Suppression of Hepatic Lipogenesis by Pectin and Galacturonic Acid Orally-Fed at the Separate Timing from Digestion-Absorption of Nutrients in Rat

Masashige SUZUKI and Takeshi KAJUU

Laboratory of Biochemistry of Exercise and Nutrition, Institute of Health and Sports Sciences, University of Tsukuba, Sakura-mura, Niihari-gun, Ibaraki 305, Japan
(Received December 8, 1982)

Summary This study was conducted to know the possibility that pectin-induced alterations in lipid metabolism of animals might be partly ascribed to galacturonic acid produced by the degradation of ingested pectin in the digestive tract. After a 4-week meal feeding twice a day, fasted rats were fed glucose and fructose and 3 h later orally administered 213 mg of pectin (from apple) or galacturonic acid per kg of body weight, or fed water alone. Significant changes in serum and liver lipids were observed 30 min and 1 h after the administration of pectin and galacturonic acid but not 5 h after the administration. Pectin and galacturonic acid showed contradictory effects on serum lipids, adipose tissue lipo-protein lipase activity and triacylglycerol (TG) production and removal rates. However, the elevation of total lipid and TG levels in liver with the sugar feeding was significantly inhibited by the administration of either pectin or galacturonic acid. These results support our hypothesis that galacturonic acid produced by the degradation of ingested pectin in the digestive tract may be partly responsible for the pectin-induced changes in lipid metabolism. This was discussed in relation to another possible regulation of lipid metabolism by short-chain fatty acids which are produced by the intestinal fermentation of pectin and galacturonic acid.

Key Words pectin, galacturonic acid, serum and liver lipids, TG production and removal, rat

Pectin, in which α-D-galacturonic acid and its methylester are principal constituents, has been reported to regulate the lipid metabolism of animals by lowering the absorption of dietary (1) and biliary (2) lipids in the digestive tract. Recently, however, Commings et al. (3) have reported that ingested pectin is almost

1 鈴木正成, 加重 剛
completely degraded in the digestive tract of humans, and Nyman and Aps (4) have also confirmed this in rats. Van Soest and Robertson (5) have demonstrated that about 90% of ingested pectin was degraded into short-chain fatty acids, CH₄, CO₂, H₂O, etc. by intestinal bacteria. Furthermore, Werch and Ivy (6, 7) have shown earlier that a portion of ingested pectin disappears before coming to the intestinum colon. On the other hand, Viola et al. (8) have demonstrated that 55–75% of orally-fed galacturonic acid could be absorbed in the small intestine of rats. Werch et al. (9) have also reported the intestinal degradation of galacturonic acid by bacteria. Thus, these findings suggest that the degradation products of pectin, including galacturonic acid, could be absorbed from the digestive tract and then exert some effects on lipid metabolism in animals.

In the present studies, pectin and galacturonic acid were orally-fed singly to rats at the separate timing from digestion-absorption of nutrients to demonstrate a new possible nutritional consequence of pectin in the regulation of lipid metabolism. This feed timing of pectin seems also important, when pectin containing foods are considered to be preferably consumed between meals in human food habits. Our results showed that the administration of either pectin or galacturonic acid lowered hepatic lipogenesis, suggesting that some parts of the nutritional effects of pectin might be exerted by galacturonic acid. Present studies also indicate the importance of the feed timing of fibers, which have been fed at the same timing with cholesterol and other nutrients in most of the previous studies, for further exploration of nutritional consequences of dietary fibers.

EXPERIMENTAL

Male JCL-Sprague-Dawley rats (CLEA Japan Inc., Tokyo) were used in all experiments. Forty-six rats of 4 weeks old (experiment 1), 42 rats of 4 weeks old (experiment 2) and 32 rats of 3 weeks old (experiment 3) were fed on a laboratory chow (CE-7, CLEA Japan Inc.) ad libitum until they were 6 weeks old and then meal-fed daily twice at 08.00-09.00 and 20.00-21.00 in a room maintained at 22 ± 2°C and illuminated from 07.00 to 19.00. Water was fed ad libitum throughout the feeding periods.

