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Fermented Tea Improves Glucose Intolerance in Mice by Enhancing Translocation of Glucose Transporter 4 in Skeletal Muscle

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ABSTRACT: The antihyperglycemic effects of tea are well documented. However, the effects of fermented tea on the translocation of glucose transporter 4 (GLUT4), the major glucose transporter for glucose uptake in the postprandial period, in skeletal muscle and the underlying molecular mechanisms are not fully understood. This study investigated the translocation of GLUT4 and its related signaling pathways in skeletal muscle of male ICR mice given fermented tea. Intake of oolong, black, or pu-erh tea for 7 days enhanced GLUT4 translocation to the plasma membrane of skeletal muscle. Each type of fermented tea stimulated the phosphorylation of phosphoinositide 3-kinase (PI3K), Akt/protein kinase B, and AMP-activated protein kinase (AMPK). Fermented tea also increased the protein expression of insulin receptor. These results strongly suggest that fermented tea activates both PI3K/Akt- and AMPK-dependent signaling pathways to induce GLUT4 translocation and increases the expression of insulin receptor to improve glucose intolerance.

KEYWORDS: *fermented tea, glucose transporter 4 (GLUT4), AMP-activated protein kinase (AMPK), phosphoinositide 3-kinase (PI3K), insulin receptor*

INTRODUCTION

Type 2 diabetes mellitus is characterized by the loss of sensitivity to insulin, and many countries are experiencing a rapid increase in its prevalence. The pathogenesis of type 2 diabetes mellitus involves the progressive development of insulin resistance in peripheral tissues. In turn, insulin resistance is associated with an increased risk of cardiovascular diseases and diabetes. Therefore, preventing excess postprandial hyperglycemia and improving insulin resistance are effective strategies to treat hyperglycemia and diabetes mellitus.

Several natural products, including tea, have the potential to prevent and treat hyperglycemia and diabetes mellitus. Tea, originating in China, is the most widely consumed beverage other than water. Tea can be classified into four major categories according to the degree of fermentation: non-fermented green tea, partially fermented oolong tea, fully fermented black tea, and postfermented pu-erh tea. The composition of catechins and other bioactive components in these tea varies with the degree of fermentation.

Several studies have shown that green, oolong, and black tea possess antihyperglycemic effects.[1−3] Tea and its components were reported to inhibit digestive enzymes in the small intestine[4] and gluconeogenic enzymes in the liver.[5] More recent studies have focused on insulin-sensitive glucose transporter 4 (GLUT4) as a novel target of food factors.[2,6,7]

Glucose transporters (GLUTs) play an important role in the regulation of blood glucose levels. GLUT4 is specifically expressed in skeletal muscle and adipose tissue, where it takes up glucose to reduce postprandial hyperglycemia.[8] GLUT4 is mainly localized in intracellular storage vesicles and translocates to the plasma membrane in response to insulin.[9] The insulin and AMP-activated protein kinase (AMPK) signaling pathways are the major regulators of GLUT4 translocation in muscle.[10] Binding of insulin to the insulin receptor (IR) induces phosphorylation of the tyrosine kinase domain, which then transmits the insulin signal to multiple tyrosine residues on insulin receptor substrate (IRS) molecules. Activated IRS-1 phosphorylates the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K), which phosphorylates phosphoinositide-dependent kinase 1 and downstream Akt and atypical protein kinase C. This pathway ultimately induces GLUT4 translocation to the cell membrane and enhancement of glucose transport.

