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쥐에서 柑橘粕 粉末 또는 펙틴을 含有한 飼料의 給與가 血液, 肝, 糞의 콜레스테롤 水準 및 콜레스테롤 吸收에 미치는 影響

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Effect of Feeding Diets Containing Tangerine Pulp Meal or Pectin on the Cholesterol Contents or Cholesterol Absorption in the Serum, Liver and Feces of Rats

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要 約

이 硏究는 飼料중 水溶性 纖維質이 쥐의 血淸, 肝, 糞 中의 cholesterol 및 脂肪 水準과 腸으로부터의 cholesterol 吸收에 미치는 影響을 糾明하기 위하여 實施되었다. 實驗 1에서는 成熟한 Sprague Dawley 암쥐 (평균 체중 219 g, 처리당 7마리)에게 對照飼料, 또는 對照飼料의 澱粉을 pectin (5%) 또는 柑橘粕 粉末 (5%)과 代替하여 給與하였다. 柑橘粕 粉末은 柑橘의 즙을 짜고 난 나머지 外皮와 內皮를 乾燥하고 粉碎하여 利用하였다. 모든 飼料에는 0.5%의 cholesterol과 10%의 豚脂를 添加하여 生體內의 cholesterol의 水準을 높였다. 이는 給與된 飼料에 의한 cholesterol低下의 效果를 分明하게 보기 위한 意圖이었다. 4週間의 飼養 後 쥐들을 屠殺하여 血淸과 肝을 採取하였으며, 糞은 屠殺 前 3日間 採取, 乾燥하여 粉碎 保管하였다. 增體量, 飼料 攝取量, 肝의 무게, 糞의 무게, 大腸 內容物의 무게는 處理間 差異가 없었다. 血淸 總 cholesterol 水準은 pectin 處理區가 對照區에 比해 顯著히 ($P < 0.05$) 낮았으며, high density lipoprotein, low density lipoprotein plus very low density lipoprotein cholesterol 水準은 處理區間에 差異가 없었다. 肝 總 cholesterol 水準도 pectin 處理區는 對照區에 比해 顯著히 ($P < 0.01$) 낮았으며, 脂肪 水準은 處理區 間의 差異가 없었다. 糞의 cholesterol 水準은 處理區 間의 差異가 없었다.

實驗 2에서는 成熟한 Sprague Dawley 암쥐 (처리당 5마리)에게 0.2 μCi의 ^{14}C-cholesterol이 添加된 對照飼料 혹은 10% pectin飼料를 胃管注入法에 의해 注入하였다. 비록 統計的인 有意性은 없었지만 給與 後 1日間 혹은 2日

間에 糞으로 排泄된 放射能의 量은 pectin區에서 對照區와 比較하여 거의 2倍였다. 이는 pectin이 cholesterol의 吸收를 抑制한다는 것을 나타내었다.

本 實驗의 結果는 飼料에 pectin 添加가 腸으로부터 cholesterol 吸收를 抑制하여 血淸과 肝의 콜레스테롤 水準을 抑制한다는 것을 보여주었다. 또한 柑橘粕 添加區에서는 有意性은 없었으나 血淸과 肝의 cholesterol 水準이 對照區에 比해 減少되는 傾向을 나타내었다. 그러므로 柑橘粕 添加 水準을 增加시켜 飼料의 pectin含量을 높일 수 있다면, 柑橘粕 粉末도 cholesterol水準을 抑制할 수 있을 것으로 豫測된다.

I . Introduction

Cholesterol has long been known as a risk factor for cardiovascular diseases including atherosclerosis, and many investigators have attempted to reduce cholesterol in the body by dietary manipulations. Soluble fibers, such as those in pectin, guar gum, psyllium, oat bran and barley, decreased plasma cholesterol level in experimental animals (Tsai *et al.* 1976, De Schrijver *et al.* 1992, Wang *et al.* 1992) and also in humans (Hopewell *et al.* 1993). Among soluble fibers, pectin invariably had a more pronounced effect on lowering serum cholesterol level in animals (Kelly and Tsai 1978, Reddy *et al.* 1980, Fernandez *et al.* 1990, Nishina *et al.* 1991, Arjmandi *et al.* 1992a and 1992b, De Schrijver *et al.* 1992) and humans (Judd and Truswell 1982, Bell *et al.* 1990).

