

Oligonol, a Low-molecular Weight Polyphenol Extracted from Lychee Fruit, Modulates Cholesterol Metabolism in Rats within a Short Period

Khota Hayashi, Saki Kawai, Ken Hinohara, and Kyoichi Osada*

Department of Agricultural Chemistry, School of Agriculture, Meiji University, 1-1-1 Higashimita, Tama-ku, Kawasaki, Kanagawa 214-8571, JAPAN

Abstract: There is growing research interest in the hypocholesterolemic effect of various food components such as polyphenols. In this study, we examined the effects of oligonol—a low-molecular weight polyphenol extracted from lychee fruit—on cholesterol metabolism in rats under short-term administration. Administration of oligonol for 3 days significantly increased cecum weight and decreased cecal n-butyric acid concentrations in rats. Oligonol also significantly lowered the levels of hepatic cholesterol and increased the levels of total neutral steroids excreted in the feces. It also increased fecal β -muricholic acid significantly, whereas the levels of total acidic steroids remained unchanged. Gene expression of hepatic CYP7A1 (cytochrome P450 family 7 subfamily A member 1) significantly increased following the administration of oligonol. This increase could be ascribed to changes in the expression of farnesoid X receptor, small heterodimer partner, and fibroblast growth factor 15 in ileum. Our data suggest that oligonol induces hypocholesterolemic effects through the inhibition of biliary cholesterol absorption from the intestine and the upregulation of cholesterol catabolism in rats even following short-term administration. Therefore, oligonol may be an important food component for reducing cholesterol level.

Key words: cholesterol metabolism, lychee, oligonol, procyanidin, rat

1 Introduction

The increase in lifestyle-related diseases following the diversification of eating habits, including dyslipidemia, cardiovascular diseases, and obesity has become a social problem in developed countries. In particular, cardiovascular disease, which causes arteriosclerosis, is one of the leading causes of death in Japan¹⁾. Therefore, the prevention or amelioration of dyslipidemias, such as hypercholesterolemia, would help to reduce the risk of cardiovascular diseases.

There is growing interest in hypocholesterolemic effect of various food components, especially polyphenols, such as tea catechin²⁾. Polyphenols are present in a wide range of food products derived from plants such as vegetables, fruits, and beans. Procyanidins are composed of catechin and epicatechin oligomers and exert many health benefits, such as cancer prevention, cardiovascular protection, and diabetes prevention³⁾. In a recent study, absorption of low molecular weight procyanidins, including dimers, was observed in rats⁴⁾, while the bioavailability of high molecular

procyanidins is limited. Moreover, several studies have shown that polyphenols from cacao polyphenols⁵⁾, grape seed⁶⁾, and apple⁷⁾, which have high level of procyanidins, exhibit hypocholesterolemic effects.

Oligonol is a commercially available phenolic product containing catechin-type monomers and low-molecular-weight oligomers derived from lychee fruit extract, which contains high molecular procyanidins⁸⁾. Recent studies showed that oligonol exerts various biological activities including antioxidant⁹⁾, anti-obesity¹⁰⁾, anticarcinogenic¹¹⁾, or anti-inflammatory¹²⁾ effects. However, the hypocholesterolemic effects of oligonol are still incompletely understood. Therefore, in this study, we focused on investigating the direct hypocholesterolemic effects of oligonol and its molecular mechanism in cholesterol-free condition as dietary cholesterol modulates various factor related to cholesterol metabolism.

*Correspondence to: Kyoichi Osada, Department of Agricultural Chemistry, School of Agriculture, Meiji University 1-1-1 Higashimita, Tama-ku, Kawasaki, Kanagawa 214-8571, JAPAN

E-mail: kyochi@meiji.ac.jp

Accepted May 11, 2020 (received for review April 9, 2020)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

2 Experimental Procedures

2.1 Reagents

Oligonol[®] was obtained from AminoUp Chemical Co., Ltd. (Sapporo, Japan). Oligonol consist of 16.3% monomeric flavan-3-ols including (-)-epicatechin, 13.8% procyanidins dimer including procyanidin A1, procyanidins trimer including (-)-epicatechin-(4 β →8, 2 β →O→7)-epicatechin-(4 β →8)-epicatechin, and 58.6% high molecular procyanidins. The structures of major polyphenols in oligonol are shown in Fig. 1¹³⁾. All other chemicals used in this study were of analytical grade.

2.2 Animals and diet

Animal experiments were conducted according to the guidelines provided by the ethical committee of experimental animal care at Meiji University (approval code: IACUC 10-0008). Wistar rats (7-weeks-old male; Japan Laboratory Animals, Inc., Tokyo, Japan) were housed individually in a temperature- (22–24°C) and light-controlled (7:00–19:00) room. After a 4-days-acclimatization period, 14 rats were divided into 2 groups. The first group (control group; 7 rats) was fed standard diet prepared according to the AIN-76 recommendations¹⁴⁾ and orally administrated

saline. The diet contained the following (g/100 g): casein (50), corn oil (5; Nissin Oil Co., Tokyo, Japan), vitamin mixture (1; AIN-76 mixture; Oriental Yeast Co., Tokyo, Japan), mineral mixture (3.5; AIN-76 mixture; Oriental Yeast Co., Tokyo, Japan), choline bitartrate (0.2; Nacalai Tesque Inc., Kyoto, Japan), DL-methionine (0.3; Nacalai Tesque Inc., Kyoto, Japan), cellulose (5.0; CLEA Japan, Inc., Tokyo, Japan), cornstarch (15; Nippon Shokuhin Kakoh Ltd., Tokyo, Japan), and sucrose (100; Nacalai Tesque Inc., Kyoto, Japan). The second group (oligonol group; 7 rats) was orally administrated oligonol (1000 mg/kg body weight, O group) for 3 days at 10 am. After feeding, rats were bled from the abdominal aorta, and liver or mucous membranes of intestines were then excised quickly under anesthesia using isoflurane. Plasma was prepared by centrifugation after allowing blood to clot at room temperature and tissues were kept at –80°C; they were transferred to RNAlater solution at 4°C for 1 day prior to RNA extraction. Feces were collected for 2 days (beginning 2 days before killing) and lyophilized.