Experiment 1. At the end of the feeding period, rats of 10 weeks old and weighing 260 ± 2 g (mean ± SEM) were fed the final meal of only 20 min at 20.40-21.00, when rats could consume only about 50% of the usual consumption at the meal time of 20.00-21.00. Then rats were fasted for 14 h until 11.00 of the next day. Five rats were killed by decapitation at 11.00, when the remaining 41 rats were orally-fed with 10 ml of a sugar solution, 30% glucose and 30% fructose, per kg of body weight. Five out of 41 rats were killed 3 h after the sugar administration at 14.00, when every 12 remaining rats were orally-fed with 213 mg of pectin (from apple, Wako Pure Chemicals Ind. Ltd., Tokyo) or 213 mg of galacturonic acid per kg of body weight, or fed water alone. Every 6 rats receiving any treatment were killed 1 h (15.00) and 5 h (19.00) after the administration. Blood and liver were taken.
from every rat for lipid determinations.

Experiment 2. At the end of the feeding period, rats of 10 weeks old were fed the final meal at 20.40–21.00 and then fasted for 14 h. At 11.00 of the next day, all rats were orally fed with the same amount of the sugar solution to that in experiment 1. Five out of 42 rats were killed 3 h after the sugar administration, at 14.00, when the remaining every 12 rats were again orally fed with pectin, galacturonic acid, or water as described in experiment 1. Every 6 rats receiving any treatment were killed 30 min (14.30) and 1 h (15.00) after the administration. Blood and liver were taken from every rat for lipid determinations. Heart and epididymal adipose tissue were obtained from rats killed at 15.00 for the measurement of lipoprotein lipase (LPL) activity.

Experiment 3. After the 2-week meal feeding, rats of 9 weeks old and weighing 250 ± 3 g were fed the final meal at 20.40–21.00 and fasted for 17 h until 14.00 of the next day. Rats were divided into 3 equal groups and orally fed with pectin, galacturonic acid, or water, respectively, as described in experiments 1 and 2. Each half of every group of rats was injected at their tail veins with 300 mg of soybean fat as 10% fat emulsion with 1.2 g of egg lecithin and 2.5 g of glycerol in 100 ml of 0.15 M NaCl solution per kg of body weight (10) at 14.45 or 17.00. Blood was collected from the tail veins of all rats 15 and 30 min after the injection to determine triacylglycerol (TG) removal rate (10). Five days after the TG removal measurement, rats were fed the final meal at 20.40–21.00 and then fasted for 14 h until 11.00 of the next day, when every rats were orally fed with a sugar solution as described in experiments 1 and 2. At 14.00, 3 h after the feeding, an equal number of rats were orally fed with pectin, galacturonic acid, or water in the same manner as in experiments 1 and 2. Blood samples were obtained from the tail veins of half the rats receiving any treatment just after (14.00) or 2 h (16.00) after the treatment. Immediately after the blood collection, rats were injected with 600 mg of Triton WR-1339 per kg of body weight. They were killed 1.5 h after the Triton injection and their blood was sampled for the determination of hepatic-intestinal VLDL (very low density lipoprotein)-TG production rate (11).

Determinations of serum and liver constituents, tissue LPL activity, TG production rate and TG removal rate. Determinations were conducted for serum glucose (12), serum TG (11), serum total cholesterol (11), liver total lipid (11), liver TG (10), liver total cholesterol (11), liver glycogen (13) and tissue LPL activity (11). Hepatic-intestinal TG production rate (mg TG/h/rat) was calculated from the difference in serum TG concentrations before and after the Triton injection and plasma volume of rats as described previously (11). Plasma TG removal rate (mg TG/min/rat) was calculated from the difference in serum TG concentrations 15 and 30 min after the injection of soybean fat emulsion and plasma volume of rats as previously described elsewhere (10).

Thus in all experiments except for the measurement of TG removal rates, effects of pectin and galacturonic acid were investigated in rats whose lipogenesis activity was stimulated by the glucose and fructose feeding 3 h before the adminis-
tration of pectin and galacturonic acid.
All data were analyzed by the Student’s $t$-test.

RESULTS

Effects of a single oral administration of pectin or galacturonic acid on serum and liver lipids (experiments 1 and 2; Figs. 1 and 2)

In experiment 1, several marked effects of the administration of pectin and galacturonic acid on the concentrations of lipids in serum and liver appeared at the initial 1h during the 5-h experimental period following the administration, therefore, similar but more precise examinations were conducted 30 min and 1h after the administration in experiment 2.

Serum TG levels were elevated with pectin administration in both experiments 1 and 2, and the levels 1h after the administration were significantly ($p<0.05$) higher in pectin-administered rats than in $H_2O$-administered control rats. Although serum total cholesterol levels considerably increased after the administration of galacturonic acid in experiment 1, this increase did not occur in experiment 2. Serum total cholesterol levels in galacturonic acid-administered rats at 15.00, however, were significantly higher than those in controls 1h after the administration in either experiment 1 ($p<0.02$) or 2 ($p<0.05$). Serum glucose levels did not significantly change during 1h after the administration of pectin and galacturonic acid (experiment 2).

