Potato Pulps Lowered the Serum Cholesterol and Triglyceride Levels in Rats

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Summary In our previous study, we demonstrated that retrograded starch, a kind of resistant starch, of beans reduced serum lipid levels in rats. In this study, we examined whether retrograded starch in potato pulps could reduce serum lipid concentrations. Rats were given diets containing 15 g of retrograded starch in potato pulps from the Benimaru potato (BM) or Hokkaikogane potato (HK) in a 100 g diet for 4 wk. At the 4th week, the total cholesterol level in the serum in the BM group and serum triglyceride (TG) level in the HK group were significantly lower than those in the control group. In the BM group, the contents of fecal bile acids were significantly higher than those in the control group. On the other hand, in the HK group, the hepatic mRNA level of fatty acid synthase (FAS) was significantly lower than that in the control group. The FAS mRNA level correlated with the mRNA level of sterol regulatory element-binding protein-1c (SREBP-1c), a regulator of expression of FAS, positively. These results suggested that BM pulp promoted the excretion of bile acids, which resulted in a low concentration of serum cholesterol. On the other hand, HK pulp inhibited the synthesis of fatty acids at the mRNA levels of FAS and SREBP-1c, which might lead to a reduction of the serum TG level.
Key Words cholesterol, triglyceride, potato pulps, liver

Excess nutrient status is involved in many diseases. For instance, hypercholesterolemia is known as a risk factor for arteriosclerosis (*1, 2*) and hypertriglyceridemia may be involved in diabetes mellitus (*3, 4*). Some foods, including dietary fibers, are candidates for pre-

Abbreviations: BM, Benimaru; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; FAS, fatty acid synthase; GAPDH, glycelaldehyde-3-phosphate dehydrogenase; HDL, high density lipoprotein; HK, Hokkaikogane; IDL, intermediate density lipoprotein; IDPS, indigestible polysaccharide; LCA, lithocholic acid; LDL, low density lipoprotein; RS, resistant starch; SCFA, short chain fatty acid; SREBP-1c, sterol regulatory element-binding protein-1c; TG, triglyceride; VLDL, very low density lipoprotein.

vention of these abnormalities (*5–7*). Retrograde starch is a kind of resistant starch that has resistance against amylase, and may act like dietary fiber in the digestive tract. Actually, we have previously reported that resistant starches of beans reduce serum cholesterol and triglyceride (TG) levels in rats (*8–11*). It is suggested that the cholesterol-lowering function by the resistant starches of beans was due to cecal fermentation products such as short chain fatty acid (SCFA) and increased fecal steroid excretion (*8–10*). Illman et al. (*12*) also reported that the SCFA content in the cecum was positively correlated with the cecal neutral steroids and bile acids. Furthermore, a putative role of SCFA in lowering cholesterol by fibers has been reported (*13*), and is probably related to inhibition of metabolism of the major lipogenic precursors such as acetate and lactate (*14, 15*). It has been also reported that some kinds of dietary fiber inhibited formation of micelle or emulsion of bile, which may be resistant to absorption of lipids in part (*16–18*).

Potatoes are a major source of starch. Potato pulp is a by-product of purifying the starch and is treated as industrial waste. The pulp contains much starch, and may be available as a source of it. Potato starches are

naturally highly phosphorylated (19), which possibly contributes to avoiding attack by digestive enzymes (20). Thus, the starch in the potato pulps may be a good source of resistant starch (RS) although it is not certain whether the resistant starches in potato pulps are useful for reduction of serum lipid levels. In this study, we investigated the effects of the retrograded starch in potato pulps on the metabolism of lipids.

