DOI:10.1021/jf102840w

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Green and Black Tea Suppress Hyperglycemia and Insulin Resistance by Retaining the Expression of Glucose Transporter 4 in Muscle of High-Fat Diet-Fed C57BL/6J Mice

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To investigate the preventive effects of tea on hyperglycemia and insulin resistance, male C57BL/6J mice were given a high-fat diet containing 29% lard and also green or black tea *ad libitum* for 14 weeks. Both teas suppressed body weight gain and deposition of white adipose tissue caused by the diet. In addition, they improved hyperglycemia and glucose intolerance by stimulating glucose uptake activity accompanied by the translocation of glucose transporter (GLUT) 4 to the plasma membrane in muscle. Long-term consumption of the high-fat diet reduced levels of insulin receptor β-subunit, GLUT4 and AMP-activated protein kinase α in muscle, and green and black tea suppressed these decreases. The results strongly suggest that green and black tea suppress high-fat diet-evoked hyperglycemia and insulin resistance by retaining the level of GLUT4 and increasing the level of GLUT4 on the plasma membrane in muscle.

KEYWORDS: GLUT4; hyperglycemia; insulin resistance; IRβ; tea

INTRODUCTION

Obesity is a problem in many developed countries and a major contributing factor in the occurrence of metabolic syndrome. Obesity is often associated with diabetes, and their development increases the risks of cancer, hypertension, neurological disorders and cardiovascular disease (*1*). The development of obesity and diabetes is characterized by hyperglycemia, which is caused by genetic, environmental and dietary factors. Glucose transporters (GLUTs) play an important role in the regulation of blood glucose levels. Muscle is the tissue consuming the greatest amount of glucose, and GLUT4 is essential for the insulin-dependent uptake of glucose into cells (*2*). An elevated blood glucose level induces the secretion of insulin from pancreatic β-cells into the blood, where it binds to insulin receptor β-subunit (IRβ), triggering a protein phosphorylation cascade consisting of the activation of insulin receptor substrate 1, phosphatidylinositol 3-kinase, Akt/protein kinase B and protein kinase C λ/ζ (*2*). Finally, the signal is transmitted to GLUT4 in storage vesicles and stimulates the translocation of GLUT4 to the plasma membrane, and subsequently the incorporation of blood glucose into cells. This system is important to maintain glucose homeostasis, and insulin is the only hormone able to reduce blood glucose levels. Therefore, a defective glucose transport system in muscle leads to hyperglycemia and subsequent insulin resistance.

There are several natural sources of potential therapeutics in the prevention and treatment of hyperglycemia and obesity including tea, the world's most widely consumed beverage. Tea is classified into four types: unfermented green tea, partially fermented oolong tea, completely fermented black tea, and drastically fermented and aged pu-erh tea. The health-promoting effects of tea, especially green tea, have been attributed to polyphenols, mainly catechins. For example, green tea or tea catechins suppressed high-fat diet-evoked increases in body weight and adipose tissue weight (*3*, *4*). A downregulation of lipogenic enzymatic activity (*5*), an upregulation of lipolytic enzymatic activity (*6*), fat oxidation (*7*) and thermogenesis in brown adipose tissue (*8*), regulation of the activity and expression of lipoproteins (*9*), decreases in cell numbers of preadipocytes and adipocytes (*10*), and inhibition of the differentiation of preadipocytes to adipocytes (*11*) have also been reported as actions of tea and its constituents. Moreover, green, oolong and black tea have hypoglycemic effects (*4*, *12*, *13*). The mechanisms underlying the hypoglycemic effects of tea or tea constituents are the inhibition of digestive enzymes in the small intestine (*14*) and gluconeogenic enzymes in the liver (*15*). We also demonstrated that *ad libitum* drinking of green tea for 3 weeks to male Wistar rats fed a commercial chow increased glucose uptake activity accompanied by the translocation of GLUT4 in muscle, while decreasing this activity in adipose tissue (*16*). These results strongly suggest that tea has an ability to improve hyperglycemia and insulin resistance through modulation of the glucose transport system. In this study, we investigated whether green and black tea consumed *ad libitum* suppress hyperglycemia and insulin resistance through regulation of the glucose transport system in muscle of mice fed a high-fat diet.

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 Published on Web 11/24/2010 © 2010 American Chemical Society

MATERIALS AND METHODS

Animal Experiments. All experiments with animals were approved by the Institutional Animal Care (Permission number: 19-5-32 and 21-07-02) and Use Committee and carried out according to the Kobe University Animal Experimentation Regulations. Male C57BL/6J mice (4 weeks old) were housed in a temperature-controlled (23−25 °C) room at 60 ± 5% humidity under a 12 h light−dark cycle. The mice were acclimatized for 7 days with access to a commercial chow diet and distilled water. They were randomly divided into 2 groups of 15 and fed a high-fat diet containing 29% lard or an AIN93M-based control diet, which is the same as each experimental diet used in the previous report by Mito et al. (*17*). The mice of each group were further divided at random into 3 subgroups of 5, and given distilled water ($n = 5 \times 2$ groups), green tea ($n = 5 \times 2$ groups), or black tea ($n = 5 \times 2$ groups). The teas were freshly prepared daily as follows. Twenty grams of green tea leaves (*Camellia sinensis*, Sen-cha) or black tea leaves (*Camellia sinensis*, Uva) was soaked in 1 L of hot water (90 °C) for 5 min and cooled at room temperature. Each aqueous solution was given to the animals for 14 weeks. During the feeding period, food intake, water/tea intake and body weight were measured once a week. At the end of the feeding period, the blood of the mice was collected via cardiac puncture under anesthesia after 18 h fasting, and then the mice were sacrificed. Mesenteric, epididymal, retroperitoneal, or subcutaneous white adipose tissue, interscapular brown adipose tissue, muscle, and small intestinal epithelial cells were taken and weighed. Each tissue was washed with 1.15% (w/v) KCl and stored at −80 °C prior to use. Blood was collected, and serum was subjected to measurements of glucose, lipid, leptin and adiponectin levels.

