Original Research

Dietary kakrol (*Momordica dioica* Roxb.) flesh inhibits triacylglycerol absorption and lowers the risk for development of fatty liver in rats

Masao Sato[1], Takatoshi Ueda[1], Kazuko Nagata[1], Sawako Shiratake[1], Hiroko Tomoyori[2], Mitsuo Kawakami[3], Yukio Ozaki[4], Hiroshi Okubo[5], Bungo Shirouchi[1] and Katsumi Imaizumi[1]

[1]Laboratory of Nutrition Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581; [2]Department of Food and Health Sciences, Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, 3-1-100 Tsukide, Kumamoto 862-8502; [3]Branch of Ishigaki, Okinawa Prefectural Agricultural Research Center, 1178-6 Hirae-Chisokobaru, Ishigaki, Okinawa 907-0003; [4]Laboratory of Agricultural Ecology, Department of Agro-environmental Sciences, Faculty of Agriculture, Graduate School, Kyushu University, 111 Harumachi Kasuya-machi, Kasuya, Fukuoka 811-2307; [5]Laboratory of Horticultural Science, Department of Agro-environmental Sciences, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
Corresponding author: Masao Sato. Email: masaos@agr.kyushu-u.ac.jp

Abstract

Kakrol (*Momordica dioica* Roxb.) is a cucurbitaceous vegetable native to India and Bangladesh. Bitter gourd (*Momordica charantia* Linn.), a species related to kakrol, has been shown to have pharmacological properties including antidiabetic and antisteatotic effects. In this study, we investigated the effect of dietary kakrol on lipid metabolism in rats. Sprague-Dawley rats were fed AIN-76 formula diets containing 3% freeze-dried powders of whole kakrol or bitter gourd for two weeks. Results showed significantly lowered liver cholesterol and triacylglycerol levels in rats fed on both diets. Fecal lipid excretion increased in rats fed the kakrol diet, and lymphatic transport of triacylglycerol and phospholipids decreased in rats fed the kakrol diet after permanent lymph cannulation. Furthermore, *n*-butanol extract from kakrol caused a significant concentration-dependent decrease in the pancreatic lipase activity *in vitro*. These results indicate that the mechanisms of action on lipid metabolism in kakrol and bitter gourd are different and that dietary kakrol reduces liver lipids by inhibiting lipid absorption.

Keywords: kakrol, *Momordica dioica* Roxb., lymph cannulation, lipid absorption, fatty liver, bitter gourd, *Momordica charantia* Linn.

Experimental Biology and Medicine 2011; **236**: 1139–1146. DOI: 10.1258/ebm.2011.011037

Introduction

Momordica dioica Roxb. is known as kakrol or teasle gourd in English and as kakora in Hindi. Kakrol is native to India and Bangladesh. In India, the vegetable is traditionally renowned for its medicinal properties.[1] Like bitter gourd (*Momordica charantia* Linn.), kakrol belongs to the genus *Momordica*. Animal studies reportedly show that bitter gourd flesh[2] and winter melon seed (*Benincasa hispida* Cogn.)[3] improve lipid metabolism in animal models. In Japan, China and India, westernized dietary habits have increased the potential for and development of metabolic syndrome. Fatty liver, in which triacylglycerol comprises more than 10% of weight, is one of the symptoms of metabolic syndrome[4] and is usually caused by high-calorie diets. Treatment and recovery generally include a relatively long-term decrease in caloric intake and an increase in exercise. Problems associated with low-calorie diets, such as low protein intake and an imbalance in minerals and vitamins,[5] can also contribute to fatty liver disease. Patients can control and cure fatty liver disease by following a dietician-recommended diet. Interest in finding new plant-based compounds and researching triacylglycerol metabolism has increased because compounds such as capsaicin[6] and catechin[7] promote fatty acid catabolism. Some saponins[8] naturally inhibit intestinal absorption of triacylglycerol and effectively ameliorate triacylglycerol metabolism.

Jain *et al.*[1] reported that ethanolic and aqueous extracts of kakrol leaves have antioxidant and hepatoprotective

properties in rats with fatty liver induced by carbon tetrachloride. Also, dietary intake of the cucurbit bitter gourd reportedly reduces hepatic triacylglycerol and steatosis.[2] Studies indicate that momordicin[9] and geranylgeraniol[10] in bitter gourd flesh are peroxisome proliferator-activated receptor α (PPARα) ligands, which promote fatty acid catabolism.[11] Thus, cucurbitaceous plants can improve lipid metabolism.

We determined the nutrient contents of an edible part of kakrol to identify its nutritional value. Moreover, we also compared the effect of freeze-dried dietary kakrol and bitter gourd powders on lipid metabolism in rats. The purpose of this study was to determine which dietary intake, kakrol or bitter gourd, ameliorates hyperlipidemia and fatty liver by improving lipid metabolism in rats.

Materials and methods

Determination of nutrients in kakrol and bitter gourd

Kakrol (*Cucurbitaceae*: *Momordica dioica* Roxb.) and bitter gourd (*Cucurbitaceae*: *Momordica charantia* Linn.; a variety of Powerful-Reishi) were developed and cultivated at the Ishigaki branch of the Okinawa Prefectural Agricultural Research Center (Okinawa, Japan) and Kyushu University (Fukuoka, Japan). Kakrol and bitter gourd were cut into small pieces, freeze-dried and powdered. These freeze-dried powders were used in the following experiments. Protein content in the freeze-dried kakrol powder was determined by the Kjeldahl method (AOAC 984.13). We applied a nitrogen–protein conversion factor of 5.71 for vegetables. Total lipids were extracted by the method of Folch *et al.*[12] and weighed. Ash content was determined by the direct ash method (AOAC 942.05). α-Tocopherol[13] and β-carotene[14] contents were measured by high-performance liquid chromatography (HPLC). The column for α-tocopherol was packed with Zorbax SIL (5 μm, 4.6 $\times$ 250 mm; Agilent Technologies, Tokyo, Japan) and the column for β-carotene was packed with Inertsil ODS-3 (5 μm, 4.6 $\times$ 250 mm; GL Sciences Inc, Tokyo, Japan). Ascorbic acid content was determined by the hydrazine method.[15] Total lipids were transmethylated using the sulfuric acid − methanol method;[16] fatty acid compositions of the total lipids in kakrol and bitter gourd were determined by packed-column gas chromatography, and the column was packed with Silar 10C (GL Sciences Inc).

