

## Sugar beet pulp and apple pomace dietary fibers improve lipid metabolism in rats fed cholesterol

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### Abstract

The effect of diets supplemented with sugar beet pulp fiber (SBP, 10%) and apple pomace fiber (AP, 10%) on lipids and lipid peroxides was investigated in 60 male Wistar rats. The rats were divided into six groups of 10 and adapted to cholesterol-free or 0.3% cholesterol diets. The basal diet (BD) contained wheat meal, barley meal, wheat hulls, meat-bone meal, barley sprouts, skimmed milk, fodder yeast, mineral and vitamin mixtures. The Control group (Control) consumed BD only. To the BD were added 3 g/kg cholesterol (Chol), 100 g/kg dry sugar beet pulp fiber (SBP), both 100 g/kg sugar beet pulp fiber and 3 g/kg cholesterol (SBP+Chol), 100 g/kg apple pomace fiber (AP), both 100 g/kg apple pomace fiber and 3 g/kg cholesterol (AP+Chol). The experiment lasted 40 days. Plasma total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), total phospholipids (TPH), HDL phospholipids (HDL-PH), lipid peroxides (LP) and liver TC concentration were measured. Groups did not differ before the experiment. In the Chol+SBP and the Chol+AP vs. Chol group the sugar beet pulp and apple pomace dietary fiber supplemented diet significantly ( $P < 0.05$ ) hindered the rise of plasma lipids: (a) TC –2.97 vs. 3.69 mmol/l, –20% and 3.01 vs. 3.69 mmol/l, –18.4%, respectively; (b) LDL-C –1.36 vs. 2.02 mmol/l, –32.6% and 1.39 vs. 2.02 mmol/l, –31.2%, respectively; (c) TG –0.73 vs. 0.88 mmol/l, and 0.75 vs. 0.88 mmol/l; –17 and –14.8%, respectively, and TC in liver (17.1 vs. 24.3  $\mu$ mol/g, –29.6% and 17.9 vs. 24.3  $\mu$ mol/g, –26.3%, respectively). Sugar beet and apple pomace fiber-supplemented diets significantly hindered the decrease in HDL-PH (0.79 vs. 0.63 mmol/l, –25.3%,  $P < 0.025$  and 0.75 vs. 0.63 mmol/l, –19%,  $P < 0.05$ , respectively) and decreased the level of TPH (1.34 vs. 1.74 mmol/l, –23%,  $P < 0.005$  and 1.37 vs. 1.74 mmol/l, –21.3%,  $P < 0.01$ , respectively). Both sugar beet pulp fiber and apple pomace fiber, in rats fed the basal diet without cholesterol, did not significantly affect the variables measured. Neither sugar beet pulp fiber or apple pomace fiber-supplemented diets influenced the level of lipid peroxides. These results demonstrate that sugar beet pulp fiber and to a lesser degree apple pomace fiber possess hypolipidemic properties. This is more evident when sugar beet pulp fiber or apple pomace fiber are added to the diet of rats fed cholesterol. The hypolipidemic effects of both sugar beet pulp fiber and apple pomace fiber can be attributed to their water-soluble parts. The sugar beet pulp and apple pomace fibers have no antioxidant properties. © 2000 Elsevier Science Ltd. All rights reserved.

### 1. Introduction

Dietary fiber has been recognized as an important dietary constituent, which possesses a wide range of positive properties (Eastwood & Kay, 1979; Rimm,

Ascherio, Giovannucci, Spiegelman, Stampfer & Willett, 1996; Shinnick, Mathews & Ink, 1991; Zarling, Edison, Berger, Leya & DeMeo, 1994). Some authors have demonstrated that components of dietary fiber, particularly those soluble in water, might influence lipid metabolism (Eastwood & Kay, 1979; Gorinstein, Bartnikowska, Kulasek, Zemser & Trakhtenberg, 1998; Gorinstein, Kulasek et al., 1998; Shinnick et al.). And as consequence, Rimm et al. found an inverse association between fiber intake and myocardial infarction. Although fiber has been recognized as an important