Determination of Catechin, Theaflavin and Caffeine Contents in Green Tea and Black Tea. Contents of catechins, theaflavins, and caffeine contents in green tea and black tea were determined according to our previous report (*18*). Briefly, green tea and black tea were filtrated, and then were injected into high-performance liquid chromatography (HPLC) using a Waters 600E multisolvent delivery system and a 486 UV/vis detector (Nihon Waters K. K., Tokyo, Japan). Analytical conditions were as follows: 250 mm × 4.6 mm i.d. Wako pack C18HG column maintained at 40 °C; mobile phase, 22% (v/v) methanol solution in 0.1% (v/v) phosphate buffer; flow rate, 1 mL/min; and wavelength of 230 and 280 nm.

Measurement of Serum Levels of Glucose, Lipids, Leptin, and Adiponectin. During the feeding period, blood was sampled by cutting the tip of the tail after 18 h fasting. An oral glucose tolerance test (OGTT) was performed by using the fasted mice at 5 and 10 weeks according to a previous report (*19*). Mice were orally administered a glucose solution at 2 g/kg BW, and blood was sampled 0, 15, 30, 60, and 120 min after the administration. The blood samples were centrifuged at 15000*g* for 15 min at 4 °C, and the supernatant was collected as serum. Levels of glucose, total cholesterol, free cholesterol, HDL cholesterol, free fatty acid and triacylglycerol were measured with commercial kits (Wako Pure Chemical Industries Ltd., Osaka, Japan) according to the manufacturer's instructions. Leptin and adiponectin levels were also measured with commercial kits from Seikagaku Co. (Tokyo, Japan) and Otsuka Pharmaceutical Co. (Tokyo, Japan), respectively.

Determination of Glucose Uptake Activity in Adipose Tissue and Muscle. The glucose uptake activity of adipose tissue and muscle was determined according to a previous report with some modifications (*16*). Briefly, small pieces of tissue (approximately 200 mg) were preincubated with 1 mL of Krebs-Ringer-HEPES (KRH) buffer (50 mM HEPES, pH 7.4, 137 mM NaCl, 4.8 mM KCl, 1.85 mM $CaCl_2$ and 1.3 mM $MgSO_4$) for 10 min at 37 °C, and incubated with 6.5 mM 3-*O*-methyl-[^{3}H]-D-glucose (0.5 μCi) in KRH buffer for a further 2 min. After the incubation, the tissue was washed with ice-cold KRH buffer 6 times and then solubilized by NCSII (GE Healthcare Bio-Science Co., Piscataway, NJ). The radioactivity of 3-*O*-methyl-[^{3}H]-D-glucose incorporated into each tissue was measured using a liquid scintillation counter with a scintillation cocktail. Nonspecific binding was determined under the same conditions in the presence of 20 μM cytocalasin B.

Measurement of α-Glucosidase Activity in the Small Intestine. Small intestinal epithelial cells were homogenated with 7 volumes of 1.15% (w/v) KCl to measure maltase and sucrase-isomaltase activities in the small intestine. The reaction mixture consisted of 0.1 M maltose or sucrose in 50 mM maleate buffer (pH 6.0), and the reaction was started by adding the homogenate. After incubation at 37 °C for 0, 5, 10, 20, and 40 min, the reaction was terminated by heating the mixture in boiling water for 5 min and keeping it on ice for 10 min. The mixture was centrifuged at 1000*g* for 10 min, and the supernatant was used to measure glucose levels with a commercial kit (Wako Pure chemical industries Ltd.). Enzymatic activity is presented as the amount of glucose (nmol/min/mg protein).

Western Blot Analysis. Preparation of the cell lysate and the plasma membrane fraction and Western blotting were performed according to our previous report (*20*). Anti-GLUT4 (Santa Cruz Biotechnology Inc.), anti-GLUT1 (Santa Cruz Biotechnology Inc.), anti-IRβ (Santa Cruz Biotechnology Inc.), anti-AMP-activated protein kinase (AMPK) α (Cell Signaling Technology Inc., Danvers, MA) and anti-β-actin (Sigma-Aldrich, St. Louis, MO) antibodies were used as primary antibodies, and the density of specific bands was determined using a Gel-Pro Analyzer (Media Cybernetics Inc., Silver Spring, MD).

Statistical Analysis. Data are expressed as the mean ± SE. Statistical significance was analyzed using a factorial ANOVA with the Tukey−Kramer multiple comparison test, and a 0.05 level of probability was used as the criterion for significance.

RESULTS

Contents of Catechins, Theaflavins, and Caffeine in Green Tea and Black Tea. Contents of catechins, theaflavins, and caffeine in green tea and black tea used in this study were investigated, and the results were as follows: In green tea, (+)-catechin, 24.6 mg/L; (−)-epicatechin, 227.5 mg/L; (−)-gallocatechin, 58.1 mg/L; (−)-epigallocatechin, 811.4 mg/L; (−)-catechin gallate, 11.7 mg/L; (−)-epicatechin gallate, 141.5 mg/L; (−)-gallocatechin gallate, 26.0 mg/L; (−)-epigallocatechin gallate, 609.7 mg/L; theaflavin, 4.9 mg/L; theaflavin-3-*O*-gallate, 8.3 mg/L; theaflavin-3′-*O*-gallate, 7.4 mg/L; theaflavin-3,3′-di-*O*-gallate, 7.6 mg/L; caffeine, 557.2 mg/L. In black tea, (+)-catechin, 22.6 mg/L; (−)-epicatechin, 58.6 mg/L; (−)-gallocatechin, 14.5 mg/L; (−)-epigallocatechin, 39.1 mg/L; (−)-catechin gallate, 35.7 mg/L; (−)-epicatechin gallate, 132.2 mg/L; (−)-gallocatechin gallate, 7.6 mg/L; (−)-epigallocatechin gallate, 130.0 mg/L; theaflavin, 25.0 mg/L; theaflavin-3-*O*-gallate, 26.7 mg/L; theaflavin-3′-*O*-gallate, 17.9 mg/L; theaflavin-3,3′-di-*O*-gallate, 30.0 mg/L; caffeine, 491.3 mg/L. Total catechin contents in green tea were almost 4.3 times as large as those in black tea, and green tea had abundant contents of (−)-epigallocatechin and (−)-epigallocatechin gallate (EGCG). On the contrary, total theaflavin contents in black tea were almost 3.5 times as large as those in green tea. The content of caffeine in green tea and black tea was almost the same.

