

# The Biological Effects of $\beta$ -Cyclodextrin on Antithrombotic Activity and Plasma Lipid Metabolism in Rats

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## 흰쥐에서 혈액지질 대사 및 항혈전작용에 관한 베타사이클로덱스트린의 생물학적 효과

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### ABSTRACT

The effect of feeding a cyclic oligosaccharide,  $\beta$ -cyclodextrin( $\beta$ CD) on plasma cholesterol and triacylglyceride concentrations and on antithrombotic activity were investigated in rats fed a control chow diet, or one either high in cholesterol or in saturated fat. The bleeding time of  $\beta$ CD-fed groups was significantly prolonged by 293%, 157% and 218% in normal, high cholesterol and high fat diet fed groups, respectively, as compared to the control group( $p < 0.05$ ). The whole blood clotting time was significantly increased by 202%, 168% and 211% in normal, high cholesterol and high fat diet fed groups as compared to control group, respectively( $p < 0.05$ ). The  $\beta$ CD diet caused a marked decrease in plasma total lipid (TL), triacylglyceride (TAG), total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) concentrations. The plasma TL concentration was significantly decreased by 70%, 82% and 87% in normal, high cholesterol and high fat diet fed groups as compared to the control group, respectively( $p < 0.05$ ). The plasma TAG concentration was significantly decreased by 89%, 43% and 59% in normal, high cholesterol and high fat diet fed groups, respectively, as compared to the control group( $p < 0.05$ ). The plasma TC concentration was significantly decreased by 28%, 62% and 36% in normal, high cholesterol and high fat diet fed groups, respectively, as compared to the control group( $p < 0.05$ ). The LDL-C concentration was significantly decreased by 39%, 54% and 25% in normal, high cholesterol and high fat diet fed groups as compared to control group, respectively( $p < 0.05$ ). The plasma total bile acids contents of  $\beta$ CD group was significantly increased by 66%, 95% and 97% in normal, high cholesterol and high fat diet fed groups as compared to control group, respectively( $p < 0.05$ ). The hepatic HMG-CoA reductase activity was significantly lowered by 41% in the  $\beta$ CD-fed group compared to normal diet fed rats( $p < 0.05$ ). The fecal steroid excretions of the  $\beta$ CD groups was significantly increased by 167% in normal diet fed rats( $p < 0.05$ ). These results suggest that the  $\beta$ CD has a biological active function on antithrombotic activity and is hypolipidemic, hypotriglyceridemic and hypocholesterolic agents. These are all effects that can help to prevent obesity and coronary heart disease in humans.

(Key words :  $\beta$ -cyclodextrin, Antithrombotic activity, Triacylglyceride, Cholesterol, Steroid)

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## I . INTRODUCTION

It is widely known that frequent and excessive intake of saturated fatty acids(SFA) and cholesterol increases plasma TAG and cholesterol concentrations, in particular LDL-C which is a risk factor for the development of coronary heart disease (Paul et al., 1979; Chen et al., 1989; McLennan et al., 1990; Reaven, 1993; Tkac, 1997).

There are some reports on decrease in plasma cholesterol concentrations that occur upon feeding of  $\beta$ CD to rats (Suzuki and Sato, 1985; Oliver et al., 1991; Frijlink et al., 1991; Hashimoto, 1991; Favier et al., 1995), hamsters (Oakenfull et al., 1991) and pigs (Ferezou et al., 1997; Juste et al., 1997; Catala et al., 2000).  $\beta$ CD is a cyclic oligosaccharide of seven glucose units all linked in a ring structure in  $\alpha(1\rightarrow4)$  position (Abadie et al., 1994).  $\beta$ CD forms an inclusion complex with cholesterol and bile acids in the gut (Riottot et al., 1993; Saenger, 1994; Yen and Chen, 2000). Its use as a food additive benefits from the advantages that  $\beta$ CD is nontoxic, palatable, non-hygroscopic, chemically stable, and easy to separate (Nagamoto, 1985). Thus, it is widely used by the food industry as an external absorbent in the removal of cholesterol from lard (Yen and Chen, 2000), dairy products (Riottot et al., 1993) and egg yolk (Newsweek, 1992).  $\beta$ CD is not absorbed from the small intestine, at least in rats (Oakenfull et al., 1991).  $\beta$ CD is poorly hydrolyzed in the human small intestine but it is fermented by the colonic flora, to yield products (e.g., fatty acids) that are known to have nutritive value (Antenucci and Palmer, 1984; Flourie et al., 1993). Recently observations of the effects of  $\beta$ CD-feeding on plasma lipid and

