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Glucose-lowering Effect of Powder Formulation of African Black Tea Extract in KK-A^y/TaJcl Diabetic Mouse

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We observed the suppressive effect of a powder formulation of African black tea extract prepared from the leaves of *Camellia sinensis* on type 2 non-insulin dependent diabetic mice, KK-A^y/TaJcl. Black tea extract significantly showed suppressive effect of the elevation of blood glucose on oral glucose tolerance test of 8 week-old KK-A^y/TaJcl mice (P<0.05). Long-term treatment with black tea extract showed significant suppression of post-prandial blood glucose and obesity (P<0.05). The weight of the intestine of mice treated with black tea extract was significantly reduced (P<0.05). From these results, African black tea used in this study showed a suppressive effect on the elevation of blood glucose during food intake and the body weight.

Key words: Black tea extract, Glucose-lowering, Theaflavin, Tea polyphenol, Adipocyte

INTRODUCTION

Tea, which originates from *Camellia sinensis*, contains a variety of biologically active components including tea polyphenols, vitamins, proteins and minerals. Tea is widely consumed in many countries on a daily basis. Beyond daily consumption of tea as a beverage, tea has drawn much attention from the health benefit point of view. Tea polyphenols have been demonstrated to have a variety of physiological functions such as antibacterial (Arakawa *et al.*, 2004), antioxidative (Leung *et al.*, 2001), antiviral (Nakayama *et al.*, 1993), anticarcinogenic (Kuroda *et al.*, 1999), and antimutagenic activity (Kuroda *et al.*, 1999). It has been reported that green tea has a glucose-lowering effect in animal models (Crespy and William, 2004; Shirai and Suzuki, 2004; Tsuneki *et al.*, 2004; Wu *et al.*, 2004). Epidemiologic studies suggest that the habit of drinking green tea prevents type 2 non-insulin dependent diabetes mellitus (NIDDM). The results of oral glucose tolerance test (OGTT) in healthy volunteers were improved by drinking of a suspension of green tea powder (Tsuneki *et al.*, 2004). Since epigallocatechin gallate (EGCG) is the most abundant tea polyphenol in green tea, EGCG and epicatechin (EC), which is structurally similar to EGCG, have been actively investigated in relation to elucidating the mechanism of the hypoglycemic activity of green tea (Kao *et al.*, 2000; Waltner-Law *et al.*, 2002).

Although oolong tea and black tea contain less catechin, they also have a glucose-lowering effect (Gomes *et al.*, 1995; Hosoda *et al.*, 2003). Hosoda *et al.* (2003) investigated the lowering effect of oolong tea on blood glucose in NIDDM patients in a randomized crossover clinical trial. They suggested that oolong tea might be effective as an oral hypoglycemic agent for NIDDM (Hosoda *et al.*, 2003). Black tea extract also significantly reduced the blood glucose level in streptozotocin (STZ)-induced diabetic rats (Gomes *et al.*, 1995). Since STZ destroys β-islets of the pancreas, this model does not accurately reflect human NIDDM. To our knowledge, no report has seriously investigated the effect of black tea on NIDDM. In this study, we used male KK-A^y/TaJcl mice, which are the most suitable polygenic mice model of NIDDM with obesity (Suto *et al.*, 1998), to examine the effect of black tea extract on the glucose level.

Since NIDDM is attributed to insulin resistance (Taylor, 1999), a major treatment strategy for diabetes is sensitization of the insulin response (Hermann, 2000; Lazar, 2005; Yale *et al.*, 2001). It has been suggested that TNF-α in adipocytes may play a central role in insulin resistance (Halle *et al.*, 1998; Ruan and Lodish, 2003; Winkler *et al.*,

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2003). At present, maintaining normal blood glucose levels is thought to be the best way to control NIDDM. Several orally active hypoglycemic drugs such as inhibitors of digestive enzymes and thiazolidinediones, insulin sensitizers, are clinically available (Rosak, 2002). Pioglitazone, a new type of antidiabetic thiazolidinediones, enhances insulin sensitivity (Tolman *et al.*, 2004). Pioglitazone enhances the differentiation of 3T3-L1 pre-adipocytes (Takamura *et al.*, 2001). It has been proposed that thiazolidinediones enhance small-size adipocytes and replace large-size adipocytes with small ones (Okuno *et al.*, 1998; Kadowaki, 2000; Yamauchi *et al.*, 2001). Other data have shown that tea polyphenols may increase insulin activity (Anderson and Polansky, 2002). We investigated the effect of black tea extract on the insulin-induced differentiation of 3T3-L1 pre-adipocytes to examine whether]black tea extract would have a similar action to an insulin sensitizer.