Effects of Tea on Body and Adipose Tissue Weights, and Serum Lipid Levels. In this study, mice were fed a control diet or high-fat diet with water, green tea, or black tea for 14 weeks. During the feeding period, the energy intake and the water/tea intake were measured once per week (data not shown). In both groups on the control and high-fat diet, the mice given green and black tea had the relative higher energy intake compared with those given water. In both control and high-fat diet groups, little difference in the water/tea intake was observed among the mice given green tea, black tea and water. These results indicate that the antiobesity effects of green and black tea do not contribute to alteration of the energy intake by both teas. To confirm the antiobesity effects of green and black tea, body and adipose tissue weights were measured (data not shown). The high-fat diet caused a significant increase in body weight from week 8 compared with the control diet. In the groups on the control diet, green and black tea did not alter body weight in the mice given water (control diet: water, 26.1 ± 0.27 g; green tea, 26.9 ± 0.93 g; black tea, 26.6 ± 1.33 g). In the groups on the high-fat diet, both teas significantly suppressed weight gain with body weight almost the same as that of mice on the control diet at the end of the feeding period (high fat diet: water, 34.6 ± 2.18 g; green tea, 27.6 ± 0.56 g; black tea, 27.4 ± 0.60 g). The high-fat diet significantly increased the relative

Table 1. Adipose Tissue Weight (% of Body Weight) of Mice Given Green and Black Tea for 14 Weeks[a]

	C-W	C-G	C-B	H-W	H-G	H-B
total WAT	6.15 ± 0.31 a	3.15 ± 0.56 a	3.41 ± 0.51 a	14.4 ± 4.05 b	3.14 ± 0.48 a	4.09 ± 0.49 a
mesenteric WAT	0.68 ± 0.04 a	0.37 ± 0.08 ac	0.43 ± 0.08 ac	1.19 ± 0.22 b	0.29 ± 0.03 c	0.35 ± 0.05 c
epididymal WAT	2.09 ± 0.08 a	1.11 ± 0.16 a	1.16 ± 0.14 a	3.73 ± 0.89 b	1.08 ± 0.11 a	1.55 ± 0.14 a
retroperitoneal WAT	0.71 ± 0.04 a	0.30 ± 0.08 a	0.43 ± 0.09 a	2.05 ± 0.63 b	0.27 ± 0.04 a	0.50 ± 0.07 a
subcutaneous WAT	2.67 ± 0.19 a	1.36 ± 0.29 a	1.39 ± 0.24 a	4.89 ± 1.93 b	1.50 ± 0.32 a	1.70 ± 0.28 a
interscapular BAT	0.24 ± 0.08 a	0.23 ± 0.07 a	0.22 ± 0.04 a	0.30 ± 0.06 a	0.22 ± 0.03 a	0.29 ± 0.03 a

[a] Mice were fed a control diet (C) or high-fat diet (H) with water (W), green tea (G), or black tea (B) for 14 weeks. Body weight and tissue weight as a percentage of body weight were measured. Data are shown as the mean ± SE ($n = 5$). Values with the same letters are not significantly different by the Tukey−Kramer multiple comparison test ($p < 0.05$). WAT and BAT; white and brown adipose tissues, respectively.

Table 2. The Serum Lipid, Leptin and Adiponectin Levels of Mice Given Green and Black Tea for 7 and 14 Weeks[a]

	C-W	C-G	C-B	H-W	H-G	H-B
			7 Weeks			
total cholesterol (mg/dL)	84.1 ± 9.6 a	82.3 ± 3.5 a	83.5 ± 3.2 a	123 ± 2.2 b	106 ± 2.8 bc	97.9 ± 3.3 ac
free cholesterol (mg/dL)	17.9 ± 1.1 ac	19.1 ± 1.1 a	18.4 ± 0.8 a	25.4 ± 0.7 b	22.1 ± 0.7 abc	19.4 ± 1.1 a
HDL cholesterol (mg/dL)	24.8 ± 3.5 a	24.2 ± 1.3 a	24.6 ± 1.2 a	39.3 ± 0.7 b	32.9 ± 1.9 bc	30.0 ± 1.3 ac
LDL cholesterol (mg/dL)	50.9 ± 3.5 a	50.1 ± 5.8 a	50.0 ± 2.4 a	74.5 ± 3.2 b	65.8 ± 1.4 bc	60.0 ± 2.4 ac
free fatty acid (meq/L)	0.68 ± 0.05 a	0.64 ± 0.05 a	0.77 ± 0.05 a	0.81 ± 0.07 a	0.70 ± 0.05 a	0.71 ± 0.05 a
triacylglycerol (mg/dL)	40.5 ± 3.3 a	40.1 ± 1.3 a	46.9 ± 3.7 a	42.6 ± 3.7 a	35.2 ± 3.3 a	39.6 ± 1.9 a
leptin (ng/mL)	1.15 ± 0.04 a	1.05 ± 0.08 a	1.22 ± 0.07 a	4.29 ± 0.86 b	1.44 ± 0.18 a	1.93 ± 0.16 a
adiponectin (ng/mL)	21.0 ± 1.2 abc	20.0 ± 0.9 ac	22.2 ± 0.7 ab	24.1 ± 0.8 b	17.7 ± 0.5 c	18.6 ± 1.1 ac
			14 Weeks			
total cholesterol (mg/dL)	95.6 ± 4.6 a	88.9 ± 7.9 a	94.8 ± 4.3 a	143 ± 7.4 b	125 ± 4.1 b	124 ± 7.5 b
free cholesterol (mg/dL)	16.8 ± 3.6 a	18.3 ± 2.0 ac	18.3 ± 1.5 ac	25.4 ± 1.3 b	22.6 ± 1.6 bc	20.5 ± 3.3 ab
HDL cholesterol (mg/dL)	39.5 ± 1.5 a	35.6 ± 2.8 a	36.3 ± 1.6 a	57.6 ± 2.7 b	54.3 ± 1.5 b	54.8 ± 2.7 b
LDL cholesterol (mg/dL)	44.0 ± 3.1 a	42.6 ± 7.4 a	47.9 ± 4.3 ab	72.2 ± 5.5 b	60.8 ± 4.2 ab	58.3 ± 9.7 ab
free fatty acid (meq/L)	1.33 ± 0.08 a	1.46 ± 0.06 a	1.41 ± 0.13 a	2.01 ± 0.08 b	1.43 ± 0.13 a	1.48 ± 0.13 a
triacylglycerol (mg/dL)	60.4 ± 4.7 a	53.7 ± 2.0 a	53.1 ± 5.5 a	67.1 ± 2.7 a	51.4 ± 3.4 a	52.6 ± 4.8 a
leptin (ng/mL)	3.29 ± 0.22 a	1.49 ± 0.20 a	1.64 ± 0.09 a	13.3 ± 5.54 b	1.37 ± 0.09 a	1.75 ± 0.15 a
leptin index	0.51 ± 0.03 a	0.45 ± 0.01 a	0.45 ± 0.06 a	0.88 ± 0.07 b	0.45 ± 0.02 a	0.41 ± 0.04 a
adiponectin (ng/mL)	25.8 ± 0.6 ae	20.2 ± 1.7 ab	20.4 ± 1.3 ad	31.1 ± 3.5 ce	17.2 ± 1.2 bd	18.3 ± 2.0 ab
adiponectin index	4.22 ± 0.18 ab	5.95 ± 0.38 a	5.40 ± 0.56 a	2.18 ± 0.62 b	5.83 ± 0.42 a	4.68 ± 0.50 a