The presence of soluble fiber in the gastrointestinal tract was known to increase viscosity and as a result to interfere with micelle formation and lipid absorption (Superko *et al.* 1988, Gallaher *et al.* 1993a). This effect was achieved by only moderate increases in viscosity (Gallaher *et al.* 1993b). In addition, certain fibers were suggested to bind or absorb

bile acids and neutral sterols, enhancing their removal from enterohepatic circulation (Story and Kritchevsky 1976, Illman and Topping 1985, Arjmandi *et al.* 1992a).

The present study was designed to evaluate the effect of dietary pectin and tangerine pulp meal on serum, liver and fecal cholesterol and triacylglycerol levels, and also on cholesterol absorption from the intestine in rats. Particularly, our study was carried out to explore tangerine pulp meal, which is a byproduct of tangerine juice manufacture and becomes a polluting waste, as a possible cholesterol-lowering agent.

II. Literature Review

1. Cholesterol metabolism (absorption, synthesis, transport and excretion) in vivo

Cholesterol is an important nutrient which is a precusor of steroid hormones, bile acids and components of the cell wall. It comes from diets and is synthesized in mitochondria of cells in live organs (mainly liver). Many studies demonstrated that a high level of cholesterol and fat in diet increased plasma and liver cholesterol levels (Hutagalung *et al.*, 1969; Kelly and Tsai 1978), which was decreased when animals were fed diet containing no cholesterol (Cromwell *et al.*, 1970; Nishina and Freedland, 1990; Topping *et al.*, 1993).

Cholesterol synthesis begins with two acetyl Co-A binding and is done through many complex reactions (Lehninger, 1982). Its synthesis is reduced by increasing dietary cholesterol but is induced by reducing dietary cholesterol (Grundy *et al.*, 1969). This mechanism allows

body to maintain relatively constant cholesterol level (i.e. homeostasis). Therefore, a feedback system exists between synthesis and absorption of cholesterol in vivo. But if absorbed cholesterol exceeds body's need, cholesterol accumulates in the body. Cholesterol accumulating in the artery is a primary cause of atherosclerosis and coronary heart disease.

Dietary cholesterol is absorbed through portal vein, and absorbed cholesterol is transported into peripheral tissues (Vahouny *et al.*, 1988). Liver cells synthesize cholesterol from acetyl Co-A and synthesized cholesterol associated with low density lipoprotein (LDL) is transported to whole body tissue. LDL receptors located in the surface of cells receive LDL, which is divided into individual components, cholesterol, triacylglycerol, phospolipid and protein (Goldstein and Brown, 1977). High density lipoprotein (HDL) transports cholesterol from peripheral tissues to the liver. In the liver, cholesterol transforms into bile salts, which is excreted into the intestine. Part of excreted bile salts is reabsorbed into the liver, and recycled (McCarthy, 1993).

Some investigators (Grundy *et al.*, 1969) reported that

the human being can absorb only limited amounts (about 300 mg/day) of dietary cholesterol and the unabsorbed cholesterol and bile salts produced from cholesterol metabolism are excreted into feces.

2. Effect of pectin on cholesterol absorption

Dietary soluble fibers such as pectin, guar gum and agar are high in viscosity and fermentability. Pectin is a material which has a high viscosity and gel-forming property, and it was known to affect lipid metabolism in vivo. Some investigators reported that dietary pectin reduced blood and liver cholesterol level because viscous pectin binds dietary cholesterol and inhibits its absorption through intestinal tract (Kelly and Tsai, 1978; Lee and Han, 1985; Vahouny *et al.*, 1988; Fernandez *et al.*, 1990).

Chen *et al.* (1984) reported that certain soluble fibers such as pectin are almost completely fermented by colonic bacteria to short chain fatty acids such as acetate, propionate and butyrate and other products, and also 0.5% dietary

propionate reduced the liver and serum cholesterol level in rats fed a diet containing 0.3% dietary cholesterol. They suggested that propionate reduced cholesterol level in vivo by inhibiting hepatic cholesterol synthesis.