cholesterol metabolism indicate that  $\beta$ CD decreases plasma TAG and cholesterol concentrations in the rat (Suzuki and Sato, 1985; Oliver et al., 1991; Frijlink et al., 1991; Favier et al., 1995).

But the hypolipidemic, hypocholesterolemic and hypotriglyceridemic effect of  $\beta$ CD is not well-established and it remains to be verified whether this effect is observed when given to rats maintained on high cholesterol or high fat diets. There are also no reports on its antithrombotic activity.

Therefore, the present study has investigated the effect of  $\beta$ CD on plasma TL, TAG and cholesterol concentrations and the antithrombotic activity in rats maintained on normal, high cholesterol and high saturated fat diets.

## II . MATERIALS AND METHODS

### 1. Animals

Two groups of thirty male Wistar rats weighing 140~150g were used. Experiment I was designed to elucidate the effect of  $\beta$ CD on antithrombotic activity and plasma lipid concentrations in rats maintained on a normal chow diet. Experiment II was designed to investigate the effect of  $\beta$ CD on the antithrombotic activity and decreasing plasma lipid concentrations in the rats fed high cholesterol- and high fat-diet. In each experiment, rats were housed in individual cages and fed *ad libitum* for 30 days on pelleted semipurified diets. In experiment I, the basal control diet (Table 1) contained the following (in grams per kilogram, AIN, 1977): casein, 200; sucrose, 500; corn starch, 150;  $\alpha$ -cellulose, 50; fat (lard and corn oil), 47.5 and 2.5; mineral and vitamins, 35 and

Table 1. Formulation of the basal control diet

Ingredients	Percentage
casein	20.0
sucrose	50.0
corn starch	15.0
$\alpha$ -cellulose	5.0
fat	5.0
AIN'77 min. mix.	3.5
AIN'77 vit. mix.	1.0
DL-methionine	0.3
choline bitartrate	0.2
Total	100

10 ; DL-methionine, 3 ; choline bitartrate, 2. The experimental diets contained 50, 100, 150 or 200g  $\beta$ CD/kg diet, as a replacement of sucrose in the basal control diet, respectively. In experiment II, high cholesterol diets(Exp. II) contained 10g cholesterol, 0, 50 or 100g  $\beta$ CD/kg basal diet, as a replacement for sucrose in the basal control diet, respectively. The high fat diets(Exp. II) contained 100 fat(lard 97.5% and corn oil 2.5%), 0, 50 or 100g  $\beta$ CD/kg diet, instead of sucrose in the basal control diet, respectively. The animals housed in controlled environment room(22~23°C, lights on 07:00 to 19:00 hr). Food intake and body weight were recorded on every 10 days. Blood was collected under light ethyl ether anaesthesia from the abdominal aorta in a syringe containing 100 $\mu$ l of EDTA solution(0.01M). Plasma was obtained by centrifugation at 3,000 rpm for 10 min at 4°C. Plasma for lipid analysis was snap-frozen in liquid nitrogen and stored at -20°C until the end of the study, when all samples were analyzed. The livers, heart, spleen and kidney were quickly removed, washed with cold saline

(9g NaCl/l), blotted dry on filter paper, and weighed.