[a] Mice were fed a control diet (C) or high-fat diet (H) with water (W), green tea (G), or black tea (B) for 7 and 14 weeks. The total cholesterol, free cholesterol, HDL cholesterol, free fatty acid, triacylglycerol, leptin and adiponectin levels in serum were measured as described in Materials and Methods. LDL cholesterol was calculated according to a previous report (*21*). A leptin or adiponectin index was calculated by dividing each serum level by relative total white adipose tissue weight (% of body weight) in this table. Data are shown as the mean ± SE ($n = 5$). Values with the same letters are not significantly different by the Tukey−Kramer multiple comparison test ($p < 0.05$).

weights of mesenteric, epididymal, retroperitoneal, and subcutaneous white adipose tissues, but not interscapular brown adipose tissue compared with the control diet (**Table 1**). In the high-fat diet-fed mice, green and black tea significantly decreased the relative weight of each white adipose tissue by more than 50%, and green tea had the stronger effect. Both teas tended to decrease the relative weight of each white adipose tissue in the mice on control diet. The relative weight of brown adipose tissue did not change in any experimental groups.

To investigate the effects of green and black tea on lipid metabolism, total cholesterol, free cholesterol, HDL cholesterol, free fatty acid and triacylglycerol level in serum were measured at 7 and 14 weeks (**Table 2**). The serum LDL cholesterol level was calculated according to a previous report (*21*). After 7 weeks, the high-fat diet had increased total cholesterol, free cholesterol, HDL cholesterol and LDL cholesterol levels compared with the control diet, while the levels of free fatty acid and triacylglycerol remained unchanged. The high-fat diet-induced increases in these levels were suppressed by black tea but not green tea. In the control diet-fed groups, there was no significant change in serum lipid levels. After 14 weeks, the high-fat diet had significantly increased the levels of all serum lipids tested except triacylglycerol compared with the control diet. Green and black tea significantly decreased the free fatty acid level, though they only tended to reduce cholesterol levels. In the control diet-fed groups, no significant changes in lipid levels caused by green and black tea were observed. These results indicate that green or black tea can suppress obesity caused by a high-fat diet, but the mode of action is not an improvement in lipid metabolism.

Effects of Tea on the Secretion of Leptin and Adiponectin. The influence of green and black tea on the secretion of leptin and adiponectin was investigated at 7 and 14 weeks (**Table 2**). Leptin has an antifeeding effect (*22*) and is secreted from adipocytes in response to the accumulation of lipids in cells (*23*). The high-fat diet increased the serum leptin level at 7 and 14 weeks compared with the control diet, and green and black tea significantly suppressed this increase. Adiponectin decreases the plasma glucose level (*24*) and enhances the utilization of fatty acids in muscle (*25*). Green and black tea tended to decrease the serum level of adiponectin at 7 and 14 weeks in both the control and high fat diet-fed groups. Since body and white adipose tissue weights differed among the experimental groups, we defined a secretion index, which was obtained by dividing the adipocytokine level by the relative weight of all white adipose tissue, to normalize levels of secretion of adipocytokines. The high-fat diet increased the leptin index to approximately 75% significantly, while it decreased the adiponectin index to approximately 50%. Green and black tea maintained both indexes at control levels. These results