MATERIALS AND METHODS

Materials and animals

Male KK-A^y/TaJcl mice (Clea Japan, Inc.) were used as a model of NIDDM with obesity (Suto *et al.*, 1998). The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of St. Marianna University, School of Medicine, Japan. Mice were maintained at an ambient temperature of 25°C under a photoperiod of 12 h of light and 12 h of darkness. They were fed a standard mouse diet and water was administered *ad libitem*.

Pioglitazone hydrochloride, an orally active hypoglycemic agent, was purchased from Sigma (MO, U.S.A.). Insulin, dexamethasone, and 3-isobutyl-methylxanthine were also purchased from Sigma (MO, U.S.A.).

Preparation of tea extract

Black tea extract powder was supplied by Sunfield Co. Inc. (Fukuoka, Japan). Briefly, the black tea leaves of *Camellia sinensis* harvested in Africa were extracted by hot water (80°C) and sterilized at 90°C for 1 h. Thereafter, the extract was sprayed and dried at a temperature of 200°C. The dried material was mechanically ground to make a powder formulation. This formulation was stored at -20°C until use.

Oral glucose tolerance test (OGTT)

Eight-week-old mice were starved one-day prior to the OGTT. First, mice were orally given 10 mg/kg black tea extract and control mice were administered water. Four hours later, they were orally administered 2 mg/kg glucose. Blood sampling was carried out from a tail vein at 0, 30, 60, 120 min after glucose administration. The glucose levels in whole blood were measured by the glucose oxidase method (Glucocard™, Aventis Pharma). After 3 months of long-term administration of black tea, the same OGTT was carried out.

Long-term treatment with tea extract in KK-A^y/TaJcl

Mice were divided into four groups for long-term administration of black tea extract. The control group was administered water, and the other groups were administered 5 mg/kg, 10 mg/kg, or 50 mg/kg of the powder formulation of black tea extract dissolved in water. The test materials were orally administered for 4 weeks. Postprandial blood glucose levels were tested once a week. Fasting blood glucose level was also measured one day after of starvation. At the same time, the amount of drinking water was measured. Body weight was monitored every day.

Biochemical analysis and histological observation of tissue after long-term treatment

After long-term administration of black tea extract, whole blood was collected under anesthesia with diethyl ether. The blood was centrifuged and serum was collected for measurement of insulin, cholesterol, triglyceride, and total lipid levels. Heart, lung, spleen, kidney, stomach, pancreas, small intestine, and fat were removed and fixed with 10% buffered formalin. Thin sections were made from paraffin blocks and eosin staining was performed. Morphological changes were observed under a light microscope.

Adipogenesis

3T3-L1 pre-adipocytes (Registered number JCRB9014, Health Science Research Resources Bank, Oosaka, Japan) were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) in 96-well flat-bottomed multiwell plates. Two days' post-confluence, cells were induced to differentiate into adipocytes by changing the medium to DMEM supplemented with 10% FCS, 1 μg/mL insulin, 0.25 μM dexamethasone, and 0.5 mM 3-isobutyl-methylxanthine as an adipogenesis induction mixture, according to the method previously described elsewhere (Tzameli *et al.*, 2004). Tea extract was added with or without adipogenesis induction mixture. Two days after induction, the medium was changed to DMEM supplemented with 10% FCS containing 1 μg/mL insulin. The medium and black tea extract were changed every other day. At 7~10 days after differentiation, lipid accumulation was colorimetrically measured by oil-red-O staining method as a marker of differentiation. Briefly, the supernatant was aspirated and each well was washed with PBS three times. Each well was fixed with 4% paraformaldehyde at 4°C for 1 h. After aspiration of paraformaldehyde from

each well, it was allowed to dry. Then 50 μL of 15 mg/mL oil-red-O staining solution was added to each well and allowed to stand at room temperature for 15 min. Each well was washed with 50 μL distilled water three times. Oil-red-O staining was extracted with 50 ml isopropanol and the absorbance at 570 nm was measured colorimetrically.

Statistical analysis

All results were expressed as Means ± S.D. Comparisons between control and treated mice were performed by one-way ANOVA or two-ways ANOVA. Significance was defined as a P value less than 0.05. All analyses were performed using the GraphPad PRISM® version 4 statistical program (GraphPad Software Inc., San DiegoCA, U.S.A.).

