Original Communication

Apple Pectin Affects the Efficacy of Epigallocatechin Gallate on Oral Sucrose Tolerance Test in Adult Mice

Motoi Tamura and Sachiko Hori

National Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

Received: March 6, 2011; Accepted: September 17, 2011

Abstract: Epigallocatechin gallate (EGCg), a dietary polyphenol and a major tea catechin, is a known sucrase inhibitor. Since dietary pectin is known to modulate some of the functions of the gastrointestinal tract, we investigated whether it could specifically affect the efficacy of EGCg on an oral sucrose tolerance test in mice. Male Crj:CD-1 (ICR) mice (seven weeks old) were randomly divided into two groups and fed a 5 % apple pectin (PE) or 5 % cellulose (CE) diet (control diet) for 28 days. After the experimental diet period, all mice were fasted overnight. A volume of 0.2 mL EGCg (20 mg/mL) was orally administered to all the mice by stainless steel feeding needle via injection syringe and a sucrose tolerance test was performed. The blood glucose levels were measured in blood collected from the tail vein using the OneTouch® Ultra® blood glucose monitoring system. Blood glucose levels at 30 minutes and 60 minutes after sucrose loading in the PE group were significantly higher than initial blood glucose levels. However, blood glucose levels at 30 minutes, 60 minutes, and 120 minutes after sucrose loading in the CE group were not significantly higher than initial blood glucose levels. After laparotomy, plasma lipids were also measured. Plasma triglyceride concentrations were significantly greater in the PE group than in the CE (control) group. This demonstrates that dietary pectin can affect the efficacy of EGCg on the oral sucrose tolerance test in mice.

Key words: blood glucose, apple pectin, epigallocatechin gallate, sucrose, absorption

Introduction

The beneficial effects of dietary polyphenols have been widely reported [1–3], and are known to modulate intestinal enzyme activities. Dietary supplementation with chokeberry fruit extract (0.2 %) decreased the activity of maltase and sucrase, as well as increased the activity of lactase in the mucosa of the rat small intestine. This chokeberry fruit extract was shown to be a condensed source of polyphenols (714 mg/g), especially anthocyanin glycosides (56.6 %) [4]. A reduction in intestinal maltase and sucrase activities in quercetin-fed diabetic rats was observed in contrast to the increased activities in starch-fed diabetic rats [5].

In an *in vitro* experiment, inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols has been reported [6]. There are also *in vivo* investigations of the reduction in blood glucose levels by tea catechin [7]. From these reports tea catechin was found to be a sucrase inhibitor. Epigallocatechin gallate (EGCg) is one of the major tea catechins and has been suggested to be a potent sucrase inhibitor [6]. Dietary pectin, a soluble polysaccharide, is also known to affect the gut environment. Animals given soluble polysaccharides had plasma enteroglucagon levels significantly higher than animals given insoluble cellulose [8]. It has been reported that a 10 % pectin diet elicited a marked enlargement of the cecum, a drop in cecal pH, and an increase in the volatile fatty acids (VFA) pool in the rat [9]. A pectin-supplemented enteral diet reduces the severity of methotrexate-induced enterocolitis in rats [10]. It has been demonstrated that pectin is used by intestinal microbes as the principal energy source to catabolize nitrogenous compounds in pigs [11]. Both pectin and EGCg seem to be important functional foods.

Health benefits of dietary pectin [12–14] and EGCg [15, 16] have been reported. Elucidating the effects of dietary pectin on physiological function of EGCg would seem to be important. However, there are few reports of the influence of dietary pectin on the effects of EGCg in mice. As dietary pectin is known to affect the general function of the gastrointestinal tract, we wanted to determine whether it could affect the efficacy of EGCg on oral sucrose tolerance test in mice.

Methods and materials

Materials

Apple pectin used in the diet of pectin (PE)-fed mice was purchased from Sigma (St. Louis, MO., USA). EGCg was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Treatment of animals and oral sucrose tolerance test