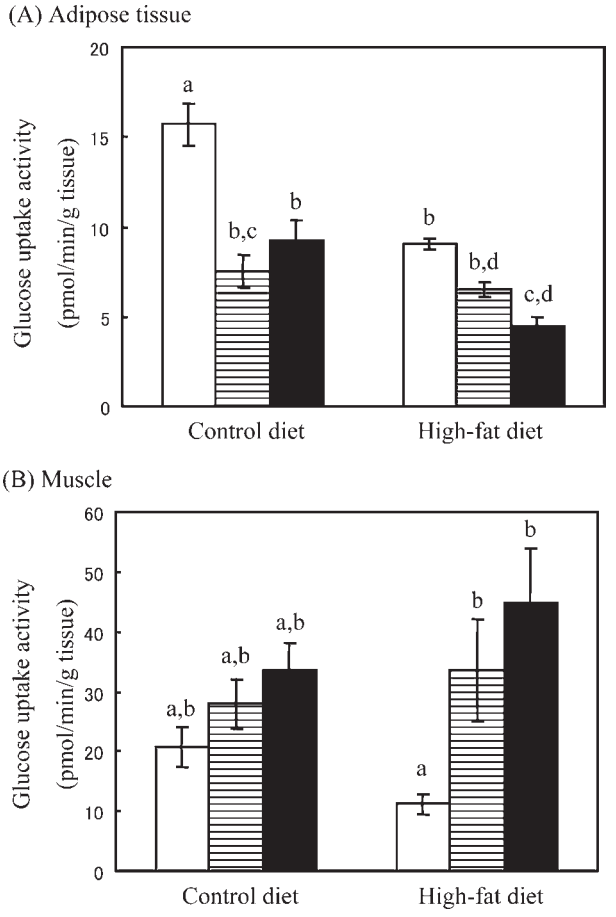

Figure 1. Effects of tea on glucose uptake activity in adipose tissue and muscle. Mice were fed a control diet or high-fat diet with water, green tea, or black tea for 14 weeks, and glucose uptake activity in adipose tissue (**A**) and muscle (**B**) was measured. Open, hatched and closed bars represent the results from mice given water, green tea and black tea, respectively. Data are shown as the mean ± SE (n = 5). Values with the same letters are not significantly different by the Tukey−Kramer multiple comparison test ($p < 0.05$).

indicate that green or black tea is able to normalize levels of adipocytokines altered by a high-fat diet.

Effects of Tea on Glucose Uptake Activity. The glucose uptake activity in muscle and adipose tissue, which is deeply involved in glucose homeostasis, was measured at 14 weeks. In adipose tissue (**Figure 1A**), a significant decrease was observed in mice on the high-fat diet. In the groups given the control diet, green and black tea decreased glucose uptake activity approximately 50% compared with water. In the groups on the high-fat diet, black tea significantly decreased the activity. In muscle (**Figure 1B**), glucose uptake activity tended to be decreased by the high-fat diet compared with the control diet, and was significantly higher in the mice fed the high-fat diet plus green or black tea.

Effects of Tea on Blood Glucose Levels. Next, time-dependent changes in fasting blood glucose levels were measured at 1, 5, 7, 10, and 14 weeks (**Figures 2A** and **2B**), and area under the curve (AUC) values were calculated (**Figure 2C**). In both the control and high-fat diet-fed groups, green and black tea maintained lower fasting blood glucose levels than water. Moreover, both teas significantly decreased the AUC values, indicating that they improve glucose metabolism. To investigate the ability of green and black tea to incorporate blood glucose into the body, OGTT was performed at 5 and 10 weeks (**Figure 3**). In the control groups at both 5 and 10 weeks, green and black tea caused lower glucose levels than water 120 min after the administration of glucose (**Figures 3A** and **3B**). In the high-fat diet-fed groups at 5 weeks, the teas had a prompt lowering effect on blood glucose levels: black and green tea showed a significant effect 60 and 120 min (for green tea), and 120 min (for black tea), after the injection of glucose (**Figure 3A**). Similar results were obtained at 10 weeks (**Figure 3B**). Regarding the control diet-fed group, neither green tea nor black tea affected the AUC values at 5 and 10 weeks (**Figures 3C** and **3D**). In the high-fat diet-fed group, a significant increase was observed in the AUC value of mice treated with water at 10 weeks (**Figure 3D**), indicating that the high-fat diet caused insulin resistance. Green and black tea significantly reduced AUC values to control levels at 10 weeks. At 5 weeks, the high-fat diet exhibited a tendency to increase the AUC value, and black tea significantly decreased this effect (**Figure 3C**). Therefore, tea, especially black tea, has the potential to improve high-fat diet-induced insulin resistance.

Tea and tea constituents were reported to inhibit the enzymatic activity of α-glucosidases, such as maltase and sucrase-isomaltase, in the small intestine (*14*), and this inhibitory effect is considered to be involved in the decrease of postprandial blood glucose levels. Therefore, we measured the enzymatic activities of maltase and sucrase-isomaltase, and found that green and black tea did not have any effects on the activity (**Table 3**). Interestingly, the high-fat diet decreased the activities of these enzymes, particularly maltase. These results indicate that green and black tea did not inhibit α-glucosidase activity in the small intestine under our experimental conditions.

Effects of Tea on the Glucose Transport System in Muscle. Finally, we investigated whether green and black tea influence the glucose transport system in muscle, because muscle is the largest tissue incorporating blood glucose into the body (**Figure 4**). In this study, we focused on GLUTs, which play an important role in glucose homeostasis and are required for the incorporation of glucose into the cells (*2*), and also examined the levels of IRβ and AMPKα, proteins essential for the translocation of GLUT4 to the plasma membrane (*2*, *26*). The high-fat diet significantly decreased the expression of GLUT4, IRβ and AMPKα accompanied by a reduction in GLUT4 on the plasma membrane compared with the control diet, but did not alter GLUT1 expression. In the control diet groups, green tea tended to upregulate GLUT4 expression (**Figure 4B**) resulting in an increase in the GLUT4 level on the plasma membrane (**Figure 4A**). In the high-fat diet-fed groups, green and black tea inhibited the high-fat diet-induced decreases in GLUT4, IRβ and AMPKα expression with and without significant differences, and the GLUT4 translocated level in muscle. These results indicate that green and black tea suppress high-fat diet-evoked hyperglycemia by promoting the expression and translocation of GLUT4 in muscle.

DISCUSSION

Tea has various beneficial properties including antiobesity and hypoglycemic effects (*3*, *4*, *12*, *13*). It has been suggested that the antiobesity effects and hypoglycemic effects of tea are mediated via multiple actions (*5*−*11*, *14*, *15*). In this study, green tea had the tendency to increase the GLUT4 level in the plasma membrane of muscle of the mice fed a control diet (**Figure 4**), consistent with our previous report (*16*). In addition, green and black tea suppressed a high-fat diet-induced increase in body weight and deposition of white adipose tissue (**Table 1**), indicating that the teas suppress high-fat diet-induced obesity. We further found that green and black tea ameliorated high-fat diet-evoked increases in blood glucose levels (**Figure 2**) and glucose intolerance (**Figure 3**), suggesting that they improve high-fat diet-evoked hyperglycemia.