2.3 Plasma and liver lipid analysis

The levels of plasma triacylglycerol, total cholesterol,

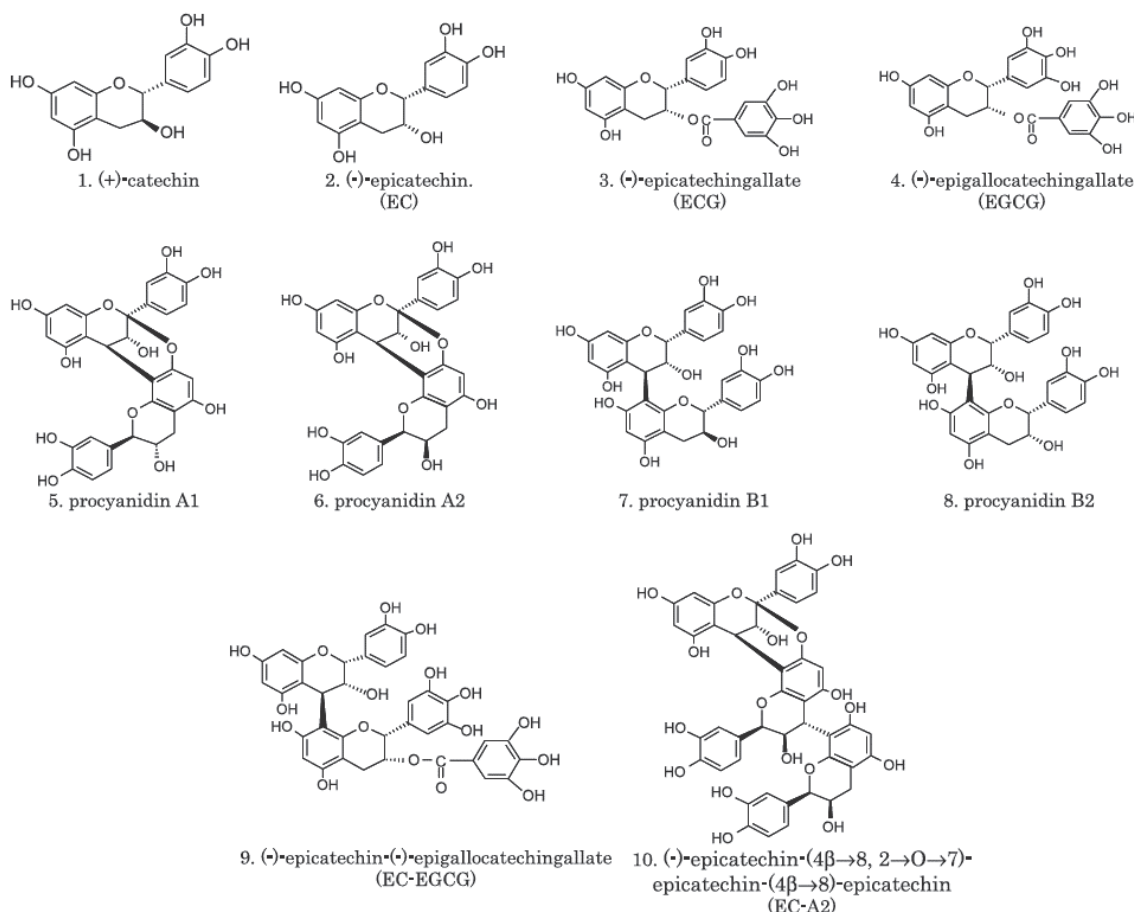


Fig. 1 Structures of major polyphenols in oligonol.

and high-density lipoprotein (HDL) cholesterol were measured using commercial kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan) after the rats were killed. Liver lipids were extracted by the method described by Folch *et al.*¹⁵⁾. The levels of liver triacylglycerol, cholesterol, and phospholipids were measured according to methods described by Fletcher¹⁶⁾, Sperry and Webb¹⁷⁾, and Rouser *et al.*¹⁸⁾, respectively.

2.4 Fecal lipid analysis

Fecal neutral steroid or acidic steroids were analyzed by GC using 5 α -cholestane or 23-nor-deoxycholic acid, respectively, as internal standards¹⁹⁾. The levels of excreted fatty acids were also analyzed according to Jeejeebhoy *et al.*²⁰⁾.

2.5 Cecal short-chain fatty acid analysis

Cecal short-chain fatty acids level was determined by gas chromatography²¹⁾. Briefly, 100 μ L of diethyl ether and 5 μ L of 35% HCl were added to 100 μ L of 15% cecal content homogenates containing 0.20 mM 2-ethylbutyric acid as an internal standard. After centrifugation at 1500 g at 4°C for 20 min, the diethyl ether layer (upper layer) was collected. The diethyl ether extract was injected into a gas chromatograph (GC-14B, Shimadzu Co., Kyoto, Japan) equipped with a DB-FFAP capillary column (15 m $\times$ 0.53 mm $\times$ 0.5 μ m, Agilent Technologies Inc., CA, USA). Injector and detector temperatures were 145 and 175°C, respectively. The initial oven temperature was 80°C for 1 min, which was subsequently increased at a rate of 10°C/min, and then maintained at 130°C for 1 min.