## 2. Antithrombotic activities

The bleeding time was measured on all rats at the end of the experimental period on the 29<sup>th</sup> day as described in Hornstra et al. (1981). Rats were anaesthetised with sodium pentobarbitone (40mg/kg BW, intraperitoneally). The tail was transected at 3mm from the tip and the distal 5cm of the tail was immersed vertically in saline at 37.5°C. The period between transection and the moment bleeding stopped was taken as the bleeding time. The whole-blood clotting time was measured as described in Han et al. (1987). Briefly, one day after the bleeding time measurement, ethyl ether-anaesthetised rats were bled from the abdominal aorta. Blood (0.9 volume) was collected in a plastic tube containing 3.13% sodium citrate solution (0.1 volume). One ml of citrated blood and 200 $\mu$ l of 1.7% calcium chloride solution was placed into glass tubes. The time until the formation of the thrombus was measured while agitating the tubes slowly.

## 3. Lipid analysis

TL concentrations in plasma was determined by extraction with a mixture of chloroform/methanol (2:1, vol/vol) as described in Frings and Dunn (1970). TAG, TC, LDL-C, high density lipoprotein-cholesterol(HDL-C) and bile acid concentrations in plasma were measured by enzymatic assays using commercially available reagent kits(Sigma, USA).

## 4. Enzyme assays

The total microsomal  $\beta$ -hydroxy- $\beta$ -methylglutaryl coenzyme(HMG-CoA) reductase activity was measured by the method described in Quin and Haslam (1979). Microsomes were prepared as described in Qureshi et al. (1983).

In brief, liver homogenates were prepared in potassium phosphate buffer (100mM, pH 7.4) containing  $MgCl_2$ (4mM), EDTA(1mM) and dithiothreitol(1mM). Homogenisation was performed at 0~4°C using a polytron homogenizer. The homogenate was centrifuged for 10min at 5000×g. The supernatant was passed through cheesecloth and then centrifuged at 20000×g(10min) and 100000×g for 60 min. The 100000×g supernatant(cytosol) and pellet (microsomes) was stored at -20°C. HMG-CoA reductase activity was determined spectrophotometrically by the rate of decrease in absorbance at 340nm caused by the oxidation of NADPH. The reaction mixture contained, in 1ml, dithiothreitol(5mM), potassium phosphate buffer (pH 7.4, 100mM), NADPH [150 $\mu$ M (P-L Biochemicals), prepared in sodium carbonate buffer (100mM, pH 10.6)],  $\beta$ -hydroxy- $\beta$ -methylglutaryl coenzyme [150 $\mu$ M(sigma), prepared in acetate buffer(200mM, pH 4.6)] and Triton × 100(0.5%, v/v). Microsomal protein was determined by the method of Lowry et al. (1951).

### 5. Steroid analysis

At the end of the experimental period of 30 days, all fecal excretions during 3 days was collected. Neutral and acidic steroids in feces were measured by gas liquid chromatography as described in Grundy et al. (1965) and Miettinen et al. (1965).

### 6. Statistical analysis

The significance of differences between treatment groups was determined by analysis of variance with Duncan's multiple-range test(SAS Institute, Cary, NC1999). Results were considered significant at  $p < 0.05$ .

## III. RESULTS

### 1. Growth performance and organ weight

There were no significant differences in food intake among the different groups of animals maintained on the control chow and high cholesterol diet fed groups( $p > 0.05$ ). But the high fat control diet fed groups had significantly lower intake than those maintained on a high fat  $\beta$ CD diet( $p < 0.05$ ). Daily food intakes per animal were approximately 22g in normal and high cholesterol diet fed groups, and 16g and 20g in high fat control and high fat  $\beta$ CD diet fed groups, respectively. There were no significant differences in body weight gains among the different groups in normal, high cholesterol and high fat diet fed groups, respectively ( $p > 0.05$ ). Body weight gains per animal were about 5.8g in control and 5%  $\beta$ CD diet fed groups, 5.3 to 5.0g in 10% to 20%  $\beta$ CD diet fed groups in normal diet fed groups, 5.0g in high cholesterol diet fed groups, and 4.3g in high fat diet fed groups, respectively.

