic C57Bl mice and returned the decreased hepatic GSH concentration in diabetic KKAy mice to the level of non-diabetic C57BL mice completely, Thus, orally administered Oct was found to be able to ameliorate not only systemically observed hyperlipidemia and oxidative stress but also hepatic lipid accumulation and oxidative stress in diabetic KKAy mice. In addition, orally administered Oct was found to exert an ameliorating effect on oxidative stress more strongly than on hyperlipidemia in diabetic KKAy mice.

It has been suggested that hypertriglycermia in diabetic KKAy mice is due to severe dyslipoproteinemia[23]. It has been shown that the level of triglyceride associated with very low-density lipoporotein (VLDL) and LDL is markedly increased in the plasma of diabetic KKAy mice[23]. It has also been implicated that triglyceride synthesis is enhanced in the liver of diabetic KKAy mice, resulting in an increase in plasma triglyceride concentration[25]. It has been shown that dietary administration of Oct to rats fed on a high-fat diet for 20 days reduces serum triglyceride concentration probably through inhibition of hepatic phosphatidate phosphohydrolase which metabolizes phosphatidate to 1,2-diacylgyceride in the pre-final step of triglyceride synthesis[28]. As described above, dietary administration of Oct (50 mg/kg BW) could not cause a complete reduction of increased serum triglyceride concentration despite causing a complete reduction of increased hepatic triglyceride concentration in diabetic KKAy mice. These findings allow us to suggest that Oct (50 mg/kg BW) administered in a dietary form could attenuate enhanced triglyceride synthesis by inhibiting phosphatidate phosphohydrolase in the liver of diabetic KKAy mice, resulting in an incomplete reduction of increased triglyceride level in the serum and a complete reduction of increased triglyceride level in the liver. The incomplete reduction of increased serum triglyceride concentration in diabetic KKAy mice administered with Oct (50 mg/kg BW) suggest that the administered Oct could have a little ameliorating effect on the efflux of VLDL-associated triglyceride into the bloodstream from the liver tissue under severe dyslipoproteinemia.

It has been suggested that hypercholesterolemia in diabetic KKAy mice is due to severe dyslipoproteinemia[23]. It has been shown that high-density lipoprotein (HDL)-cholesterol level rather than LDL-cholesterol level is markedly increased in the plasma of diabetic KKAy mice[23]. It has been suggested that policosanol administered in a dietary form reduces increases in plasma total-cholesterol and LDL-cholesterol levels by inhibiting hepatic cholesterol synthesis in rabbits with casein-induced hypercholesterolemia[6]. It has also been suggested that policosanol and tiracontanol decrease the activity of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in cholesterol synthesis, in an indirect manner, i.e., by activating AMP-kinase, in rat hepatoma cells[35]. However, it has been shown that Oct cannot decrease HMG-CoA reductase activity in rat hepatoma cells[35]. As described above, Oct (50 mg/kg BW) administered in a dietary form could not cause a complete reduction of increased serum total-cholesterol concentration despite causing a complete reduction of increased hepatic cholesterol concentration in diabetic KKAy mice. Therefore, it is assumable that administered Oct (50 mg/kg BW) attenuates increased cholesterol concentration in the liver of diabetic KKAy mice by inhibiting cholesterol synthesis without affecting the rate-limiting step of cholesterol synthesis, in which HMG-CoA reductase takes part, in the liver, resulting in an incomplete reduction of increased total-cholesterol level in the serum and a complete reduction of increased cholesterol level in the liver. The incomplete reduction of increased serum total-cholesterol concentration in diabetic KKAy mice administered with Oct (50 mg/kg BW) suggests that the administered Oct could have a low ability to reduce increased serum HDL-cholesterol level under severe dyslipoproteinemia.

As described above, orally administered Oct (50 mg/kg BW) attenuated increased serum and hepatic LPO concentrations in diabetic KKAy mice to the levels of non-diabetic C57BL mice completely. In addition, orally administered Oct (50 mg/kg BW) returned decreased hepatic GSH concentration in diabetic KKAy mice to the level of non-diabetic C57BL mice completely. Our previous report[29] has shown that Oct cannot inhibit lipid peroxidation induced by a free radical generator in rat liver microsomes in a direct manner, although a single oral administration of Oct can reduce increased hepatic LPO level with recovering decreased hepatic GSH level in rats intoxicated with carbon tetrachloride. Therefore, these findings suggest that Oct (10 or 50 mg/kg BW) administered in a dietary form attenuates systemically observed oxidative stress and hepatic oxidative stress in diabetic KKAy mice through its indirect antioxidant action to sustain GSH level in the liver tissue rather than its direct antioxidant action

to scavenge free radicals participating in lipid peroxidation.

In conclusion, the results of the present study indicate that Oct administered in a dietary form exerts an ameliorating effect on hyperlipidemia and oxidative stress in diabetic KKAy mice without affecting diabetic status itself. However, the mechanism(s) by which administered Oct ameliorates hyperlipidemia and oxidative stress in diabetic KKAy mice is unclear at present. Therefore, further studies are needed to clarify the mechanism(s) underling the ameliorating effect of Oct on hyperlipidemia and oxidative stress in diabetic KKAy mice.

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