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dietary constituent, controversy and confusion still exist about its physiological effects. Specifically, the independent ability of dietary fiber to lower serum lipid levels is controversial (Glore, Van Treeck, Knehens & Guild, 1994). Only some researchers support the suggestion that dietary fiber also exerts an antioxidant effect (Larrauri, Goni, Martin-Carron, Ruperez & Saura-Calixto, 1996; Lin, 1994). The above-mentioned controversial points demand further clarification. The objective of this study was to clarify the possible influence of different sources and different quantities of dietary fibers on plasma lipids and lipid peroxides in rats fed cholesterol-containing and cholesterol-free diets. In previous experiments, we used semipurified basal diet (Gorinstein, Bartnikowska et al., 1998; Gorinstein, Kulasek et al., 1998). Low quantities of dietary fiber were used by some investigators who deny this independent ability of dietary fiber to lower serum lipid levels (Bobek, Ozdin & Hromadova, 1998). Therefore it was decided to feed the rats of the experimental groups BD enriched with higher quantity of dietary fiber from two different sources. Investigators who deny the independent ability of dietary fiber to lower plasma lipid levels have only used dietary fiber of apple pomace (Bobek et al.). Therefore, dietary fibers of both apple pomace and sugar beet pulp were chosen (Bobek, et al.; Guillon, Auffret, Robertson, Thibault & Barry, 1998; Langkilde, Andersson & Bosaeus, 1993).

## 2. Materials and methods

### 2.1. Rats and diets

The Animal Care Committee of Warsaw Agricultural University approved this study. The Institute of Animal Physiology and Nutrition of Polish Academy of Sciences (Jablonna, Poland) provided 6 month-old male Wistar rats ( $n=60$ ) with a mean weight of 120 g. They were housed in stainless steel metabolic cages and were divided into six groups of 10. During the first 5 days of the adaptation period to the new condition in the animal house, all six groups were fed BD, received from Quarto factory (Warsaw-Kabaty, Poland). After 5 days of the adaptation period, the rats of the Control group (Control) were fed BD and 10% of cellulose. To the BD were added 10% of cellulose and 0.3% nonoxidized cholesterol, 10% of apple pomace fiber, 10% apple pomace fiber and 0.3% cholesterol, 10% of dry sugar beet pulp fiber and 10% sugar beet pulp fiber and 0.3% cholesterol for the cholesterol group (Chol), apple pomace fiber group (AP), cholesterol/apple pomace fiber group (Chol+AP), sugar beet pulp group (SBP) and cholesterol/sugar beet pulp group (Chol+SBP), respectively (Arjmandi, Ahm, Nathami & Reeves, 1992; Bobek et al., 1998). Dietary fiber was determined

according to Prosky, Asp, Schweizer, De Vries and Furda (1992). Total, soluble and insoluble dietary fibers were (in g/kg of dry matter): 113.04, 18.0, 99.05; 117.04, 18.0, 95.05; 164.19, 30.0, 134.14; 164.19, 30.0, 134.14; 189.5, 39.2, 150.27 and 189.5, 39.2, 150.27 for Control, Chol, AP, Chol+AP, SBP and Chol+SBP diet groups, respectively. The sugar beet pulp fiber was supplied by the sugar factory Lapy (Poland) and the apple pomace fiber by fruit-vegetable plant Dwikozy (Poland). The sugar beet pulp and the apple pomace fiber were mixed with the BD before the rats were fed. Cholesterol of analytical grade (USP) was obtained from Sigma Chemical, St Louis, MO. The dietary cholesterol was checked according to the HPLC method of Ansari, Smith and Smart (1979) and was found not to contain cholesterol oxides. The cholesterol batches were mixed carefully with the BD of the Chol, Chol+AP and Chol+SBP diet groups (0.3:99.7) immediately before the diet was offered to the rats. The diets contained, as percentage of energy, 66% of carbohydrates, 25% of protein and 9% of fat. The calculated energy values of all diets were not significantly different. The exact compositions of the diets are presented in Table 1.