GLUT4 translocation in skeletal muscle is also stimulated by exercise via the activation of AMPK, an insulin-independent signaling pathway.[9,11] AMPK acts as a cellular energy sensor and regulates metabolic homeostasis. Consequently, there is much interest in developing AMPK activators as potential therapies for type 2 diabetes mellitus and obesity.[12,13] Previously, we demonstrated that ad libitum drinking of green tea increased skeletal muscle glucose uptake and GLUT4 translocation in male Wistar rats, whereas its activity was decreased in adipose tissue.[14] We also found that green and black tea suppressed high-fat diet-induced hyperglycemia and insulin resistance by maintaining GLUT4 expression and increasing its translocation to the plasma membrane in skeletal muscle of mice. These results strongly suggest that tea may improve hyperglycemia and insulin resistance by modulating the function and expression of GLUT4. However, the effects of fermented tea, other than black tea, on the translocation of GLUT4 in skeletal muscle and its underlying molecular

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Table 1. Catechin and Theaflavin Composition of Fermented Tea[a]

constituent	oolong tea	black tea	pu-erh tea
catechins (mg/L)			
C	1.54 ± 0.26a	7.55 ± 0.25b	3.26 ± 0.48c
EC	3.86 ± 0.43a	6.50 ± 0.09b	5.16 ± 0.24c
GC	6.61 ± 0.99a	4.60 ± 0.20b	6.51 ± 0.08a
EGC	14.09 ± 0.30a	4.56 ± 0.14b	10.34 ± 0.16c
ECg	5.78 ± 1.59a	20.11 ± 0.29b	0.68 ± 0.08c
Cg	0.05 ± 0.01a	0.17 ± 0.00b	0.02 ± 0.00c
EGCg	28.41 ± 3.82a	25.51 ± 0.84a	3.41 ± 0.90b
GCg	0.54 ± 0.12a	0.09 ± 0.01b	0.28 ± 0.05c
total catechins	60.88 ± 3.94a	69.09 ± 0.95b	29.66 ± 0.47c
theaflavins (mg/L)			
TF	0.14 ± 0.08a	19.38 ± 0.66b	0.03 ± 0.01a
TF3g	0.03 ± 0.01a	11.12 ± 0.32b	0.01 ± 0.00a
TF3′g	0.02 ± 0.01a	7.07 ± 0.39b	0.01 ± 0.00a
TF3,3′dg	0.02 ± 0.01a	7.91 ± 0.23b	0.01 ± 0.00a
total theaflavins	0.20 ± 0.06a	45.49 ± 0.09b	0.06 ± 0.01c
catechins plus theaflavins (mg/L)	61.08 ± 3.98a	114.58 ± 1.04b	29.72 ± 0.46c
total polyphenol[b] (mequiv/L)	295	1351	320

[a]The catechin and theaflavin composition of fermented tea was determined by LC-MS/MS. Data are the mean ± standard deviation (n = 3). The total polyphenol content was measured by the Folin−Ciocalteu method. [b]Total polyphenol content is expressed as mequiv gallic acid/L.

mechanism are not fully understood. Therefore, in this study, we investigated the effects of fermented tea on GLUT4 translocation and its related signaling pathways.

MATERIALS AND METHODS

Materials. Catechins (C, GC, EC, EGC, Cg, GCg, ECg, and EGCg) were purchased from Kurita Analysis Service Co. Ltd. (Tukuba, Japan), and theaflavins (TF, TF-3-g, TF-3′-g, and TF-3,3′-Dg) were provided from ITO EN, Ltd. (Makinohara, Japan). Blood glucose levels were measured using a commercially available kit from Wako Pure Chemical Industries (Osaka, Japan). For Western blotting, anti-GLUT4 goat IgG, anti-GLUT1 goat IgG, anti-IRβ rabbit IgG, anti-p-PI3K goat IgG, anti-mouse IgG, anti-goat IgG, and anti-rabbit IgG antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-Akt rabbit IgG, anti-p-Akt rabbit IgG, anti-AMPK rabbit IgG, and anti-p-AMPK rabbit IgG antibodies were from Cell Signaling Biotechnology (Tokyo, Japan). Anti-PI3K mouse IgG antibody was obtained from BD Transduction Laboratories (Tokyo, Japan). Blocking-One solution was from Nacalai Tesque (Kyoto, Japan). Polyvinylidene difluoride (PVDF) membrane and Immunostar were products of Pall Gelman Laboratory (Tokyo, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively. All of the other reagents used were of the highest grade available from commercial sources.