MATERIALS AND METHODS

Preparation of potato pulp. Potato pulps derived from the residuum of starch production from Benimaru (BM) and Hokkaikogane (HK) potatoes were obtained from Jinno Potato Starch Factory Co., Ltd. (Sarabetsu, Japan). Primary potato pulps contained some dietary fiber and starch. The retrogradation of starch in potato pulps was carried out as reported previously (8, 9). Briefly, the potato pulps were boiled for 2 h, left at room temperature, and then freeze-dried. In this study, "potato pulp" is used to mean the residuum of potato composed of dietary fiber, resistant starch and digestive starch. Indigestive matter in the potato pulps, including dietary fiber and resistant starch, was analyzed by a modification of the Prosky method (21), and the protein, lipid, carbohydrate, moisture, and ash contents in the potato pulps were determined by the Association of Official Analytical Chemists (AOAC) procedure (22). The compositions of BM pulp and HK pulp were as follows (g/100 g powder): moisture, 14.0 and 2.4; protein (calculated by multiplying nitrogen contents by 6.25), 4.0 and 4.4; lipid, 2.0 and 0.9; ash, 1.6 and 1.5; fiber (including RS), 47.0 (insoluble fiber, 37.6; soluble fiber, 9.4) and 37.5 (insoluble fiber, 28.3; soluble fiber, 9.2); carbohydrate, 78.4 and 90.8, respectively. The phosphorus contents in the BM and HK pulps and corn starch were 580, 937 and 137 μg/g, respectively.

Animals and experimental design. This experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine. Male Crj:Wistar rats (5 wk old) were purchased from Charles River Japan, Inc. (Yokohama, Japan) and used in this study. Rats were housed individually under conditions of $23 \pm 1°C$, $60 \pm 5\%$ humidity, and a 12:12-h light:dark cycle, and were allowed access to diets and water freely. After acclimation for 7 d, rats were divided into 3 groups ($n=5$) without a difference of body weight. During the experimental period of 28 d, rats were given the diets shown in Table 1, with a control based on the AIN-93G composition. Potato pulps of BM and HK were replaced by α-corn starch in the control diet. Body weight and food intake were recorded weekly. Each week, blood was collected from the jugular vein of an overnight-fasted rat without anesthesia. Serum was obtained from the blood by centrifugation at 1,300 $\times g$ for 20 min and was used for analyses of the cholesterol and triglyceride (TG) contents. Feces were collected in the final 3 d of the experiment. On the 28th day, the rats were killed under pentobarbital anesthesia, and the liver and cecum were quickly removed, weighed, frozen and stored at $-80°C$ until analysis.

Table 1. Compositions of the experimental diets.[1]

	Control	BM (g/kg diet)	HK
Casein	200	200	200
Sucrose	100	100	100
α-Corn starch	529.5	379.5	379.5
BM pulp[2]	—	150	—
HK pulp[2]	—	—	150
Soybean oil	70	70	70
Cellulose	50	50	50
Mineral mix[1]	35	35	35
Vitamin mix[1]	10	10	10
L-Cystine	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5
TBHQ[3]	0.014	0.014	0.014

[1] These diets were based on the AIN-93G diet composition.
[2] BM: Benimaru; HK: Hokkaikogane. Potato pulps were retrograded.
[3] *tert*-Butyl hydroquinone.

Lipid analyses. Total cholesterol, high density lipoprotein (HDL)-cholesterol and TG concentrations in the serum were evaluated with commercially available kits for the TDX system (Abbott Laboratory Co., Irving, TX, USA). The sum of very low, low and intermediate density lipoprotein (VLDL, LDL and IDL, respectively) cholesterol contents was calculated by subtraction of the HDL-cholesterol content from the total cholesterol content.

In the liver and feces, neutral sterols were extracted with chloroform:methanol=2:1 (v/v), acetylated and analyzed by gas liquid chromatography (GLC) using a Shimadzu 14A chromatograph (Kyoto, Japan) with a DB17 capillary column (0.25 mm$\times$30 m, J and W Scientific, Folsom, CA, USA). Fecal bile acids were measured by the methods of Grundy et al. (23). A part of the cecum was infused out into deionized water and suspended. The suspension of cecal contents was deproteinized with ice-cooled perchloric acid (final concentration 50 g/L), and the resulting supernatant was neutralized with NaOH so as to precipitate perchloric acid and change SCFA into sodium salts. Individual SCFA were measured by GLC using a Shimadzu 14A chromatograph with a ZEBRON ZB FFAP capillary column (0.53 mm$\times$30 m, Phenomenex, CA, USA) with H_3PO_4 (100 mL/L) as the liquid phase.