Animals and diets

Five-week-old male Sprague − Dawley rats (Kud:SD) obtained from Kyudo Co (Kumamoto, Japan) were divided into three groups. The rats were housed individually in an air-conditioned room (21–24°C, with light conditions from 08:00 to 20:00 h). Before the experiment, all rats were allowed free access to commercial chow (type NMF; Oriental Yeast, Tokyo) for one week. The experimental diets were prepared according to AIN-76.[17] The control diet contained 200 g/kg casein, 150 g/kg cornstarch, 50 g/kg cellulose, 50 g/kg corn oil, 35 g/kg mineral mixture (AIN-76), 10 g/kg vitamin mixture (AIN-76), 3 g/kg DL-methionine, 2 g/kg choline bitartrate and 500 g/kg sucrose. In the kakrol and bitter gourd groups, sucrose was replaced with 30 g/kg of freeze-dried kakrol and bitter gourd powders. The rats were pair-fed the experimental diets for 14 d. They were sacrificed by decapitation without starvation, and blood was collected. Liver and white adipose tissues (epididymal, mesenteric, perirenal and retroperitoneal) were excised and weighed. This experiment was conducted under the Guidelines for Animal Experiments at Kyushu University (Fukuoka, Japan) and under Law no. 105 and Notification no. 6 of the Government of Japan. The authorization number was A20-041-0.

Serum and hepatic lipids

Serum total cholesterol and triacylglycerol concentrations were determined using enzyme assay kits (Cholesterol E test and Triglyceride E test; Wako Pure Chemicals, Osaka, Japan). Liver lipids were extracted with chloroform:methanol (2:1, v/v) according to the method of Folch *et al.*[12] The concentrations of hepatic total cholesterol, triacylglycerol and phospholipids were determined by the method of Sperry and Web,[18] Fletcher[19] and Wootton,[20] respectively. Cholesterol ester levels were calculated as the difference between total and free cholesterol.

Fecal steroids

Feces were collected for three days at the end of the feeding period, lyophilized and weighed. Powdered feces were extracted with absolute ethanol at 80°C, and the extract was hydrolyzed in sodium hydroxide solution at 120°C.[21] After extraction of the neutral steroids with diethyl ether, the solution was acidified with HCl, and the acidic steroids were extracted with diethyl ether. Fecal neutral sterols were derivatized into trimethylsilyl ethers and quantified by gas − liquid chromatography on a Supelco SPB-1 column (0.25 μm, 0.25 mm $\times$ 60 m; Sigma-Aldrich Japan, Tokyo, Japan). Fecal acidic steroids were methylated, derivatized into trimethylsilyl ethers and quantified by gas–liquid chromatography on a Supelco SPB-1 column. 5α-Cholestane (Nacalai Tesque, Kyoto, Japan) and 23-nordeoxycholic acid (Steraloids, Wilton, NH, USA) were used as internal standards for the neutral sterols and bile acids, respectively.[22] Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid; Gasukuro Kogyo, Tokyo, Japan), deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid; Calbiochem, Behring, CA, USA), chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid; Sigma-Aldrich, St Louis, MO, USA), ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid; Sigma-Aldrich), hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholan-24-oic acid; Applied Science Laboratories, Bedford, MA, USA), α-muricholic acid (3α,6β,7α-trihydroxy-5β-cholan-24-oic acid; Steraloids), β-muricholic acid (3α,6β,7β-trihydroxy-5β-cholan-24-oic acid; Steraloids) and cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; Wako Pure Chemicals) were used to determine peaks. Neutral steroid and acidic steroid levels were calculated based on the sum of the identified peaks.

Fecal free fatty acid

Fecal free fatty acid was analysed by the method of van de Kamer[23] modified by Jeejeebhoy.[24] In brief, fecal lipids were extracted three times with heptane:diethyl ether:ethanol (1:1:1, v/v) (10 mL × 2) and the upper phase consisted of heptane:diethyl ether:ethanol:deionized water (1:1:1:1, v/v) (10 mL × 1) from 1 g of powdered feces acidified with two drops of concentrated HCl. The lipids were dried under a stream of nitrogen and then redissolved in 10 mL of hexane as fecal lipid solution. Five milliliter of 1 N NaOH in 90% ethanol was added to the dried fecal lipid solution and refluxed for 45 min at 80°C. Five milliliter of 1.05 N H_2SO_4 in saturated NaCl and 2.5 mL of deionized water were added to the hydrolysis mixture after cooling. At this stage, the pH of the mixture was checked. The acidified hydrolysate (pH 3) was extracted twice with 12 mL of the upper phase consisting of heptane:diethyl ether:ethanol:-deionized water (1:1:1:1, v/v). The extracts were dried under a stream of nitrogen and then added to 3 mL of ethanol and titrated with 0.01 mol/L NaOH using phenolphthalein as an indicator. The equivalence of NaOH to fatty acid was determined by estimating the volume of 0.01 mol/L NaOH used to neutralize 0.1 mmol of oleic acid in 3 mL of ethanol.