RESULTS

Effect of short-term treatment with black tea extract on glucose tolerance test

Blood glucose level in the control group was elevated within 30 min after the administration of glucose to mice. Blood glucose level had nearly recovered to the original level after 120 min, but was still higher than the original level. Blood glucose level in mice treated with black tea extract was also elevated 30 min after glucose administration. However, as shown in Fig. 1, the blood glucose level in mice treated with 10 mg/kg black tea extract was significantly (P<0.05) suppressed at each time point of blood sampling compared to that in control mice. As shown in Fig. 1, the area under the curve (AUC) in mice treated with black tea extract was significantly (P<0.05) smaller than that in control. The elevation of blood glucose in mice treated with 5 mg/kg was slightly suppressed but the effect was not statistically significant (data not shown).

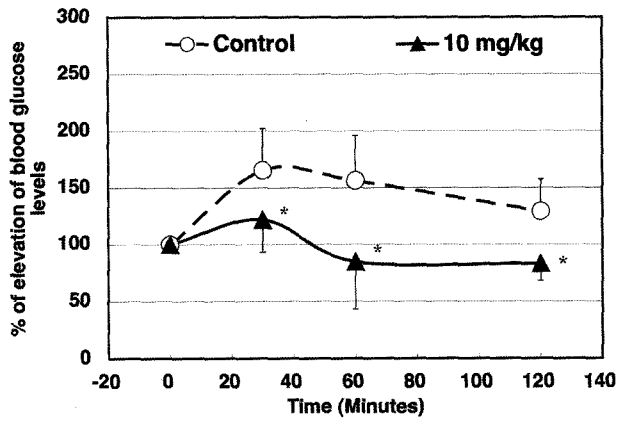

Fig. 1. Suppressive effect of black tea extract on OGTT in 8-week-old KK-A^y/TaJcl mice. Mice were administrated black tea extract. 4 h later, 2 mg/kg glucose was administered to mouse. The blood glucose levels were monitored at 0,30,60,120 min after glucose administration by Glucocard™. Elevation of blood glucose levels was expressed as the percentage of the initial glucose level of each mouse. Blood glucose levels in mice treated 10 mg/kg black tea extract were significantly lower than those in control at each time point. AUC in the control group was significantly (P<0.05) smaller than that in the group treated with 10 mg/kg black tea extract. *: P<0.05 vs. control.

Effect of long-term treatment of black tea extract on glucose tolerance test

After 3 months' treatment with black tea extract, OGTT was performed. As shown in Fig. 2, the blood glucose level in the control group was elevated after 120 min and it did not recover to the original level. The elevation of blood glucose level in mice treated with a low dose of tea extract was not changed compared to that of control. On the contrary, the elevation of blood glucose level in mice treated with a high dose of tea extract (50 mg/kg) was slightly suppressed, but the effect was not statistically significant.

Effect of long-term treatment with tea extract on blood glucose

In the next experiment, the glucose-lowering effect of long-term treatment with tea extract was investigated. Generally, KK-A^y/TaJcl mice develop diabetes at 8-10 weeks of age, with high blood glucose and obesity. The experiment started from 8 weeks age. Post-prandial blood glucose levels in the control mice increased during the experiment, as shown in Fig. 3. The blood glucose levels in mice treated with black tea extracts were suppressed, especially at a dose of 50 mg/kg. At each time point, the blood glucose level in the group treated with black tea

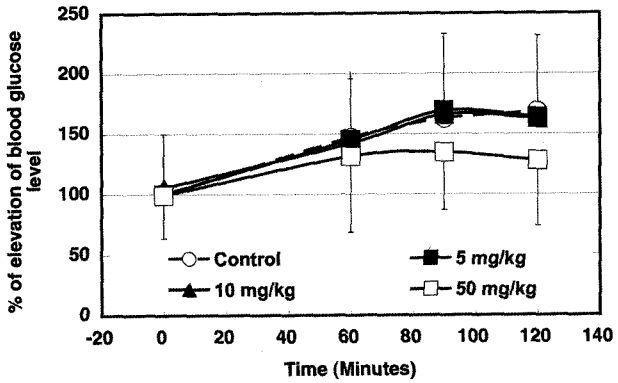

Fig. 2. OGTT in 20-week-old KK-A^y/TaJcl mice. Mice were administered black tea extract for 4 weeks. Glucose 2 mg/kg was administered to mice. The blood glucose levels were monitored at 0, 60, 90, 120 min after the glucose administration by Glucocard™. Elevation of blood glucose levels was expressed as the percentage of the initial glucose level of each mouse. AUC in the control group was slightly smaller than that in the group treated with 50 mg/kg black tea extract, but the difference was not significant.

