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Epigallocatechin gallate promotes GLUT4 translocation in skeletal muscle

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ABSTRACT

In this study, we investigated whether epigallocatechin gallate (EGCg) affects glucose uptake activity and the translocation of insulin-sensitive glucose transporter (GLUT) 4 in skeletal muscle. A single oral administration of EGCg at 75 mg/kg body weight promoted GLUT4 translocation in skeletal muscle of rats. EGCg significantly increased glucose uptake accompanying GLUT4 translocation in L6 myotubes at 1 nM. The translocation of GLUT4 was also observed both in skeletal muscle of mice and rats *ex vivo* and in insulin-resistant L6 myotubes. Wortmannin, an inhibitor of phosphatidylinositol 3′-kinase, inhibited both EGCg- and insulin-increased glucose uptakes, while genistein, an inhibitor of tyrosine kinase, failed to inhibit the EGCg-increased uptake. Therefore, EGCg may improve hyperglycemia by promoting GLUT4 translocation in skeletal muscle with partially different mechanism from insulin.

Glucose transporter (GLUT) 4 plays a pivotal role in regulating insulin-stimulated glucose transport in skeletal muscle and adipose tissue [1]. The conditional depletion of GLUT4 caused insulin resistance and chronic hyperglycemia; eg. muscle-specific GLUT4 knockout mice revealed hyperglycemia [2], and the overexpression of GLUT4 in adipose tissue of these mice overcame glucose intolerance and diabetes [3]. Long-term feeding of a fructose-rich diet caused fasting hyperglycemia, hyperinsulinemia, and elevated blood pressure due to a decrease in the expression level of GLUT4 in adipose tissue of Sprague-Dawley rats [4]. A high-fat diet also caused declines in GLUT4 and insulin receptor (IR) levels in skeletal muscle of C57BL/6 mice [5]. These results suggest that GLUT4 is a target for improvement in hyperglycemia.

Skeletal muscle accounts for nearly 40% of body mass and is the most important tissue for glucose utilization [6]. Our previous report demonstrated that the intake of green tea for 3 weeks increased glucose uptake activity accompanied by the translocation of GLUT4 in skeletal muscle of male Wistar rats, while it decreased uptake activity and the translocation in adipose tissue [7]. Green tea improved lowered-GLUT4 expression, elevated-blood pressure and hyperinsulinemia in Sprague-Dawley rats fed a high-fructose diet [4]. These reports suggested that green tea regulates the expression and function of GLUT4 resulting in improvements in hyperglycemia, although the active component of green tea is not clear yet.

Epigallocatechin gallate (EGCg) is a major polyphenol in green tea [8,9] and is reported to have various beneficial biological functions including anti-tumor [8], anti-oxidative, anti-inflammatory [10], and anti-obesity [11] activities. EGCg was reported to inhibit the differentiation of 3T3-L1 preadipocytes into adipocytes by suppressing the expression of peroxisome proliferator-activated receptor-γ2 and CCAAT/enhancer-binding protein-α, key transcription factors at an early stage of differentiation, and the expression of GLUT4 at a later stage [12]. Oral administration of EGCg to obese Zucker rats, a model of type-2 diabetes, significantly lowered blood glucose and insulin levels [13]. In the present study, we investigated whether EGCg stimulated glucose uptake accompanying the translocation of GLUT4 in skeletal muscle.

Materials and methods

Chemicals and antibodies. EGCg was a gift from Dr. Suong-Hyu Hyon (Kyoto University, Japan). Dried green and black tea leaves (*Camellia sinensis*) were manufactured in Japan and Sri Lanka, respectively. These leaves (2 g) were extracted with 100 ml of hot water for 5 min, and then cooled to room temperature. For the glucose uptake assay, [^{3}H]-3-*O*-methyl-D-glucose (3-OMG) was obtained from DuPont/NEN Research Products (Boston, MA). For the Western blot analysis, anti-GLUT4 goat IgG, anti-IR$_\beta$ rabbit IgG, anti-goat IgG, and anti-rabbit IgG antibody were purchased from

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Santa Cruz Biotechnology Inc. (Santa Cruz, CA), and anti-β-actin mouse IgG, from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of the highest grade available commercially.