Organ weight were unaffected by inclusion of  $\beta$ CD in the diet of all treatment groups. When the values were expressed per 100g body weight, relative liver, heart, spleen and kidney weights were about 3.33g, 0.35g, 0.36g and 0.70g respectively.

### 2. Antithrombotic activities

The effect of the  $\beta$ CD-feeding on bleeding- and whole blood clotting- times were investigated in normal, high cholesterol and high fat diets fed animals for 30 days. As is shown in Table 2, bleeding time was significantly increased by 293%, 157% and 218% in normal, high cholesterol and high fat diet fed groups with  $\beta$ CD, when compared to each control groups, respectively ( $p < 0.05$ ). whole blood clotting time was also significantly increased by

$\beta$ CD-feeding, by 202%, 168% and 211% in normal, high cholesterol and high fat diet fed groups, respectively ( $p < 0.05$ ).

### 3. Plasma lipid concentrations

The TL, TAG, TC, HDL-C, LDL-C and bile acid concentrations in plasma of rats fed experimental diets are given in Table 3. The TL concentrations were significantly decreased by 17

Table 2. Bleeding time and whole blood clotting time in rats fed normal, high cholesterol and high fat diets with  $\beta$ CD for 30 days

Rats	Bleeding time (sec.)	Whole blood clotting time (sec.)
Normal diet fed <sup>1)</sup>		
control	69.93 <sup>c</sup>	79.35 <sup>c</sup>
5% $\beta$ CD	78.39 <sup>c</sup>	90.78 <sup>c</sup>
10% $\beta$ CD	158.70 <sup>b</sup>	123.15 <sup>b</sup>
15% $\beta$ CD	205.35 <sup>a</sup>	137.70 <sup>ab</sup>
20% $\beta$ CD	170.40 <sup>ab</sup>	160.35 <sup>a</sup>
SEM <sup>4)</sup>	13.4763	7.7027
High cholesterol diet fed <sup>2)</sup>		
control	77.0 <sup>b</sup>	85.75 <sup>c</sup>
5% $\beta$ CD	85.0 <sup>b</sup>	115.00 <sup>b</sup>
10% $\beta$ CD	121.5 <sup>a</sup>	144.00 <sup>a</sup>
SEM	6.0321	7.8242
High fat diet fed <sup>3)</sup>		
control	57.25 <sup>b</sup>	67.50 <sup>c</sup>
5% $\beta$ CD	118.50 <sup>a</sup>	123.25 <sup>b</sup>
10% $\beta$ CD	124.75 <sup>a</sup>	142.25 <sup>a</sup>
SEM	9.7396	9.8002

<sup>1)</sup> Normal diets contained 5% fat.

<sup>2)</sup> High cholesterol diet contained 5% fat and 1% cholesterol.

<sup>3)</sup> High fat diets contained 10% fat.

<sup>4)</sup> Standard error of means.

<sup>a,b,c</sup> Values within the same columns with different superscript are significantly different ( $p < 0.05$ ).

Table 3. Plasma TL, TAG, TC, HDL-C, LDL-C, and Bile acids in rats fed normal, high cholesterol and high fat diets with  $\beta$ CD for 30 days<sup>1)</sup>