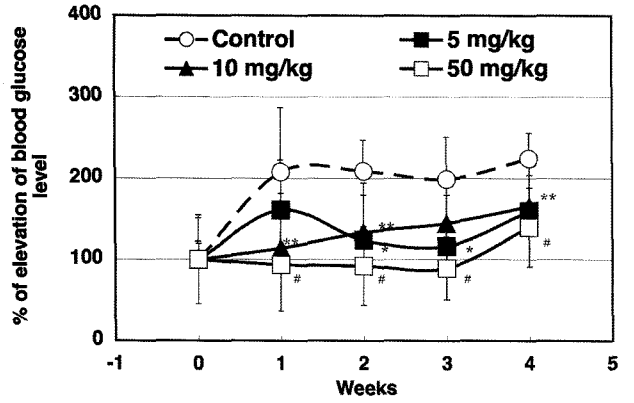

Fig. 3. Changes of post-prandial blood glucose levels during long-term treatment with black tea extract. Male KK-A^y/TaJcl mice were administered black tea extract (5, 10, 50 mg/kg) in each group. Post-prandial blood glucose levels were monitored once a week by Glucocard™. Elevation of blood glucose level was expressed as the percentage of the initial glucose level. The blood glucose levels in the group treated with black tea extract were significantly suppressed at each time point. AUC of blood glucose in mice treated with black tea extract at a concentration of 5, 10, 50 mg/kg was significantly (P<0.05) smaller than that in control. *: P<0.05 for 5 mg/kg vs. control; **: P<0.05 for 10 mg/kg vs. control; # : P<0.05 for 50 mg/kg vs. control.

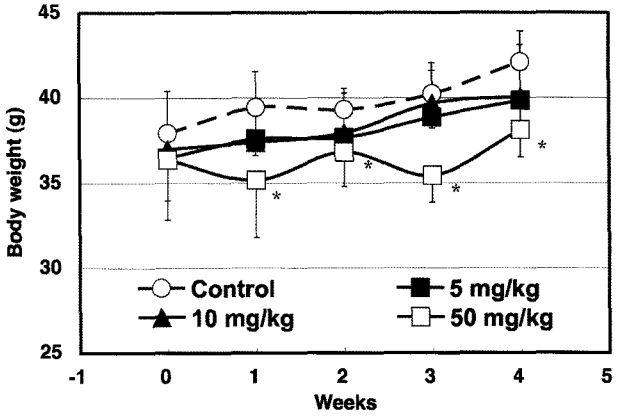

Fig. 4. Changes in body weight during long-term treatment of tea extract. Male KK-A^y/TaJcl mice were divided into four groups for long-term treatment with black tea extract. The control group was administered water, and the other groups were administered 5, 10, or 50 mg/kg of powder formulation of black tea extract dissolved in water. Body weight of mice treated with 50 mg/kg black tea extract was significantly suppressed compared to the control group (P<0.05). The body weight of the other groups was slightly suppressed but the difference was not significant.

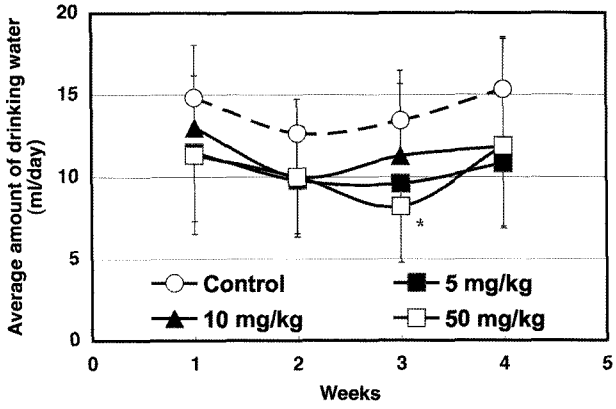

Fig. 5. Water intake during long-term treatment with tea extract. Male KK-A^y/TaJcl mice were divided into four groups for long-term treatment with black tea extract. The control group was administered water, and the other groups were administered 5, 10, or 50 mg/kg of powder formulation of black tea extract dissolved in water. The amount of drinking water was monitored once a week. The amount of drinking water in mice treated with black tea extract was slightly lower than that in control, but the difference was not significant. Only at the 3rd week, the water intake of mice treated with 50 mg/kg black tea extract was significantly lower than that in control. *: P<0.05 vs. control.

extract was significantly suppressed compared to that of control. 4 weeks after the treatment, blood glucose level in the mice treated with black tea extract started to slightly increase. The AUC in mice treated with black tea extract at a dose of 5, 10, and 50 mg/kg was significantly (P<0.05) smaller than that in control mice. Fasting blood glucose levels in mice treated with black tea extract was slightly suppressed compared to that in control mice, but the difference was not significant (data not shown).