Falk *et al.* (1982) also reported that binding between pectin and taurocholate, which is a product of cholesterol metabolism, was present in in vitro experiment. A 20-mg pectin in 2 ㎖ 0.001 M imidazole buffer (pH 6.8) with and without addition of $FeSO_4$ (5 mg) was added to 5 ㎖ buffer containing Na-taurocholate (1,200, 4,000 and 8,200 ㎍) in a dialysis bag, and dialyzed for 2 days at 5℃. A significant binding between pectin and taurocholate was observed.

Gallaher et. al. (1993a) examined the effect of feeding diets containing 0.12% cholesterol and 5% fiber on plasma and liver cholesterol concentrations in hamsters. They reported that high-viscosity hydroxypropyl methylcellulose reduced plasma and liver cholesterol concentrations compared with low viscosity hydroxypropyl methylcellulose, and also suggested viscous materials could inhibit intestinal absorption of cholesterol by slowing diffusion of the cholesterol-containing micelles to the intestinal mucosal cells

(the site of cholesterol absorption) and/or by interfering with the formation of mixed micelles. Gallaher et. al. (1993b) also reported that above effect was achieved by only moderate increases in viscosity. Arjmandi et. al. (1992a) reported the effect of feeding diets containing 0.3% cholesterol and 7.5% dietary fiber as cellulose (control), pectin, psyllium or oat bran on serum, liver cholesterol level and fecal neutral sterol levels in rats. Rats fed diet containing pectin had lower serum, liver cholesterol levels, and increased fecal neutral sterol levels than those fed diet containing cellulose. They suggested that soluble dietary fibers such as pectin may exert their hypocholesterolemic effect by increasing excretion of fecal neutral sterols.

Vahouny *et al.* (1988) reported that highly methoxylated citrus pectin (5% in diet) caused decreased lymphatic absorption of cholesterol in the intestinal tract of rats. Nonfasted rats were subjected to cannulation of the left thoracic lymph duct, and 1.5 ㎖ of the test emulsion diet which had ^{3}H-cholesterol and other radioisotope was administered via the duodenal catheter. After administration of the test emulsion, lymph was collected for 4 h and then

as a single 4-24 h collection. Dietary pectin resulted in significantly lower absorption of cholesterol during the initial 4 h and 4-24 h of lymph collection than a fiber-free diet did.

3. Effect of tangerine pulp on cholesterol metabolism

Suh *et al.* (1985) reported effect of tangerine pulp, cellulose, wheat bran, ultrasonically treated tangerine pulp and acetone-extracted tangerine pulp, all added to a diet at 17%, on cholesterol deposition in carcass and steroid excretion in chicks. Tangerine pulp lowered total carcass cholesterol level (922 mg, in whole carcass) more than acetone-extracted tangerine pulp (1149 mg). Since orange (tangerine) pulp contained 30% pectin on a DM base (Yang, 1985), the effect of tangerine pulp was considered due to pectin in the pulp.

III. Materials and Methods

1. Animals and diets

In experiment 1, twenty-one female Sprague Dawley rats (mean initial weight, 219g) were housed individually in suspended wire cages in a room maintained at 20-25℃ with a 12-hr light (07:00 to 19:00) and 12-hr dark (19:00 to 07:00) cycle. Diets and water were provided for *ad libitum* consumption. Rats were divided into three groups and each group was fed a control or diets containing 5% tangerine pulp meal or 5% pectin. The composition of the experimental diets is given in Table 1.

After a 3-day adjustment period, rats were fed experimetal diets for four weeks. Feces was collected daily during the last three days of the feeding experiment and were stored at -20℃ until analysis. At the end of the experiment, rats were fasted for 16 hours and killed by decapitation, and blood and liver samples were collected.

Serum was obtained from the blood which was centrifuged (1,100 × g) for 10 min. The liver samples were weighed and frozen at -20℃ until analysis.

In Exp. 2, ten female Sprague Dawley rats were housed in the same condition as in Exp. 1. Rats were divided into two groups, each group was fed a control or 10% pectin diet for 3 days. After fasted for 16 hours, rats were fed through a stomach tube the control or diet containing 10% pectin, both mixed with 0.2 μCi [4-^{14}C]-cholesterol (Amersham International Plc, Buckinghamshire, England) which was pre-mixed with 20 ㎕ corn oil. Feces was collected daily for 4 days. During the first day after fed, rats were fasted, and then fed their diet containing no ^{14}C-cholesterol.