Male Crj:CD-1 (ICR) mice (seven weeks old) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). All mice were specific pathogen-free (SPF), and the animals were housed in conventional conditions in our laboratory. The mice were randomly divided into two groups of seven animals each and housed in suspended stainless-steel cages with wire mesh bottoms, in a room kept at 24 ± 0.5 °C and at a relative humidity of 65 %, with 12-hour periods of light and dark. They were fed a pelleted diet (MF, Oriental Yeast Co., Ltd, Tokyo, Japan) for one week. After one week, the diet was replaced with an AIN-93M diet for one week. Then the diet was replaced with a 5 % pectin (PE) diet or 5 % cellulose (CE) diet (control diet) for 28 days. All mice were pair-fed. Table I presents the composition of each diet. Body weight and food consumption were measured during the experiment (three times a week). At the end of the feeding trial, all mice were fasted overnight and the oral sucrose tolerance test was carried out. A volume of 0.2 mL EGCg (20 mg/mL) was orally administered to all the mice by stainless steel feeding needle via injection syringe. Immediately after administration of EGCg, blood were collected from the tail vein and blood glucose levels were measured as initial glucose levels. After measurements of initial blood glucose levels, the sucrose tolerance test was carried out. A 20 % sucrose solution in distilled water was administered by stainless steel feeding needle via injection syringe. Blood was collected from the tail vein and blood glucose levels were measured 30 minutes, 60 minutes, and 120 minutes later after sucrose administration. The blood glucose levels were measured in blood collected from the tail vein using the OneTouch® Ultra® blood glucose monitoring system (Johnson & Johnson Lifescan, Inc., Milpitas, CA, USA).

Table I: Composition of the experimental diet.

Ingredient	CE diet (g/kg diet)	PE diet (g/kg diet)
Cornstarch	465.692	465.692
Casein	140.0	140.0
α-Cornstarch	155.0	155.0
Sucrose	100.0	100.0
Soybean oil	40.0	40.0
Cellulose	50.0	–
Apple pectin*	–	50.0
Mineral mix (AIN-93M-MX)	35.0	35.0
Vitamin mix (AIN-93-VX)	10.0	10.0
L-Cystine	1.8	1.8
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.008	0.008

* Purchased from Sigma (St. Louis, MO, USA).

A total of 3.5 hours after administration of EGCg, all mice were anesthetized with diethyl ether and blood samples were taken from the abdominal aorta and placed in heparinized tubes. The plasma was separated from whole blood by centrifugation and stored at –80 °C until analysis of plasma triglyceride, total cholesterol, and phospholipids was carried out. The liver and cecal contents were collected. Cecal contents and livers were weighed. All animals were then euthanized with diethyl ether. This study was carried out in accordance with the Guidelines for Animal Care and Experimentation of the National Food Research Institute. The animal studies were reviewed and approved by the Animal Care and Use Committee of the National Food Research Institute, National Agriculture and Food Research Organization (NARO), Japan.

Measurement of plasma cholesterol, triglyceride, phospholipids

The total plasma cholesterol concentrations were measured using a cholesterol E-test Wako kit (Wako Pure Chemical Industries Ltd., Osaka, Japan) based on cholesterol oxidase [17]. The plasma triglyceride concentrations were measured using a triglyceride E-test Wako kit (Wako Pure Chemical Industries Ltd., Osaka, Japan) based on the glycerol-3-phosphate oxidase method [18]. The plasma phospholipid concentrations were measured using a phospholipid C-test Wako kit (Wako Pure Chemical Industries Ltd., Osaka, Japan) based on the choline oxidase method [19].

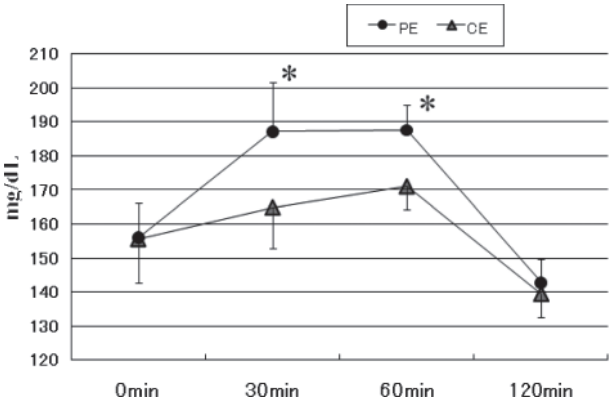

Figure 1: Effects of EGCg on changes in blood glucose levels of mice with oral loading of sucrose. X-axis shows the time after sucrose loadings. EGCg solution was administered 30 minutes before sucrose administration to the mice in the PE group or the CE group. Values are means ± SE (n = 7). The data were analyzed using one- way Repeated Measures Analysis of Variance. We used multiple comparisons versus control group (Holm-Sidak method). The control group was defined as an initial blood sugar levels (0 minutes).
*Statistical significance when p value < 0.05.