Permanent lymph duct cannulation

Male Sprague-Dawley rats (Kud:SD), aged eight weeks and obtained from Kyudo Co, were maintained in an air-controlled room. The rats were trained to consume the AIN-76 formula diet as a basal diet twice a day from 10:00 to 11:00 h and 16:00 to 17:00 h for five days. Deionized water was freely available throughout the feeding periods. All rats were anesthetized with Nembutal prior to permanent cannulation of the thoracic lymph duct as described previously.[25] A cannula (SH silicon tube, 0.5 mm inner diameter [i.d.] and 1.0 mm outer diameter [o.d.]; Kaneka Medics, Osaka, Japan) filled with heparinized saline was inserted into the thoracic duct and secured within the abdominal cavity. The rats were returned to their cages and fed a basal diet twice a day, as described. On the third day, the rats were attached to a long polyethylene cannula (0.58-mm i.d. and 0.97-mm o.d.; Becton Dickinson and Company, Sparks, MD, USA) to collect the lymph. The end of the cannula was placed at 5–10 cm below the bottom of the cage to allow the lymph to drain into the cannula. The lymph was collected for 20 min, and the rats were then given free access to the experimental diets for 30 min. Lymph was collected every hour for seven hours. After removing the fibrin, ethylenediaminetetraacetic acid at a final concentration of 0.01% was added to the lymph solutions. Each lymph solution was stored at −30°C for up to 12 h. The rats had free access to deionized water throughout the feeding periods and during lymph collection. This experiment was conducted under the Guidelines for Animal Experiments at Kyushu University (Fukuoka, Japan) and under Law no. 105 and Notification no. 6 of the Government of Japan. The authorization number was A19-195-0.

Lymphatic lipids transport

Lymphatic total cholesterol and triacylglycerol concentrations were measured using enzyme assay kits (Cholesterol E test and Triglyceride E test; Wako Pure Chemicals) similar to the analysis of serum lipids. The concentration of lymphatic phospholipids was determined using an enzyme assay kit (Phospholipid C test; Wako Pure Chemicals). If lymphatic lipid concentrations exceeded the range of a standard curve, the lymph was diluted with physiological saline.

α-Tocopherol levels in liver and lymph

The lymph solutions collected hourly were pooled into a total lymph solution in a ratio of the lymph solution volume at every hour. After lymphatic lipid extraction with hexane, α-tocopherol levels in the total lymph solution were determined by HPLC.[13] The column was packed with Zorbax SIL (5 μm, 4.6 × 250 mm; Agilent Technologies). α-Tocopherol in the liver lipid extract was determined in the same manner.

Preparation of saponin fraction (butanol-soluble fraction)

Saponin fractions (butanol-soluble fractions) of kakrol and bitter gourd were prepared according to the method of Oishi *et al.*[26] Freeze-dried kakrol and bitter gourd powders (50 g each) were combined with 70% methanol and maintained at room temperature for two days. The extracts were filtered, and the residues were combined with 70% methanol for re-extraction. The two extracts were combined, filtered, concentrated using a rotary evaporator and freeze-dried, and methanol extracts (kakrol: 12 g, 24%; bitter gourd: 10 g, 20%) were obtained. The extracts were dissolved in pure water and partitioned twice with diethyl ether, and the partitioned ether layers were removed. The water layers were partitioned twice with *n*-butanol, and the partitioned butanol layers were separated. The extracts were concentrated and dried under reduced pressure. Saponin fractions (kakrol: 0.94 g, 1.9%; bitter gourd: 0.99 g, 2.0%) were prepared as butanol-soluble components from the butanol layers.

Pancreatic lipase activity *in vitro*

Pancreatic lipase activity was measured according to the method of Han *et al.*[27] An emulsion (9 mL) of 80 mg trioleoylglycerol, 10 mg phosphatidylcholine and 5 mg sodium taurocholate in 0.1 mol/L *N*-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid (TES) buffer (pH 7.0) containing 0.1 mol/L sodium chloride was prepared by sonication and maintained at 37°C. A total of 100 μL of the emulsion was incubated at 37°C for 30 min with 5 U of porcine pancreatic lipase (Nacalai Tesque) solubilized in 0.1 mol/L TES buffer containing 0.1 mol/L sodium chloride and various concentrations of the *n*-butanol-soluble fractions from kakrol or bitter gourd (100 μL). Released fatty acids were extracted with chloroform:heptane:methanol (49:49:2, v:v:v) and measured colorimetrically at 480 nm.

Pancreatic lipase activity was calculated for each sample, and an absence of fatty acid production in the sample was assumed to indicate 100% activity.

Plant sterol content in kakrol and bitter gourd

Lipids in the kakrol and bitter gourd powders were extracted and purified by the method of Folch *et al.*[12] After saponification of the lipids, unsaponifiable matter was derivatized into trimethylsilyl ethers and quantified by gas–liquid chromatography on a Supelco SPB-1 column (0.25 μm, 0.25 mm $\times$ 60 m; Sigma-Aldrich Japan) using 5α-cholestane (Nacalai Tesque) as an internal standard.[22]

Statistical analysis

The data were expressed as means $\pm$ SEM and analyzed by Tukey–Kramer's multiple comparison *post hoc* test to identify differences among groups. Differences were considered significant at $P < 0.05$.

Results

Nutrient content of kakrol and bitter gourd

Nutrient contents and fatty acid composition of total lipids in kakrol and bitter gourd are represented in Table 1. It deserves special mention that the contents of ascorbic acid and unsaturated fatty acid ratio in kakrol flesh were higher than in bitter gourd. However, these differences were small in the diets.

Rat growth parameters

No differences in final body weights, food intakes or food efficiencies were found among the three groups (Table 2).