Rats	TL	TAG	TC	HDL-C	LDL-C	Bile acid
..... mg/dl .....						
Normal diet fed						
control	311.52 <sup>a</sup>	192.26 <sup>a</sup>	91.62 <sup>a</sup>	47.07 <sup>c</sup>	10.88 <sup>a</sup>	12.34 <sup>d</sup>
5% $\beta$ CD	259.72 <sup>b</sup>	63.97 <sup>b</sup>	74.93 <sup>b</sup>	51.73 <sup>a</sup>	8.03 <sup>b</sup>	13.82 <sup>c</sup>
10% $\beta$ CD	131.35 <sup>c</sup>	31.60 <sup>c</sup>	69.95 <sup>bc</sup>	49.98 <sup>ab</sup>	7.46 <sup>bc</sup>	14.94 <sup>c</sup>
15% $\beta$ CD	129.32 <sup>c</sup>	31.22 <sup>c</sup>	67.27 <sup>bc</sup>	51.60 <sup>a</sup>	7.76 <sup>b</sup>	20.49 <sup>a</sup>
20% $\beta$ CD	92.42 <sup>d</sup>	21.23 <sup>d</sup>	65.91 <sup>c</sup>	47.93 <sup>bc</sup>	6.67 <sup>c</sup>	17.14 <sup>b</sup>
SEM <sup>2)</sup>	16.0112	11.8692	2.0914	0.4621	0.3037	0.5546
High cholesterol diet fed						
control	404.63 <sup>a</sup>	121.31 <sup>a</sup>	188.74 <sup>a</sup>	42.73 <sup>b</sup>	43.21 <sup>a</sup>	31.85 <sup>b</sup>
5% $\beta$ CD	260.80 <sup>b</sup>	77.66 <sup>b</sup>	92.02 <sup>b</sup>	54.07 <sup>a</sup>	26.82 <sup>b</sup>	62.04 <sup>a</sup>
10% $\beta$ CD	73.05 <sup>c</sup>	69.74 <sup>b</sup>	72.68 <sup>c</sup>	42.46 <sup>b</sup>	19.86 <sup>c</sup>	32.03 <sup>b</sup>
SEM	41.2514	5.9614	12.6323	1.6348	2.7484	4.0228
High fat diet fed						
control	475.85 <sup>a</sup>	118.02 <sup>a</sup>	100.01 <sup>a</sup>	48.30 <sup>a</sup>	9.49 <sup>a</sup>	21.52 <sup>b</sup>
5% $\beta$ CD	215.25 <sup>b</sup>	88.64 <sup>b</sup>	74.75 <sup>b</sup>	49.07 <sup>a</sup>	8.32 <sup>b</sup>	21.63 <sup>b</sup>
10% $\beta$ CD	63.22 <sup>c</sup>	47.96 <sup>c</sup>	64.00 <sup>c</sup>	46.53 <sup>a</sup>	7.11 <sup>c</sup>	42.26 <sup>a</sup>
SEM	16.0112	11.8692	2.0914	0.4621	0.3037	0.5546

<sup>1)</sup> TL, total lipid; TAG, triacylglycerides; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. <sup>2)</sup> Standard error of means.

<sup>a,b,c,d</sup> Values within the same columns with different superscript are significantly different ( $p < 0.05$ ).

~70% in normal diet fed groups, 36~82% in high cholesterol diet fed groups and 55~87% in high fat diet fed groups, when compared to each control groups, respectively( $p < 0.05$ ). The TAG concentrations were significantly decreased by 67~89%, 36~43% and 25~59% in normal, high cholesterol and high fat diet fed groups, when compared to the respective control groups ( $p < 0.05$ ). The TC concentration was also signi-

ficantly decreased by 18~28%, 51~62% and 26~36% in normal, high cholesterol and high fat diet fed groups, respectively( $p < 0.05$ ). The HDL-C concentrations were significantly increased by 26% in high cholesterol diet feed groups additionally maintained on 5%  $\beta$ CD, although the effect was not observed on 10%  $\beta$ CD( $p < 0.05$ ).

There were significant differences in HDL-C

concentrations among the high fat- diet fed groups ( $p < 0.05$ ). The LDL-C concentrations were significantly decreased by 27~39%, 38~54% and 12~25% in normal, high cholesterol and high fat diet groups, when compared to each control groups, respectively ( $p < 0.05$ ). The bile acids were increased by 12~66%, 95% and 97% in normal diet fed rats, and also in high cholesterol diet fed group with 5%  $\beta$ CD, and in high fat diet fed group with 10%  $\beta$ CD, when compared to their respective control groups ( $p < 0.05$ ).