RNA isolation, reverse transcriptase-polymerase chain reaction (RT-PCR) and Southern blot analysis. As described previously (8, 10, 11), total RNA in the liver was isolated using Isogen (Nippongene, Tokyo, Japan) according to the user's manual. The amounts of mRNA encoding fatty acid synthase (FAS), sterol regulatory element-binding protein-1c (SREBP-1c) and glycelaldehyde-3-phosphate dehydrogenase (GAPDH, as an internal standard) were estimated by semi-quantitative RT-PCR and subsequent Southern blot analysis (8–10). The sets of oligonucleotide primers used for the PCR of

Table 2. Body weight gain, food intake, liver weight and feces excretion of the rats fed the control, Benimaru (BM) or Hok-kaikogane (HK) diet for 4 wk.[1]

	Initial body wt. (g)	Body wt. gain (g)	Food intake (g/4 wk)	liver wt. (g/100 g b.w.)	Cecum wt. (g/100 g b.w.)	Feces wt. (wet g/d)
Control	183±4	181±14	726±43	4.0±0.2	1.4±0.2	2.3±0.5
BM	182±8	182±22	756±17	4.3±0.3	1.6±0.3	3.3±1.1
HK	183±9	189±15	778±64	4.0±0.2	1.7±0.4	3.2±0.9

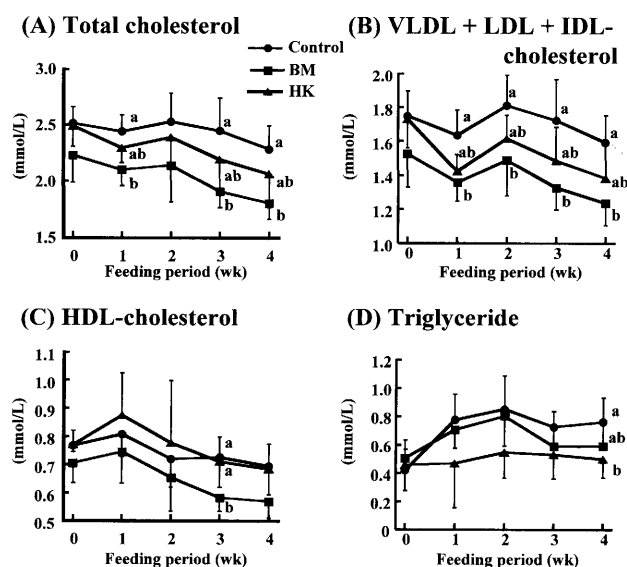[1] Values are means±SD of 5 rats in each group.

Fig. 1. Changes in the total (A), VLDL+IDL+LDL (B) and HDL (C) cholesterol, and triglyceride (D) concentrations in the rats fed the control, Benimaru (BM) or Hokkaikogane (HK) diet for 4 wk. Values are means±SD of 5 rats in each group. Values not sharing a common letter within the same time point significantly differ ($p < 0.05$).

Table 3. Changes in neutral sterols in the liver and feces in the rats fed the control, Benimaru (BM) or Hokkaikogane (HK) diet for 4 wk.[1]

	Liver cholesterol (μmol/g)	Feces	
		Cholesterol (μmol/dry g)	Coprostanol (μmol/dry g)
Control	5.44±1.73	4.3±1.31	3.76±0.99
BM	5.65±0.68	5.0±1.15	2.67±1.11
HK	6.22±1.92	4.4±1.07	3.60±1.25

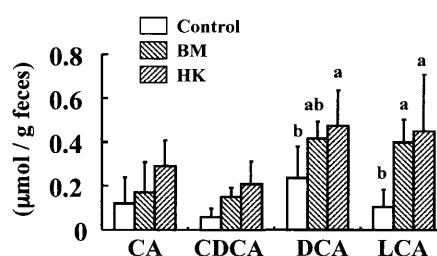[1] Values are means±SD of 5 rats in each group.