Animal treatment. All animal treatments was approved by the Institutional Animal Care and Use Committee and carried out according to the Kobe University Animal Experimentation Regulations. For *in vivo* experiments, male Sprague-Dawley rats (six-weeks-old, 140–170 g; Japan SLC, Shizuoka, Japan) were housed in an air-conditioned room (25 ± 1 °C) under a 12-h light-dark cycle with free access to water and commercial chow, and acclimatized for 1 week. Rats were divided at random into three groups of 3 each. Each group was given freshly prepared green or black tea or water *ad libitum* daily for 7 days, and sacrificed under anesthesia on day 7. Another series of 6 rats were divided at random into two groups of 3 each. After fasting for 12 h, they were given orally EGCg at 75 mg/kg body weight or 0.85% NaCl (2 ml/kg body weight) as a vehicle control, and sacrificed under anesthesia 1 h later. From both series of rats, the soleus muscle was trimmed and lysates and a plasma membrane fraction were prepared [5].

For the *ex vivo* experiment, soleus muscles of Sprague-Dawlay rats and C57BL/6 mice were chopped into pieces as small as possible with scissors. Aliquots of 100 mg of muscle were incubated with 1 or 100 nM EGCg in 3 ml of Krebs-Ringer phosphate-HEPES buffer (KRH; 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 15 min at 37 °C with continuous shaking, and then immediately washed twice with ice-cold KRH buffer. As positive and negative controls, 100 nM insulin and DMSO (final 0.1%) were added for 15 min, respectively. GLUT4 translocation in these muscles was detected by Western blot analysis [5].

Cell culture and treatments. Cultures and the differentiation of L6 myoblasts into myotubes were performed as described [5]. Insulin-resistant L6 myotubes were prepared by treatment with palmitate [13]. Briefly, a stock solution of palmitate (75 mM) in ethanol was diluted 1:100 in modified Eagle's medium (MEM) containing 2% fetal bovine serum and 2% bovine serum albumin (BSA) just prior to use. L6 myotubes were cultured in the presence of 0.75 mM palmitate for 14 h, and then kept in serum-starved medium without palmitate for a further 18 h. These cells were treated with 100 nM EGCg and/or of 100 nM insulin for 15 min to detect the translocation of GLUT4.

Glucose uptake assay. L6 myotubes on 24-well plates were serum-starved for 18 h in MEM containing 0.2% BSA at 37 °C. The cells were incubated with various catechins in KRH buffer. Then a final concentration of 6.5 mM (0.5 μCi) [^{3}H]-3-OMG was added and incubated for 1 min at 37 °C. As controls, 100 nM insulin and DMSO (final concentration, 0.1%) as a vehicle were given to the cells for 15 min. The uptake was terminated by washing the myotubes immediately four times with ice-cold KRH, and the cells were solubilized with 0.05 N NaOH. Non-specific uptake was measured in the presence of 20 μM cytochalasin B as an inhibitor of glucose transporters. The radioactivity was measured by a liquid scintillation counter with the scintillation cocktail.

Statistical analysis. Statistical analyses were performed with Student's *t*-test. Significance was defined as $p < 0.05$.

Results

EGCg promoted GLUT4 translocation in vivo

The translocation of GLUT4 was investigated in skeletal muscle of rats given green and black teas for 1 week. During feeding period, we did not observe any differences of a body weight gain and the intake of diet between control and tea-given groups (data not shown). Significant translocation was observed in the plasma membrane of skeletal muscle (Fig. 1). Neither green nor black tea affected the levels of IR_β and GLUT4. Significant GLUT4 translocation was also observed in skeletal muscle of rats dosed with EGCg (Fig. 1B). These results indicate that EGCg is one of the active constituents in tea.