#### 4. Enzyme activities

The hepatic microsomal HMG-CoA reductase activities are shown in Table 4. HMG-CoA reductase activities in normal diet fed rats were significantly lowered by 22~41% with 5% and 10%  $\beta$ CD, compared to the control group ( $p < 0.05$ ).

#### 5. Fecal steroid excretion

Table 5 shows fecal excretion rates of neutral and acidic steroids by rats fed normal diets with  $\beta$ CD, at the end of the experimental period. These have shown that the effect of  $\beta$ CD on fecal steroid excretions was greatest under this experimental regime. The daily excretion rates of total steroids were significantly increased by 46~167% in normal diet with 5~20%  $\beta$ CD groups, when compared to the control group ( $p < 0.05$ ).

### IV. DISCUSSION

$\beta$ CD-feeding had a large antithrombotic effect. Bleeding time and wholeblood clotting

times were prolonged with dietary  $\beta$ CD supplementation when compared to the control group (Table 2). The bleeding time is thought to be a measure of the combined reaction of platelets and the blood-vessel wall. The prolongation of bleeding time results from the inhibition of platelets aggregation (Han et al., 1987). The present data show that  $\beta$ CD-supplementation has strong antithrombotic activity, possibly due to the hypocholesterolemic effects shown in Table 3 (McDonald et al., 1989). Several studies have shown that bleeding time is prolonged by a fish oil (Thorngren and Gustafson, 1981; Sanders and Roshanai, 1983), canola oil diet (McDonald et al., 1989) and aspirin or aspirin-like drugs (Moncada and Vane, 1979).

From the results of plasma lipid concentrations (Table 2), it is suggested that the lower TL, TAG, TC and LDL-C of group fed the  $\beta$ CD diets was due primarily to the lowering of HMG-CoA reductase activity (Table 4) and increased fecal steroid excretions (Table 5). Similar responses in plasma TC and TAG concentrations have been observed in male genetically hypercholesterolemic rick rats maintained on 10% fat plus 0.5% cholesterol diets (Oakenfull et al., 1991). However, there are no reports that relate to the findings in this study.

A dose-related decrease in activity was observed with the rate-limiting enzymes for the synthesis (HMG-CoA reductase) of cholesterol over the range of  $\beta$ CD concentrations used (Table 4). The reduction rates of the enzyme activity ranged from 22% to 41%, respectively, when compared to the control group. This observation may reflect only an *in vivo* response to the lowering of the substrate pool in liver effected by the inhibition of the biosynthetic

Table 4. HMG-CoA reductase activities in rats fed normal diets with  $\beta$ CD for 30 days

Rats	HMG-CoA reductase activities <sup>1)</sup>
Normal diet fed	
control	314.38 <sup>a</sup>
5% $\beta$ CD	247.27 <sup>b</sup>
10% $\beta$ CD	189.07 <sup>c</sup>
15% $\beta$ CD	195.27 <sup>c</sup>
20% $\beta$ CD	184.74 <sup>c</sup>
SEM <sup>2)</sup>	12.3904

<sup>1)</sup> Specific activities of HMG-CoA are expressed as pmol NADPH oxidized  $\text{min}^{-1} \cdot \text{mg}^{-1}$  microsomal protein.

<sup>2)</sup> Standard error of means.

<sup>a,b,c</sup> Values within the same columns with different superscript are significantly different ( $p < 0.05$ ).

activities (Qureshi et al., 1983). These effects were accompanied by significant decreases in plasma cholesterol concentrations (18% to 28%), when compared to the control in normal diet-fed rats (Table 3). More importantly, there was a strong suppressive effect of  $\beta$ CD feeding on plasma LDL-C concentration. The relationship between hepatic HMG-CoA reductase activity and plasma LDL-C concentration has recently been demonstrated (Balasubramaniam et al., 1977; Qureshi et al., 1983).