Preparation of Fermented Tea. Oolong, black, and pu-erh tea leaves were purchased from a local market in Kobe. Tea was freshly prepared as follows. Briefly, 2 g of tea leaves was extracted in boiled water for 2 min. For oolong and pu-erh tea, the tea leaves were washed with 100 mL of boiled water for 15 s before extraction. The resulting extracts were cooled to room temperature and used for experiments within 1 day.

Measurement of Total Polyphenols. The total polyphenol content in each tea was measured by using the Folin−Ciocalteu method.[15] Briefly, 10 μL of tea and 50 μL of Folin−Ciocalteu reagent were mixed with 790 μL of distilled water. After 1 min, 150 μL of 20% (w/v) aqueous Na_2CO_3 solution was added, mixed, and left at room temperature in the dark for 120 min. The absorbance of the mixture was measured at 750 nm. The total polyphenol content was calculated from a calibration curve using gallic acid as a standard compound.

Measurement of Tea Catechins in Fermented Tea by Lipid Chromatography−Tandem Mass Spectrometry (LC-MS/MS). Catechins and theaflavins were analyzed by LC-MS/MS according to the previous method.[16] Tea extract was first centrifuged at 10000g for 10 min at 4 °C. The resulting supernatant was then subjected to reverse-phase high-performance liquid chromatography (HPLC) and quadrupole tandem mass spectrometry (4000 Q_TRAP, AB Sciex, Foster City, CA, USA). HPLC separation was done with a gradient system using solvent A [0.1% (v/v) formic acid] and solvent B (acetonitrile) using a COSMOSIL Cholester column (2.0 × 150 mm; Nacalai Tesque) at a flow rate of 0.2 mL/min. The gradient program was as follows: 0−2 min, 100% A; 2−20 min, linear gradient to 70% A; 20−30 min, linear gradient to 50% A; 30−40 min, linear gradient to 0% A; 40−41 min, linear gradient to 100% A; 41−48 min, 100% A hold. The catechins and theaflavins were detected by multiple reaction monitoring as follows: catechin (C) and epicatechin (EC), 291.2/139.0 $[M + H]^+$; gallocatechin (GC) and epigallocatechin (EGC), 307.1/139.0 $[M + H]^+$; catechin gallate (Cg) and epicatechin gallate (ECg), 443.1/139.0 $[M + H]^+$; gallocatechin gallate (GCg) and epigallocatechin gallate (EGCg), 459.3/139.0 $[M + H]^+$; theaflavin (TF), 565.0/139.0 $[M + H]^+$; theaflavin-3-gallate (TF3g) and theaflavin-3′-gallate (TF3′), 717.0/139.0 $[M + H]^+$; and theaflavin-3,3′-digallate (TF-3,3′-dg), 869.0/139.0 $[M + H]^+$.

Animal Treatments. Animal experiments were carried out according to the Guidelines for the Care and Use of Experimental Animals at Kobe University Rokkodai Campus (Permission 21-07-02). Male ICR mice (6 weeks old) were obtained from Japan SLC (Shizuoka, Japan) and maintained in a temperature-controlled room (23 ± 2 °C) with a 12:12 h light/dark cycle (lights on at 9:00 a.m.). In this study, we carried out two animal experiments. In both experiments, 16 mice were given free access to tap water and a commercial chow and were acclimatized for 7 days before the experiments. The mice were divided into four groups (n = 4 mice/group), to receive oolong tea, black tea, pu-erh tea, or water for 7 days. The fermented tea was supplied instead of drinking water. All animals were given free access to commercial chow during this time.

Assessment of Glucose Tolerance (Experiment 1). Mice in this experiment underwent oral glucose tolerance tests on day 7. The mice were fasted for 12 h and then orally administered a glucose solution (1 g/kg body weight). Blood samples were collected in heparinized tubes from a tail vein before (0 min) and 15, 30, 60, and 120 min after glucose administration.