All rats consumed food ad libitum, once a day, beginning at 1000 h. All rats had unrestricted access to drinking water. The food intake was monitored daily and body weight gains every 10 days. Before the experiment, the blood samples were taken from the tail vein. At the end of the experiment the rats were anaesthetized using diethyl ether and the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests. After anesthesia, the abdomen was opened to take samples of the liver for determination of TC. The experiment lasted 40 days. Two time points were used in the experiment: before and after 40 days of feeding. At these time points a wide range of laboratory tests were performed. Total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), total phospholipids (TPH), HDL-phospholipids (HDL-PH) and triglycerides (TG) were determined enzymatically. TC and TG were measured as described by Trinder and Webster (1984) with kits (PAP 100, #6.122.4 and 6.123.6, respectively); TPH according to a combined enzymatic method using phospholipase D, choline oxidase and peroxidase (Takayama, Itoh, Nagasaki & Tanimizu, 1977) with a kit (#6.149.1) from Bio Merieux (Marcy l'Etoile, France). HDL-C and HDL-PH were determined by the same enzymatic methods after the precipitation of LDL-C and VLDL-cholesterol (VLDL-C) fractions with phosphotungstic acid in the presence of magnesium ions with kit (#6.159.1) from Bio Merieux (Marcy l'Etoile, France). Lipid peroxide (LP) was determined colorimetrically (Tateishi, Yoshima & Kuzuya, 1987) in direct reaction between the methylene blue derivative

Table 1  
Composition of the diets

Ingredients	Control	Chol	AP	Chol + AP	SBP	Chol + SBP
<i>(g/kg diet)</i>						
Wheat meal	230	227	230	227	230	227
Wheat hulls	140	140	140	140	140	140
Barley meal	180	180	180	180	180	180
Meat-bone meal	90	90	90	90	90	90
Barley sprouts	70	70	70	70	70	70
Skimmed milk	65	65	65	65	65	65
Fodder yeast	80	80	80	80	80	80
Limestone	5	5	5	5	5	5
Cholesterol <sup>a</sup>	–	3	–	3	–	3
Apple pomace fiber <sup>b</sup>			100	100		
Sugar beet pulp fiber <sup>c</sup>					100	100
Cellulose	100	100				
Vitamin mixture <sup>d</sup>	5	5	5	5	5	5
Mineral mixture <sup>e</sup>	35	35	35	35	35	35
Soluble fiber <sup>f</sup>	17.99	17.99	39.23	39.23	30.05	30.05

<sup>a</sup> Sigma Chemical (St. Louis, MO).

<sup>b</sup> Phenol free apple pomace fiber was prepared and supplied by Quarto factory (Warsaw-Kabaty, Poland).

<sup>c</sup> Phenol free sugar beet pulp fiber was prepared and supplied by Quarto factory (Warsaw-Kabaty, Poland).

<sup>d</sup> Vitamin mixture: thiamin, riboflavin, pyridoxin, nicotinamide, calcium pantothenate, folic acid, biotin, cyanocobalamin, retinyl palmitate, DL- $\alpha$ -tocopherol acetate, cholecalciferol, menadione, ascorbinic acid and myo-inositol.

<sup>e</sup> Mineral mixture: CaHPO<sub>4</sub>, K<sub>2</sub>HPO<sub>4</sub>, KCl, NaCl, MgCl<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, MnSO<sub>4</sub>, CuSO<sub>4</sub> · 7H<sub>2</sub>O, ZnSO<sub>4</sub> · 7H<sub>2</sub>O, KIO<sub>3</sub>.

<sup>f</sup> In g/100 g of dry matter.

(MCDP, 0-*N*-methyscarbonyl-3,7-dimethylamino-10H-phenothiazine), catalyzed by hemoglobin, using kit (9#CC-004) from Kamiya Biomedical Company. LDL-C was calculated according to the Friedewald formula (Friedewald, Levy & Fredrickson, 1972). TC in liver was analyzed according to Mazur, Remesy, Gueux, Levrat and Demigne (1990).