2.6 RNA extraction

Total RNA was extracted from mice liver tissue using Sepasol-RNA I super G (Nacalai Tesque Inc., Kyoto, Japan). RNA concentration was determined by measuring the absorbance at 260 nm using a BioSpec-nano (SHIMADZU Co., Kyoto, Japan).

2.7 Oligonucleotide primer sequences

The primers for reverse transcription polymerase chain reaction (RT-PCR) amplification of the rat cytochrome P450 family 7 subfamily A member 1 (CYP7A1), fibroblast growth factor receptor 4 (FGFR4), fibroblast growth factor 15 (FGF15), farnesoid X receptor (FXR), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and small heterodimer partner (SHP) gene were designed using Primer3Plus software (<http://www.bioinformatics.nl/cgi-bin/primer3plus.cgi>). The primers, synthesized by Eurofins Genomics (Tokyo, Japan), were designed to flank known or putative introns of these genes, thereby preventing the amplification of any contaminating genomic DNA. The primer sequences were as follows: CYP7A1 (Gene ID: 25428),

forward 5'-ACCATTCCTGCAACCTTTTG-3' and reverse 5'-GTACCGGCAGGTCATTCAGT-3'; FGFR4 (Gene ID: 25114), forward 5'-AGAGGTGGAGGTGCTGTATC-3' and reverse 5'-TCTTCCTCTGCTTCGGGTA-3'; FGF15 (Gene ID: 170582), forward 5'-TCCCAGTCTGTGTGCGGATGA-3' and reverse 5'-AGTTGGAGACGTAGGGACCA-3'; FXR (Gene ID: 60351), forward 5'-AGGAGGAGCTCTGTGACTGA-3' and reverse 5'-GATCCTTCATCGGGAAGTCA-3'; GAPDH (Gene ID: 23483), forward 5'-CTCATGACCACAGTCCATGC-3' and reverse 5'-TTCAGCTCTGGGATGACCTT-3'; HMGCR (Gene ID: 25675), forward 5'-GCTTGAGCATCCTGACATAC-3' and reverse 5'-GAACCATAGTTCCCACGTCT-3'; SHP (Gene ID: 117274), forward 5'-GACCTGTAGAATGGGGTC-3' and reverse 5'-GCCTGCTGGACAGTTAGTCA-3'.

2.8 Real-time quantitative polymerase chain reaction

One microgram of RNA was incubated at 65°C for 5 min, and then quickly cooled on ice. Reverse transcription of RNA was performed using a ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd., Osaka, Japan) and by heating the sample to 37°C for 15 min, followed by heating at 98°C for 5 min. An aliquot of the generated cDNA samples was mixed with 5 μ L THUNDERBIRD SYBR qPCR MIX (Toyobo Co., Ltd., Osaka, Japan) in the presence of 0.3 μ mol each of the forward and reverse primers for each gene. This reaction mix was then subjected to the following cycling conditions in a Chromo 4 Sequence Detection System (Bio-Rad Laboratories, Inc., CA, USA): 1 cycle at 95°C for 1 min, and thereafter, 40 cycles at 95°C for 15 sec and 58.5°C for 1 min. The results (fold-changes) were expressed as relative fold by comparing the amount of RNA of the target gene to that of GAPDH as an internal control, as determined by the equation $2^{-C(t)_{\text{target}} + C(t)_{\text{GAPDH}}}$.

2.9 Statistical analyses

The data are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using the Student's *t*-test to evaluate significant differences between values obtained for the 2 groups²²⁾.

3 Results

3.1 Effects of oligonol on growth parameters

There were no significant differences in food intake, body weight, and liver weight between the 2 groups (Table 1). However, cecum weight was significantly higher in oligonol group (O group) (2.23 ± 0.27 g/100 g body weight) than that in control group (C group) (1.39 ± 0.22 g/100 g body weight).

3.2 Effects of oligonol on plasma and liver lipid levels

Levels of plasma total cholesterol, HDL-cholesterol, triacylglycerol, and phospholipids were not significantly differ-

Table 1 Effects of orally administrated oligonol on growth parameter and lipid levels in the plasma and liver.

	Group	
	C	O
Growth parameter		
Primary B.W. (g)	239 ± 6	237 ± 5
Final B.W. (g)	267 ± 11	263 ± 12
Total food intake (g)	128 ± 9	124 ± 6
Liver weight (g/100g B.W.)	5.24 ± 0.43	4.83 ± 0.48
Plasma		
Triacylglycerol (mg/dL)	135 ± 20	135 ± 19
Total cholesterol (mg/dL)	104 ± 6	107 ± 3
HDL-cholesterol (mg/dL)	60.5 ± 4.2	59.2 ± 3.3
Phospholipids (mg/dL)	97.0 ± 25.8	140 ± 16
Liver		
Triacylglycerol (mg/g)	7.31 ± 1.38	5.40 ± 0.74
Cholesterol (mg/g)	2.52 ± 0.10	1.88 ± 0.55*
Phospholipids (mg/g)	20.5 ± 1.3	22.1 ± 0.7

Data are presented as the mean ± SD of 7 rats in each group.
*Significantly different from the corresponding C group at $p < 0.05$ (Student's *t*-test).

B.W., body weight; C, rats administrated saline; O, rats administrated oligonol.

ent between the 2 groups (Table 1).