Statistics

The data are expressed as the mean ± standard error (SE). All data were analyzed using the Sigma Plot 11 (Systat Software, Inc., CA, USA). The data from the sucrose tolerance test were analyzed using one-way Repeated Measures Analysis of Variance. The data from plasma lipids were analyzed using *t*-test analysis. Statistical significance was reached with a p value of less than 0.05.

Results

No significant differences were observed between the PE group and the CE group in final body weight (g) (PE 38.4 ± 0.5; CE 38.7 ± 0.5; p = 0.614), in food consumption (g/day) (PE 4.1 ± 0.2; CE 4.3 ± 0.1; p = 0.225). There were no significant differences in the cecal contents (grams) between the PE (0.33 ± 0.03) and CE (0.32 ± 0.04) groups (p = 0.902). No significant differences were observed in the liver weight (grams) of the PE (1.30 ± 0.03) and CE (1.39 ± 0.05) groups (p = 0.121).

Effects of EGCg on changes in blood glucose levels of mice with oral loading of sucrose are shown in Figure 1. Blood glucose levels at 30 minutes and 60 minutes after sucrose loading in the PE group were significantly higher than initial blood glucose levels. However, no significant differences were observed in the blood glucose levels between the initial blood glucose levels and blood glucose levels at 120 minutes after sucrose loading in the PE group. On the other hand, blood glucose levels at 30 minutes, 60 minutes, and 120 minutes after sucrose loading in the CE group were not significantly higher than initial blood glucose levels. In the measurements of plasma lipids, no significant differences in the plasma cholesterol or plasma phospholipid concentrations were observed between the two groups (Figure 2). However, plasma triglyceride concentrations were found to be significantly greater in the PE group than in the CE (control) group (Figure 2).

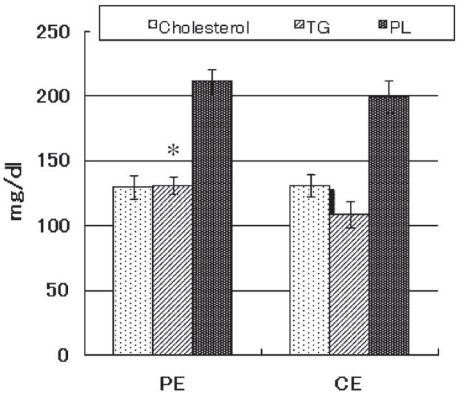

Figure 2: Plasma total cholesterol, phospholipids, and triglyceride concentrations of mice in the PE and CE groups. Values are means ± SE (n = 7). *Significantly different ($p < 0.05$) from the CE group. The data were analyzed using *t*-test analysis.

Discussion

It has been reported using a sucrose tolerance test that EGCg reduces blood glucose levels in the rat [7]. However, using a sucrose tolerance test, an apple pectin diet did not strongly reduce blood glucose levels in mice over time. On the other hand, in the CE group, blood glucose levels tended to be low during the sucrose tolerance test. The reason for this may be that EGCg administered to the PE group might have been less effective than in the CE group. It has been reported that orally administered water-soluble green tea extract (GTE), containing EGCg, maintained blood glucose at lower levels than the control during an oral glucose tolerance test, when glucose was taken immediately after GTE administration [20]. From this report, EGCg seems to affect glucose absorption. In an *in vitro* experiment, inhibition of rat small intestinal sucrase and α-glucosidase activities by EGCg has been reported [6]. EGCg inhibits enzyme activities in the gut. On the other hand, many investigations have suggested that pectin affects the absorption of dietary components and general metabolism of the digestive tract. For example, dietary pectin has been shown to promote gastric emptying and have a pronounced effect on increasing the distal accumulation of a meal [21]. It has been reported that the bioavailability of beta-carotene, lycopene, and lutein given within a mixed supplement is reduced by pectin [22]. It has also been reported that pectin increases zinc absorption [23]. Additionally, dietary pectin seems to increase the bioavailability of rutin in mice [24]. Health effects of apple pectin fiber are related not only to the pectin source but also to its physicochemical properties. Gel-forming properties in apple pectin are related to the degree of methoxylation [25], and it has been demonstrated that high-methoxyl pectins have a greater inhibitory effect on glucose uptake than low-methoxyl pectins [26]. We used high-methoxyl pectins in our experiment. So, viscosity-related increase of mucosal unstirred layer thickness may have occurred in the PE group. Viscosity-related increase of mucosal unstirred layer thickness may affect the efficacy of EGCg on the gut function by changing the mucosal environment in the PE group.

