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Note

Evaluation of the Correlation Between Amount of Curcumin Intake and its Physiological Effects in Rats

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We examined the correlation between amount of curcumin intake and its physiological effects on indices of liver function, serum and liver lipid profiles in rats. Animals were fed diets containing 0.5, 5 and 50 mg curcumin per 100 g body weight for 28 days.

HDL-cholesterol concentrations of rats fed curcumin diets were significantly higher ($P<0.05$) than those of the control group, and serum TG concentration of rats fed the x100 curcumin diets was significantly lower ($P<0.05$) than that of the x1 curcumin group. Serum TG concentration of rats fed curcumin diets tended to decrease in a curcumin dose-dependent manner. These results indicate that curcumin intake can improve serum lipid profiles effectively.

Keywords: Curcumin, Rat serum, Triglyceride, HDL-cholesterol, Liver function.

Introduction

Turmeric has long been used as a traditional remedy in Asia. Its commercial derivatives currently demand high prices in the so-called health food market of Japan because it is well known that they have various beneficial effects on human health. These physiological effects are emphasized by manufacturers and promote sales in the so-called health food market.

The main physiological ingredient of turmeric is considered to be the 3–5% of curcumin contained in *Curcuma longa*. Curcumin is a well-known natural anti-oxidant (Sharma, 1976). In addition to such a function, it has been reported that curcumin has various physiological functions such as lowering cholesterol (Ramirez-Tortosa *et al.,* 1999), improving liver function (Park *et al.,* 2000), suppressing tumor activity (Surh, 2002), and it can be used as an anti-inflammatory (Rao *et al.,* 1982).

Although curcumin is thought to be the main active ingredient of turmeric, whether there is a clear relation between the physiological function of turmeric and curcumin content has not yet been established. This is because turmeric consists of various natural materials such as minerals, dietary fiber, tannin, curcumin, flavonoids, camphor, azulene and similar compounds. In addition, the curcumin content in turmeric is about 5% at most (Hiserodt *et al.,* 1996), and absorptivity is considered to be very low (Asai and Miyazawa, 2000). Therefore, it seems unlikely that curcumin acts independently when turmeric is ingested because similar effects are observed in *Curcuma aromatica* (Salisb) and *Curcuma zedoaria* (Roscoe), which hardly contain curcumin. Thus, the relation between the amount of curcumin contained in turmeric and substantial physiological effects are still poorly understood. Therefore, it is important to examine the actual effects associated with ingestion of a certain amount or excessive intake of curcumin.

In the current study, to investigate the relation between amount of curcumin intake and its physiological effects on indices of liver function, serum and liver lipid profiles and other biochemical parameters, male Wistar rats were fed 0.5 mg (x1), 5 mg (x10), 50 mg (x100) curcumin-containing diets or a curcumin-free diet for 28 days. In addition, we also conducted histological observations of metabolic organs such as liver and kidney to gain more insight into the metabolic state resulting from excessive curcumin.

Materials and Methods

Male Wistar rats (8 weeks old) were purchased from Japan SLC Inc. (Shidzuoka, Japan). After acclimation for 1 week, rats were randomly divided into four groups ($n=6$/group). Rats were fed experimental diets ad libitum for 4 weeks. Experimental diets were based on AIN-93G formula of the following ingredients (g/kg diet): casein, 200; corn starch, 150; test oil, 100; AIN-93G mineral mixture, 35; AIN-93G vitamin mixture, 10; cellulose, 50; D, L-methionine, 3; choline bitartrate, 2 and ～1000 sucrose. The experimental diets contained 0.5 (x1), 5 (x10) or 50 (x 100) mg curcumin per 100 g body weight, while the control group received the AIN-93G type diet without curcumin. The reagent-grade curcumin was purchased from Wako Pure Chemical Industries, Ltd. The basal daily amount of curcumin in rats was estimated by weight conversion based on the recommended daily intake for humans. At the end of the experimental period, after overnight fast-

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ing, rats were anesthetized and sacrificed for analysis. Care and use of laboratory animals was in accordance with the guidelines of the National Institute of Health and Nutrition.