Fig. 2. Concentrations of the bile acids, cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and lithocholic acid (LCA), in the feces of the last 3 d of the experiment. Rats were fed the control, Benimaru (BM) or Hokkaikogane (HK) diet for 4 wk. Values are means±SD of 5 rats in each group. Values not sharing a common letter within columns of the same bile acids significantly differ ($p < 0.05$).

GAPDH were as reported previously (8), and those of the FAS and SREBP-1c were: sense primer of FAS, 5′-GCTGGAGCCCCTTTTTGTCTT-3′; anti-sense primer of FAS, 5′-ACCCCAGCACTGCAGTTTTCT-3′; sense primer of SREBP-1c, 5′-GAGCCACAATGAAGACCGCA-3′; anti-sense primer of SREBP-1c, 5′-CAAGGACAAGGGGC-TACTCT-3′. The probe for GAPDH was reported previously (8); that for FAS was 5′-CTGCTCTCTGTGGATAG-GACTGAATGCTGTGGCCTTCTGATAGACTCTTCTGGA-3′ and that for SREBP-1c was 5′-GCCGGCGTCTGAGG-GTGGAGGGGTCAGCGTTTCTACCACTTCAGGTTTCATG CC-3′. Probes with digoxigenin (DIG) were hybridized to blots. DIG was detected using an anti-DIG antibody (Boehringer Mannheim, Mannheim, Germany). The values of FAS and SREBP-1c mRNAs were normalized to the value of GAPDH mRNA. These values were expressed relative to the average values of the control group as 1.

Statistical analysis. Data are expressed means ± SD. For comparison between groups, the Turkey-Kramer test (StatView, SAS Institute, Cary, NC, USA) was carried out. $p < 0.05$ was regarded as significant.

RESULTS

Body weight, food intake and liver weight

There were no differences among the dietary groups in the body weight gain and the total food intake for 4 wk, liver weight or cecum weight (Table 2).

Serum lipids

Changes in serum lipid levels are shown in Fig. 1. The concentrations of total and VLDL+IDL+LDL-cholesterol in the BM group were significantly lower than those in the control group throughout the feeding period except at week 2. At week 4, their values in the BM group reached 81% of those at week 0, whereas those in the control group were at 91%. The HDL cholesterol level in the BM group was also lower than in the control group at week 3, but there was no difference among the groups at week 4. In the HK group, these

indicators tended to be lower than in the control group but not significantly. On the other hand, serum TG levels in the HK group tended to be lower than those in the control from week 1 to week 3, and were significantly lower than in the control group at week 4, and those in the BM group tended to be low compared with those in the control group throughout the experiment.

Neutral sterols and bile acids in the liver and feces

The concentrations of neutral sterols in the liver and feces did not differ among groups (Table 3). Fecal deoxycholic acid (DCA) and lithocholic acid (LCA) concentrations in the HK group were significantly higher than those in the control group (Fig. 2). In the BM group, fecal LCA concentration was significantly higher and DCA concentration tended to be higher than those in the control group. Fecal CA and CDCA contents were

not different among groups. Total contents of the 4 bile acids in the feces in the BM and HK groups were significantly higher than in the control group ((in μmol/g feces) 0.52±0.33, 1.13±0.22 and 1.42±0.45 for the control, BM and HK groups, respectively).

