J Nutr Sci Vitaminol, **56**, 68–71, 2010

Note

Consumption of Curcumin Elevates Fecal Immunoglobulin A, an Index of Intestinal Immune Function, in Rats Fed a High-Fat Diet

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(Received July 17, 2009)

Summary This study was conducted to elucidate the effects of dietary polyphenols on intestinal immunoglobulin A (IgA) response and mucin levels in rats fed a high-fat diet. In experiment 1, rats were fed a high-fat diet with or without several polyphenols including curcumin, rutin, D(+)-catechin, ellagic acid and quercetin at the level of 0.5%. Among the polyphenols examined, consumption of curcumin markedly elevated the level of IgA in feces and colon contents. In experiment 2, rats were fed a high-fat diet or a low-fat diet with or without 0.5% curcumin. Fecal level of IgA was higher in the high-fat diet group than in the low-fat diet group. In the rats fed the high-fat diet, dietary curcumin elevated fecal IgA, but not in those fed the low-fat diet. These results imply a novel effect of curcumin on intestinal IgA in animals fed a high-fat diet.

Key Words curcumin, immunoglobulin A, mucin, high-fat diet, rat

Consumption of polyphenols has been associated with various beneficial effects on human health. One current interest is their attribution to the prevention or cure of various diseases related to immune function, including cancers, allergies and infectious diseases (*1–6*). Secretory immunoglobulin (Ig) A is the principal immunoglobulin in mucosal surfaces and exerts a protective role against the invasion of harmful microorganisms and toxins by blocking their attachment to epithelial cells, resulting in less inflammatory response (*7, 8*). This immunoglobulin binds to mucin glycoproteins (mucins), the primary components of the mucus gel, through hydrogen or disulfide bonds (*9, 10*). The IgA level in the colon of ulcerative colitis patients is significantly decreased, leading to an increase in tissue injury (*11*). Recent study further suggests an association of the IgA production with the prevention of colon cancer (*12*). On the other hand, a high-fat diet increases the risk of ulcerative colitis and colon cancer (*13, 14*). However, limited information is available on the effects of dietary polyphenols on intestinal immune function, especially intestinal immunoglobulin A (IgA) response in humans or animals (*15*). Thus, the present study was performed to examine the effects of several dietary polyphenols on fecal IgA response and mucin levels in rats fed a high-fat diet.

Male Sprague Dawley rats (4 wk of age) were purchased from Hiroshima Laboratory Animal Center (Hiroshima, Japan) and maintained according to the "Guide for the Care and Use of Laboratory Animals" established by Hiroshima University and approved by the ethics committee of the same university. Animals were individually housed in an air-conditioned room at 23 to 24°C with a 12-h light cycle (light, 8:00–20:00). Composition of the basal diet (% w/w) was beef tallow, 30 or 5; casein, 20; L-cystine, 0.2; cellulose, 5; sucrose, 20; vitamin mixture, 1; salt mixture, 3.5; and corn starch, 20.3 or 45.3 to made up to 100% (*16*). The high-fat diet (30% beef tallow) and low-fat (5% beef tallow) diet provided 21.68 and 16.45 kJ/g of diet, respectively. In experiment 1, curcumin, rutin, D(+)-catechin, ellagic acid or quercetin was added to the high-fat diet at 0.5% (w/w). This dietary level is within the levels of polyphenols (0.1–2%) in animal studies reported (*17–19*). The polyphenols were supplemented at the expense of corn starch. Rats were fed a high-fat diet with or without the polyphenols. These experimental diets were incorporated into the individual food cups at 9 g, 10 g, 12 g, 14 g and 15 g for day(s) 1, 2–4, 5–7, 8–13 and 14–21, respectively, at 7:00 p.m. All of the diets were consumed by 12:00 a.m. the next day. These amounts of diets maintained approximately 80% of gains in body weight for 3 wk compared to ad libitum feeding (Kato et al. unpublished data). Curcumin, rutin, D(+)-catechin and quercetin were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Ellagic acid was obtained from Wako

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Table 1. Effects of dietary addition of some polyphenols on fecal dry weight, fecal IgA and mucin levels in rats fed a high-fat diet (experiment 1).[1]

	Fecal dry weight (g/3 d)	Fecal IgA (mg/3 d)	Fecal mucin (mg/3 d)
Control	3.51±0.17	2.38±0.23	3.27±0.32
Curcumin	3.88±0.17	5.29±1.11*	3.93±0.64
Rutin	3.52±0.34	2.65±0.38	5.25±1.06
Catechin	3.66±0.22	2.10±0.31	4.14±0.50
Ellagic acid	4.31±0.60	1.98±0.53	5.48±1.26
Quercetin	3.98±0.46	1.74±0.23	5.55±1.08

IgA, immunoglobulin A.
[1] Values are presented as means±SE (n=7).
*Significantly different from the control by Steel's multiple comparison test (p<0.05).