Levels of liver total cholesterol were significantly lower in O group than in C group. However, triacylglycerol levels were not significantly different between the 2 groups, although they showed a trend towards reduced levels following administration of oligonol (Table 1).

3.3 Effects of oligonol on the excretion of steroids and fatty acids in the feces

Fecal weight was increased by administration of oligonol. Levels of cholesterol excreted in the feces were significantly higher in O group than in C group, although excreted coprostanol was significantly decreased by administration of oligonol. Therefore, excreted levels of total neutral steroids were significantly higher in O group than in C group. Levels of excreted acidic steroids were the same in both groups. However, levels of the individual bile acids were modulated by administration of oligonol as follows: levels of chenodeoxycholic acid and β -muricholic acid were significantly higher in O group than in C group. On the other hand, levels of lithocholic acid and ursodeoxycholic acid were significantly lower in O group than in C group. Therefore, excreted level of primary bile acids was significantly higher in O group than in C group, whereas that of secondary bile acids was significantly lower in O group

Table 2 Effects of orally administrated oligonol on fecal lipid excretion.

	Group	
	C	O
Dry weight		
	g/day	
	1.51 ± 0.09	2.03 ± 0.16*
mg/day		
Coprostanol	1.85 ± 0.20	0.21 ± 0.06*
Cholesterol	1.28 ± 0.22	5.47 ± 0.17*
Total neutral steroids	3.13 ± 0.30	5.68 ± 0.17*
mg/day		
Cholic acid	0.73 ± 0.19	0.89 ± 0.11
Chenodeoxycholic acid	0.12 ± 0.02	0.21 ± 0.03*
α -Muricholic acid	0.82 ± 0.16	1.09 ± 0.10
β -Muricholic acid	0.39 ± 0.05	1.16 ± 0.26*
Total primary bile acid	2.06 ± 0.30	3.35 ± 0.19*
Deoxycholic acid	0.53 ± 0.07	0.32 ± 0.07
Lithocholic acid	0.29 ± 0.03	0.12 ± 0.01*
Hyodeoxycholic acid	1.19 ± 0.23	0.91 ± 0.19
Ursodeoxycholic acid	1.44 ± 0.13	0.48 ± 0.10*
ω -Muricholic acid	0.33 ± 0.07	0.54 ± 0.17
Total secondary bile acid	3.77 ± 0.34	2.38 ± 0.27*
Total bile acid	5.83 ± 0.44	5.73 ± 0.31
mg/day		
Fatty acid	7.66 ± 1.01	21.3 ± 2.4*

Data are presented as the mean ± SD of 7 rats in each group.
*Significantly different from the corresponding C group at $p < 0.05$ (Student's *t*-test).

C, rats administrated saline; O, rats administrated oligonol.

than in C group. Additionally, excreted levels of fatty acids in the feces were significantly higher in O group than in C group (Table 2).

3.4 Effects of oligonol on cecal short-chain fatty acid levels

The levels of total cecal short chain fatty acids were significantly lowered by administration of oligonol. Especially, *n*-butyric acid level was significantly lower in O group than in C group (Fig. 2).

3.5 Effects of oligonol administration on the expression of factors involved in cholesterol metabolism in the liver

The mRNA expression of HMGCR, which is the rate-limiting enzyme for cholesterol synthesis, was not modulated by the administration of oligonol. On the other hand, the mRNA expression of CYP7A1, which plays an important

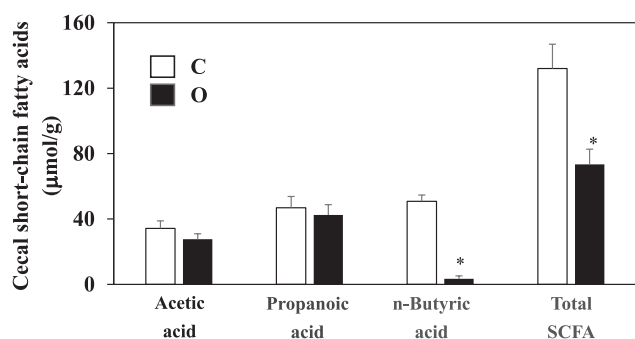


Fig. 2 Effects of orally administrated oligonol on cecal short-chain fatty acid level. Data are presented as the mean $\pm$ SD of 7 rats in each group. *Significantly different from the corresponding C group at $p < 0.05$ (Student's *t*-test). C: rats administrated saline; O: rats administrated oligonol; SCF: short-chain fatty acids.

role in regulation of bile acid biosynthesis and cholesterol homeostasis, was significantly lowered by the administration of oligonol. The mRNA expression of FXR, which regu-

lates the expression of CYP7A1, was significantly higher in O group than in C group. However, the mRNA expression of SHP, which negatively regulates CYP7A1 and FGFR4—the receptor of FGF15, remained unchanged (Fig. 3).

3.6 Effects of oligonol on the expression of factors involved in bile acid metabolism in the ileum

Significant differences in the mRNA expression of FXR, which is a ligand of bile acids and upregulates the expression of SHP or FGF15—a negative regulator of hepatic CYP7A1 through the binding to hepatic FGFR4, was not observed. However, the expression of SHP was significantly lower in O group than in C group. The same trend was observed in the mRNA expression of FGF15, although it was not significant (Fig. 4).

4 Discussion

In this study, we evaluated the effect of oligonol that was administered for 3 days on cholesterol metabolism in rats fed cholesterol-free diet.