Table 1 Nutrient contents and fatty acid compositions of kakrol and bitter gourd*

	Kakrol	Bitter gourd
Crude protein (mg/g d.w.)	92.6	133.4
Ash (mg/g d.w.)	77.0	71.3
α-Tocopherol (μg/g d.w.)	121.5	196.1
β-Carotene (μg/g d.w.)	10.5	36.4
Ascorbic acid (mg/g d.w.)	25.1	11.0
Cholesterol (μg/g d.w.)	7.4	25.9
Total plant sterol (μg/g d.w.)	335.9	279.8
Campesterol (μg/g d.w.)	n.d.	27.1
Stigmasterol (μg/g d.w.)	n.d.	20.4
β-Sitosterol (μg/g d.w.)	251.1	195.5
Sitostanol (μg/g d.w.)	84.9	36.8
Total lipid (mg/g d.w.)	35.0	100.4
Fatty acid composition (wt %)		
Palmitic acid (16:0)	23.4	10.7
Stearic acid (18:0)	5.7	51.0
Oleic acid (18:1)	9.7	10.3
Linoleic acid (18:2)	43.3	13.5
α-Linolenic acid (18:3)	17.9	14.7

d.w., dry weight
*Mean values with three determinations

A major effect exhibited by rats fed on the bitter gourd diet was an increase in the relative total white adipose tissue weight. Comparison of the two diets showed that liver weight was significantly higher in rats fed on the kakrol diet than in rats fed on the bitter gourd diet.

Lipid concentration in serum and liver

No significant differences in serum lipid concentrations were found among the three groups (Table 2). Hepatic cholesterol concentrations decreased significantly in rats fed on the kakrol or bitter gourd diet compared with those fed on the control diet because of decreasing esterified cholesterol. Also, both the kakrol and bitter gourd diets significantly decreased hepatic triacylglycerol and α-tocopherol concentrations, and the kakrol diet significantly diminished hepatic phospholipid concentration.

Feces, fatty acid and bile acid excretion per day

A significant difference in feces' dry weights was found in rats fed on the kakrol and bitter gourd diets compared with those fed on the control diet; the kakrol and bitter gourd diets showed higher decreases in feces' dry weights than the control diet (1.42 ± 0.03, 1.78 ± 0.05 and 1.70 ± 0.02 g/d for the control diet, kakrol diet and bitter gourd diet, respectively). The kakrol diet markedly increased fatty acid excretion compared with the control and bitter gourd

Table 2 Growth parameters, organ weight and lipid concentrations in rat liver and serum*

	Control	Kakrol	Bitter gourd
Initial body weight (g)	199.4 ± 2.8	199.9 ± 2.3	199.8 ± 2.3
Final body weight (g)	284.3 ± 3.3	294.1 ± 3.4	281.2 ± 2.2
Food intake (g/d)	20.9 ± 0.0	20.9 ± 0.2	20.8 ± 0.0
Food efficiency (mg weight gain/g food intake)	290.6 ± 12.8	323.1 ± 18.1	278.9 ± 12.1
Relative tissue weights (g/100 g body weight)			
Liver	4.52 ± 0.17^{ab}	4.72 ± 0.12^{a}	4.12 ± 0.06^{b}
Total white adipose tissues†	2.86 ± 0.21^{a}	3.02 ± 0.12^{ab}	3.42 ± 0.11^{b}
Liver (mg/g liver)			
Cholesterol			
Total	3.24 ± 0.34^{a}	1.92 ± 0.09^{b}	2.46 ± 0.11^{b}
Free	1.92 ± 0.19	1.55 ± 0.08	1.67 ± 0.09
Esterified	1.32 ± 0.20^{a}	0.37 ± 0.11^{b}	0.78 ± 0.16^{ab}
Triacylglycerol	47.78 ± 11.72^{a}	20.48 ± 3.43^{b}	19.11 ± 2.37^{b}
Phospholipids	32.79 ± 0.64^{a}	28.92 ± 0.65^{b}	33.17 ± 0.80^{a}
α-Tocopherol (μg/g liver)	48.27 ± 6.38^{a}	20.35 ± 1.34^{b}	32.27 ± 2.81^{b}
Serum (mg/dL)			
Cholesterol			
Total	86.1 ± 5.7	78.6 ± 3.1	89.9 ± 5.1
HDL	55.6 ± 4.6	50.7 ± 1.6	57.5 ± 3.8
VLDL + LDL	30.5 ± 2.9	27.9 ± 1.6	32.4 ± 4.8
Triacylglycerol	213.5 ± 34.7	230.5 ± 26.0	251.9 ± 67.4

Different characters indicate significant difference at $P < 0.05$
*Mean values with their standard errors for six rats per group
†Total white adipose tissue weight is the sum of epididymal, mesenteric and perirenal plus retroperitoneal
HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein

diets (Figure 1). In addition, neutral steroid and bile acid excretions increased in rats fed on the kakrol diet compared with the control diet.

Lymphatic transport

Before and after the cannulation procedure, no differences were found in body weight or food intake (4.6 ± 0.5, 4.1 ± 0.4 and 4.4 ± 0.5 g for the control diet, kakrol diet and bitter gourd diet, respectively). No difference in lymph flow for seven hours was found among the three groups (11.3 ± 0.5, 10.7 ± 0.7 and 11.1 ± 0.7 mL for the control diet, kakrol diet and bitter gourd diet, respectively). Lymphatic triacylglycerol transport was markedly inhibited by the kakrol diet compared with the control and bitter gourd diets (Figure 2). Also, lymphatic phospholipid transport was inhibited by the kakrol diet. However, cholesterol transport for seven hours was equal among the three groups (5.56 ± 0.22, 4.78 ± 0.19 and 5.26 ± 0.46 mL for the control diet, kakrol diet and bitter gourd diet, respectively). We also measured α-tocopherol level in the pooled lymph for seven hours (13.43 ± 1.45, 8.26 ± 0.91 and 13.85 ± 0.83 μg/mL of lymph for the control diet, kakrol diet and bitter gourd diet, respectively). Significant differences were found between the control diet and the kakrol diet ($P < 0.05$) and between the kakrol diet and the bitter gourd diet ($P < 0.01$).