2. Analysis of cholesterol and triacylglycerol in the serum

Total cholesterol was determined by using commercial assay kit (International Regent Corp., Tokyo, Japan), following the method in manual of kit. Sixty ㎕ of serum was used for determining of total cholesterol. High-density

lipoprotein (HDL) cholesterol (50 ㎕ of serum was used) and triacylglycerol (60 ㎕) were also determined by using commercial assay kits (WAKO Pure Chemical Ind., Osaka, Japan), respectively. Low-density lipoprotein + very low-density lipoprotein cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

3. Analysis of cholesterol and triacylglycerol in the liver

Liver samples were prepared by modifying the method described in De Hoff *et al.* (1978) to determine cholesterol and triacylglycerol. Liver tissue (1 g) was homogenized in 6 ㎖ chloroform/methanol mixture (2/1 by volume) and 2 ㎖ distilled water using a tissue homogenizer. The chloroform fraction was located under the methanol + water fraction when centrifuged (1,100 × g) for 5 min. A 0.5-㎖ aliquot of the chloroform fraction was taken from the chloroform fraction and dried under flowing nitrogen gas. A 1 : 1 Triton X-100/chloroform solution (50 ㎕, roughly six times the weight of the dried residue) was added. The mixture was diluted to 0.5 ㎖ with chloroform and thoroughly mixed.

Then a 50-㎕ sample was transferred to a test tube and the chloroform was evaporated under flowing nitrogen. Total cholesterol in the residue was determined as described for the determination of serum cholesterol.

The method used for extracting liver cholesterol was checked by measuring radioactivity recovered in chloroform fraction after a known amount of ^{14}C-cholesterol was added to 1 g liver sample. The radioactivity in the chloroform fraction (1 ㎖) was determined using a liquid scintillation counter (L.S.C., Wallac Dy, Turku, Finland), and an internal standard (^{14}C-toluene) after 15 ㎖ of Biosafe II (Research Products International Corp. Illinois) was added. All the added radioactivity added to the liver sample was recovered in 4-㎖ chloroform fraction.

Liver triacylglycerol was also determined in the chloroform extract. A 20-㎕ aliquot of the chloroform fraction was taken and dried under flowing nitrogen. The residue was dissolved in 100 ㎕ methanol and triacylglycerol was determined in the residue as described above for serum triacylglycerol assay.

4. Analysis of cholesterol and triacylglycerol in the feces

Cholesterol and triacylglycerol contents of dried feces (0.3 g) were determined using the same method used for the liver cholesterol and triacylglycerol assay.

5. Determination of radioactivity in the feces

Radioactivity excreted into feces was used to determine absorption of cholesterol and its metabolites from the gastrointestinal tract (Exp. 2). Feces collected during a first, second, third and fourth 24-hour period after the stomach feeding of a slurry diet containing ^{14}C-cholesterol were dried in air and ground, respectively. The ground feces was mixed with 15-㎖ Bio-safe II and the radioactivity was determined as described above in the cholesterol recovery test for liver sample preparation.

6. Statistical analysis

Data were analyzed by the analysis of variation (Minitab Inc., 1987). When the differences were significant, the Newman-Keuls test was applied to compare individual means (Snedecor and Cochran 1980).

Ⅳ. Results

Weight gain and liver weight were not significantly affected by diet (Table 2). The large intestinal content also was not significantly influenced by diet perhaps because rats were fasted about 16 hours before being killed. But dietary pectin usually increases the cecal weight (Hove and King 1979).

Serum total cholesterol level was significantly ($P < 0.05$) lower in rats fed the pectin diet (81.7 mg /100 mℓ) than in the control (119.2 mg) (Table 3). Dietary tangerine pulp meal tended to decrease the serum cholesterol level, as compared to the control (93.8 vs 119.2 mg /100 mℓ serum), but the difference was not significant ($P > 0.05$) due to the large individual variation. HDL-cholesterol/total cholesterol ratio in the serum tended to increase with the tangerine pulp meal or pectin (0.36 for control vs 0.39 and 0.46 for tangerine and pectin, respectively), although the difference was not significant. Serum triacylglycerol levels were not different among the dietary groups.