Assessment of GLUT4 and Its Related Signaling Pathways (Experiment 2). Another set of mice were sacrificed on day 7 without fasting, and the soleus muscle were collected. Muscle was washed in

1.15% (w/v) KCl, immediately frozen in liquid nitrogen, and stored at −80 °C until assessment of GLUT4 and its related signaling pathways by Western blot analysis.

Measurement of Plasma Glucose Levels. Blood samples were collected and centrifuged at 9600*g* for 10 min at 4 °C. The supernatant was collected as plasma and glucose levels were measured using a commercial kit, as described above.

Western Blot Analysis. We prepared plasma membrane fractions and tissue lysates from skeletal muscle as previously described.[17] The plasma membrane fraction was used to detect GLUT4 translocation and the expression of IRβ and GLUT1. The tissue lysate was used to detect the expression of GLUT4, AMPK, p-AMPK, Akt, p-Akt, PI3K, p-PI3K, and actin. The protein bands were visualized using ImmunoStar LD (Wako Pure Chemical Industries) and detected with a light-Capture II (ATTO Corp., Tokyo, Japan). The density of specific bands was determined using Image J image analysis software provided from the National Institutes of Health (NIH, Bethesda, MD, USA).

Statistical Analysis. The results are presented as the mean ± standard error (SE). Differences among each group were analyzed using Dunnett's test. The level of significance was set at $p < 0.05$.

RESULTS

Total Polyphenol Content and Catechin Composition of Fermented Tea. The total polyphenol content was measured by using the Folin−Ciocalteu method (Table 1). The total polyphenol contents were similar in oolong and pu-erh teas (295 and 320 mg/L, respectively), whereas black tea contained a much higher polyphenol content (1351 mg/L). The composition of catechins in each tea was determined by LC-MS/MS. As shown in Table 1, oolong tea mainly contained significantly much greater amounts of EGC and EGCg than the other teas. Black tea significantly contained abundant catechins, particularly EGCg and ECg, as well as TF and TF3g. By contrast, pu-erh tea contained much lower levels of catechins, particularly gallate-type catechins, than the other teas. These results indicate that the total amounts of catechins were similar in oolong tea and black teas, but their content was approximately 50% lower in pu-erh tea than in the other teas. Black tea also contained high levels of TFs specifically. On the basis of this analysis, the abundance of catechins and TFs was greatest in black tea and lowest in pu-erh tea.

Fermented Tea Improves Glucose Intolerance in ICR Mice (Experiment 1). During the feeding period, body weight gain, food intake, and beverage intake were not affected by fermented tea compared with the water-given control group. Figure 1a shows the changes in the plasma glucose levels during the oral glucose tolerance test performed on day 7. The initial glucose levels were lower in black tea group without significance. The result shows that plasma glucose levels were significantly lower in the black tea group than in the control group 15 min after glucose loading. The area under the curve (AUC) was also significantly lower in the oolong and black tea groups than in the control group (Figure 1b). The AUC for the pu-erh tea group was lower than that in the control group, but not significantly.

Fermented Tea Promotes GLUT4 Translocation by Activating the PI3K/Akt- and AMPK-Dependent Signaling Pathways (Experiment 2). In our previous study, consumption of green tea for 3 weeks enhanced GLUT4 translocation in the skeletal muscle of rats.[14] In this study, we investigated whether fermented tea also enhances GLUT4 translocation in the skeletal muscle of nonfasted ICR mice. Plasma glucose levels were generally similar in the control, oolong, black, and pu-erh tea groups (227 ± 7.2, 213 ± 13.4,