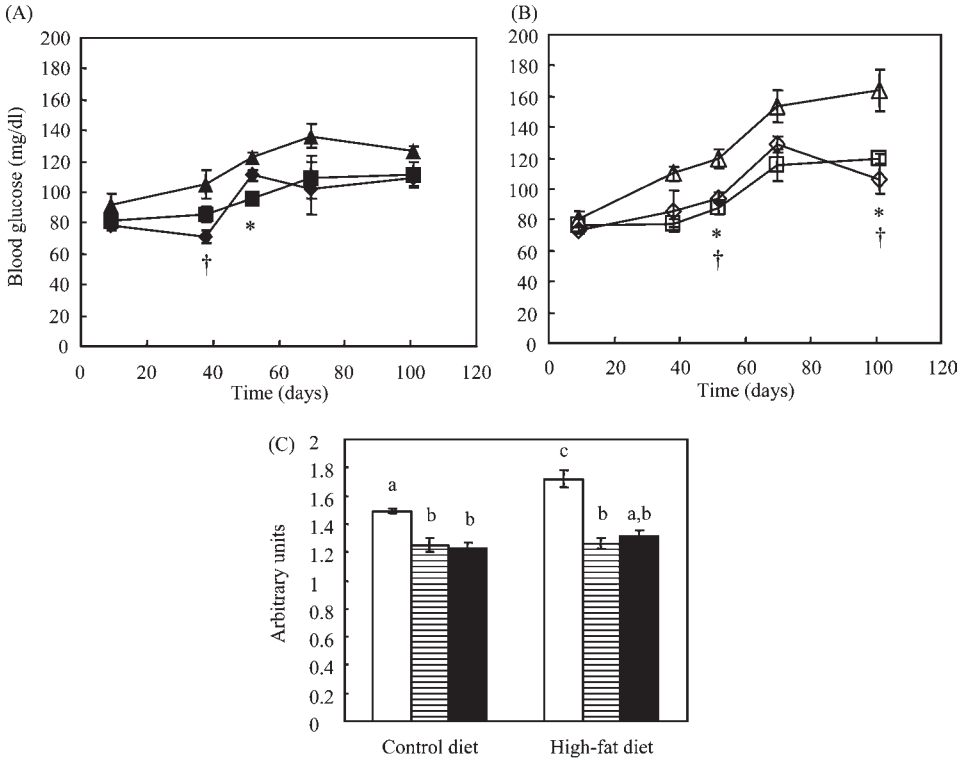

Figure 2. Effects of tea on fasting blood glucose levels. Mice were fed a control diet or high-fat diet with water, green tea, or black tea for 1, 5, 7, 10, and 14 weeks, and blood glucose levels were measured. Closed (**A**) and open (**B**) symbols represent the results from animals fed the control diet and high-fat diet, respectively. Diamonds, squares and triangles represent the results from mice given green tea, black tea and water, respectively. Asterisks and daggers indicate a significant difference for green and black tea, respectively, against the corresponding control. (**C**) The AUC values were calculated from the results in (**A**) and (**B**). Open, hatched and closed bars represent the results from mice given water, green tea and black tea, respectively. Data are shown as the mean ± SE ($n = 5$). Values with the same letters are not significantly different by the Tukey−Kramer multiple comparison test ($p < 0.05$).

Our findings indicate a hypoglycemic mechanism by which green and black tea increased glucose uptake activity (**Figure 1**) accompanied by activation of the glucose transport system in muscle of mice fed the high-fat diet (**Figure 4**) without inhibiting α-glucosidases in the small intestine *in vivo* (**Table 3**). Despite the large difference in the catechin contents between green and black tea, these teas exhibited similar effects, and black tea seems to be better than green tea. The high contents of (−)-epigallocatechin and (−)-epigallocatechin gallate are characteristic of green tea compared with black tea, while black tea especially has the higher contents of theaflavins. These results indicate that the effective constituents of green tea in this study are different from those of black tea, and a combination of some constituents in tea may be much important to exert the effects.

In this study, the high-fat diet decreased levels of GLUT4, IRβ, and AMPKα accompanied by a decline in the GLUT4 level on the plasma membrane in muscle (**Figure 4**). These results were consistent with our previous report (*20*). GLUT4 is translocated from storage vesicles to the plasma membrane of muscle cells and adipocytes and plays an important role in the insulin-stimulated incorporation of glucose into cells (*2*). IRβ exists on the plasma membrane of muscle cells and adipocytes, and binding of insulin to IRβ is the initial step in the insulin-dependent translocation of GLUT4 (*2*). By contrast, AMPKα is activated by exercise and the contraction of muscle leading to the insulin-independent translocation of GLUT4 (*26*). Thus, IRβ and AMPKα are closely involved in the GLUT4-related glucose transport system. Therefore, a defective glucose transport system in muscle, due to a deficiency of GLUT4, IRβ and AMPKα, would lead to hyperglycemia and subsequently insulin resistance. In this study, green and black tea suppressed the high-fat diet-induced downregulation of GLUT4, IRβ and AMPKα expression in muscle (**Figure 4**). Although green tea was reported to diminish a high-fructose diet-induced downregulation of GLUT4 expression in muscle of rats (*27*), there is, to our knowledge, no report of the effects of tea on IRβ and AMPKα in muscle. From our results and previous reports, green and black tea have the potential to prevent reduction in GLUT4, IRβ and AMPKα and subsequently maintain the GLUT4 level on the plasma membrane in muscle leading to hypoglycemic effects, and green and black tea may exert these effects through an enhancement of not only the insulin sensitive pathway but also the insulin insensitive pathway.