Growth parameters such as body weight and liver weight

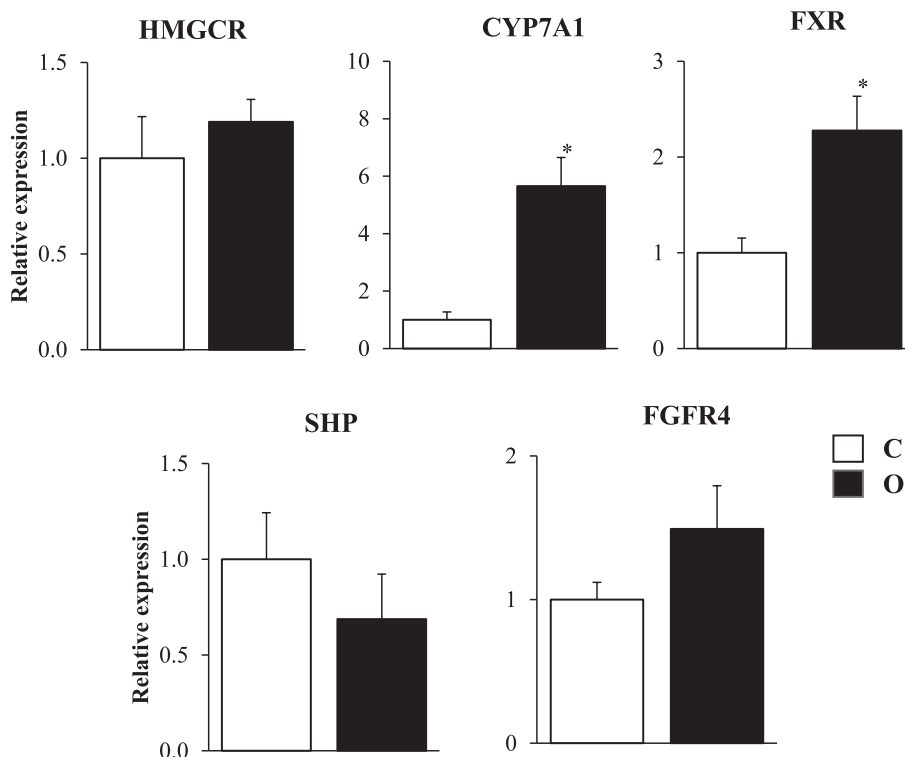


Fig. 3 Effects of orally administrated oligonol on mRNA expression of genes involved in cholesterol metabolism in liver. Data are presented as the mean $\pm$ SD of 7 rats in each group. *Significantly different from the corresponding control group at $p < 0.05$ (Student's *t*-test). The abbreviations: C: rats administrated saline; CYP7A1: cytochrome P450 family 7 subfamily A member 1; FXR: farnesoid X receptor; SHP: small heterodimer partner; FGFR4: fibroblast growth factor receptor 4; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; O: rats administrated oligonol.

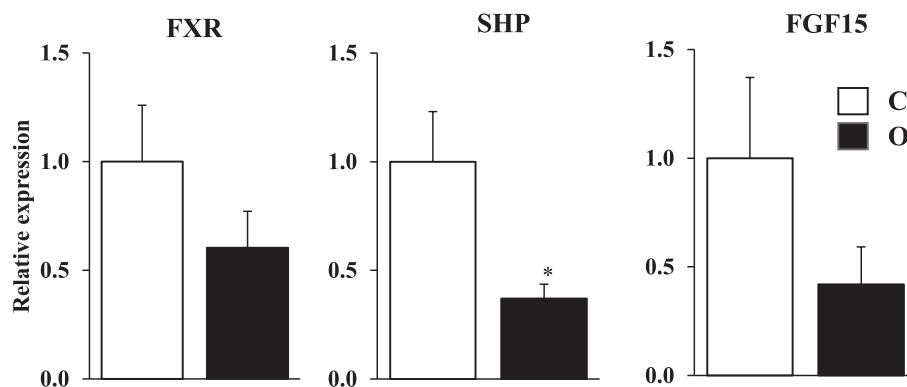


Fig. 4 Effects of orally administrated oligonol on mRNA expression of genes involved in bile acid metabolism in ileum. Data are presented as the mean $\pm$ SD of 7 rats in each group. *Significantly different from the corresponding control group at $p < 0.05$ (Student's *t*-test). C: rats administrated saline; FGF15: fibroblast growth factor 15; FXR: farnesoid X receptor; SHP: small heterodimer partner; O: rats administrated oligonol.

did not change. However, cecum weight significantly increased in rats given oligonol due to unabsorbed oligonol. In fact, cecal polyphenols amounted to 7.54 mg/g cecum in rats administered with oligonol, whereas it was not detected in the cecum of the control group. The accumulated oligonol may have a negative impact on fermentation by microorganisms in cecum. In a previous study, Unno *et al.* found that the tea polyphenol(-)-epigallocatechin gallate affected the growth of certain species of gut microbiota in rats²³⁾. This observation may relate to the modulation of cholesterol metabolism.

Lipid levels in plasma were not changed following the administration of oligonol in rats. However, liver cholesterol levels were significantly lowered despite the short-term administration of oligonol. This observation could be attributed to the inhibition of biliary cholesterol absorption from the intestine and the upregulation of cholesterol catabolism in the liver, as described below.