Hepatic mRNA

The relative quantities of mRNAs of PCR-amplified FAS cDNA, and SREBP-1c cDNA in the rat liver are shown in Fig. 3. The mRNA level of FAS was significantly lower in the HK group and tended to be lower in the BM group than in the control group (Fig. 3), but those of SREBP-1c did not differ among the groups. There was a positive correlation between the mRNA levels of FAS and SREBP-1c ($r=0.630$, $p<0.05$).

SCFA concentrations in the cecum

As shown in Table 4, there were no differences of total SCFA or acetate among the groups. Butyrate concentration in the cecum in the HK group was significantly higher and that in the BM group tended to be higher than in the control group. The cecal butyrate level had a negative correlation to the serum TG level of week 4 ($r=-0.540$, $p<0.05$). In contrast, propionate content was lower in the BM and tended to be lower in the HK group than in the control group.

DISCUSSION

In the present study, we found that feeding rats BM and HK potato pulps reduced serum cholesterol and TG levels, respectively. The serum cholesterol level was significantly lower in the BM group and tended to be lower in the HK group than in the control group at week 1, 3 and 4. The levels of total and VLDL+IDL+LDL-cholesterol in the serum tended to fall gradually in all groups. At week 4, these values in the BM group were 81% of the initial values; whereas those in the control group were 91%. This difference might have contributed to the reduction of serum total cholesterol in the BM group. Fecal concentrations of primary and secondary bile acids in the HK group and those of secondary bile acids in the BM group were significantly higher than in the control group in the last 3 d of the experiment. These data indicated that the lowering of the serum cholesterol levels in the BM group might have been due to acceleration of bile acid excretion. Potato starches are known to be phosphorylated more than other starches of cereals (24), and it is also shown that phosphates in starch, especially in C-3 position of the glu-

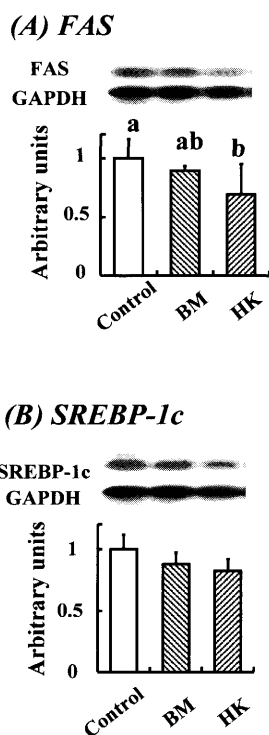### (A) FAS

(B) SREBP-1c

Fig. 3.   Hepatic mRNA levels of FAS (A) and SREBP-1c (B) and the representative blots for the rats fed the control, Benimaru (BM) or Hokkaikogane (HK) diet for 4 wk. Values are means±SD of 5 rats in each group. Values not sharing a common letter significantly differ ($p<0.05$).

Table 4.   Short chain fatty acid (SCFA) concentrations and values of pH in the cecal contents of the rats fed the control, Benimaru (BM) or Hokkaikogane (HK) diet for 4 wk.[1]

	Total[2]	Acetate	Propionate	Butyrate	pH
	(μmol/g content)				
Control	3.12±0.75	2.05±0.42	0.629±0.182[a]	0.441±0.238[b]	7.65±0.36[a]
BM	3.91±0.42	2.84±0.35	0.362±0.026[b]	0.711±0.102[ab]	7.20±0.24[ab]
HK	4.65±1.38	3.34±1.22	0.447±0.123[ab]	0.868±0.198[a]	7.01±0.20[b]

[1] Values are means±SD of 5 rats in each group. Values not sharing a common superscript significantly differ.
[2] Total=Acetate+Propionate+Butyrate.