Effect of long-term treatment with tea extract on body weight and drinking water intake

Fig. 4 shows the changes in body weight after long-term treatment with black tea extract. The body weight of mice in the control group gradually increased during the experiment. The body weight of mice treated with 50 mg/kg was significantly less than that of control mice at each time point (P<0.05). The increase of body weight in other groups was suppressed compared to the increase in control, but the change was not significant. Fig. 5 shows the changes in amount of drinking water during long-term treatment with black tea. Throughout the experiment, there was no significant change in the amount of drinking water, except in mice treated with 50 mg/kg black tea extract in the 3rd week.

Insulin, total lipid, and cholesterol levels after long-term treatment with black tea extract

In addition to its glucose-lowering effects, black tea may have cholesterol-lowering activity. After 3 months of treatment with black tea extract, mice were sacrificed under the excess anesthesia and whole blood was collected. Plasma was used for measurement of insulin, cholesterol, triglyceride, and total lipid levels. As shown in Table I, none of them was significantly changed. In fact, cholesterol level in mice treated with tea extract was slightly higher than that in control, but the difference was not significant.

Table I. Changes of biological makers after long-term treatment with black tea extract in male KK-A^y/TaJcl mice

Tea extract (mg/kg)	Insulin (ng/mL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Total lipid (mg/dL)
0	2.034±0.154	85.50± 4.80	85.00±21.37	348.75±19.81
5	2.400±0.646	100.70±11.09	72.75±30.76	398.50±64.23
10	1.450±0.773	94.50±19.23	80.25±49.16	396.75±98.21
50	1.580±0.885	106.20±12.28	80.75±41.15	397.25±75.60

3 months after treatment of black tea extract, mice were sacrificed and the whole blood was collected. Plasma was used for the measurement of insulin, cholesterol, triglyceride and total lipid were measured. None of them was significantly reduced.

Organ weights after long-term treatment with black tea extract

Table II shows the organ weights after long-term treatment with black tea extract. The weights of most organs were not changed. Fat in the group treated with a high dose of black tea extract was suppressed, but the difference was not significant. However, regarding the individual changes, body weight paralleled the decrease in fat weight. The weight of the spleen in mice treated with 5 mg/kg black tea extract was significantly suppressed (P<0.05). The average weight of the small intestine was significantly reduced at dose of 5 (P<0.01), 10 (P<0.05), and 50 mg/kg (P<0.01) compared to control.

Table II. Changes on organ weight of mice treated with black tea

Black tea (mg/kg)	Body weight (g)	Organ weight (mg)							
		Heart	Lung	Liver	Spleen	Kidney	Stomach	Intestine	Fat
0	42.1 ± 1.8	168.1 ± 11.2	237.1 ± 68.5	2082 ± 179	142.4 ± 24.8	550.8 ± 130.2	390.2 ± 72.3	2487 ± 582	2794 ± 889
5	39.9 ± 3.3	169.6 ± 21.1	234.8 ± 79.8	1646 ± 387*	88.1 ± 9.3**	518.2 ± 84.5	248.6 ± 42.3**	1455 ± 448*	2507 ± 1813
10	40.1 ± 2.2	160.0 ± 13.2	200.0 ± 30.3	2027 ± 124	230.3 ± 215.2	624.3 ± 48.5	343.7 ± 75.9	1555 ± 577*	2171 ± 454
50	38.1 ± 1.6	164.5 ± 17.8	221.4 ± 36.6	1916 ± 232	122.4 ± 46.7	595.5 ± 24.0	377.5 ± 70.2	1617 ± 116**	2130 ± 776

*: P<0.05, **: P<0.01 vs. control

3 months after the treatment of black tea extract, mice were sacrificed under the excess anesthesia and organs were collected. The weight of most of tissues of mice treated black tea extract did not change. Compared to the control group, the weight of liver (P<0.05), spleen (P<0.05), stomach (P<0.01), and intestine (P<0.05) in mice treated with 5 mg/kg black tea extract was significantly reduced. Regarding the weight of intestine, mice treated with black tea extract at 5, 10, and 50 mg/kg of black tea extract were significantly reduced. The weight of fat of mice treated with black tea extract slightly decreased, but the difference was not significant.