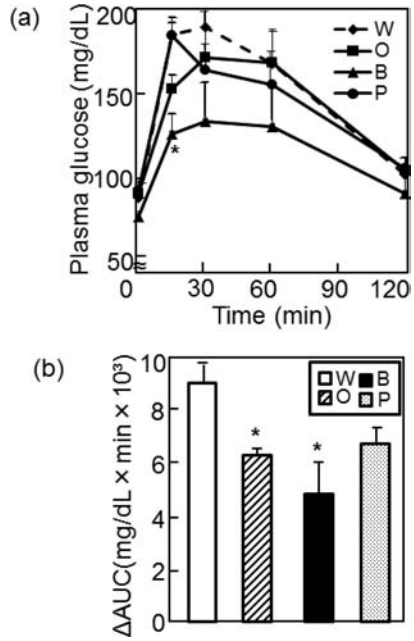

Figure 1. Effects of fermented tea on glucose intolerance: (a) ICR mice were given water (W), oolong tea (O), black tea (B), or pu-erh tea (P) for 7 days, and then oral glucose tolerance tests were performed; (b) areas under the curve for the results shown in panel a; the white, slashed, black, and hatched bars represent the results for mice given W, O, B, and P, respectively. Values are the mean ± SE (n = 4). ∗, significantly different from the corresponding control (W) group ($p < 0.05$; Dunnett's test).

238 ± 4.4, and 245 ± 3.8 mg/dL, respectively). Fermented tea significantly increased plasma membrane GLUT4 levels without affecting the GLUT1 level (Figure 2a). However, fermented tea did not affect the expression level of GLUT4 in the tissue lysate (Figure 2b). It was noteworthy that the consumption of black or pu-erh tea significantly increased the expression of IRβ in the plasma membrane fraction compared with that in the control group. These results suggest that fermented tea promotes GLUT4 translocation, without affecting its expression level, and increases IRβ expression.

GLUT4 translocation to the plasma membrane is mainly regulated by two distinct signaling pathways, the PI3K/Akt- and AMPK-dependent signaling pathways.[9,11] To elucidate the mechanisms by which fermented tea promotes GLUT4 translocation, we determined the phosphorylation status of PI3K, Akt, and AMPK in skeletal muscle. As shown in Figure 3, black and pu-erh teas significantly increased PI3K phosphorylation, whereas oolong tea elicited a nonsignificant increase in PI3K phosphorylation. All three fermented teas significantly increased the phosphorylation of Akt. These results indicate that fermented tea activates the PI3K/Akt-dependent signaling pathway. In terms of the AMPK-dependent signaling pathway, black tea and pu-erh tea significantly increased the phosphorylation of AMPK, whereas oolong tea increased its phosphorylation, albeit not significantly (Figure 4). These results indicate that fermented teas, especially black and pu-erh teas, promote GLUT4 translocation by activating both PI3K/Akt- and AMPK-dependent signaling pathways.

DISCUSSION

Tea contains abundant polyphenols such as catechins and TFs. These compounds contribute to the various health-promoting