The weight of the excreted feces as well as the level of excreted neutral steroids containing cholesterol significantly increased after oligonol administration. Some polyphenols, including tea catechin²⁴⁾, green tea catechin²⁵⁾, and grape seed polyphenols²⁶⁾, have been shown to inhibit lipid absorption from the intestine. In a previous study, we also observed inhibition of cholesterol micelles formation by apple procyanidin *in vitro*⁷⁾, further confirming that oligonol inhibits biliary cholesterol absorption from the intestine. Moreover, the results of the present study suggest that oligonol inhibits the absorption of dietary cholesterol from intestine. Of note, the excreted levels of total acidic steroids did not change in the presence of oligonol. Administration of oligonol significantly increased the levels of total primary bile acids, while decreasing the levels of total secondary bile acids. In each bile acids, the levels of chenodeoxycholic acid and β -muricholic acid significantly in-

creased by the administration of oligonol while the levels of lithocholic acid and ursodeoxycholic acid were significantly lowered. These effects are likely to be modulated by changes in bile acid metabolism and intestinal microflora. Similarly, the level of cecal short-chain fatty acids were reduced by the administration of oligonol. These data suggest that oligonol exerts bactericidal effects against bacterium of the genus *Clostridium*, as these bacteria are closely related to the production of n-butyric acid²⁷⁾. This effect can, in turn, modulate cholesterol metabolism because short-chain fatty acids are linked to fatty acid metabolism²⁸⁾. In further studies, we will examine in detail the effects of short chain fatty acid on cholesterol metabolism.

In the liver, CYP7A1 catalyzes the rate limiting step in the bile acid synthesis from cholesterol. Liver cholesterol levels were lowered in rats given oligonol. This may be caused by the promotion of cholesterol catabolism accompanying upregulation of hepatic CYP7A1 gene expression, although levels of bile acid in feces was not changed between the two groups. The levels of bile acids in feces may have remained unchanged because the administration period was too short. Another limitation of this study was that we did not analyzed feces in colon.

The nuclear bile acid receptor and transcription factor FXR controls bile acid homeostasis²⁹⁾. FXR is expressed at high levels in liver and intestine³⁰⁾. Generally, activated FXR indirectly represses CYP7A1 transcription through the induction of SHP, which binds to the orphan nuclear receptor LRH-1 (liver receptor homolog 1) and inhibits activation of the CYP7A1 promoter³¹⁾. Despite increased liver FXR expression, SHP gene expression was not increased by administration of oligonol. The lack of suppression of CYP7A1 might be explained by the modulation of gene expression described above. Therefore, ileal FXR gene expression may be strongly linked to changes in hepatic

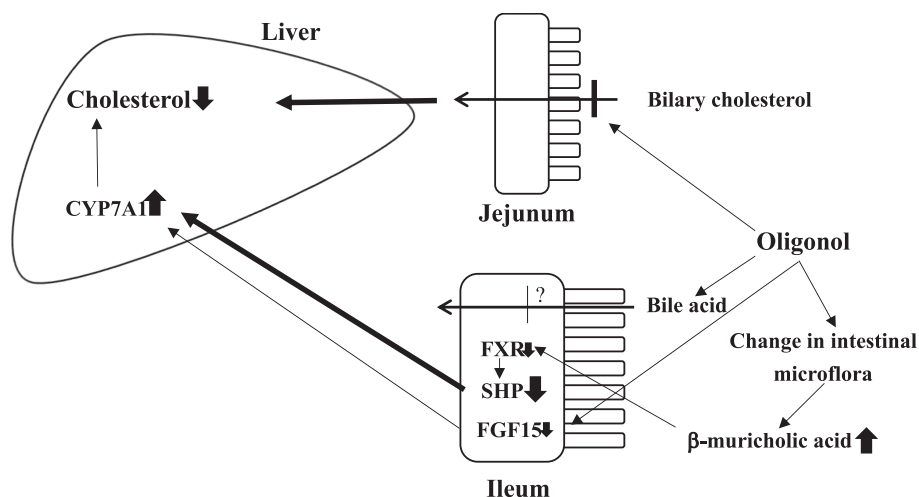


Fig. 5 Expected mechanism of hypocholesterolemic effect by oligonol administration.

Oligonol may exert hypocholesterolemic effect through upregulation of CYP7A1 and inhibition of biliary cholesterol absorption from the intestine. Upregulation of CYP7A1 gene expression may be caused by changes in FXR, SHP and FGF15 expression in the ileum. The oligonol-mediated increase of β -muricholic acid level in the ileum may be associated with modulation of these genes via changes in the intestinal microflora.

CYP7A1: cytochrome P450 family 7 subfamily A member 1; FGF15: fibroblast growth factor 15; FXR: farnesoid X receptor; SHP: small heterodimer partner; ?: an unclear point.

CYP7A1 gene expression because FXR gene expression showed a trend to reduced levels and SHP gene expression decreased in rats given oligonol.

HMGCR gene expression was not changed between the 2 groups despite the lower levels of hepatic cholesterol following administration of oligonol. This observation may have been affected by diurnal variation of cholesterol biosynthesis in the liver³²⁾.

In ileum, SHP gene expression was significantly lowered by administration of oligonol through downregulation of FXR gene expression, although changes in the expression of FXR were not significant. EGF15 activation negatively regulates CYP7A1 expression by binding to hepatic FGFR4 without FXR or SHP³³⁾. EGF15 gene expression in ileum showed a trend towards reduced levels by administration of oligonol. The effects may also contribute to upregulation of hepatic CYP7A1 gene expression.

Sayin *et al.* identified tauro-conjugated β -muricholic acid as an FXR antagonist³⁴⁾. Interestingly, β -muricholic acid levels in feces significantly increased in rats given oligonol. Moreover, levels of total secondary bile acids and coprostanol lowered in these rats. Therefore, downregulation of FXR gene expression in ileum and liver may be caused by changes of bile acid composition due to changes in intestinal microflora by oligonol administration.