## 2.2. Statistical analysis

Values are given as means  $\pm$ SD: where appropriate, data were tested by two-way ANOVA using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA), followed by Duncan's new multiple range test to assess differences between group means. Differences of  $P < 0.05$  were considered significant.

## 3. Results

The food intake, body weight gain and feed efficiency were 379.9 $\pm$ 33.8, 91.9 $\pm$ 9.1, 0.24 $\pm$ 0.02; 381.6 $\pm$ 34.1, 92.0 $\pm$ 8.9, 0.24 $\pm$ 0.02; 380.6 $\pm$ 34.2, 92.1 $\pm$ 8.9, 0.24 $\pm$ 0.02; 380.9 $\pm$ 34.0, 91.9 $\pm$ 8.9, 0.24 $\pm$ 0.02; 381.3 $\pm$ 34.1, 91.8 $\pm$ 8.9, 0.24 $\pm$ 0.02; 381.7 $\pm$ 34.1, 92.0 $\pm$ 8.9, 0.24 $\pm$ 0.02 for Control, Chol, AP, Chol + AP, SBP and Chol + SBP diet groups, respectively. ANOVA calculations show that addition of sugar beet pulp, apple pomace fiber or cholesterol to the diets did not affect food intake, body weight gain or feed efficiencies.

At baseline, the six diet groups did not differ from one another in plasma lipid concentration and lipid

peroxides (data not shown). The results of the examination of all studied indices after the experiment are shown in Table 2. According to this table, the sugar beet pulp fiber- and apple pomace dietary fiber-supplemented diet for the Chol + SBP and the Chol + AP significantly hindered the rise of plasma lipids ( $P < 0.05$ ): (a) TC – 2.97 vs. 3.69, –20% and 3.01 vs. 3.69 mmol/l, –18.4%, respectively; (b) LDL-C – 1.36 vs. 2.02, –32.6% and 1.39 vs. 2.02 mmol/l, –31.2%, respectively; (c) TG – 0.73 vs. 0.88 and 0.75 vs. 0.88 mmol/l, –17 and –14.8%, respectively. Therefore, the sugar beet pulp fiber and to less degree apple pomace fiber-supplemented diets significantly hindered the cholesterol-induced increase in plasma TC, LDL-C and TG. Sugar beet pulp- and apple pomace fibers-supplemented diets significantly reduced the decrease in HDL-PH (0.79 vs. 0.63, –25.3%, and 0.75 vs. 0.63 mmol/l, –19%,  $P < 0.025$  and  $P < 0.05$ , respectively) and decreased the level of TPH (1.34 vs. 1.74, –23%, and 1.37 vs. 1.74 mmol/l, –21.3%,  $P < 0.005$  and  $P < 0.01$ , respectively). Liver weight was  $\pm$ 4.37 g in all groups. Liver TC concentration in rats of the Chol group was 4.15 times higher than in the Control group. Liver TC concentrations in rats of the Chol + SBP and the Chol + AP groups were higher than in the Control group only 2.9 and 3.05 times, respectively. Liver TC concentration in rats of the Chol group was significantly higher than in the Chol + SBP and the Chol + AP ( $P < 0.0005$  in both cases). These results show that the sugar beet pulp- and apple pomace fibers-supplemented diets hindered an increase in the liver TC concentration in rats fed dietary cholesterol (17.1 vs. 24.3  $\mu$ mol/g, –29.6% and 17.9 vs.