The long-term consumption of a high-fat diet is known to induce oxidative stress (*28*). Oxidative stress causes insulin resistance accompanied by the inhibition of GLUT4's translocation in muscle cells and adipocytes (*29*). Oxidative stress was also reported to alter the secretion of adipocytokines from adipocytes (*30*, *31*). Moreover, reductions in GLUT4 and IRβ were induced by treatment with tumor necrosis factor (TNF) α and free fatty acids, which are adipocytokines, in experiments using cultured adipocytes and muscle cells (*32*, *33*). These results indicate the high-fat diet-evoked oxidative stress in various tissues to be due to the secretion of TNFα and free fatty acids from adipocytes followed by the downregulation of GLUT4, IRβ and AMPKα expression. Regarding other adipocytokines except TNFα and free fatty acids, the serum level of leptin, which is secreted from adipocytes in response to an accumulation of triacylglycerol in cells, positively correlated with body mass index in humans (*34*). The adiponectin level correlated negatively with obesity and positively with insulin sensitivity (*35*), and adiponectin-deficient mice developed both insulin resistance and diabetes (*36*). The serum resistin level is elevated in patients with

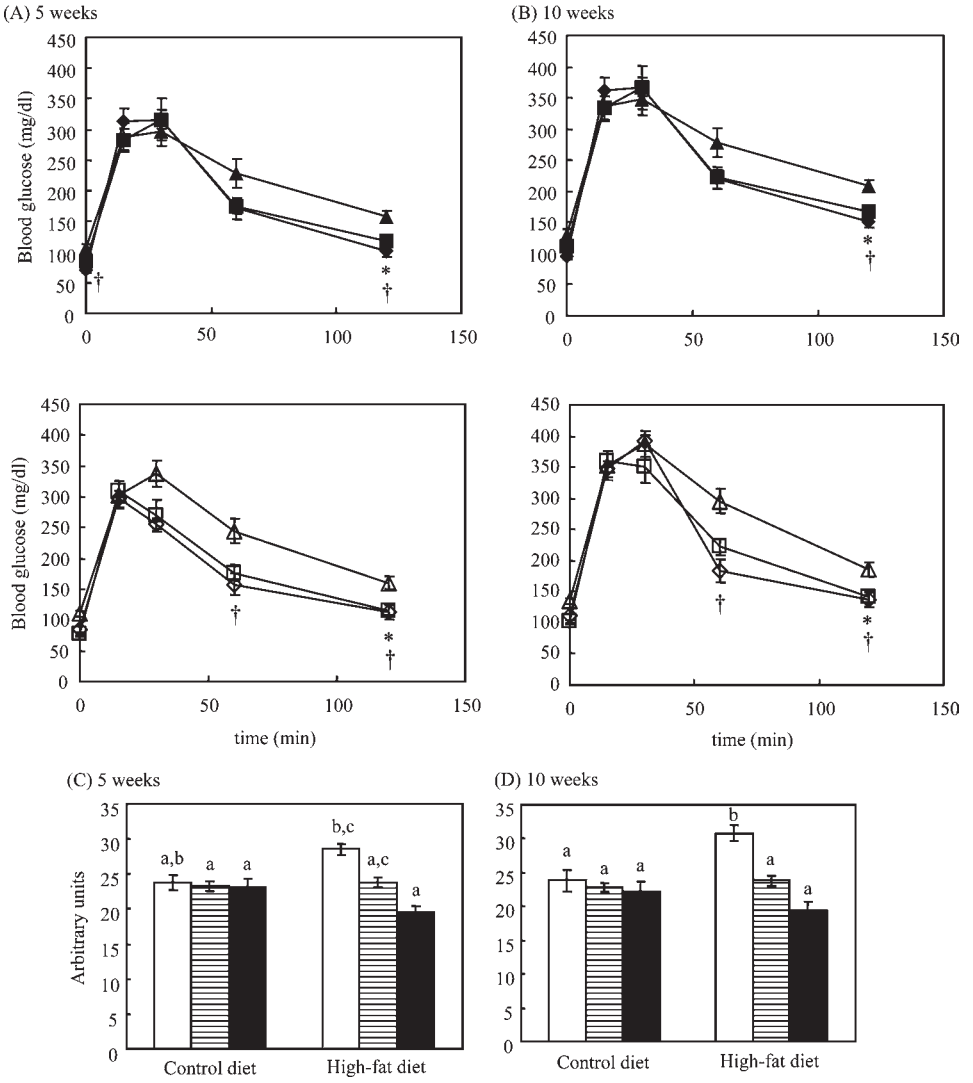

Figure 3. Effects of tea on OGTT. Mice were fed a control diet or high-fat diet with water, green tea, or black tea. OGTT was performed at 5 (**A**) and 10 (**B**) weeks. Closed and open symbols represent the results from animals fed the control diet and high-fat diet, respectively. Diamonds, squares and triangles represent the results from mice given, green tea, black tea and water, respectively. Asterisks and daggers indicate a significant difference for green and black tea, respectively, against the corresponding controls. (**C** and **D**) The AUC values were calculated from the results in (**A**) and (**B**). Open, hatched and closed bars represent the results from mice given water, green tea and black tea, respectively. Data are shown as the mean ± SE ($n = 5$). Values with the same letters are not significantly different by the Tukey−Kramer multiple comparison test ($p < 0.05$).