Taken together, our results show that oligonol may exert hypocholesterolemic effect by activating cholesterol catabolism through the modulation of the gene expression of factors modulating CYP7A1 in ileum and the inhibition of biliary cholesterol absorption from the intestine as shown

in Fig. 5. In this study, the administration period of oligonol was too short and its dosage was very high. Further, the effect of oligonol on hypercholesterolemia induced by high cholesterol diet was not investigated. Therefore, we will examine the effect of dietary oligonol on cholesterol metabolism under long-term feeding, low dose feeding, or high cholesterol diet conditions in future studies.

5 Conclusion

We found that oligonol, which contains low molecular procyanidins, exerted hypocholesterolemic effects in rats under short-term administration. This effect may be caused by upregulation of cholesterol catabolism in liver and interference of biliary cholesterol absorption from the intestine. Consequently, oligonol could be used as a component for reducing cholesterol level.

References

- 1) Suzuki, A.; Kosuge, K.; Nanyu, O.; Watanabe, H.; Watanabe, T.; Usuda, Y.; Sasaki, S.; Aizawa, Y. Five year study of cardiovascular risk factors in Japanese people: Implications concerning new onset of metabolic syndrome. *Intern. Med.* **49**, 1-6 (2010).
- 2) Ikeda, I.; Kobayashi, M.; Hamada, T.; Tsuda, K.; Goto, H.; Imaizumi, K.; Nozawa, A.; Sugimoto, A.; Kakuda, T. Heat-epimerized tea catechins rich in gallic acid

- gallate and catechin gallate are more effective to inhibit cholesterol absorption than tea catechins rich in epigallocatechin gallate and epicatechin gallate. *J. Agric. Food Chem.* **51**, 7303-7307 (2003).
- 3) Gonzalez-Quilen, C.; Rodriguez-Gallego, E.; Beltran-Debon, R.; Pinent, M.; Ardevol, A.; Blay, M.T.; Terra, X. Health-promoting properties of proanthocyanidins for intestinal dysfunction. *Nutrients* **12**, 130; doi:10.3390/nu12010130 (2020).
 - 4) Zhang, L.; Wang, Y.; Li, D.; Ho, C.T.; Li, J.; Wan, X. The absorption, distribution, metabolism and excretion of procyanidins. *Food Funct.* **7**, 1273-1281 (2016).
 - 5) Yasuda, A.; Natsume, M.; Sasaki, K.; Baba, S.; Nakamura, Y.; Kanegae, M.; Nagaoka, S. Cacao procyanidins reduce plasma cholesterol and increase fecal steroid excretion in rats fed a high-cholesterol diet. *Biofactors* **33**, 211-223 (2008).
 - 6) Quifer-Rada, P.; Choy, Y.Y.; Clavert, C.C.; Waterhouse, A.L.; Lamuela-Raventos, R.M. Use of metabolomics and lipidomics to evaluate the hypocholesterolemic effect of proanthocyanidins from grape seed in a pig model. *Mol. Nutr. Food Res.* **60**, 2219-2227 (2016).
 - 7) Osada, K.; Suzuki, T.; Kawakami, Y.; Kasai, A.; Sami, M.; Ohta, Y.; Kanda, T.; Ikeda, M. Dose-dependent hypocholesterolemic actions of dietary apple polyphenol in rats fed cholesterol. *Lipids* **41**, 133-139 (2006).
 - 8) Fujii, H.; Sun, B.; Nishioka, H.; Hirose, A.; Aruoma, O.I. Evaluation of the safety and toxicity of the oligomerized polyphenol Oligonol. *Food Chem. Toxicol.* **45**, 378-387 (2007).
 - 9) Sakurai, T.; Nishioka, H.; Fujii, H.; Nakano, N.; Radak, Z.; Izawa, T.; Haga, S.; Ohno, H. Antioxidative effects of a new lychee fruit-derived polyphenol mixture, oligonol, converted into a low-molecular form in adipocytes. *Biosci. Biotechnol. Biochem.* **72**, 463-476 (2008).
 - 10) Bahijri, S.M.; Ajabnoor, G.; Hegazy, G.A.; Alsheikh, L.; Moumena, M.Z.; Bashanfar, B.M.; Alzahrani, A.H. Supplementation with Oligonol, prevents weight gain and improves lipid profile in overweight and obese Saudi females. *Curr. Nutr. Food Sci.* **14**, 164-170 (2018).
 - 11) Kim, M.; Park, W.H.; Lee, S.; Suh, D.H.; Kim, K.; No, J.H.; Kim, Y.B. Oligonol, a low molecular weight polyphenol, enhances apoptotic cell death in ovarian cancer cells via suppressing NF- κ B activation. *Nutr. Cancer* **71**, 141-148 (2019).
 - 12) Lee, N.; Shin, M.S.; Kang, Y.; Park, K.; Maeda, T.; Nishioka, H.; Fujii, H.; Kang, I. Oligonol, a lychee fruit-derived low-molecular form of polyphenol mixture, suppresses inflammatory cytokine production from human monocytes. *Hum. Immunol.* **77**, 512-515 (2016).
 - 13) Ogasawara, J. Oligonol, a new lychee fruit-derived low-molecular form of polyphenol, enhances lipolysis in primary rat adipocytes through activation of the ERK1/2 pathway. *Phyther. Res.* **23**, 1626-1633 (2009).
 - 14) American Institute of Nutrition. Report of the AIN Ad Hoc committee on standards for nutritional studies. *J. Nutr.* **107**, 1340-1348 (1977).
 - 15) Folch, J.; Ascoli, I.; Lees, M. Preparation of lipid extracts from brain tissue. *J. Biol. Chem.* **191**, 833-841 (1951).
 - 16) Fletcher, M.J. A colorimetric method for estimating serum triglycerides. *Clin. Chim. Acta* **22**, 393-397 (1968).
 - 17) Rouster, G.; Siakotos, A.N.; Fleischer, S. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorous analysis of spots. *Lipids* **1**, 85-86 (1966).
 - 18) Sperry, W.M.; Webb, M. A revision of the schoenheimer-sperry method for cholesterol determination. *J. Biol. Chem.* **187**, 97-106 (1950).
 - 19) Sugano, M.; Yamada, Y.; Yoshida, K.; Hashimoto, Y.; Matsuo, T.; Kimoto, M. The hypocholesterolemic action of the undigested fraction of soybean protein in rats. *Atherosclerosis* **72**, 115-122 (1988).
 - 20) Jeejeebhoy, K.N.; Ahmad, S.; Kozak, G. Determination of fecal fats containing both medium and long chain triglycerides and fatty acids. *Clin. Biochem.* **3**, 157-163 (1970).
 - 21) Duan, J.; Ishida, M.; Aida, K.; Tsuduki, T.; Zhang, J.; Manabe, Y.; Hirata, T.; Sugawara, T. Dietary cerebroside from sea cucumber (*Stichopus japonicus*): Absorption and effects on skin barrier and cecal short-chain fatty acids. *J. Agric. Food Chem.* **21**, 7014-7021 (2016).
 - 22) Fisher, R.A. *Statistical methods for research workers*. 14th ed., Oliver and Boyd, Edingburgh, Scotland, pp.140-142 (1970).
 - 23) Unno, T.; Sakuma, M.; Mitsuhashi, S. Effect of dietary supplementation of (-)-epigallocatechin gallate on gut microbiota and biomarkers of colonic fermentation in rats. *J. Nutr. Sci. Vitaminol.* **60**, 213-219 (2014).
 - 24) Yang, T.T.; Koo, M.W. Chinese green tea lowers cholesterol level through an increase in fecal lipid excretion. *Life Sciences* **66**, 411-423 (1999).
 - 25) Ashigai, H.; Taniguchi, Y.; Suzuki, M.; Ikeshima, E.; Kanaya, T.; Zembutsu, K.; Tomita, S.; Miyake, M.; Fukushima, I. Fecal lipid excretion after consumption of a black tea polyphenol containing beverage-randomized, placebo-controlled, double-blind, crossover study. *Biol. Pharm. Bull.* **39**, 699-704 (2016).
 - 26) Nakamura, Y.; Tonogai, Y. Effects of grape seed polyphenols on serum and hepatic lipid contents and fecal steroid excretion in normal and hypercholesterolemic rats. *J. Health Sci.* **48**, 570-578 (2002).
 - 27) Okereke, A.; Montville, T.J. Bacteriocin-mediated inhibition of *Clostridium botulinum* spores by lactic acid