Pancreatic lipase activity *in vitro*

The amounts of *n*-butanol extract from kakrol and bitter gourd were the same (18.8 and 19.8 g/kg, respectively). We measured the inhibitory activity of pancreatic lipase derived from porcine pancreas *in vitro*. The *n*-butanol extract from kakrol powder inhibited lipase activity dose dependently, but the extract from bitter gourd did not (Figure 3).

Discussion

In the present study, we evaluated the effects of freeze-dried dietary kakrol powder on lipid metabolism in rats. Our results identified several medical properties of kakrol, including the inhibition of triacylglycerol and phospholipid intestinal absorption and subsequent reduction of hepatic triacylglycerol and cholesterol contents in rats. These effects most likely are the result of the inhibition of pancreatic lipase activity by *n*-butanol-soluble fractions from the powder. Moreover, triacylglycerol and phospholipid absorption was inhibited more effectively by the kakrol powder than by the bitter gourd powder. However, both powders lowered the hepatic levels of triacylglycerol and cholesterol equally well. Generally, when dietary triacylglycerol absorption is inhibited in this manner, plasma triacylglycerol levels in a postprandial state are attenuated. Fasting plasma triacylglycerol levels are used as a diagnosis criterion of hypertriacylglycerolemia. The fasting levels are associated with the risk of coronary artery disease (CAD).[28] Patsch *et al.* investigated the relation of triacylglycerol metabolism in the postprandial state and CAD in the

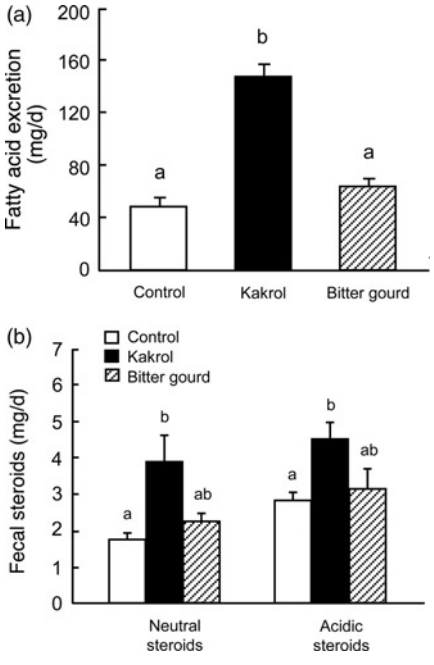

Figure 1 Fecal fatty acid and fecal steroid excretions in the rats fed on the control diet (blank bar), kakrol diet (solid bar), and bitter gourd diet (shaded bar). (a) Fatty acid excretion levels measured by titration. (b) Neutral steroid and acidic steroid excretion levels determined by gas chromatography. Values are means ± SEM, $n = 6$. Different characters indicate significant difference at $P < 0.05$

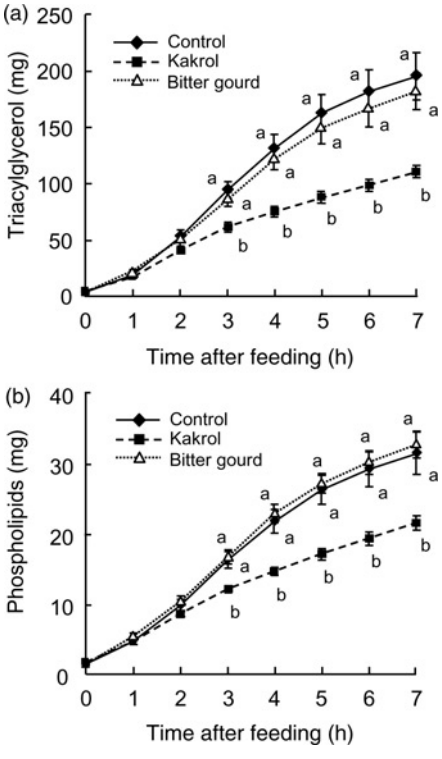

Figure 2 Cumulative transports of triacylglycerol (a) and phospholipids (b) into lymph in rats fed on the control diet (closed rhombus), kakrol diet (closed square) and bitter gourd diet (blank triangle). Mean values were significantly lower in rats fed on the kakrol diet than in rats fed on the control and bitter gourd diets at three hours after feeding. Values are means ± SEM, $n = 6$. Different characters indicate significant difference at $P < 0.05$

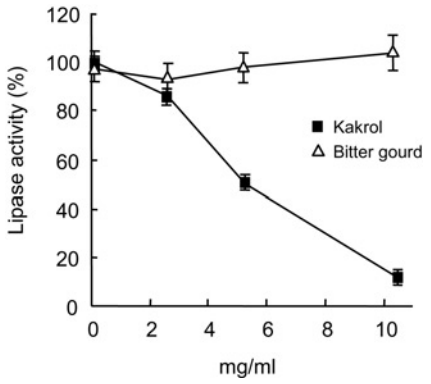

Figure 3 Effects of *n*-butanol extract from kakrol and bitter gourd powders on the activity of pancreatic lipase *in vitro* (closed square, *n*-butanol extract from kakrol powder; blank triangle, *n*-butanol extract from bitter gourd powder). Enzyme activity at 0 mg/mL was considered to be 100%. Values are means ± SEM, $n = 3$

case-control study. The plasma triacylglycerol levels in the CAD subjects after loading the test diet were significantly higher than in the control subjects.[29] They suggested that relative higher postprandial triacylglycerol levels lead to higher fasting plasma triacylglycerol levels. As the lymphatic triacylglycerol transport reduces with a subsequent decrease of plasma postprandial triacylglycerol levels, the hepatic triacylglycerol accumulation decreases.