Table 2

Plasma lipids and total cholesterol concentration in liver of rats fed diets with and without 0.3% cholesterol (Chol) and with and without 10% apple pomace fiber (AP) or sugar beet pulp fiber (SBP)<sup>a,b,c</sup>

Plasma lipids							Liver
Diets	TC (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)	TPH (mmol/l)	HDL-PH (mmol/l)	TC (μmol/g)
Control	2.86±0.15c	1.23±0.05c	1.62±0.07	0.69±0.04b	1.76±0.08a	1.05±0.06a	5.86±0.24c
Chol	3.69±0.21a	2.02±0.12a	1.61±0.07	0.88±0.05a	1.74±0.08a	0.63±0.04c	24.3±0.31a
SBP	2.74±0.14c	1.12±0.05c	1.60±0.07	0.68±0.04b	1.71±0.07a	1.03±0.06a	5.81±0.24c
SBP+Chol	2.97±0.17b	1.36±0.05b	1.59±0.07	0.73±0.05b	1.34±0.06b	0.79±0.04b	17.1±0.25b
AP	2.81±0.15c	1.19±0.05c	1.61±0.07	0.70±0.05b	1.72±0.08a	1.04±0.06a	5.74±0.24c
AP+Chol	3.01±0.18b	1.39±0.05b	1.60±0.07	0.75±0.05b	1.37±0.06b	0.75±0.04b	17.9±0.25b
2-way ANOVA							
(P-value)							
SBP	NS	NS	NS	NS	NS	NS	NS
AP	NS	NS	NS	NS	NS	NS	NS
Chol	<0.001	NS	<0.001	NS	<0.001	<0.0005	
SBP+Chol	<0.001	<0.001	NS	<0.001	<0.005	<0.025	<0.0005
AP+Chol	<0.05	<0.05	NS	<0.05	<0.01	<0.05	<0.0005

<sup>a</sup> Values are means ± S.D., *n* = 10.

<sup>b</sup> Means without letters in common differ significantly (*P* < 0.05).

<sup>c</sup> Abbreviations used: AP, apple pomace fiber; Chol, nonoxidized cholesterol; HDL-C, HDL cholesterol; HDL-PH, HDL phospholipids; LDL-C, LDL cholesterol; NS, not significant (*P* ≥ 0.05); SBP, sugar beet pulp fiber; TC, total cholesterol; TG, triglycerides; TPH, total phospholipids.

24.3 μmol/g, –26.3%, for Chol + SBP and the Chol + AP, respectively).

The results of the examination of plasma LP concentration are summarized in Fig. 1. According to Fig. 1, plasma LP concentration did not differ in Control, SBP and AP groups. Plasma LP concentrations in Chol + SBP and Chol + AP were significantly higher than in the Control group (*P* < 0.001 in both cases), but equal to the Chol group; thus sugar beet pulp- and apple pomace fibers-supplemented diets did not affect plasma LP concentration. Neither sugar beet pulp fiber, or apple pomace fiber in rats fed the basal diet without cholesterol, affected the variables measured.

#### 4. Discussion

Interest in dietary fiber arose following epidemiological studies indicating low prevalence of coronary heart disease, diabetes, gallstones, obesity, appendicitis, diverticulosis, varicose veins and hemorrhoids in countries, with high consumption of foods, rich in fiber (Bartnikowska, 1999). It was “unusual for a single nutritional concept to have such an immediate and widespread impact as the dietary fiber hypothesis” (Chesson, 1995). And indeed, many investigators experimented with dietary fibers on animals and humans (Bagger, Andersen, Nielsen & Rytting, 1996; Eastwood & Kay, 1979; Jackson, Suter & Topping, 1994; Rimm et al., 1996; Zarling et al., 1994). On the basis of these experiments it would appear that dietary fiber, if not panacea, is at least a universal prophylactic (Chesson). But the independent ability of dietary fiber to low plasma lipid levels is still

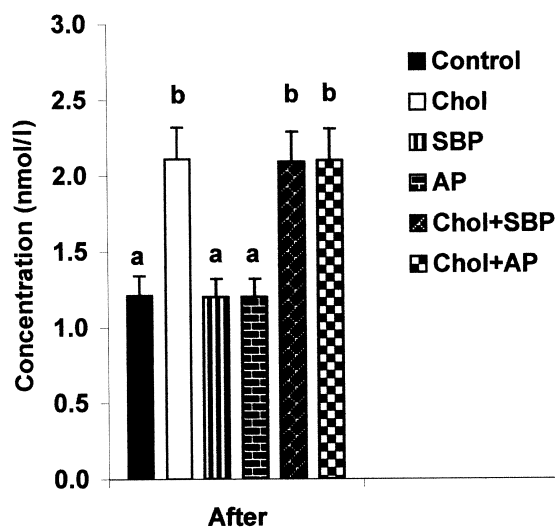


Fig. 1. Lipid peroxide concentrations in plasma of rats fed different diets for 40 days. Values are means ± S.D. (vertical lines), *n* = 10. Bars with different letters are significantly different (*P* < 0.05). Abbreviations: AP, apple pomace dietary fiber; Chol, cholesterol; SBP, sugar beet pulp dietary fiber.