Table 3. α-Glycosidase Activity in the Small Intestine of Mice Given Green and Black Tea for 14 Weeks[a]

α-glycosidase act. (nmol/min/mg protein)	C-W	C-G	C-B	H-W	H-G	H-B
maltase act.	36.4 ± 0.9 a	35.6 ± 0.9 ac	49.2 ± 0.7 a	17.9 ± 0.7 bc	18.4 ± 0.5 b	18.6 ± 0.3 b
sucrase-isomaltase act.	2.9 ± 0.2 ac	2.7 ± 0.1 ab	3.3 ± 0.1 a	1.7 ± 0.0 bcd	1.7 ± 0.1 bd	1.7 ± 0.2 bd

[a] Mice were fed a control diet (C) or high-fat diet (H) with water (W), green tea (G), or black tea (B) for 14 weeks, and maltase and sucrase-isomaltase activities in the small intestine were measured. Data are shown as the mean ± SE ($n = 5$). Values with the same letters are not significantly different by the Tukey−Kramer multiple comparison test ($p < 0.05$).

obesity (*37*), and resistin suppressed the insulin-stimulated glucose uptake in muscle cells (*38*) and adipocytes (*39*). Therefore, the long-term consumption of high-fat diet would cause oxidative stress followed by the enlargement of adipocytes, leading to changes in adipocytokine levels, namely, increases in TNFα, free fatty acids, resistin, and leptin, and a decrease in adiponectin. In the present study, green and black tea reduced serum free fatty acid and leptin levels in both the control and high-fat diet-fed groups, and tended to increase the adiponectin index (**Table 2**). Previous studies found that (−)-catechin, a green tea polyphenol, increased the expression and secretion of adiponectin in mouse 3T3-L1 adipocytes (*40*), and EGCG, the most abundant catechin in green and black tea, reduced the mRNA level of resistin in 3T3-L1 adipocytes (*41*). EGCG and green tea leaf extract increased the serum adiponectin level *in vivo* (*42*), and EGCG also decreased the serum leptin level *in vivo* (*43*). Moreover, oolong tea increased the serum adiponectin level in patients with coronary artery disease (*44*). Our previous report demonstrated that catechins delayed adipocyte differentiation involved in the suppression of peroxisome proliferated-activated receptor γ and CCAAT/enhancer binding protein α expression in 3T3-L1 cells (*11*). Therefore, green and black tea would prevent the enlargement of adipocytes accompanied by a

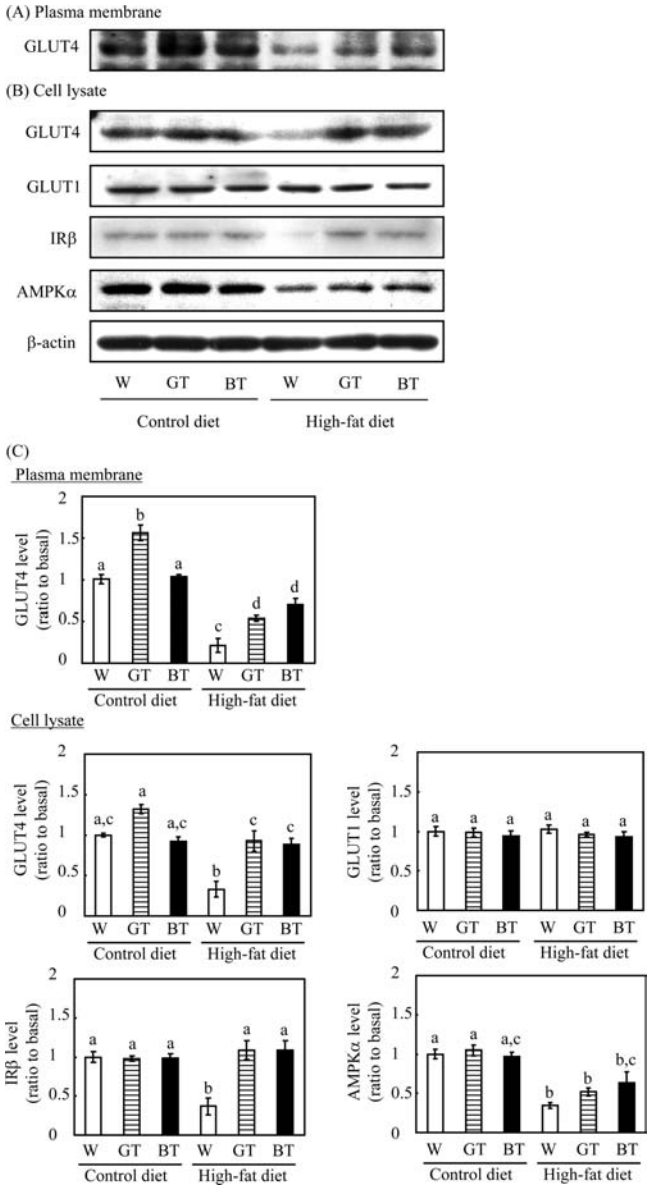

Figure 4. Effects of tea on the glucose transport system in muscle tissue. Mice were fed a control diet or high-fat diet with water, green tea, or black tea for 14 weeks, and the GLUT4 protein in a plasma membrane fraction (**A**) obtained from muscle, and the GLUT4, GLUT1, IRβ, AMPKα and β-actin proteins in cell lysate (**B**) were detected by Western blotting. Each panel shows a typical result. (**C**) The density of each band was normalized to that of β-actin, and values are presented as a ratio to the results from mice fed the control diet plus water. Open, hatched and closed bars represent the results from mice given water, green tea and black tea, respectively. Data are shown as the mean $\pm$ SE ($n = 3$). Values with the same letters are not significantly different by the Tukey−Kramer multiple comparison test ($p < 0.05$).

change in adipocytokine levels, probably due to antioxidative activity, resulting in suppression of the decline in GLUT4, IRβ and AMPKα levels. In addition, suppression of the enlargement of adipocytes would be also concerned with the decrease in white adipose tissue weight by green and black tea (**Table 1**).

In conclusion, the intake of green and black tea suppressed obesity and hyperglycemia caused by a high-fat diet by preventing the impairment of GLUT4-dependent glucose transport in muscle, probably due to their antioxidative activity. This is a proposed mechanism underlying the hypoglycemic effects of green and black tea.

ABBREVIATIONS USED

AMPK, AMP-activated protein kinase; AUC, area under the curve; EGCG, (−)-epigallocatechin gallate; GLUT, glucose transporter; IRβ, insulin receptor β-subunit; KRH, Krebs-Ringer-HEPES; OGTT, oral glucose tolerance test; TNF, tumor necrosis factor.

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Received for review July 30, 2010. Revised manuscript received October 7, 2010. Accepted November 11, 2010.