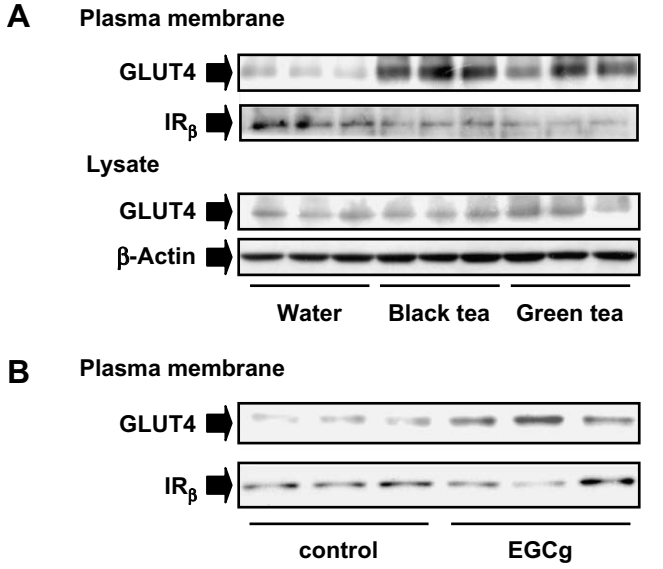

Fig. 1. Tea and its constituent EGCg promoted GLUT4 translocation in rat skeletal muscle. (A), GLUT4 and IRβ levels in the plasma membrane and lysate in skeletal muscle of rats given green and black tea and water *ad libitum* for 1 week. (B), translocated GLUT4 on the plasma membrane of skeletal muscle of rats given EGCg at 75 mg/kg body weight.

EGCg stimulated glucose uptake in skeletal muscle cells

Next, the effect of 8 catechins at 5 μM on the glucose uptake activity in L6 myotubes was investigated by using [^{3}H]-3-OMG. Treatment with 100 nM insulin as a positive control increased the glucose uptake activity approximately 2.6-fold compared to the DMSO-treated negative control (Fig. 2A). All catechins tested except catechin and catechin gallate increased the glucose uptake activity in L6 myotubes compared that in the DMSO-treated cells. When EGCg was added to L6 myotubes at various concentrations for 15 min, glucose uptake increased in a dose-dependent manner, with a significant increase observed at 1 nM (Fig. 2B). EGCg at 10 nM had almost the same effect as insulin at 100 nM. After treatment with EGCg at 100 nM, a rapid increase in the uptake activity was observed within 1 min. The activity reached a maximum at 15 min, and had slightly decreased by 60 min (Fig. 2C).

EGCg promoted GLUT4 translocation in normal and insulin-resistant L6 myotubes

We confirmed whether EGCg promotes GLUT4 translocation in muscle cells. At 1 nM, EGCg promoted the translocation in skeletal muscle of rats and mice in *ex vivo* experiments (Fig. 3A and B) and in L6 myotubes (Fig. 3C). When EGCg and insulin were added simultaneously to the cells, neither a synergistic nor an additive effect on GLUT4 translocation (Fig. 3D) or the glucose uptake activity (data not shown) was observed.

To prepare insulin-resistant skeletal muscle cells, L6 myotubes were treated with 0.75 mM palmitate for 14 h then cultured for 18 h without palmitate [13]. Under these conditions, we confirmed the downregulation of IR_β expression as a marker for insulin resistance (date not shown). When EGCg was added to insulin-resistant L6 myotubes, GLUT4 translocation was observed, although insulin failed to promote the translocation.