Pectin is also known to affect the small intestinal morphology. It has been reported that the jejunal crypt depth, and both the ileal villus height and crypt depth of the mice fed the pectin diet were significantly greater than those of the mice fed the cellulose diet [27]. Rats fed a 5 % pectin diet had heavier and longer small intestines and heavier mucosa than the rats fed a fiber-free diet [28]. From these reports, pectin seems to change the structure of the mucosa of the small intestine. Changes in the morphology of intestine might have been induced in the gut of the PE group. The efficacy of EGCg on the sucrose tolerance test might have been changed by means of the altered structure of the mucosa of the small intestine in the PE group.

Pectin has also been shown to affect lipid and bile acid metabolism in rats. Dietary pectin increases bile acid pool size and changes bile acid composition in rats [29]. Cholesterol concentration in feces showed a significant increase by week 3 in rats fed 5 % orange or apple pectin. The hepatic cholesterol concentration declined significantly in all pectin-fed groups. Serum cholesterol only declined significantly in apple-fed groups [30]. It has been reported that cholesterol absorption remained depressed in rats pre-fed pectin for 4 weeks [31]. From these reports, it can be concluded that a pectin diet affects lipid metabolism; however, in this experiment, no significant differences were observed in the plasma cholesterol between the PE and the CE diet groups. The apple pectin in the diet should not have strongly impacted endogenous cholesterol. However, plasma triglycerides were significantly greater in the PE group than in the CE group. Plasma triglyceride has been shown previously to be significantly lower in pectin- and rutin-supplemented mice [24]. Further studies are needed to clarify the relationship between plasma triglyceride and dietary pectin.

In summary, we demonstrated that dietary pectin affected the efficacy of EGCg on the oral sucrose tolerance test in mice. Investigation of the interaction between dietary fiber and EGCg would lead to elucidation of the composite effects of functional foods.

Acknowledgments

This study was financially supported by a Grant-in-Aid for Scientific Research© from the Japan Society for the Promotion of Science (JSPS).

References

1. Del Rio, D., Borges, G. and Crozier, A. (2010) Berry flavonoids and phenolics: bioavailability and evidence of protective effects. Br. J. Nutr. 104. Suppl. 3, S 67.
2. Sies, H. (2010) Polyphenols and health: update and perspectives. Arch. Biochem. Biophys. 501, 2.
3. Das, D.K., Mukherjee, S. and Ray, D. (2010) Resveratrol and red wine, healthy heart and longevity. Heart Fail. Rev. 15, 467.
4. Jurgoński, A., Juśkiewicz, J. and Zduńczyk, Z. (2008) Ingestion of black chokeberry fruit extract leads to intestinal and systemic changes in a rat model of prediabetes and hyperlipidemia. Plant Foods Hum. Nutr. 63, 176.
5. Ramachandra, R., Shetty, A.K. and Salimath, P.V. (2005) Quercetin alleviates activities of intestinal and renal disaccharidases in streptozotocin-induced diabetic rats. Mol. Nutr. Food Res. 49, 355.
6. Honda, M. and Hara, Y. (1993) Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. Biosci. Biotech. Biochem. 57, 123.
7. Matsumoto, N., Ishigaki, F., Ishigaki, A., Iwashita, H. and Hara, Y. (1993) Reduction of blood glucose levels by tea catechin. Biosci. Biotech. Biochem. 57, 525.
8. Johnson, I.T., Gee, J.M. and Brown, J.C. (1988) Plasma enteroglucagon and small bowel cytokinetics in rats fed soluble nonstarch polysaccharides. Am. J. Clin. Nutr. 47, 1004.
9. Demigné, C., Levrat, M.A., and Rémésy, C. (1989) Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between the cecum and blood plasma in the rat. J. Nutr. 119, 1625.
10. Mao, Y., Kasravi, B., Nobaek, S., Wang, L.Q., Adawi, D., Roos, G., Stenram, U., Molin, G., Bengmark, S. and Jeppsson, B. (1996) Pectin-supplemented enteral diet reduces the severity of methotrexate induced enterocolitis in rats. Scand. J. Gastroenterol. 31, 558.
11. Mosenthin, R., Sauer, W.C. and Ahrens, F. (1994) Dietary pectin's effect on ileal and fecal amino acid digestibility and exocrine pancreatic secretions in growing pigs. J. Nutr. 124, 1222.
12. Jackson, C.L., Dreaden, T.M., Theobald, L.K., Tran, N.M., Beal, T.L., Eid, M., Gao, M.Y., Shirley, R.B., Stoffel, M.T., Kumar, M.V. and Mohnen, D. (2007) Pectin induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure. Glycobiology 17, 805.
13. Dongowski, G. and Lorenz, A. (2004) Intestinal steroids in rats are influenced by the structural parameters of pectin. J. Nutr. Biochem. 15, 196.
14. Wu, H., Dwyer, K.M., Fan, Z., Shircore, A., Fan, J. and Dwyer, J.H. (2003) Dietary fiber and progression of atherosclerosis: the Los Angeles Atherosclerosis Study. Am. J. Clin. Nutr. 78, 1085.
15. Wolfram, S. (2007) Effects of green tea and EGCG on cardiovascular and metabolic health. J. Am. Coll. Nutr. 26, 373 S–388 S.
16. Hill, A.M., Coates, A.M., Buckley, J.D., Ross, R., Thielecke, F. and Howe, P.R. (2007) Can EGCG reduce abdominal fat in obese subjects? J. Am. Coll. Nutr. 26, 396 S-402 S.
17. Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. and Fu, P.C. (1974) Enzymatic determination of total serum cholesterol. Clin. Chem. 20, 470.
18. Spayd, R.W., Bruschi, B., Burdick, B.A., Dappen, G.M., Eikenberry, J.N., Esders, T.W., Figueras, J., Goodhue, C.T., LaRossa, D.D., Nelson, R.W., Rand, R.N. and Wu, T.W. (1978) Multilayer film elements for clinical analysis: applications to representative chemical determinations. Clin. Chem. 24, 1343.
19. Takayama, M., Itoh, S., Nagasaki, T. and Tanimizu, I. (1977) A new enzymatic method for determination of serum choline-containing phospholipids. Clin. Chim. Acta 79, 93.
20. Park, J.H., Jin, J.Y., Baek, W.K., Park, S.H., Sung, H.Y., Kim, Y.K., Lee, J. and Song, D.K. (2009) Ambivalent role of gallated catechins in glucose tolerance in humans: a novel insight into non-absorbable gallated catechin-derived inhibitors of glucose absorption. J. Physiol. Pharmacol. 60, 101.
21. Brown, N.J., Greenburgh, A. and Tomlin, J. (1994) The effects of pectin and wheat bran on the distribution of a meal in the gastrointestinal tract of the rat. Br. J. Nutr. 72, 289.
22. Riedl, J., Linseisen, J., Hoffmann, J. and Wolfram, G. (1999) Some dietary fibers reduce the absorption of carotenoids in women. J. Nutr. 129, 2170.
23. Kondo, H. and Osada, A. (1996) Influence of dietary fiber on the bioavailability of zinc in rats. Biomed. Environ. Sci. 9, 204.