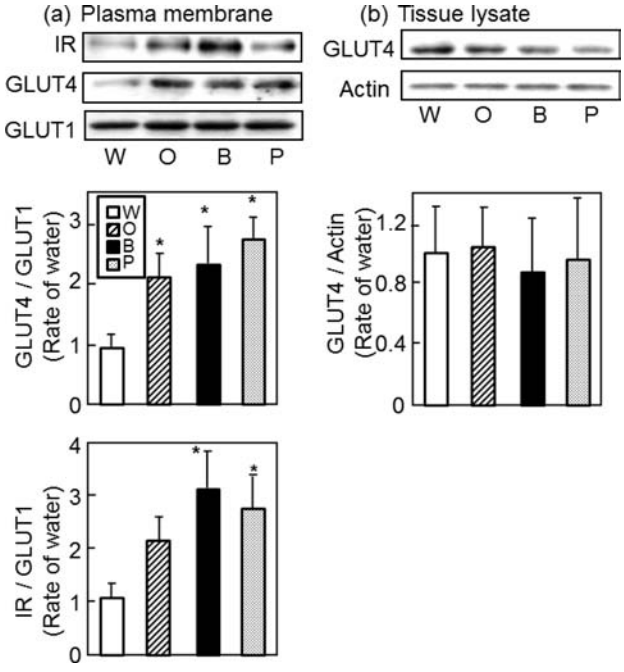

Figure 2. Effects of fermented tea on GLUT4 translocation in skeletal muscle. ICR mice were given water (W), oolong tea (O), black tea (B), or pu-erh tea (P) for 7 days. (a) The plasma membrane fraction was prepared and subjected to Western blotting analysis to determine IR, GLUT4, and GLUT1 expression. (b) The lysate of skeletal muscle was prepared and subjected to Western blotting analysis to determine GLUT4 and actin expression. Each panel shows a typical result from four animals. The white, slashed, black, and hatched bars represent the band density for mice given W, O, B, and P, respectively. Values are the mean ± SE (n = 4). ∗, significantly different from the corresponding control (W) group ($p < 0.05$; Dunnett's test).

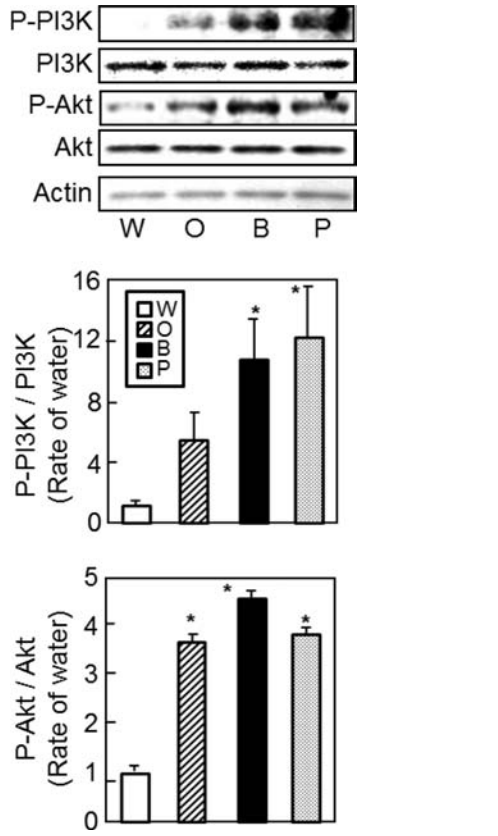

Figure 3. Effect of fermented tea on phosphorylation of PI3K and Akt in skeletal muscle. ICR mice were given water (W), oolong tea (O), black tea (B), or pu-erh tea (P) for 7 days. Skeletal muscle tissue lysate was prepared and subjected to Western blotting analysis to detect p-PI3K, PI3K, p-Akt, Akt, and actin expression. Each panel shows a typical result from four animals. The white, slashed, black, and hatched bars represent the band density for mice given W, O, B, and P, respectively. Values are the mean ± SE (n = 4). ∗, significantly different from the corresponding control (water) group ($p < 0.05$; Dunnett's test).

effects of tea.[18,19] In this study, we found that oolong, black, and pu-erh teas prevented postprandial hyperglycemia (Figure 1) by enhancing GLUT4 translocation to the plasma membrane of skeletal muscle in ICR mice (Figure 2). The plasma glucose levels were significantly lower in the black tea group than in the control group 15 min after glucose loading. The pu-erh tea group was lower than that in the control group, but not significantly (Figure 1). Fermented tea, in particular black and pu-erh teas, activated both the PI3K/Akt- and AMPK-dependent pathways to induce GLUT4 translocation (Figures 3 and 4). We also found that fermented tea increased the IRβ expression in skeletal muscle (Figure 2). Our findings are consistent with previous studies in which EGCg was reported to promote GLUT4 translocation to the plasma membrane in skeletal muscle,[6] and green and black teas attenuated high-fat diet-induced suppression of IRβ, GLUT4, and AMPK expression in the skeletal muscle of C57BL/6J mice.[2] In addition, black tea revealed relatively stronger effects than green tea,[2] indicating that fermented tea will contain more effective compounds than EGCg. Therefore, in this study, we focused on only fermented tea antihyperglycemic effects. Our current results and these previous results indicate that fermented teas, in particular black and oolong teas, prevent and ameliorate hyperglycemia by stimulating GLUT4 translocation in skeletal muscle. Pu-erh tea tended to lower the blood glucose level after glucose load without significance. On the other hand, pu-erh tea significantly activated both the PI3K/Akt- and AMPK-dependent pathways to induce GLUT4 translocation. Moreover, pu-erh tea did not contain catechins and TFs abundantly. Thus, pu-erh tea will contain unknown compound(s) that may be able to help prevent hyperglycemia.