- bacteria at refrigeration and abuse temperatures. *Appl. Environ. Microbiol.* **57**, 3423-3428 (1991).
- 28) Miyoshi, M.; Iizuka, N.; Sasaki, S.; Fujiwara, M.; Aoyama-Ishikawa, M.; Maeshige, N.; Hamada, Y.; Takahashi, M.; Usami, Y. Oral tributyrin prevents endotoxin-induced lipid metabolism disorder. *Clin. Nutr. ESPEN* **10**, e83-e88 (2015).
- 29) Kok, T.; Hulzebos, C.V.; Wolters, H.; Havinga, R.; Agellon, L.B.; Stellaard, F.; Shan, B.; Schwarz, M.; Kuipers, F. Enterohepatic circulation of bile salts in farnesoid X receptor-deficient mice: efficient intestinal bile salt absorption in the absence of ileal bile acid-binding protein. *J. Biol. Chem.* **278**, 41930-41937 (2003).
- 30) Lu, T.T.; Repa, J.J.; Mangelsdorf, D.J. Orphan nuclear receptors as eLiXiRs and FiXeRs of sterol metabolism. *J. Biol. Chem.* **276**, 37735-37738 (2001).
- 31) Goodwin, B.; Jones, S.A.; Price, R.R.; Watson, M.A.; McKee, D.D.; Moore, L.B.; Galardi, C.; Wilson, J.G.; Lewis, M.C.; Roth, M.E.; Maloney, P.R.; Willson, T.M.; Kliewer, S.A. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol. Cell.* **6**, 517-526 (2000).
- 32) Shefer, S.; Hauser, S.; Lapar, V.; Mosbach, E.H. Diurnal variation of HMG CoA reductase activity in rat intestine. *J. Lipid Res.* **13**, 571-573 (1972).
- 33) Iagaki, T.; Choi, M.; Moschetta, A.; Peng, L.; Cummins, C.L.; McDonald, J.G.; Luo, G.; Jones, S.A.; Goodwin, B.; Richardson, J.A.; Gerard, R.D.; Repa, J.J.; Mangelsdorf, D.J.; Kliewer, S.A. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab.* **2**, 217-225 (2005).
- 34) Sayin, S.I.; Wahlström, A.; Felin, J.; Jäntti, S.; Marschall, H.U.; Bamberg, K.; Angelin, B.; Hyötyläinen, T.; Orešič, M.; Bäckhed, F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* **17**, 225-235 (2013).
-