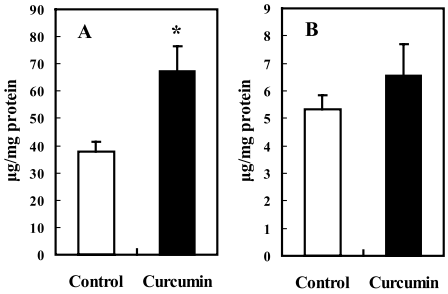

Fig. 1. Effect of dietary addition of curcumin on IgA level in colon contents (A) and colonic mucosa (B) in rats fed a high-fat diet (experiment 1). Values are presented as means±SE (n=7). *Significantly different from control group (p<0.005) by Mann-Whitney U test.

Table 2. Effect of dietary addition of curcumin on serum immunoglobulin levels in rats fed a high-fat diet (experiment 1).[1]

	IgA (μg/mL)	IgE (ng/mL)	IgG (mg/mL)
Control	30.4±5.3	11.7±3.7	1.21±0.19
Curcumin	19.9±1.3*	19.8±3.7	1.53±0.33

IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G.
[1] Values are presented as means±SE (n=7).
*Significantly different from the control by Mann-Whitney U test for IgA or Student's-t test for IgE and IgG (p<0.05).

Pure Chemical Industries, Ltd. (Osaka, Japan). In experiment 2, 0.5% (w/w) curcumin was added to either the high-fat diet (30% beef tallow) or low-fat diet (5% beef tallow). Spontaneously defecated feces from each animal were collected for the final 3 d. At the end of the feeding period, the rats were sacrificed by decapitation with anesthesia of diethyl ether. Whole blood was collected and serum was separated by centrifugation at 3,000 rpm for 20 min. The colon was excised and opened longitudinally and the contents and mucosa were collected. All samples were stored at −70°C prior to analyses. Total IgA concentrations in feces, colon contents and colonic mucosa were measured by using an ELISA quantitation kit (Bethyl Laboratories, Montgomery, Texas, USA) (*20*). Fecal mucins were extracted by the methods of Bovee-Oudenhoven et al. (*21*) and quantitated using a fluorometric assay (*22*). Serum Ig levels were determined using ELISA quantitation kits (Bethyl Laboratories, Montgomery, Texas, USA). Protein concentrations of colon contents and the mucosa were determined by the Bradford method (*23*). Data are expressed as the means±SE. In experiment 1, statistical analysis was performed with one-way ANOVA or the Kruskal-Wallis test. Dunnett's or Steel's multiple comparison test (Excel Statistics 2008 for Windows, Social Survey Research Information Co., Ltd, Tokyo, Japan) was performed if significant effects were determined. Differences between two groups were evaluated with the Student's t test or Mann-Whitney U test, as appropriate. In experiment 2, Two-way ANOVA was used to determine the effect of dietary fat level or curcumin and their interaction. When a p-value in the ANOVA was <0.05, Scheffé's test was used for multiple comparisons. Statistical significance of the difference among means was estimated at p<0.05.

In experiment 1, food intake and final body weight did not vary among treatment groups (data not shown). Fecal dry weight was not affected by dietary manipulation (Table 1). Fecal IgA was significantly greater only in rats fed the curcumin-supplemented diet than in those fed the control diet (p<0.05). Supplemental curcumin enhanced IgA concentration in the colon contents (p<0.005) relative to the control group (Fig. 1A), but did not in colonic mucosa (Fig. 1B). These results imply that dietary curcumin enhances secretion of IgA into the gut lumen or suppresses degradation of IgA. The serum IgA level was significantly lower in rats fed the curcumin-supplemented diet than in those fed the control diet (Table 2). It has been reported that the decrease of serum IgA might be due to a lesser burden on the whole body immunity by the activation of intestinal immune responses (*24*), which is consistent with the results of the present study. Our results also indicated that dietary curcumin did not affect serum levels of IgE or IgG, suggesting that the effect of curcumin might be specific for IgA. These findings imply a novel effect of curcumin on intestinal immune response to IgA, which is a major component of intestinal barrier. In the present study, there was no influence of other polyphenols examined on fecal IgA (Table1). Thus, among various polyphenols, curcumin appears to have specific effect on fecal secretion of IgA.