GLUT4 is specifically expressed in adipose tissue, skeletal muscle, and cardiac muscle. Of these, skeletal muscle is the most important therapeutic target for hyperglycemia because it accounts for approximately 80% of insulin-stimulated glucose uptake in the postprandial state.[20] The two main pathways that regulate GLUT4-mediated glucose uptake are the PI3K/Akt- and AMPK-dependent signaling pathways. To clarify the mechanism by which fermented teas, in particular black and pu-erh teas, promote GLUT4 translocation, we determined the phosphorylation status of PI3K, Akt, and AMPK in the skeletal muscle of ICR mice and found that fermented tea activated both the PI3K/Akt- and AMPK-depdendent signaling pathways. Polyphenols have already been reported to promote GLUT4 translocation through the PI3K/Akt- and/or AMPK-depdendent signaling pathways in skeletal muscle. For example, the effects of procyanidin,[7] resveratrol,[21] and anthocyanin[22] on GLUT4 translocation were dependent on the activation of the AMPK pathway. Meanwhile, EGCg inhibited dexamethasone-induced insulin resistance by activating both PI3K/Akt and AMPK pathways in rat L6 cells.[23] It was also reported that EGCg improves glucose uptake and Akt phosphorylation by

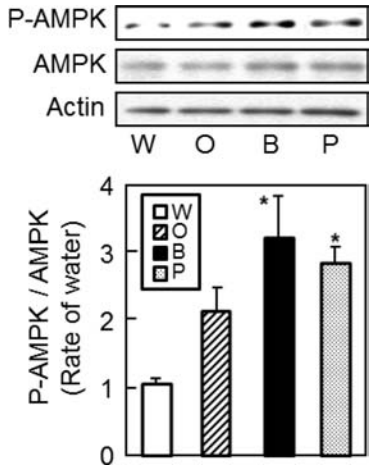

Figure 4. Effects of fermented tea on AMPK phosphorylation in skeletal muscle. ICR mice were given water (W), oolong tea (O), black tea (B), or pu-erh tea (P) for 7 days. Skeletal muscle tissue lysate was prepared and subjected to Western blotting analysis to detect p-AMPK and AMPK expression. Each panel shows a typical result from four animals. White, slashed, black, and hatched bars represent the band density for mice given W, O, B, and P, respectively. Values are the mean ± SE (n = 4). *, significantly different from the corresponding control (W) group (p < 0.05; Dunnett's test).

activating AMPK in high-glucose-induced insulin-resistant HepG2 cells.[24] Thus, the reports describing interactions between the PI3K/Akt- and AMPK-dependent signaling pathways are controversial. To our knowledge, this is the first report to show that black and pu-erh teas enhance GLUT4 translocation by activating both PI3K/Akt- and AMPK-dependent signaling pathway in the skeletal muscle of mice. Further studies are needed to understand the complexity of the crosstalk between these two signaling pathways. Oolong tea also enhanced GLUT4 translocation and tended to decrease the plasma glucose levels (Figures 1 and 2). However, oolong tea-promoted GLUT4 translocation was independent of insulin and the AMPK pathway (Figures 3 and 4). Our previous study demonstrated that 4-hydroxyderricin and xanthoangelol promote GLUT4 translocation in skeletal muscle by a mechanism that is independent of insulin and the AMPK pathway.[25] Thus, there is a possibility that an unknown unique mechanism exists to promote GLUT4 translocation. It is necessary to perform a comprehensive analysis of the signaling affected by oolong tea.