The effect of feeding diet containing pectin or tangerine pulp meal on liver total cholesterol and triacylglycerol levels are presented in table 4. Total cholesterol level (14.2 mg/g fresh liver) in the control was higher ($P < 0.05$) than that found with the pectin diet (7.94 mg/g). Dietary tangerine pulp meal also tended to reduce the liver cholesterol level, but not significantly. Liver triacylglycerol level was not influenced by the dietary treatments.

Fecal cholesterol levels were not different among the dietary groups as shown in Table 5. Fecal triacylglycerol content was higher in the control (1.44 mg) than in rats fed the pectin diet (1.01 mg) or the tangerine pulp meal diet (0.91 mg). However, the amount of triacylglycerol excreted daily was relatively small and perhaps insignificant.

Radioactivity excreted (as percentage of intake) during the first day in the feces of rats fed the control or the pectin diet is 3.08% and 5.43%, and 9.84% and 16.5% during the first two days after feeding, respectively (Table 6). Rats fed the pectin diet excreted about twice radioactivity as compared with the control, but the difference was not significant. Radioactivity excreted in feces of rat fed pectin

diet during the three or four days after the feeding was similar to that of the control group.

V. Discussion

Many investigators have reported that dietary pectin has a hypocholesterolemic effect (Leveille and Sauberlich, 1966; Tsai *et al.*, 1976; Kelly and Tsai, 1978; Fernandez *et al.*, 1990; Arjmandi *et al.*, 1992a and 1992b). Most of these studies were done with diets supplemented with cholesterol. However, when animals were fed diets unsupplemented with cholesterol, the influence was not found (Kelly and Tsai 1978; Nishina and Freedland 1990; Arjmandi *et al.*, 1992a), with some exceptions especially when young rats were used in studies (e.g., Reddy *et al.*, 1980) or when rats were fed high fat diets (e.g., Vigne *et al.*, 1987). Most studies done on humans have also shown a positive effect of dietary soluble fibers on lowering blood cholesterol level (Hopewell et el., 1993), possibly because human diets normally contain significant amounts of inherent added cholesterol.

The differences between the results on the hypocholesterolemic effect of dietary pectin found with and

without cholesterol supplementation suggest that dietary soluble fibers are more effective in binding with, and excreting, exogenous cholesterol. This contention has been supported by many investigators (Reddy *et al.*, 1980; Illman and Topping 1985; Arjmandi *et al.*, 1992a; Gallaher *et al.*, 1993a), who reported that dietary soluble fibers increased fecal steroids. Turley *et al.*, (1991) indicated that a hydrophilic, gel-forming polymer decreases cholesterol absorption from the intestine and prevented accumulation of cholesterol in the liver as well as in the plasma.

Reddy *et al.* (1980) found that feeding a diet containing 15% pectin increased the level of bile acid in feces (9.61 mg /g dry feces), compared with that found in the control rats (4.92 mg). Blood cholesterol level was increased in guinea pigs by feeding a diet with added cholesterol (0.25%), and the increase was negated by adding 1% pectin to the diet (Fernandez *et al.*, 1990). Similar results were reported by Ebihara and Schneeman (1989) who found decreased levels of cholesterol and triacylglycerol absorption from the small intestine of rats fed a diet containing 5% guar gum, as compared with a control. Turley *et al.* (1991) observed a

similar result with hamsters fed diets containing 0.2% cholesterol and 7.5% psyllium mucilloid, indicating that a hydrophilic, gel-forming polymer decreased cholesterol absorption from the intestine and prevented accumulation of cholesterol in the liver as well as in the plasma.

By contrast, Nishina and Freedland (1990) were not able to demonstrate the cholesterol-lowering effect of pectin when Sprague Dawley rats fed diets containing no cholesterol: 66 mg/100 ㎖ plasma for control, 64 mg for pectin, 52 mg for cellulose, 61 mg for oat bran and 86 mg for wheat bran diet groups. Similarly, Topping *et al.* (1988) reported that the viscosity of methylcellulose (80g/kg, in diet) did not influence plasma or liver cholesterol concentration in rats fed diet without added cholesterol.