controversial. Only few dietary studies support the claim that dietary fiber also exerts an antioxidant effect (Lin, 1994; Larrauri et al., 1996). The above-mentioned two points of disagreement demand further clarification. Therefore, it was decided to study the influence of dietary fiber on plasma lipids and lipid peroxides. The objective of this study was to clarify the possible influence of different sources and various quantities of dietary fibers on plasma lipids and lipid peroxides in rats fed cholesterol-containing and cholesterol-free diets. It

was supposed that controversy about the ability of dietary fiber to lower plasma lipid levels arose from low quantities of dietary fiber used by some investigators (Bobek et al., 1998). Therefore, we supplemented the BD for the experimental groups with higher quantity of dietary fibers from two different sources: apple pomace and sugar beet pulp (Bobek et al., 1998; Guillon et al., 1998; Langkilde et al., 1993). The influence of these fibers on lipids and lipid peroxides was studied in rats adapted to cholesterol-free or 0.3% cholesterol diets. The sugar beet pulp and apple pomace dietary fibers were added to these diets in the proportion of 100 g/kg basal diet. After 40 days of the experiment, we found that the sugar beet pulp- and apple pomace dietary fiber-supplemented diets significantly hindered a rise of plasma lipids and TC in liver. Sugar beet pulp- and apple pomace fibers-supplemented diets significantly hindered the decrease in HDL-PH and decreased the level of TPH. We found that neither sugar beet pulp fiber or apple pomace fiber, in rats fed the basal diet without cholesterol, affected the variables measured. Neither sugar beet pulp fiber- or apple pomace fiber-supplemented diets influenced the level of lipid peroxides. These results demonstrate that sugar beet pulp fiber, and to a lesser degree apple pomace fiber, possess hypolipidemic properties. In this experiment we have tried to clarify two points of disagreement: the independent ability of dietary fiber to low serum lipid levels and its antioxidant properties. The results of our investigation are different from the results of previous authors, who deny hypolipidemic ability of dietary fiber (Bobek et al.). We found that sugar beet pulp fiber and apple pomace fiber do possess hypolipidemic properties and that different dietary fibers have significantly different effects on plasma and liver lipids. However, this is only evident when sugar beet pulp fiber or apple pomace fiber are added to the diet of rats fed cholesterol (Anderson, Jones & Riddell-Mason, 1994; Kiryama, Okazaki & Joshida, 1969). These results conflict the results of Bobek et al: they did not find that apple pomace fiber possessed hypolipidemic properties. We suppose that these opposite results arise from the small quantity of apple pomace fiber used in their experiment. The hypolipidemic effect of both sugar beet pulp fiber and apple pomace fiber can be attributed to their water-soluble parts. In contrast to Lin (1994) and Larrauri et al. (1996), sugar beet pulp fiber and apple pomace fiber apparently have no antioxidant properties.

## 5. Conclusion

1. Sugar beet pulp fiber, and to a less degree apple pomace fiber, possess hypolipidemic properties. This is more evident when sugar beet pulp fiber or

apple pomace fiber are added to the diets of rats fed cholesterol.

2. The hypolipidemic effect of fiber can be attributed to the water-soluble parts of dietary fiber. Thus, the influence of diet supplemented with sugar beet pulp is more profound.
3. Different dietary fibers have significantly different effects on plasma lipids and TC in liver.
4. The sugar beet pulp fiber and the apple pomace fiber have no antioxidant properties.

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