It is also notable that the intake of fermented tea increased IRβ expression in skeletal muscle, except oolong tea (Figure. 2). Binding of insulin to IRβ is the initial step in the insulin-dependent GLUT4 translocation pathway.[20] It was reported that insulin resistance decreases IRβ expression in skeletal muscle.[26] We previously reported that black and green tea preserved IRβ expression in high-fat diet-induced insulin-resistant C57BL/6J mice.[2] Therefore, fermented tea may be able to maintain or increase the IRβ protein expression, although the underlying molecular mechanism is currently unclear. We suspect that this phenomenon contributes to the preventive effects of tea on hyperglycemia, in addition to enhanced GLUT4 translocation in skeletal muscle.

In the present study, we demonstrated that black and pu-erh teas enhanced GLUT4 translocation and activated both PI3K/Akt- and AMPK-dependent signaling pathways (Figures 3 and 4), even though their polyphenol contents and compositions were very different (Table 1). The polyphenol contents differ between individual species of tea. In the present study, of the three types of tea extracted from 2 g of tea leaves, black tea contained the highest amount of polyphenols, whereas oolong and pu-erh teas contained similar amounts of polyphenols (Table 1). Catechins, particularly EGCg, have various beneficial health-promoting effects, such as anticancer activity, hepatoprotective effect, antiobesity effect, and antiatherosclerotic effect.[27] TFs, which are mostly found in black tea, also have beneficial effects, such as decreasing hepatic lipid accumulation.[28] Catechins and TFs were also reported to inhibit intestinal α-glucosidase activity in vitro. In vivo, inhibition of α-glucosidase activity is an important mechanism that suppresses postprandial hyperglycemia.[8] In our previous paper, we showed that long-term intake of green or black tea suppressed hyperglycemia by modulating the expression and translocation of GLUT4 but did not affect α-glucosidase activity.[2] Our current findings provide strong evidence to support that black tea improves glucose tolerance mainly by promoting GLUT4 translocation and enhancing glucose uptake in skeletal muscle. This beneficial action is probably due to the abundance of polyphenols in black tea under almost the same brewing conditions of tea. Although pu-erh tea contains the lowest amounts of catechins and TFs of the three types of fermented teas tested in this study, it nevertheless significantly enhanced GLUT4 translocation and the phosphorylation of PI3K, Akt, and AMPK, comparable to the effects of black tea. These results indicate that unknown compound(s) in pu-erh tea may stimulate GLUT4 translocation. Taken together, TFs were not the most effective compounds, but catechins and TFs did, at least in part, contribute to promoting GLUT4 translocation in skeletal muscle. Further experiments using various tea extracts containing the same amounts of polyphenols are needed to compare the effects of GLUT4 translocation and antihyperglycemia.

In conclusion, dietary supplementation of fermented tea, particularly black tea, in addition to green tea improved postprandial hyperglycemia by promoting GLUT4 translocation to the plasma membrane and activating both PI3K/Akt- and AMPK-dependent signaling pathways. Therefore, consumption of fermented tea may be able to help prevent diabetes mellitus.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AMPK, AMP-activated protein kinase; AUC, area under the curve; C, catechin; Cg, catechin gallate; EC, epicatechin; ECg, epicatechin gallate; EGC, epigallocatechin; EGCg, epigallocatechin gallate; GCg, gallocatechin; GCg, gallocatechin gallate; GLUT4, glucose transporter 4; HPLC, high-performance liquid chromatography; IR, insulin receptor; IRSs, insulin receptor substrate; LC-MS/MS, lipid chromatography–tandem mass

spectrometry; PI3K, phosphoinositide 3-kinase; TF, theaflavin; TF3g, , theaflavin-3-gallate; TF3′g, theaflavin-3′-gallate; TF-3,3′-dg, theaflavin-3,3′-digallate.

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