The effect of tangerine pulp meal, if any, is considered due to pectin contained in the tangerine pulp meal. Because tangerine pulp meal contains about 30% pectin (Yang, 1985), the amount of pectin in the tangerine pulp meal diet used in the present study is approximately 1.5%. Therefore, if the level of tangerine pulp meal was increased to 15% of the diet, the cholesterol-lowering effect of the tangerine pulp

meal diet would have been equivalent to that of the pectin diet.

An alternative suggestion that the hypocholesterolemic ability of dietary pectin or certain soluble fibers may in part be ascribed to their fermentation products has been supported by the observation that blood serum cholesterol level was decreased by feeding propionic acid (Boila *et al.*, 1981; Thacker *et al.*, 1981; Thacker and Bowlland ., 1981; Chen *et al.*, 1984; Illman *et al.*, 1988) or by infusing propionate solution into the rat cecum (Ebihara *et al.*, 1993). Lactose is not totally digested in the small intestine and enters the large intestine (Kim *et al.*, 1978) where it is fermented by microflora, producing lactic acid and volatile fatty acids (VFA), especially increasing propionate fraction (Kim *et al.*, 1979).

However, other investigators reported that VFA produced by fermentation would not be responsible for the hypocholesterolemic effects of certain water-soluble fibers, because tissue concentrations and rates of cholesterol synthesis were unaffected by dietary propionate in the rat (Illman *et al.*, 1988), and serum cholesterol level was even

increased (15%) when propionate solution was infused into the pig cecum (Beauleu and McBurney, 1992) or a mixture of acetate and propionate (3:1 by mole fraction) into human rectum (Wolever *et al.*, 1991), as compared to that found with saline infusion.

These reports support the hypothesis that propionate or VFA produced in the large intestine from the fermentation of dietary fibers are an indicator but not a specific regulator of cholesterol metabolism as suggested by Illman *et al.* (1993). Some of the non-specific hypocholesterolemic activity of VFA produced in the large intestine may be attributable to their cathartic effect and decreased transit time possibly by the osmotic effect and acidic pH, resulting in decreased absorption of nutrients (Kelsay *et al.*, 1978).

Fecal cholesterol level in rats fed the diet containing pectin was not different from that in the control (Table 5). Similarly, Jonnalagadda *et al.* (1993) reported that the fecal cholesterol level was not influenced by feeding a control diet containing no soluble fibers or diet containing 10% pectin or 12% guar gum in hamsters. These results of the present study and Jonnalagadda *et al.* (1993) make it difficult to

interpret the data on the serum and liver cholesterol levels decreased by the dietary pectin. The main cause of the reduced cholesterol level was assumed to be due to reduced absorption of cholesterol through the intestine. If this were true, the fecal excretion of cholesterol should increase with dietary pectin.

A few possible explanations for this irresponsiveness can be considered : 1) the analytical method of fecal cholesterol is not an appropriate method as a measure of cholesterol absorption, because it does not include a wide variety of cholesterol metabolites including microbial metabolites. In fact, dietary pectin markedly increased fecal excretion of coprostanol, a microbial metabolite of cholesterol in rats, as compared to the control (Reddy *et al.*, 1980; Arjmandi *et al.*, 1992a), although other investigators (Abraham and Mehta, 1988) found an insignificant increase in fecal excretion of steroids and bile acids in men fed psyllium. 2) pectin-bound cholesterol may not be extractable with the organic solvent chloroform. Possible solution to this problem may be the use of labelled cholesterol in the diet to study the absorption rate of cholesterol and its metabolites as a whole. Our second

experiment proves this possibility.