24. Tamura, M., Nakagawa, H., Tsushida, T., Hirayama, K. and Itoh, K. (2007) Effect of pectin enhancement on plasma quercetin and fecal flora in rutin-supplemented mice. J. Food Sci. 72, S 648.

25. Judd, P.A. and Truswell, A.S. (1985) The hypocholesterolaemic effects of pectins in rats. Br. J. Nutr. 53, 409.

26. Kim, M. (2005) High-methoxyl pectin has greater enhancing effect on glucose uptake in intestinal perfused rats. Nutrition 21, 372.

27. Tamura, M. and Suzuki, H. (1997) Effects of pectin on jejunal and ileal morphology and ultrastructure in adult mice. Ann. Nutr. Metab. 41, 255.

28. Chun, W., Bamba, T. and Hosoda, S. (1989) Effect of pectin, a soluble dietary fiber, on functional and morphological parameters of the small intestine in rats. Digestion 42, 22.

29. Matheson, H.B. and Story, J.A. (1994) Dietary psyllium hydrocolloid and pectin increase bile acid pool size and change bile acid composition in rats. J. Nutr. 124, 1161.

30. González, M., Rivas, C., Caride, B., Lamas, M.A. and Taboada, M.C. (1998) Effects of orange and apple pectin on cholesterol concentration in serum, liver and faeces. J. Physiol. Biochem. 54, 99.

31. Vahouny, G.V., Satchithanandam, S., Chen, I., Tepper, S.A., Kritchevsky, D., Lightfoot, F.G. and Cassidy, M.M. (1988) Dietary fiber and intestinal adaptation: effects on lipid absorption and lymphatic transport in the rat. Am. J. Clin. Nutr. 47, 201.

Dr. Motoi Tamura

National Food Research Institute
National Agriculture and Food Research Organization
2–1–12 Kannondai
Tsukuba
Ibaraki 305–8642
Japan
Tel.: +81–298–38–8089
Fax: +81–298–38–7996
motoita@affrc.go.jp