The administration of pectin and galacturonic acid caused decreases in liver total lipid contents, and, 1h after the administration, the levels in pectin- and galacturonic acid-administered rats were significantly lower than those in controls in either experiment 1 ($p<0.02$) or 2 ($p<0.05$). Liver TG contents increased during 1h after $H_2O$-administration in both experiments 1 and 2, however, the increase was inhibited with the administration of either pectin or galacturonic acid. Especially in the rats administered with galacturonic acid, liver TG contents 1h after the administration were significantly lower than those in control rats in both experiments 1 ($p<0.05$) and 2 ($p<0.02$). On the other hand, liver total cholesterol and glycogen contents were not significantly influenced by the administration of pectin and galacturonic acid. Liver weights of animals in experiments 1 and 2 were $7.6 \pm 0.2$ and $7.8 \pm 0.3$ g, respectively, and there was no significant difference in the weights obtained before and after administration of pectin or galacturonic acid.

Effects of a single oral administration of pectin or galacturonic acid on tissue LPL activity, TG removal rate and TG production rate (experiments 2 and 3; Table 1)

Heart LPL activity was slightly higher in pectin- and galacturonic acid-administered rats than in control rats, but the difference was not significant. LPL activity of epididymal adipose tissue was significantly ($p<0.05$) decreased with galacturonic acid administration. TG removal rates at 15.00–15.15 were not significantly but considerably decreased with the administration of galacturonic...
Fig. 1. Effects of the single oral administration of pectin and galacturonic acid on serum glucose, total cholesterol and TG concentrations in rats (experiments 1 and 2). At 11.00, after the 14-h fasting, rats were fed with 10 ml of sugar solution, 30% glucose and 30% fructose, per kg of body weight. At 14.00, rats were orally administered with 213 mg of pectin or galacturonic acid per kg of body weight, or fed water alone. Rats were killed at the times indicated. Each point and vertical bar represent mean and SEM respectively, for 5–6 rats. a,b,c Significantly different from water- and pectin-treatment (a: p < 0.02), from water- and galacturonic acid-treatment (b: p < 0.05), or from water-treatment (c: p < 0.05)
Fig. 2.

J. Nutr. Sci. Vitaminol.
Table 1. Tissue LPL activity, TG removal rate and TG production rate after a single oral administration of pectin and galacturonic acid to rats (experiments 2 and 3).

| Treatment                  | H₂O       | Pectin    | Galacturonic acid |
|----------------------------|-----------|-----------|-------------------|
| Experiment 2               |           |           |                   |
| LPL activity (15.00)       |           |           |                   |
| Heart                      | 90.8 ± 10.8 | 95.9 ± 7.9 | 94.0 ± 7.4        |
| Epididymal fat pad         | 30.0 ± 1.8  | 31.7 ± 2.5  | 22.1 ± 2.0*       |
| TG removal rate            |           |           |                   |
| 15.00–15.15                | 0.44 ± 0.11 | 0.44 ± 0.06 | 0.24 ± 0.10       |
| 17.15–17.30                | 0.25 ± 0.08 | 0.27 ± 0.07 | 0.28 ± 0.06       |
| TG production rate         |           |           |                   |
| 14.00–15.30                | 38.2 ± 2.1  | 41.6 ± 3.2  | 35.0 ± 4.4        |
| 16.00–17.30                | 33.5 ± 0.9  | 34.2 ± 2.0  | 28.5 ± 2.5        |

Values are means ± SEM for 4–6 rats. At 11.00, after 14-h fasting, every rat except for rats for the measurement of TG removal rate was orally fed with 10 ml of sugar solution, 30% glucose and 30% fructose, per kg of body weight. At 14.00, rats were administered with 213 mg of pectin or 213 mg of galacturonic acid per kg of body weight or fed water alone. Then rats were killed at 15.00 for the measurement of tissue LPL activity (experiment 2), injected with 300 mg of soybean fat emulsion per kg of body weight at 14.45 or 17.00 for the measurement of TG removal rate at 15.00–15.15 or 17.15–17.30, respectively, or injected with 600 mg of Triton WR-1339 per kg of body weight at 14.00 or 16.00 for the measurement of TG production rate at 14.00–15.30 or 16.00–17.30, respectively. *Significantly different from H₂O and pectin treatment (p<0.05).