Radioactivity excreted into feces in rats fed with diet containing 10% pectin with ^{14}C-cholesterol was twice the control (Table 6). Leveille and Sauberlich (1966) reported similar result. Rats were given a 2.8 μCi of ^{14}C-cholesterol in 1 g of corn oil through a stomach tube after feeding a diet containing 1% cholesterol and 0 or 5% pectin. The feces were collected for 24 hours and radioactivity in the feces was determined. Rats fed diet containing pectin excreted more than twice the radioactivity found in rats fed no pectin (13.1 vs 6.3% of the administered dose). Ikeda *et al.* (1989) suggested that the gel-forming fibers, such as guar gum and pectin, reduce rapid accessibility of the micelle to intestinal absorptive surface and may decrease the absorption of lipids. In their study, lymph-cannulated rats were given 3 ㎖ test emulsion containing 1 μCi ^{14}C-cholesterol, 25 mg cholesterol, 50 mg dietary fiber (cellulose, guar gum or chitosan) via stomach tube, and the lymph was collected for 24 h. Absorption tended to be low in rats fed chitosan, high in rats fed cellulose and intermediate in the guar gum group. Ikeda *et al.* (1989) suggested various mechanisms by which

dietary fibers interfere with cholesterol absorption. These include 1) the reduction in gastric emptying and intestinal transit times; 2) the absorption of bile salts and, hence, the inhibition of micellar solubility of cholesterol and glyceride digestion products; and 3) the reduction of accessibility of micelles to the surface of intestinal absorptive cells, particularly by viscous fibers.

The present study demonstrated the feasibility and efficacy of pectin or tangerine pulp meal as a cholesterol-lowering agent, possibly through decreased absorption from the intestine. Therefore, a serious consideration should be given to the use of tangerine pulp meal, so that it can be incorporated into regular food items. This will also help alleviate pollution resulting from the tangerine juice manufacture.

Ⅵ. Summary

This study was conducted to determine the effect of dietary pectin or tangerine pulp on cholesterol and triacylglycerol levels in the serum, liver and feces, and cholesterol absorption in rats. Twenty-one adult female Sprague Dawley rats (initial mean weight 219 g) were divided into three groups, and each group was fed with or without pectin or tangerine pulp in the diet. All the diets contained 0.5% added cholesterol and 10% lard. Rats were killed after a 4-week feeding period, serum and liver samples were collected. Feces was collected during the last three days of the feeding experiment. Body weight gain, diet consumption, liver weight, feces weight and large intestinal content were not different among the diet groups. Serum total cholesterol level was significantly lower ($P < 0.05$) in rats fed with the pectin diet than in the control. The cholesterol levels of high density lipoprotein (HDL), low density lipoprotein plus very low density lipoprotein (total amount minus HDL) were not different ($P > 0.05$) among the diet groups. Liver total cholesterol level was also lower

($P < 0.05$) in rats fed with the pectin diet than that in the control, but liver triacylglycerol level was not influenced by the diets. Fecal cholesterol content was not affected by diets.

In other experiment, ten adult Sprague Dawley rats were divided into two groups, and fed with or without 10% pectin diet through stomach tube, both containing 0.2 μCi ^{14}C-cholesterol. Radioactivity of the pectin diet excreted into the feces of rats fed was about twice than that of the control during the first or two days after feeding. This result shows that dietary pectin depressed cholesterol absorption from the gastrointestinal tract.

Our data indicate that dietary pectin decreases serum and liver cholesterol levels, possibly by decreasing absorption of cholesterol and its metabolites from the gatrointestinal tract. The serum and liver cholesterol levels in rats fed with diet containing tangerine pulp meal tended to be lower than those in the control, although the difference was not significant ($P > 0.05$).

Table 1. Composition of three diets fed to rats during the experiment

Ingredient	Control	Tangerine	Pectin
	%		
Casein[a]	20.0	20.0	20.0
Lard[b]	10.0	10.0	10.0
Cholesterol[c]	0.5	0.5	0.5
Sucrose[d]	10.0	10.0	10.0
Corn starch[e]	55.5	50.5	50.5
Tangerine pulp meal[f]	-	5.0	-
Pectin[g]	-	-	5.0
L-Methionine[a]	0.3	0.3	0.3
Choline chloride[a]	0.5	0.5	0.5
Vitamin mix[h]	1.0	1.0	1.0
Mineral mix[h]	3.5	3.5	3.5
Total	100	100	100

[a] United States Biochemical Corp., Cleveland, Ohio.

[b] Samlip Yugi Co., Seoul, Korea.

[c] Fluka Chemie, Switzerland.

[d] Jeil Jedang Co., Seoul, Korea.

[e] Sunil Pododang Co., Seoul, Korea.

[f] Collected from local tangerine juice manufacture, dried and ground.