Table III. Morphological findings of KK-A^y/TaJcl mice after the long-term treatment with black tea extract

Organ	Findings		Control	5 mg/kg	10 mg/kg	50 mg/kg
Liver	Fatty change, hepatocyte	centrilobular	+++	+++	++	+++
	〃	intermediate zone	-	-	-	-
	Hepatocellular necrosis	focal	-	-	-	-
	Ossification	focal	-	-	-	-
Kidney	Basophilic change, renal tubules		+	+	+	+
	Extramedullary hematopoiesis		-	-	+	-
	Mesangial expansion		+	+	+	+
	Pelvis dilatation		-	-	-	-
Lung	Accumulation, foamy macrophage		-	-	-	-
	Foreign body aspiration		-	-	-	-
	Granuloma		-	-	-	-
	Hemorrhage		+	+	-	+
	Inflammatory cell infiltration		-	+	-	-
	Muscular hypertrophy, pulmonary artery		+	+	+	+
	Ossification		-	-	-	-
Spleen	Increase, extramedullary		-	-	-	-
Heart	Calcium deposition		++	+	-	-
	Fibrosis, interstitium		-	-	-	-
	Lipid accumulation		-	-	-	-
	Myocardial degeneration		++	+	+	+
Stomach	Glandular atrophy		-	-	-	-
	Inflammatory cell infiltration		-	-	-	-
Small intestine	No abnormality					
Pancreas	Lipid accumulation		+	+	+	

-: negative, +: slight, ++: moderate, +++: severe

Morphological changes in tissue section

An overall summary of the morphological changes in each tissue after long-term treatment with black tea extract is listed in Table III. Since KK-A^y/TaJcl mice develop diabetes spontaneously, most morphological findings were related to the typical histopathological features of diabetes. Lipid accumulation was observed in the centrilobular and intermediate zones of liver, both in control and treated mice. Long-term treatment with black tea extract did not affect the morphological changes except for calcium deposition in the heart. Calcium deposition was not observed in the heart of mice treated with black tea at a high dose (50 mg/kg). A low dose of black tea slightly reduced the deposition of calcium in the heart. There were no memorable morphological changes in the pancreas.

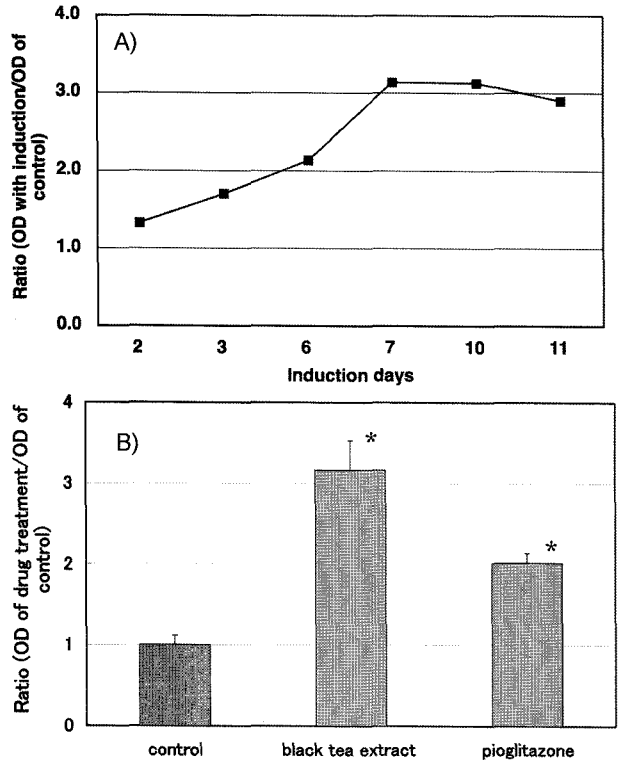

Fig. 6. Effect of black tea extract on adipogenesis. 3T3-L1 pre-adipocytes were incubated with adipogenesis reagents (IB, Dex, Ins) from day 0~2. The medium was changed to include only 1mg/ml insulin. The medium was every other day. After adipogenesis, oil-red-O was extracted with isoprapanol and measured absorbance at 570 nm. Data were expressed as the ratio against the differentiated 3T3-L1 cells with adipogenesis reagents only. Data were presented as Mean ± S.D. When cells with adipogenesis reagent showed more than 2-fold higher absorbance compared to that of non-treated cells, they were defined as differentiated. A) Time course of adipogenesis of 3T3-L1 pre-adipocyte. From the time course of adipogenesis, drug treatment was carried out for 7~10 days. B) Effect of black tea extract on insulin-induced differentiation of 3T3-L1 pre-adipocytes. Drug treatment started from day 0 for black tea extract (8 ng/ml) and from day 2 for pioglitazone (25 mM). Data were expressed as the percentage of the non-treated cells with adipogenesis reagents. *: P<0.01 DEX: dexamethazone, IB: 3-isobuthyl-methylxanthine, Ins: insulin.