Pectin and galacturonic acid, when administered at separate times from digestion-absorption of nutrients, showed slightly different effects on serum choles-

DISCUSSION

Pectin and galacturonic acid, when administered at separate times from digestion-absorption of nutrients, showed slightly different effects on serum choles-
terol and TG levels, LPL activity in epididymal adipose tissue, TG removal rates and TG production rates. However, during 1 h after the administration of pectin and galacturonic acid, when TG production rates and liver total cholesterol and glycogen contents did not show any marked change, an inhibition in the accumulation of total lipid and TG was clearly observed in liver. Thus, although unfortunately the determination of liver phospholipid contents were not conducted in this study, these findings support a hypothesis that pectin and galacturonic acid depresses in vivo hepatic lipogenesis including TG synthesis in rats. Such effects of pectin and galacturonic acid were observed 30 min and 1 h after their administration but were not detected 5 h after the administration, indicating that the inhibition of hepatic lipogenesis seems to be not due to long-lasting but due to acute effects by pectin and galacturonic acid.

There are few studies on pectin of galacturonic acid and hepatic lipid metabolism, but our findings are partly supported by the findings of Kelley and Tsai (14) who have reported on decreased synthesis of long-chain fatty acids from glucose in liver from rats fed on a 5% pectin diet for 2–3 weeks. Our results are also partly in agreement with those of Mokady (15) who showed the decreased hepatic synthesis of TG and phospholipids from intraperitoneally injected \(^{14}\)Cacetate in pectin-fed rats, but in which cholesterol synthesis was increased.

For the resolution of mechanisms of regulation of hepatic lipogenesis by pectin it is noteworthy to describe the recent work of Illman et al. (16) who have explored much transport of volatile short-chain fatty acids such as acetate, propionate and butyrate from the digestive tract to liver through the hepatic portal vein in rats fed a 10% citrus pectin diet for 2 weeks. These authors (17) have further revealed by the perfusion study that hepatic lipogenesis is strongly regulated by acetate, a major short-chain fatty acid detected in the portal vein of rats (16). These findings by others and our results suggest that pectin-induced changes in lipid metabolism of animals may be partly caused by the degradation products from pectin, such as galacturonic acid as well as short-chain fatty acids. In addition to these, alterations of hormonal balance by pectin ingestion must be taken into account, because the decrease in the postprandial insulin response by pectin ingestion has been reported by Jenkins et al. (18). This is beneficial for suppressing the elevation of hepatic lipogenesis after the sugar ingestion as observed in the present study.

As compared with H₂O-treatment, serum total cholesterol levels were significantly elevated 1 h after the administration of galacturonic acid, when there were no marked differences in the levels of liver total cholesterol and serum TG between these two treatments but had slightly lower VLDL-TG production rates and TG removal rates in the galacturonic acid treatment. This suggests that a possible decrease in serum cholesterol removal by peripheral tissues might be occurring with the administration of galacturonic acid.

Pectin-administered rats showed significantly higher serum TG levels, as compared with controls, 1 h after the pectin administration, when there were significantly lower liver TG levels, slightly higher TG production rates and almost
equal TG removal rates in the pectin treatment. Thus, pectin seems to stimulate hepatic TG secretion resulting in the elevated serum TG concentrations.

TG removal rates were slightly increased with the administration of pectin but inversely slightly decreased with the administration of galacturonic acid. We have confirmed this in another experiment using rats equally fed and treated as animals of this study (experiment 3); TG removal rates 1 h after the administration of H₂O alone, pectin and galacturonic acid were 33.1±2.4, 38.6±3.8 and 27.8±2.2 mg TG/h/rat, respectively. These results suggest that pectin and galacturonic acid might inversely affect TG removal in rats.

Several contradictory effects above mentioned between pectin and galacturonic acid on serum lipids and TG production and removal could be partly ascribed to differences in net absorbed amounts of galacturonic acid as well as short-chain fatty acids after the administration of pectin and galacturonic acid.

In conclusion, singly administered pectin may rapidly reduce hepatic lipogenesis in rats. This may be directly caused by galacturonic acid, and also possibly by other compounds such as short-chain fatty acids, produced by the intestinal fermentation of the ingested pectin. As other dietary fibers, pectin has been mostly investigated from the view point that pectin plays nutritional activities in the digestive tract. Thus, our present results indicate an additional manner probably played by pectin in the regulation of lipid metabolism of animals. In addition, for further investigations of fibers, it is suggested to be important to consider the fiber feed timing as to whether or not fiber should be given alone or together with other nutrients.