These data indicated that the decrease in TC and LDL-C concentrations in response to  $\beta$ CD intake could be caused by changes of some parameters in cholesterol metabolism including fecal excretions of neutral and acidic steroids (Fukushima et al., 1996). There were significant differences in the fecal steroid excretions

between the control and  $\beta$ CD-fed groups (Table 5). These results show that the effect of decreasing plasma TC and LDL-C concentrations of  $\beta$ CD tended to be high due to increased fecal excretions of neutral and acidic steroids (Myant and Eder, 1961; Hostmark et al., 1989). The entero-hepatic circulation of bile acids is of great importance, not only for the feedback regulation of their own synthesis, but also for the overall metabolism of cholesterol in the liver. Interruption of the hepatic circulation of bile acids will therefore influence the hepatic metabolism of cholesterol in several ways (Einarsson et al., 1991). Some investigations demonstrated that treatment with bile acid-binding resins, which bind bile acids in the intestine and prevent their reabsorption, increase fecal bile acid excretions (Grundy et al., 1971; Einarsson et al., 1991).

In this study, the lowered plasma concentrations of LDL-C suggest that  $\beta$ CD-feeding causes increased metabolism of cholesterol to bile acids, as reflected in the increased excretion of fecal steroid (Table 4 and Table 5). As shown in Table 4. The plasma concentration of total bile acids was significantly increased in  $\beta$ CD groups when compared to the control group (chow-diet fed rats).

In conclusion, an important set of findings of the present study was that  $\beta$ CD feeding resulted in combined hypolipidemic (87% reduction in high fat diet fed group), hypotriacylglyceridemic (89% reduction in normal diet fed group) and hypocholesterolemic effects (62% and 54% reduction in high cholesterol diet fed group, TC and LDL-C, respectively) of a  $\beta$ CD-fed groups compared with a controls.

Moreover,  $\beta$ CD appears to have an anti-



Table 5. Fecal steroid excretions in rats fed normal diets with  $\beta$ CD for 30 days

Rats	Steroids <sup>1)</sup>							
	TS	NS	TC	CS	AS	TBA	LCA	CA
	..... mg/day/head .....							
Normal diet fed								
control	22.98 <sup>e</sup>	18.25 <sup>d</sup>	8.26 <sup>d</sup>	9.98 <sup>d</sup>	4.73 <sup>b</sup>	3.82 <sup>b</sup>	0.24 <sup>c</sup>	0.67 <sup>b</sup>
5% $\beta$ CD	33.47 <sup>d</sup>	26.68 <sup>c</sup>	11.52 <sup>cd</sup>	15.16 <sup>cd</sup>	6.79 <sup>b</sup>	4.48 <sup>ab</sup>	0.93 <sup>b</sup>	1.37 <sup>ab</sup>
10% $\beta$ CD	43.69 <sup>c</sup>	32.91 <sup>c</sup>	16.08 <sup>bc</sup>	16.82 <sup>bc</sup>	10.77 <sup>a</sup>	7.43 <sup>a</sup>	1.80 <sup>a</sup>	1.54 <sup>a</sup>
15% $\beta$ CD	53.92 <sup>b</sup>	42.76 <sup>b</sup>	20.67 <sup>ab</sup>	22.09 <sup>ab</sup>	11.16 <sup>a</sup>	7.62 <sup>a</sup>	1.93 <sup>a</sup>	1.60 <sup>a</sup>
20% $\beta$ CD	61.13 <sup>a</sup>	50.18 <sup>a</sup>	26.64 <sup>a</sup>	23.54 <sup>a</sup>	10.94 <sup>a</sup>	7.90 <sup>a</sup>	1.91 <sup>a</sup>	1.12 <sup>ab</sup>
SEM <sup>2)</sup>	3.2800	2.7305	1.6205	1.3245	0.7689	0.6016	0.1623	0.1295

<sup>1)</sup> TS, total steroids (neutral steroid + acidic steroids); NS, neutral steroid; TC, total cholesterol; CS, coprostanol; AS, acidic steroids; TBA, total bile acids; LCA, lithocholic acids; CA, cholic acid.