Blood samples were obtained from the rat abdominal aorta after overnight fasting, and centrifuged at 3,000 rpm for 15 min. Sera were stored at −80℃ before analysis. Serum lipids (total cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride (TG)), liver function indices (GOT, GPT, γ-GPT, ALP and LDH) and other biochemical parameters (total protein and blood glucose) were analyzed enzymatically using commercially available assay kits (Wako Pure Chemical, Osaka). Serum insulin concentrations were measured using a commercially available EIA kit (Biotrak, Amersham Pharmacia Biotech, MO).

Liver was excised, weighed and stored at −80℃ until analysis. The liver lipids were extracted with chloroform-methanol (2: 1 v/v) (Folch *et al.,* 1957). Liver cholesterol and TG concentrations were determined using a Cholesterol E-test and Triglyceride E-test (Wako, Osaka) as described elsewhere with minor modifications (Carr *et al.,* 1993).

The liver and kidneys obtained from rats fed control and x100 curcumin diets were fixed with 10% formaldehyde solution (pH=7.4) and embedded in paraffin. Paraffin-embedded specimens were prepared, and stained with hematoxylin and eosin. Sample preparation and microscopic examination was performed at the Sapporo Pathology Research Institute.

Data are presented as means±SEM (standard error of the mean). The statistical significance of the difference was evaluated by ANOVA followed by Fisher's PLSD test. Differences at $P<0.05$ were considered to be significant.

Results and Discussion

There were no significant differences in body weight gain, food intake, and relative weights of liver, kidney, spleen, testis, epididymal and perirenal adipose tissue between groups by curcumin intake. These findings indicate that excessive curcumin intake did not affect the growth of rats. Therefore, it is thought that curcumin as a component of food is not harmful on rat growth in the range of curcumin consumption measured in the present study. Pathological examination revealed lipid deposition or extramedullary hematopoiesis in one sample and small granuloma in the livers of two rats fed the x100 curcumin diet. However, these manifestations were not considered a pathological problem.

Serum HDL-cholesterol concentrations of rats fed curcumin diets were significantly higher ($P<0.05$) than that of control group. Serum TG concentration of rats fed the x100 curcumin diets was significantly lower ($P<0.05$) than that of the x1 curcumin group. Serum TG concentration tended to decrease in a curcumin dose-dependent manner (Fig. 1). No significant differences in serum and liver cholesterol, and liver TG concentrations were observed between groups. The hypocholesterolemic effect of curcumin can probably be explained by its effect

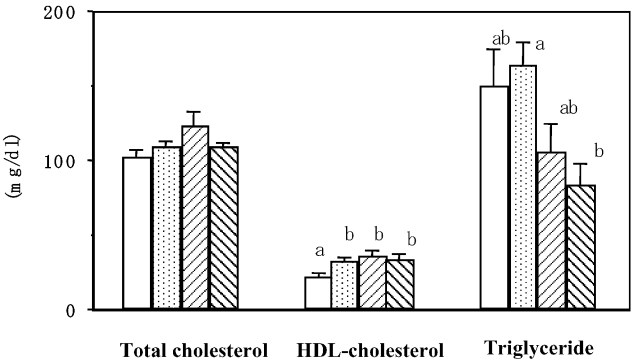

Fig. 1. Serum lipid profiles of rats maintained on different curcumin diets.
Values are means±SEM for 6 rats. Values not sharing a common letter differ, $P<0.05$.
□: Control diet, ⬚: x1 Curcumin diet, ▨: x10 Curcumin diet, ▧: x100 Curcumin diet.