Adipogenesis

Fig. 6 shows the effect of black tea extract on adipogenesis in 3T3-L1 pre-adipocytes. Adipogenesis reagent and black tea extract were added to 3T3-L1 pre-adipocytes simultaneously from day 0~11. Compared to the control cells, many lipid droplets were observed after differentiation. In 3T3-L1 cells treated with black tea extract from day 0 of adipogenesis, many lipid droplets accumulated from day 2~11. The time course of lipid accumulation during adipogenesis in 3T3-L1 pre-adipocytes stained with oil-red-O was shown in Figure 6A. Differentiation was defined as two-fold greater lipid accumulation in 3T3-L1 pre-adipocytes compared to control. As shown in Fig. 6A, 3T3-L1 pre-adipocytes differentiated after 5 days and reached a plateau at 7 days. As shown in Figure 6B, black tea extract (8 ng/mL) significantly enhanced adipogenesis (P<0.01). At the same time, black tea extract itself stimulated the differentiation of 3T3-L1 to adipocytes without insulin (data not shown). As a control, Pioglitazone (25 μM) was added to 3T3-L1 pre-adipocytes from day 2 of differentiation. Pioglitazone also significantly enhanced adipogenesis compared to control (P<0.01). It has been reported that black, oolong, and green tea increased insulin activity in an *in virto* assay (Anderson, Polansky, 2002). These data suggest that black tea extract could play a role in the induction of differentiation of pre-adipocytes.

DISCUSSION

Consumption of green tea substantially improves insulin resistance and regulates the glucose transporter IV (Tzameli *et al.*, 2004). While the glucose-lowering effect of EGCG in green tea has been actively investigated, less is known about the biological activities of black tea compared to other tea polyphenols. The content of polyphenols may vary depending on the extraction or brewing conditions. To avoid variation in each experiment, first of all we made a powder formulation of tea extract from the leaves of *Camellia sinensis*. The amount of polyphenols in a powder formulation of black tea extract is consistent when the solution is freshly prepared. In this study, we used a powder formulation to investigate the short- and long-term glucose-lowering effect of black tea extract in a NIDDM model. Gomes *et al.* (1995) reported that black tea reduced the blood glucose level on STZ-induced diabetes in rats. However, STZ-induced diabetes does not reflect human NIDDM. To our knowledge, few reports demonstrated the glucose-lowering effect of black tea on NIDDM. Therefore, we assessed the glucose-lowering effect of black tea extract on a NIDDM model, KK-A^y/TaJcl

mouse.

In the first experiment, we tested the powder formulation of African black tea extract prepared from the fresh leaves of *Camellia sinensis* to observe the glucose-lowering effect in KK-A^y/TaJcl mice. We observed that African black tea extract suppressed the elevation of blood glucose levels in KK-A^y/TaJcl mice with continuous food access. As shown in Fig. 1, short-term treatment with black tea extract in 8-week-old mice significantly suppressed the glucose levels in OGTT at each time point of measurement. Compared to the AUC of blood glucose levels in control mice, the AUC in mice treated with 10 mg/kg black tea extract was significantly reduced ($P< 0.05$). After long-treatment with black tea extract, the same OGTT was carried out in 20-week-old mice. Only mice treated with 50 mg/kg black tea extract showed lowering of the elevation of blood glucose; however, the difference was not significant (Fig. 2).

As shown in Fig. 3, blood glucose levels of mice treated with 5, 10 or 50 mg/kg was significantly reduced but blood glucose levels started slightly increasing from the 4th week. Black tea extract contains caffeine (approximately 7 g/100 g tea powder) as well as theaflavin, based on HPLC data. The long-term efficacy of caffeine for glucose-lowering is still unknown; long-term administration of caffeine could have a negative effect on the blood glucose level. Most tea such as green tea, oolong tea, and black tea, but not herbal tea, has shown a glucose-lowering effect (Ryan *et al.*, 2000). Since herbal tea does not contain catechin, catechin is thought to be responsible for the glucose-lowering effect. Epidemiologic studies suggest that drinking multiple cups of tea per day lowers glucose, but the hypoglycemic activity of EGCG is still controversial (Chakravarthy *et al.*, 1982; Kolb *et al.*, 2004). Although black tea contains less catechin, it is necessary to elucidate the component responsible for the glucose-lowering effect.

It has been suggested that tea catechins could play a role in controlling the dietary glucose uptake through the intestine tract (Shimizu *et al.*, 2000). It has been demonstrated that tea catechins inhibit glucose uptake by human intestinal epithelial Caco-2 cells (Shimizu *et al.*, 2000). Black tea polyphenols contain abundant theaflavin and other unknown components. Since theaflavin extract suppresses digestive enzyme activity such as α-amylase and α-glucosidase (Honda and Hara, 1993; Matsui *et al.*, 1996), black tea extract would be effective for the suppression of carbohydrate digestion. The black tea extract used in this study showed partial inhibition of α-glucosidase activity *in vitro* (data not shown). Since the weight of the intestine was reduced, the α-glucosidase activity in the small intestine would be reduced. It appears likely that part of the glucose-lowering effect could be attributed to the suppression of digestive enzymes. Black tea extract, however, suppressed the elevation of blood glucose induced by external glucose administration. Thus, it was suggested that the glucose-lowering effect of black tea extract was not solely due to inhibition of α-glucosidase.