Results of the permanent lymph cannulation and fatty acid excretion measurements in feces showed that the absorption of triacylglycerol and phospholipids, which are dietary fatty acid sources, was inhibited more effectively by kakrol than by bitter gourd. Plant components known to have the ability to decrease intestinal lipid absorption are polyphenols, saponins and plant sterols.[30,31] Fernandez *et al.*[32] showed that following lymph cannulation, 10^{-4} mol/L tetrahydrolipstatin as a lipase inhibitor prevents the transport of fat to thoracic duct chyle. Results showed that kakrol decreased α-tocopherol absorption in the lymph and its accumulation in the liver. Reboul *et al.*[33] reported that polyphenols do not affect α-tocopherol absorption. Hepatic α-tocopherol level is considered a suitable marker for triacylglycerol absorption.[34] In this study, the kakrol diet may inhibit absorption of α-tocopherol in the intestine, but the bitter gourd diet may inhibit accumulation of α-tocopherol in the liver. Thus, the mechanisms for decreasing α-tocopherol appear to be different between the two vegetables. In addition, saponin fractions from other plants, such as chikusetsusaponins,[31] have the ability to inhibit lipase activity. Figure 3 shows that a fraction rich in saponin *n*-butanol extract from kakrol powder inhibited pancreatic lipase activity. Activity per unit as a lipase inhibitor was more efficient in the kakrol extract than in the bitter gourd extract. We found that kakrol and bitter gourd both yielded similar amounts of *n*-butanol extract. Saponin structure may be related to inhibitory activity,[35] and the saponins in kakrol may be more potent than the saponins in bitter gourd. The nutrients in kakrol were different from those in bitter gourd. Specifically, kakrol contained about one-third of both the chloroform-soluble fraction of the lipid content and the β-carotene content compared with bitter gourd. Ascorbic acid content was twice as high in kakrol as in bitter gourd. However, these differences in basic nutrients, which comprised only 3% of the powders in the diets, may be too small to explain the effects in the study rats. Therefore, saponins may be the main components in powdered kakrol flesh that inhibit absorption of fatty acids. In order to determine the molecular species of saponins, we used the Liebermann–Burchard colorimetric assay[36] to investigate the *n*-butanol extracts from the kakrol and bitter gourd powders; no differences were found between the coloration and the color optical density (data not shown). Further studies are needed to identify the active components in the *n*-butanol extract from kakrol using the pancreatic lipase activity assay.

Senanayake *et al.*[2] reported that two of three varieties of bitter gourd lowered serum and liver triacylglycerol levels in rats fed a cholesterol-free diet but not serum and liver cholesterol levels. We used the Powerful-Reishi variety of bitter gourd, which showed the lowering effect in this study but not in Senanayake's report. Liver triacylglycerol content was 35.8 mg/g liver in Senanayake's report and 19.11 mg/g liver in our study, despite similarities in diets, breeding periods and rat strains as well as hepatic triacylglycerol contents in rats fed the control diet. Our study on bitter gourd has provided more operative data than Senanayake's report. Nutritional differences in food intakes were found between Senanayake's report and our studies;[2] the differences were approximately 5 or 7 kcal/d and 98 kcal per two weeks in a feeding period, which resulted in an increase in calories of approximately 25%. The reason for this difference may be that we used the paired-feeding method for all rats to ensure the same caloric intake. The AIN-76G rodent diet is high in sucrose (50% by weight). Several investigators found that increasing dietary fructose intake leads to hypertriglyceridemia under certain conditions[37] and may affect serum triacylglycerol levels. In fact, serum triacylglycerol levels were around 500 mg/dL in that report and around 200 mg/dL in our study. Hellerstein[38] reported that serum triacylglycerol level can be changed by dietary calories. Inconsistencies in the two studies may be the result of differences in harvest seasons and fruit production areas. As described in the introduction of this paper, pharmacological and food chemical industries have conducted more extensive research on bitter gourd flesh[2,39] and seeds[40] than on kakrol flesh and seeds.

Bitter gourd fruits are known to contain several active components for lipid metabolism: geranylgeraniol, a diterpene, which may be a PPARα ligand;[10] charantin, a sterol glycoside, which may have a hypoglycemic effect;[9] and plant sterols, which have a hypocholesterolemic effect. The main effect of plant sterols on lipid absorption is through the intestinal absorption of cholesterol via a stage of bile acid micellization.[41] However, plant sterol contents were lower in both the kakrol and bitter gourd powders than in the corn oil[42] used in the diets. The effects of geranylgeraniol were investigated using cell cultures;[10] geranylgeraniol acts inside of the body after intestinal absorption. Dietary bitter gourd powder did not inhibit intestinal absorption of dietary lipid according to the permanent lymph duct

cannulation data from this study. In summary, the effect of bitter gourd on liver triglyceride level, as discussed in Senanayake's[2] report, is through the intestinal absorption of compounds contained in the powder without the inhibition of lipid absorption. In addition, the bitter gourd powders changed the hepatic α-tocopherol status as described above. Sülzle *et al.*[43] reported that the dietary oxidized fats caused an activation of a series of target PPARα genes with a decrease in hepatic α-tocopherol levels. We represent a hypothesis that when hepatic triacylglycerol oxidation is promoted by PPARα activation by some ligands like geranylgeraniol, hepatic α-tocopherol levels may decrease. Understandably, α-tocopherol in hepatocytes is an antioxidant. Therefore, the decrease in the hepatic α-tocopherol involves in disappearance of lipid peroxides. In order to prove this hypothesis, we should perform an experiment by means of which hepatic α-tocopherol levels are measured when PPARα ligands like troglitazone and geranylgeraniol are administrated to rats.