In the intestine, mucins act as a protective barrier against pathogens and antigens and also provide binding sites for immunoglobulins, especially secretory IgA (*9*, *10*). So we hypothesized that dietary polyphenols may increase fecal mucins as well as IgA. The present results indicated no effects of dietary polyphenols on fecal mucins (Table 1). There was no correlation

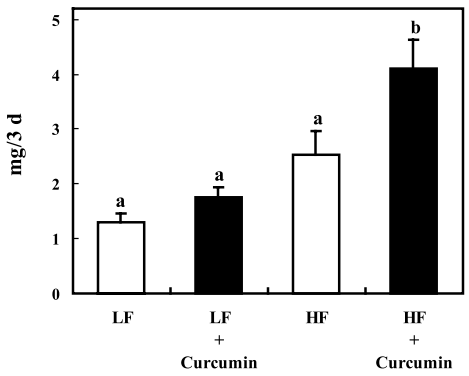

Fig. 2. Effect of dietary addition of curcumin on fecal IgA level in rats fed either a low-fat diet or high-fat diet (experiment 2). Values are presented as means±SE (n=8). Means not sharing a common superscript differ significantly by Scheffe's post-hoc test (p<0.05). LF, low-fat diet; HF, high-fat diet.

between fecal IgA and the mucins (r=0.102, n=42, p=0.519). Accordingly, the possibility that the elevation in fecal IgA by curcumin affects fecal mucin levels appears to be negated.

In experiment 2, food intake and fecal dry weight did not differ among the groups fed the 4 diets (data not shown). There were no significant interactions between dietary fat level and curcumin to affect final body weight, food efficiency or fecal IgA (ANOVA, p=0.453, p=0.337 and p=0.143, respectively). The final body weight of the low-fat diet group, low-fat diet with curcumin group, high-fat diet group and high-fat diet with curcumin group was 206±2 g, 201±1 g, 247±3 g and 246±2 g, respectively, and the high-fat diet caused 21% higher final body weight (ANOVA, p<0.001). Food efficiency was also significantly higher (ANOVA, p<0.001) in the high-fat diet groups (0.512±0.007 g/g) than in the low-fat diet groups (0.356±0.007 g/g), and was unaffected by dietary curcumin (ANOVA, p=0.345). Fecal IgA was greater in rats fed the high-fat diets than in those fed the low-fat diets (ANOVA, p<0.001) (Fig. 2). Supplemental curcumin significantly elevated fecal IgA in rats fed the high-fat diet (p<0.05).

High-fat diets are associated with increased risks of inflammation, ulcerative colitis and colorectal cancer (*13*, *14*). It is of interest to examine if fecal IgA can be influenced by a high-fat diet. In this study, we found that a high animal fat diet enhanced secretory IgA in feces (ANOVA, p<0.001) (Fig. 2). A recent study suggests that a high-fat diet changes intestinal microflora towards a decreased number of bifidobacteria (*25*). Furthermore, the alteration of the microflora induced by high-fat feeding strongly increases intestinal permeability, by reducing the expression of genes coding for intestinal tight junction proteins (*26*). Disturbance in the intestinal tight junction barrier allows increased tissue penetration of luminal antigens. Thus, it is of interest to test if the elevation in fecal IgA from a high-fat diet relates to alterations in permeation of antigens and/or microflora. It is also possible that the difference in energy intake might affect fecal IgA. Further study is necessary to examine if the elevation in IgA level by a high-fat diet might be mediated by mechanisms including intestinal IgA producing cells, cytokines, etc.

Curcumin is a highly pleiotropic molecule and executes its function by modulating multiple targets (*27*). A recent clinical trial suggests a potential therapeutic role for curcumin in colon cancer (*28*). Churchill et al. have reported that curcumin supplementation increased numbers of $CD4^+$ T cells and B cells in the intestinal mucosa of colorectal cancer-model mice (*29*). Activations of mucosal $CD4^+$ T cells and IgM^+ B cells are associated with preferential switching to IgA^+ B cells. Recently, a study in rats with chemically-induced colitis has suggested that curcumin increased intestinal expression of interleukin (IL)-10, which is an anti-inflammatory cytokine and is involved in the differentiation of B cells to IgA plasma cells (*29*–*31*). Thus, the elevation in fecal IgA from curcumin intake might be mediated by these alterations. In the present study, dietary curcumin did not affected IgA concentration in colonic mucosa. Our findings raise a possibility that curcumin supplementation may improve intestinal immune function in high-fat-fed animals through elevation in luminal IgA by increasing IgA production and/or suppressing IgA degradation. Intestinal IgA level can be affected by some intestinal microflora (*32*, *33*). Therefore, it is necessary to test if dietary curcumin enhances intestinal IgA response by affecting the profile of intestinal microflora.

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