This work was presented in part at the 36th Meeting of Japan Nutrition and Food Society (Abstract No. 3F-4p), Tokyo, May, 1982. This work was partly supported by the U.S.-Japan Cooperative Medical Science Program for Malnutrition.

REFERENCES

1) Vahouny, G. V., Roy, T., Gallo, L. L., Story, J. A., Krichevsky, D., and Cassidy, M. (1980): Dietary fibers III. Effects of chronic intake on cholesterol absorption and metabolism in the rat. Am. J. Clin. Nutr., 33, 2182–2191.
2) Miettinen, T. A., and Tarpila, S. (1977): Effects of pectin on serum cholesterol, fecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals. Clin. Chim. Acta, 79, 471–477.
3) Commings, J. H., Southgate, D. A. T., Branch, W. J., and Wiggins, H. S. (1979): The digestion of pectin in the human gut and its effect on calcium absorption and large bowel functions. Brit. J. Nutr., 41, 477–489.
4) Nyman, M., and Asp, N-G. (1982): Fermentation of dietary fibre components in the rat intestinal tract. Brit. J. Nutr., 47, 357–366.
5) Van Soest, P. J., and Robertson, J. B. (1977): What is fibre and fibre in food? Nutr. Rev., 35, 12–22.
6) Werch, S. C., and Ivy, A. C. (1941): On fate of ingested pectin. Am. J. Digest. Dis., 8, 101–105.
7) Werch, S. C., and Ivy, A. C. (1941): A study of metabolism of ingested pectin. *Am. J. Dis. Child.*, 62, 499–511.

8) Viola, S., Zimmermann, G., and Mokady, S. (1970): Effect of pectin and algin upon protein utilization, digestibility of nutrients, and energy in young rats. *Nutr. Rep. Int.*, 1, 367–375.

9) Werch, S. C., Young, R. W., Day, A. A., Friedmann, T. E., and Ivy, A. C. (1942): Decomposition of pectin and galacturonic acid by intestinal bacteria. *J. Infect. Dis.*, 70, 231–242.

10) Katamine, S., Hoshino, N., Totsuka, K., and Suzuki, M. (1983): Effects of the long-term (7–9 months) feeding of iodine-enriched eggs on lipid metabolism of rats. *J. Nutr. Sci. Vitaminol.*, 29, 23–33.

11) Suzuki, M., Hashiba, N., and Kajuu, T. (1982): Influence of timing of sucrose meal and physical activity on plasma triacylglycerol levels in rats. *J. Nutr. Sci. Vitaminol.*, 28, 295–310.

12) Suzuki, M., Shimomura, Y., and Satoh, Y. (1983): Diurnal changes in lipolytic activity of isolated fat cells and their increased responsiveness to epinephrine and theophylline with meal feeding in rats. *J. Nutr. Sci. Vitaminol.*, 29, 399–411.

13) Saitoh, S., Yoshihake, Y., and Suzuki, M. (1983): Enhanced glycogen repletion in liver and skeletal muscle with citrate orally fed after an exhaustive treadmill running and swimming. *J. Nutr. Sci. Vitaminol.*, 29, 45–52.

14) Kelley, J. J., and Tsai, A. C. (1978): Effect of pectin, gum arabic and agar on cholesterol absorption, synthesis and turnover in rats. *J. Nutr.*, 108, 630–639.

15) Mokady, S. (1974): Effect of dietary pectin and algin on the biosynthesis of hepatic lipids in growing rats. *Nutr. Metab.*, 16, 203, 217.

16) Illman, R. J., Trimble, R. P., Snoswell, A. M., and Topping, D. L. (1982): Daily variations in the concentrations of volatile fatty acids in the splanchnic vessels of rats fed diet high in pectin and bran. *Nutr. Rep. Int.*, 26, 439–446.

17) Snoswell, A. M., Trimble, R. P., Fishlock, R. G., Storer, G. B., and Topping, D. L. (1982): Metabolic effects of acetate in perfused rat liver: studies on ketogenesis, glucose output, lactate uptake and lipogenesis. *Biochim. Biophys. Acta*, 716, 290–297.

18) Jenkins, D. J. A., Leeds, A. R., Gassull, M. A., Cochet, B., and Alberti, K. G. M. M. (1977): Decrease in postplandial insulin and glucose concentrations by guar and pectin. *Ann. Int. Med.*, 86, 20–23.