on the stimulation of bile fluid and biliary cholesterol secretion and enhanced excretion of bile acids and cholesterol in feces (Ramprasad and Sirsi, 1957; Patil and Srinivasan, 1971; Srinivasan and Sambaiah, 1991). Although, in this study, we did not observe a marked reduction in serum cholesterol concentrations in rats fed curcumin diets, the results agree with a previous report, which indicated that the plasma cholesterol levels of animals fed cholesterol-free diet were not affected by curcumin intake (Rao *et al.,* 1970). Furthermore, curcumin ingestion contributed to an increase in HDL-cholesterol concentration regardless of the amount of curcumin ingestion. These results indicate that curcumin intake modulated HDL- and LDL-cholesterol concentrations, maintaining proportions at desirable levels. It is known that a decrease in the ratio of LDL- to HDL-cholesterol concentrations leads to improvement in the arteriosclerosis index (Hostmark *et al.,* 1990). Moreover, although Asai *et al.* also observed a reduction in serum TG concentration (Asai and Miyazawa, 2001), the critical regulatory mechanism of curcumin on the reduction in serum TG concentration has not yet been clarified. Thus, the alterations in serum lipid profiles observed in the present study could not be explained by the increase in fecal bile acid excretion alone, and further detailed study will be required to examine the regulatory mechanism of curcumin on serum lipid profiles.

Liver function indices were not significantly influenced by curcumin ingestion, although GOT and GPT slightly increased, and γ-GTP and ALP tended to decrease in accordance with an increase in curcumin intake (Table 1). It is well known that effects of liver function improvement are the typical physiological effect associated with curcumin (Nirmala and Puvanakrishnan, 1996; Rukkumani *et al.,* 2004). Since the present study was carried out using a normal animal model, a marked improvement in liver function might not be observed under these experimental conditions. However, use of an impaired liver function animal model, a heavy cholesterol diet, or over-

Table 1. Serum biochemical parameters in rats maintained on different curcumin diets.

	Control	Curcumin		
		x1	x10	x100
		(IU/L)		
GOT	41.2±4.54	54.9±2.25	49.7±1.84	52.8±5.26
GPT	1.57±0.45	3.53±0.53	0.47±0.30	2.59±0.59
γ-GTP	6.11±0.76	5.67±0.51	4.11±0.25	3.93±0.47
		(K-A unit)		
ALP	88.5±13.6	63.9±8.28	56.1±10.1	57.2±4.68
		(mg/dl)		
LDH	736.2±3.23	727.9±5.99	731.1±6.66	672.2±67.3
		(g/dl)		
Total protein	6.33±0.20	6.16±0.11	5.84±0.18	5.97±0.06
		(mg/dl)		
Blood glucose	96.9±12.9a	120.6±4.89ab	140.6±8.68bc	159.0±9.00c
		(ng/ml)		
Insulin	1.13±0.11	1.11±0.18	1.75±0.34	2.36±0.63

Values are means±SEM, n=6. Values not sharing a common letter differ significantly ($P<0.05$).

dose of curcumin under the cruel experimental conditions in animal models might be better suited for illustrating the effect of the amount of curcumin intake on improved liver function (Park *et al.,* 2000; Patil and Srinivasan, 1971; Rao *et al.,* 1970). In the present study, however, we found that moderate amounts of curcumin consumption improved the ratio of HDL- and LDL-cholesterol concentrations, even in a healthy animal model. While curcumin intake increased both blood glucose and serum insulin concentrations in this study (Table 1), previous studies on blood glucose, serum insulin or diabetes found that the antioxidant properties of curcumin had antidiabetic effects (Srivivasan *et al.,* 2003; Arun and Nalini, 2002; Nishizono *et al.,* 2000). It may therefore be necessary to consider changes in these biochemical characteristics in future research.

In conclusion, it is noteworthy in this study that curcumin intake improved the proportion of HDL- and LDL-cholesterol concentrations, even at curcumin concentrations found in turmeric, and excessive curcumin intake reduced the serum TG concentration in healthy rats. These results imply that curcumin may contribute to the regulation of lipid metabolism. The mechanisms of action are not yet clear and further study will be necessary to understand how curcumin affects liver function. In addition, the physiological effect of other turmeric ingredients, such as natural polyphenolic compounds, needs to be considered.

To clarify the substantial physiological benefits of turmeric or curcumin, we intend to study the effects of long-term or excessive feeding in an impaired animal model, such as animals with liver dysfunction and hyperlipidemia. A series of such experiments will increase our understanding of the regulatory mechanisms of lipid metabolism and the correlation between physiological function and amount of turmeric intake.

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