Various hypothetical mechanisms of the glucose-lowering effect of tea polyphenols have been proposed. Some data suggested that EGCG, a major tea polyphenol of green tea, depressed hepatic glucose production (Waltner-Law *et al.*, 2002). *In vitro* insulin activity test suggested that black tea might improve glucose and insulin metabolism (Kadowaki, 2000). Plants containing EC were found to increase insulin secretion from isolated rat Langerhans islets in the presence of either glucose or static incubation (Hii and Howell, 1984). The black tea extract used in this study did not change the fasting blood glucose levels (data not shown), and insulin levels after long-term treatment were not significantly different compared to the control. Furthermore, there were no remarkable morphological changes in the pancreas. From these results, it is not likely that black tea extract directly stimulates the secretion of insulin from the pancreas. Broadhurst *et al.* (2000) showed that green tea and black tea have high insulin-like activity, following cinnamon and witch hazel by *in vitro* screening adipocyte assay using a rat epididymal.

In the last several years, recovery of insulin resistance has become one of the major treatments for NIDDM (Lohray and Bhushan, 2004). Thiazolidinediones, promising antidiabetic agents in clinical usage, are known as insulin-sensitizing agents (Bloomgarden *et al.*, 1998). Thiazolidinediones bind to peroxisome proliferators-activated receptor (PPARγ) and act as potent regulators of the differentiation of adipocytes (Lehmann *et al.*, 1995). It has been proposed that thiazolidinediones induce the differentiation of pre-adipocytes into small adipocytes, and replace large adipocytes by small ones (Okuno *et al.*, 1998; Kadowaki, 2000; Yamauchi *et al.*, 2001). This qualitative alteration of adipocytes would affect the secretion of adiponectin from large adipocytes ones (Okuno *et al.*, 1998; Kadowaki, 2000; Yamauchi *et al.*, 2001). The suppressive effect of black tea in body weight might be related to this qualitative alteration of adipocytes. Pioglitazone augments the differentiation of 3T3-L1 pre-adipocytes (Takamura *et al.*, 2001). However, the reason why drugs that induce adipogenesis improve insulin resistance has not been fully elucidated. It is hypothesized that TNF-α is produced in adipocytes, which would induce insulin resistance (Ruan and Lodish, 2003). Thiazolidiones block TNF-α, and reduced TNF-α induces insulin signaling (Peraldi and Spiegelman, 1997). It has been suggested that thiazolidinediones induce recovery of insulin resistance through the regulatory activity of NF-κB in adipocytes (Ruan *et al.*, 2003).

In the next experiment, we assessed the adipogenic effect of black tea extract on 3T3-L1 pre-adipocytes and compared to that of pioglitazone. Staining with oil-red-O is a maker of adipogenesis. We defined differentiated cells as those with a more than two-fold increment of absorbance of oil-red-O. As shown in Fig. 6A, cells were differentiated from day 7. To assess the effect of black tea extract on adipogenesis, the incubation time was set at 7~10 days. As shown in Fig. 6B, black tea extract enhanced lipid accumulation, as effectively as pioglitazone.

Tea consumption has been gradually increasing since many people have become aware of its health benefit. A powder formulation of African black tea extract can provide steady glucose-lowering effects both in short- and long-term treatment. Although black tea extract itself does not have strong activity to reduce the blood glucose level in diabetic models, it could play a role as an alternative supplement to keep glucose levels normal rather than to improve the secretion of insulin. It is interesting that black tea extract augmented the differentiation of 3T3-L1 pre-adipocyte. It could have a regulatory effect on adipogenesis, although we need to define the responsible component and further investigate this phenomenon in the molecular basis. Black tea consumption is expected to act in a preventive rather than curative manner in NIDDM patients. Furthermore, the calcium deposition of the heart in mice treated with black tea extract for long-term was less than that in control. Which implies that tea drinking may have a preventive effect on diabetes induced cardiovascular complications.

CONCLUSIONS

A powder formulation of African black tea extract can provide steady glucose-lowering effects in NIDDM model mice. In this study, we demonstrated the short- and long-term effect of African black tea in suppressing the elevation of blood glucose levels. Black tea extract may not directly stimulate pancreas to secret insulin. Rather, it could have a preventive action to control hyperglycemia. Daily consumption of black tea would be expected to control blood glucose.

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