cose structure, were resistant to digestion by α-amylase (20). It has been reported that some indigestive polysaccharides (IDPS) promote excretion of bile acids into the feces (25–27). These data may support the idea that more IDPS exists in BM and HK pulps than in the α-corn starch in the control diet, and might be involved in the excretion of the bile acids. It is suggested that suppression of micellar solubility of cholesterol or micelle formation lead to a decrease in serum cholesterol by some elements of foods such as soy protein peptic hydrolysate (17) and pectin (18). Interaction between the indigestive fraction derived from potato pulps and micelle formation is possibly involved in excretion of the bile acid. Additionally, it has been reported that ingestion of SCFA lowers plasma cholesterol in rats without any influence on fermentation (28). Some SCFA in the cecum was possibly associated with lowering the serum cholesterol content. It is known that the bile acid level in the body is maintained by degradation of cholesterol (29, 30), as bile acids are necessary for absorption of fat-soluble substances. In the BM group, more total bile acid was excreted into the feces than the control group, which may promote the recruitment of bile acids. Cholesterol in the BM group was possibly converted into bile acids for the compensation of plasma cholesterol, unlike in the control group. Concerning the reduction of the serum cholesterol level, however, some explanations for the BM group did not necessarily match the results in the HK group. To determine this mechanism, further studies on the relations between cholesterol metabolism and resistant starches should be done.

On the other hand, HK pulp might reduce the serum TG concentration, although no significant influence on the serum cholesterol level was observed. As shown in Fig. 3, the mRNA level of FAS was lower in the HK group than in the control group. Fatty acid synthesis in the HK group may have been lower in the liver than that in the control group. Fatty acids are the major components of TG. In the HK group, the serum level of TG may reflect the reduction of fatty acid synthesis. The mRNA level of SREBP-1c was not statistically different among the groups, but had a positive correlation between the mRNA level of FAS ($r=0.630$, $p<0.05$). It is known that the expression of FAS is promoted by activation of SREBP-1c (31, 32). In this study, the diet containing HK pulp inhibited the expression of SREBP-1c, which might have influenced the FAS mRNA level in the HK group. Since the reason for the low expression of SREBP-1c in the HK group was unclear in the present experiment, we hypothesized the following explanation. Kim et al. (33) reported that SREBP-1c mRNA expression decreased in a diet containing a low carbohydrate level. Additionally, the absence of glucose in a medium might inhibit the expression of SREBP-1c in vitro (34). That is, expression of SREBP-1c may be regulated by intake of carbohydrate. It is reported that phosphate contents in the starch of potatoes are higher than those in other cereals (24), and phosphorylated starch is resistant to digestion by α-amylase (20). Much phosphorylation of HK pulp may lead to less carbohydrate

absorption in the body than with the control diet. This carbohydrate deficiency possibly caused low expression of SREBP-1c in the HK group. The butyrate concentration had a negative correlation to the serum TG content at week 4 ($r=-0.540$, $p<0.05$). It has been reported that butyrate inhibits the permeability of TG through a layer of Caco 2 cells (35). It is thus possible that the butyrate content in the cecum was also involved in the reduction of the serum TG content in the HK group, partly by suppressing absorption of fat in this study. Moreover, it is reported that some dietary fibers affected lipase activities by the change of emulsion stability in vivo and in vitro (16, 18). This effect of dietary fibers is possibly associated with absorption of TG in this study.

However, the reasons why the cholesterol level in the HK group and the TG level in the BM group were not lowered remained unclear in this study. It is possible that the difference of these results involves a difference of phosphate contents or/and oligosaccharide concentrations avoiding attack by digestive enzymes in BM and HK pulps. Kok et al. (36) have reported that long-term feeding of oligofructose protected rats against liver TG accumulation induced by fructose, and the lower lipogenic capacity of the liver could be the key event in this protection, since even after the fructose load, FAS activity remained significantly lower in oligofructose-fed rats. It has also been reported that dietary sucrose promotes increases in hepatic concentrations of total lipids, TG and cholesterol when compared to a starch-based diet (37).

In conclusion, the BM pulp lowered the serum cholesterol level; while the HK pulp reduced the serum TG content. These results suggest that retrograded starches in potato pulps are useful for reduction of serum lipid levels.

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