<sup>2)</sup> Standard error of means.

<sup>a,b,c,d</sup> Values within the same columns with different superscript are significantly different ( $p < 0.05$ ).

thrombotic activity, extrapolating to the situation *in vivo*, it can be of help to prevent coronary heart disease associated with disorders of lipid metabolism in humans.

## V. 요약

일반사료, 고콜레스테롤사료 또는 포화지방 함량이 높은 사료를 섭취한 흰쥐에서 항혈전작용과 혈액중성지방 및 콜레스테롤 수준에 관한  $\beta$ -cyclodextrin( $\beta$ CD)의 급여효과를 조사하였다. 출혈시간은 일반사료, 고콜레스테롤 및 고지방사료 섭취군 모두에서  $\beta$ CD 첨가구가 대조구에 비해서 각각 293%, 157% 및 218% 까지 유의적으로 연장되었다( $p < 0.05$ ). 전혈응고시간은 일반사료, 고콜레스테롤 및 고지방사료 섭취군에서  $\beta$ CD 첨가구가 대조구와 비교할 때 각각 202%, 168% 및 211%까지 유의적으로 높았다( $p < 0.05$ ).  $\beta$ CD 첨가사료는 흰쥐 혈액내 총지질, 중성지방, 총콜레스테롤 및 저밀도지질 단백질 콜레스테롤 함량을 현저하게 낮추는 원

인이 되었다. 혈액내 총지질 함량은 일반사료, 고콜레스테롤 및 고지방사료 섭취군에서  $\beta$ CD 첨가구가 대조구에 비해서 각각 70%, 82% 및 87%까지 유의적으로 낮아졌다( $p < 0.05$ ). 혈액내 중성지방함량은 일반사료, 고콜레스테롤 및 고지방사료 섭취군에서  $\beta$ CD 첨가구가 대조구와 비교할 때 각각 89%, 43% 및 59%까지 유의적인 감소를 나타냈다( $p < 0.05$ ).

혈액내 총콜레스테롤 함량은 일반사료, 고콜레스테롤 및 고지방사료 섭취군에서  $\beta$ CD 첨가구가 대조구에 비해서 각각 28%, 62% 및 36%까지 유의적으로 낮아졌다( $p < 0.05$ ). 혈액내 저밀도 지질단백질 콜레스테롤 함량은 일반사료, 고콜레스테롤 및 고지방사료 섭취군에서  $\beta$ CD 첨가구가 대조구에 비해서 각각 39%, 54% 및 25%까지 유의적인 감소를 나타냈다( $p < 0.05$ ). 혈액내 총담즙산 함량은 일반사료, 고콜레스테롤 및 고지방사료 섭취군에서  $\beta$ CD 첨가구가 대조구에 비해서 각각 66%, 95% 및 97% 까지 유의적인 증가를 보였다( $p < 0.05$ ). 일반사료 섭취군에서  $\beta$ CD 첨가구의 HMG-CoA reductase

활성도는 대조구와 비교할 때 41%까지 유의적으로 낮아졌으며 분을 통한 스테로이드의 배설량은 167%까지 유의적인 증가를 나타냈다( $p < 0.05$ ).

이 결과는  $\beta$ CD가 사람의 심장혈관계질환과 비만을 예방하는데 도움이 될 수 있는 혈액내 지질, 중성지방 및 콜레스테롤의 감소효과 그리고 항혈전작용에 관한 생리활성 기능을 갖는다는 것을 시사해 주고 있다.

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