Cholesterol metabolism was different between rats fed on kakrol diets and those fed on bitter gourd diets. Based on the increased excretion of cholesterol into feces, cholesterol absorption was inhibited by the kakrol diet but not by the bitter gourd diet. Also, the level of excretion of bile acids into feces increased in the rats fed the kakrol diet but not in the rats fed the bitter gourd diet. In the permanent lymph cannulation experiment, there was no difference in lymphatic cholesterol transport among the three diets. The reasons for the diets to not contain cholesterol and the rats to be fed only a single meal of the diet before lymph was collected in the lymph cannulation experiment may be questioned; in addition, because the intake of cholesterol was low, all cholesterol in the lymph was derived from the bile or intestinal cells. The amount of cholesterol transported in lymph was 5 mg for seven hours in this study. Tomoyori *et al.*[25] reported in a study performed in our laboratory that the transport level of cholesterol in lymph was around 10 mg under the same operation conditions, time schedules and food intakes for seven hours in rats fed on a 0.5% cholesterol diet. Therefore, the kakrol diet promotes the excretion of cholesterol and bile acid into feces and decreases liver cholesterol levels. Both diets decreased cholesterol levels in the liver but not in serum. The difference of serum cholesterol among the groups may be too low to be detected. Further experiments to reveal more effects of both vegetables should be conducted using rats fed on a diet containing cholesterol to induce hypercholesterolemia. Liver cholesterol content also decreased in the bitter gourd diet compared with the control diet despite the lack of differences in the neutral sterol content in feces. This decrease was not observed in Senanayake's report. Another study reports that the basic activity of liver X receptor α (LXRα), a nuclear receptor that plays an important role in the regulation of hepatic lipid metabolism,[44] is inhibited by geranylgeraniol contained in bitter gourd flesh. Thus, lipid synthesis may be downregulated by the compound. However, this result may not apply directly to this animal model. Considering the absorption and behavior in blood of this compound when taken orally, the compound should be administered to animals.

Thus, dietary kakrol powder containing components like saponin(s) prevents the development of fatty liver through the inhibition of intestinal lipid absorption as a lipase inhibitor in rats. Dietary bitter gourd powder does not inhibit lipid absorption. Therefore, the prevention of fatty liver development by dietary bitter gourd powder may be performed in different manners from kakrol powder.

Author contributions: Conception and design: MS, TU, KN, BS and KI; analysis and interpretation: MS, TU, KN, SS, HT, MK, YO and HO; data collection: MS, TU, KN, SS, HT, MK, YO and HO; writing the article: MS, KN, BS and KI; critical revision of the article: MS, TU and KN; final approval of the article: MS, TU, KN, SS, HT, MK, YO, HO, BS and KI; statistical analysis: MS, TU, KN and BS; overall responsibility: MS, TU, KN, SS, HT, MK, YO, HO, BS and KI.

REFERENCES

1 Jain A, Soni M, Deb L, Jain A, Rout SP, Gupta VB, Krishna KL. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. leaves. *J Ethnopharmacol* 2008; **115**:61–6

2 Senanayake GV, Maruyama M, Shibuya K, Sakono M, Fukuda N, Morishita T, Yukizaki C, Kawano M, Ohta H. The effects of bitter melon (*Momordica charantia*) on serum and liver triglyceride levels in rats. *J Ethnopharmacol* 2004;**91**:257–62

3 Lee KH, Choi HR, Kim CH. Anti-angiogenic effect of the seed extract of *Benincasa hispida* Cogniaux. *J Ethnopharmacol* 2005;**97**:509–13

4 Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management. *Hepatology* 2009;**49**:306–17

5 Gabuzda GJ. Fatty liver in man and the role of lipotropic factors. *Am J Clin Nutr* 1958;**6**:280–97

6 Kawada T, Hagihara K, Iwai K. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *J Nutr* 1986;**116**:1272–8

7 Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int J Obes Relat Metab Disord* 2002;**26**:1459–64

8 Han LK, Kimura Y, Kawashima M, Takaku T, Taniyama T, Hayashi T, Zheng YN, Okuda H. Anti-obesity effects in rodents of dietary teasaponin, a lipase inhibitor. *Int J Obes Relat Metab Disord* 2001;**25**:1459–64

9 Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*: a review. *J Ethnopharmacol* 2004;**93**:123–32

10 Takahashi N, Kawada T, Goto T, Yamamoto T, Taimatsu A, Matsui N, Kimura K, Saito M, Hosokawa M, Miyashita K, Fushiki T. Dual action of isoprenols from herbal medicines on both PPARgamma and PPARalpha in 3T3-L1 adipocytes and HepG2 hepatocytes. *FEBS Lett* 2002;**514**:315–22

11 Keller H, Mahfoudi A, Dreyer C, Hihi AK, Medin J, Ozato K, Wahli W. Peroxisome proliferator-activated receptors and lipid metabolism. *Ann NY Acad Sci* 1993;**684**:157–73

12 Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;**226**:497–509

13 Zommara M, Toubo H, Sakono M, Imaizumi K. Prevention of peroxidative stress in rats fed on a low vitamin E-containing diet by supplementing with a fermented bovine milk whey preparation: effect of lactic acid and beta-lactoglobulin on the antiperoxidative action. *Biosci Biotechnol Biochem* 1998;**62**:710–7

14 Kishi T, Carvajal O, Tomoyori H, Ikeda I, Sugano M, Imaizumi K. Structured triglycerides containing medium-chain fatty acids and linoleic acid differently influence clearance rate in serum of triglycerides in rats. *Nutr Res* 2002;**22**:1343–51