[g] Sigma Chemical Co., St. Louis, Mo.

[h] AIN-76A, Harlan, Madison, WI.

Table 2. Body weight, diet consumption, liver weight, large intestine content weight and fecal weight in rats fed control or diets containing tangerine (5%) and pectin (5%)†

Item	Control	Tangerine	Pectin
Body weight, g			
Initial	220 ± 4	223 ± 8	215 ± 5
Final	237 ± 7	234 ± 15	217 ± 5
Diet consumption, g	335 ± 19	342 ± 29	314 ± 17
Liver weight, g			
g fresh liver	7.0 ± 0.5	6.3 ± 0.6	6.1 ± 0.7
g/100g body weight	2.9 ± 0.2	2.7 ± 0.2	2.9 ± 0.2
Large intestinal content, g	1.4 ± 0.1	1.9 ± 0.3	2.1 ± 0.2
Fecal weight, g ‡	1.1 ± 0.1	1.2 ± 0.2	1.1 ± 0.2

† Values are mean ± SE of 7 rats.

‡ D M base.

Table 3. Effect of feeding diets containing tangerine pulp meal (5%) and pectin (5%) on the serum cholesterol or triacylglycerol content in rats†

Item	Control	Tangerine	Pectin
	mg/100 ㎖		
Total cholesterol	119.2 ± 9.1[a]††	93.8 ± 12.0[ab]	81.7 ± 5.4[bc]
HDL[1] cholesterol	40.1 ± 4.2	33.7 ± 3.6	36.7 ± 4.1
LDL+VLDL[2] cholesterol	79.1 ± 11.9	60.1 ± 11.2	45.0 ± 6.7
Triacylglycerol	91.0 ± 18.4	75.6 ± 15.0	82.8 ± 7.4
HDL /total cholesterol	0.36 ± 0.13	0.39 ± 0.13	0.46 ± 0.14

† Values are mean ± SE of 7 rats.

†† Values in the same row not sharing the same superscripts differ significantly ($P < 0.05$).

1. HDL=high density lipoprotein.

2. LDL+VLDL=low density lipoprotein + very low density lipoprotein.

Table 4. Effect of feeding diets containing tangerine pulp meal (5%) and pectin (5%) on the liver cholesterol or triacylglycerol level in rats†

Item	Control	Tangerine	Pectin
	mg/g fresh liver		
Total Cholesterol	14.2 ± 1.2[a]‡	12.6 ± 1.6[ab]	7.9 ± 1.0[bc]
Triacylglycerol	24.7 ± 5.1	17.6 ± 4.4	14.9 ± 2.7

† Values are mean ± SE of 7 rats.

‡ Values in the same row not sharing the same superscripts differ significantly ($P < 0.01$).

Table 5. Effect of feeding diet containig tangerine pulp meal (5%) and pectin (5%) on the fecal cholesterol and triacylglycerol levels in rats†

Item	Control	Tangerine	Pectin
Cholesterol excreted			
(mg/day)	31.8 ± 3.7	27.0 ± 4.4	25.8 ± 3.4
(mg/g DM)	91.6 ± 12.1	69.0 ± 6.0	72.4 ± 8.5
Triacylglycerol excreted			
(mg/day)	1.4 ± 0.2[a]‡	0.9 ± 0.1[b]	1.0 ± 0.1[b]
(mg/g DM)	4.0 ± 0.2[a]¶	2.3 ± 0.2[b]	3.0 ± 0.4[b]

† Values are mean ± SE of 7 rats.

‡ Values in the same row not sharing the same superscripts differ significantly ($P < 0.05$).

¶ Values in the same row not sharing the same superscripts differ significantly ($P < 0.01$).

Table 6. Effect of feeding diet containing pectin (10%) on radioactivity excreted into feces during one, two, three and four days after gavage feeding of a emulsion diet labelled with ^{14}C-cholesterol in rats†

Diet	Period after feeding (days) 1	2	3	4
	% of the infused dose			
Control	3.08 ± 2.1	9.84 ± 2.8	19.20 ± 4.6	25.62 ± 3.9
Pectin	5.43 ± 3.0	16.50 ± 6.3	21.60 ± 6.4	26.76 ± 4.3

† Values are mean ± SE of 5 rats.

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