15 Roe JH, Mills MB, Oesterling MJ, Damron CM. The determination of kiketo-1-gulonic acid, dehydro-1-ascorbic acid, and 1-ascorbic acid in the

same tissue extract by the 2,4-dinitrophenylhydrazine method. *J Biol Chem* 1948;**174**:201–8
16 Carvajal O, Nakayama M, Kishi T, Sato M, Ikeda I, Sugano M, Imaizumi K. Effect of medium-chain fatty acid positional distribution in dietary triacylglycerol on lymphatic lipid transport and chylomicron composition in rats. *Lipids* 2000;**35**:1345–51
17 American Institute of Nutrition. Report of the American Institute of nutrition ad hoc committee on standards for nutritional studies. *J Nutr* 1977;**107**:1340–8
18 Sperry WM, Webb M. A revision of the Schoenheimer-Sperry method for cholesterol determination. *J Biol Chem* 1950;**187**:97–106
19 Fletcher MJ. A colorimetric method for estimating serum triglycerides. *Clin Chim Acta* 1968;**22**:393–7
20 Wootton DG. Semiautomated spectrophotometry of total phospholipids in plasma. *Clin Chem* 1977;**23**:1766–8
21 Uchida K, Nomura Y, Kadowaki M, Takase H, Takano K, Takeuchi N. Age-related changes in cholesterol and bile acid metabolism in rats. *J Lipid Res* 1978;**19**:544–52
22 Ikeda I, Nakagiri H, Sugano M, Ohara S, Hamada T, Nonaka M, Imaizumi K. Mechanisms of phytosterolemia in stroke-prone spontaneously hypertensive and WKY rats. *Metabolism* 2001;**50**:1361–8
23 van de Kamer JH, ten Bokkel Huinink H, Weyers HA. Rapid method for the determination of fat in feces. *J Biol Chem* 1949;**177**:347–55
24 Jeejeebhoy KN, Ahmad S, Kozak G. Determination of fecal fats containing both medium and long chain triglycerides and fatty acids. *Clin Biochem* 1970;**3**:157–63
25 Tomoyori H, Carvajal O, Nakayama M, Kishi T, Sato M, Ikeda I, Imaizumi K. Lymphatic transport of dietary cholesterol oxidation products, cholesterol and triacylglycerols in rats. *Biosci Biotechnol Biochem* 2002;**66**:828–34
26 Oishi Y, Sakamoto T, Udagawa H, Taniguchi H, Kobayashi-Hattori K, Ozawa Y, Takita T. Inhibition of increases in blood glucose and serum neutral fat by Momordica charantia saponin fraction. *Biosci Biotechnol Biochem* 2007;**71**:735–40
27 Han LK, Takaku T, Li J, Kimura Y, Okuda H. Anti-obesity action of oolong tea. *Int J Obes Relat Metab Disord* 1999;**23**:98–105
28 Hulley SB, Rosenman RH, Bawol RD, Brand RJ. Epidemiology as a guide to clinical decisions. The association between triglyceride and coronary heart disease. *N Engl J Med* 1980;**302**:1383–9
29 Patsch JR, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, Gotto AM Jr, Patsch W. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler Thromb* 1992;**12**:1336–45
30 Nakai M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, Mitsunaga T, Hashimoto F, Kiso Y. Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. *J Agric Food Chem* 2005;**53**:4593–8
31 Han LK, Zheng YN, Yoshikawa M, Okuda H, Kimura Y. Anti-obesity effects of chikusetsusaponins isolated from *Panax japonicus* rhizomes. *BMC Complement Altern Med* 2005;**5**:9
32 Fernandez E, Borgström B. Effects of tetrahydrolipstatin, a lipase inhibitor, on absorption of fat from the intestine of the rat. *Biochim Biophys Acta* 1989;**1001**:249–55
33 Reboul E, Thap S, Perrot E, Amiot MJ, Lairon D, Borel P. Effect of the main dietary antioxidants (carotenoids, gamma-tocopherol, polyphenols, and vitamin C) on alpha-tocopherol absorption. *Eur J Clin Nut* 2007;**61**:1167–73
34 Bieri JG, Wu AL, Tolliver TJ. Reduced intestinal absorption of vitamin E by low dietary levels of retinoic acid in rats. *J Nutr* 1981;**111**:458–67
35 Pillion DJ, Amsden JA, Kensil CR, Recchia J. Structure-function relationship among Quillaja saponins serving as excipients for nasal and ocular delivery of insulin. *J Pharm Sci* 1996;**85**:518–24
36 Ng TB, Li WW, Yeung HW. A steryl glycoside fraction with hemolytic activity from tubers of Momordica cochinchinensis. *J Ethnopharmacol* 1986;**18**:55–61
37 Hill P. Effect of fructose on rat lipids. *Lipids* 1970;**5**:621–7
38 Hellerstein MK. Carbohydrate-induced hypertriglyceridemia: modifying factors and implications for cardiovascular risk. *Curr Opin Lipidol* 2002;**13**:33–40
39 Huang HL, Hong YW, Wong YH, Chen YN, Chyuan JH, Huang CJ, Chao PM. Bitter melon (*Momordica charantia* L.) inhibits adipocyte hypertrophy and down regulates lipogenic gene expression in adipose tissue of diet-induced obese rats. *Br J Nutr* 2008;**99**:230–9
40 Sathishsekar D, Subramanian S. Beneficial effects of *Momordica charantia* seeds in the treatment of STZ-induced diabetes in experimental rats. *Biol Pharm Bull* 2005;**28**:978–83
41 Ikeda I, Tsuda K, Suzuki Y, Kobayashi M, Unno T, Tomoyori H, Goto H, Kawata Y, Imaizumi K, Nozawa A, Kakuda T. Tea catechins with a galloyl moiety suppress postprandial hypertriacylglycerolemia by delaying lymphatic transport of dietary fat in rats. *J Nutr* 2005;**135**:155–9
42 Ellegård LH, Andersson SW, Normén AL, Andersson HA. Dietary plant sterols and cholesterol metabolism. *Nutr Rev* 2007;**65**:39–45
43 Sülzle A, Hirche F, Eder K. Thermally oxidized dietary fat upregulates the expression of target genes of PPAR alpha in rat liver. *J Nutr* 2004;**134**:1375–83
44 Steffensen KR, Gustafsson JA. Putative metabolic effects of the liver X receptor (LXR). *Diabetes* 2004;**53**:S36–S42

(Received February 2, 2011, Accepted May 5, 2011)