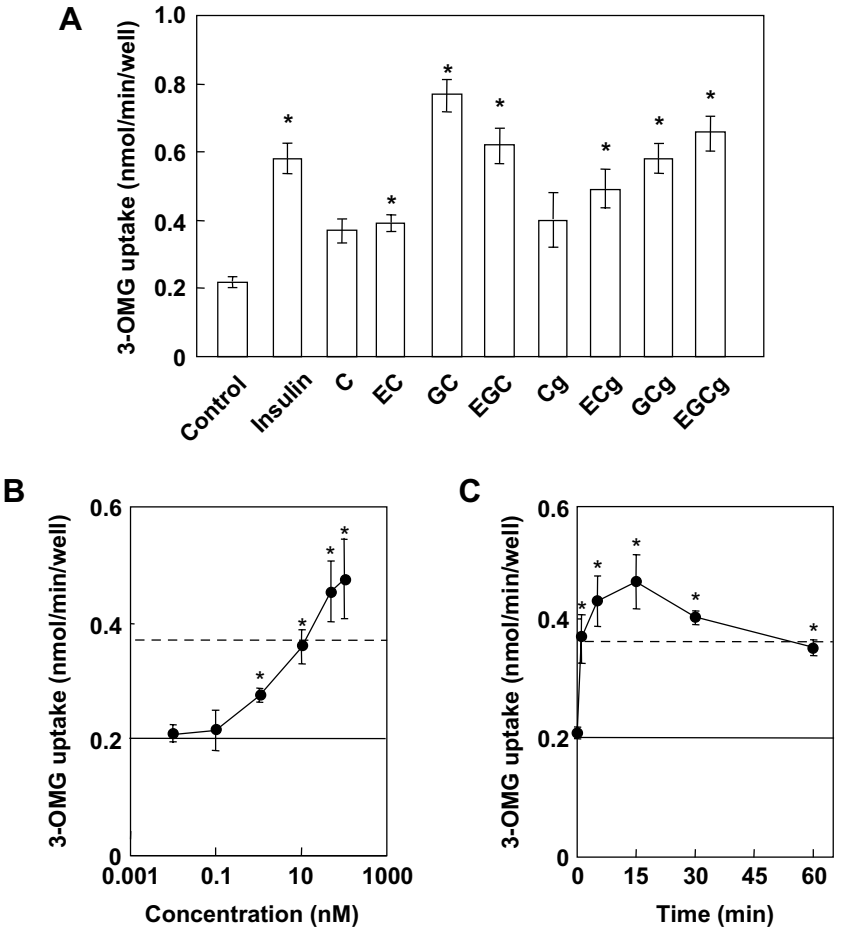

Fig. 2. The effects of catechins on the glucose uptake activity in L6 myotubes. (A), the glucose uptake activity was measured in serum-starved cells treated with various catechins at 5 μM for 15 min. DMSO and 100 nM insulin were also added to the cells for as a negative and positive control, respectively. (B), dose-dependency of EGCg after 15 min, (C), time-dependency of 100 nM EGCg. Broken and solid lines indicate the activity of 100 nM insulin- and DMSO-treated cells, respectively. Data are expressed as the means ± SE. *Significant difference from control, $p < 0.05$ by Student's *t*-test. C, Catechin; EC, epicatechin; GC, gallocatechin; EGC, epigallocatechin; Cg, catechin gallate; ECg, epicatecin gallate; GCg, gallocatechin gallate; and EGCg.

Effects of genistein and wortmannin on the increased glucose uptake activity in L6 myotubes

To investigate how EGCg increased glucose uptake activity, genistein as a tyrosine kinase inhibitor and wortmannin as phosphatidylinositol 3′-kinase inhibitor were introduced. As shown in Fig. 4, genistein inhibited the insulin-induced glucose uptake activity expectably, but it did not inhibit the EGCg-induced glucose uptake. In the case of wortmannin, this inhibitor suppressed both insulin- and EGCg-induced glucose uptake.

Discussion

In this study, we showed that EGCg stimulated the uptake of glucose accompanying the translocation of GLUT4 in skeletal muscle cells at a concentration as low as 1 nM. Several papers have reported that the plasma concentration of EGCg after the drinking of tea or injection of EGCg. In human subjects, it was reported that the plasma concentration of conjugated EGCg ranged from 0.1 to 0.6 μM, 1 h after the injection of 1.2 g of decaffeinated green tea containing 88 mg of EGCg [14]. The same research group showed that conjugated forms of EGCg reached a peak level of 0.26 μM, 1.5–2.5 h after the consumption of 1.5 g of decaffeinated green tea [9]. In rats, the plasma concentration of conjugated EGCg was approximately 0.04 μM, 1 h after the oral administration of EGCg at 75 mg/kg body weight [15]. It was reported that the concentration of EGCg aglycone reached 0.28 μM in plasma of male CF-1 mice after the administration of EGCg at 75 mg/kg body weight [16]. These results indicated that the concentration of EGCg ranged from 0.04 to 0.6 μM in plasma. Hence, EGCg is able to stimulate glucose uptake and GLUT4 translocation at a physiological concentration.

In this study, EGCg also promoted GLUT4 translocation in insulin-resistant L6 myotubes (Fig. 4E). This result suggests that EGCg might improve hyperglycemia in type-2 diabetes mellitus, because skeletal muscle is a major glucose-utilizing tissue [6]. However, little is known about dietary components affecting GLUT4 translocation in muscle cells. This is the first report that tea and its component EGCg increased glucose uptake accompanied by GLUT4 translocation. In contrast, some food extracts including green tea extract were reported to improve hyperglycemia by preventing the depletion of GLUT4 expression or promoting GLUT4 translocation in adipose tissue [4,17,18]. Since it is known that EGCg is easily oxidized in culture medium [19,20], we checked a remaining amount of EGCg by a high-performance liquid chromatography and confirmed over 60% of EGCg was remained in the medium and cells as the aglycone form (data not shown).

Although, the mechanism by which EGCg promotes GLUT4 translocation in skeletal muscle cells was not clarified in this

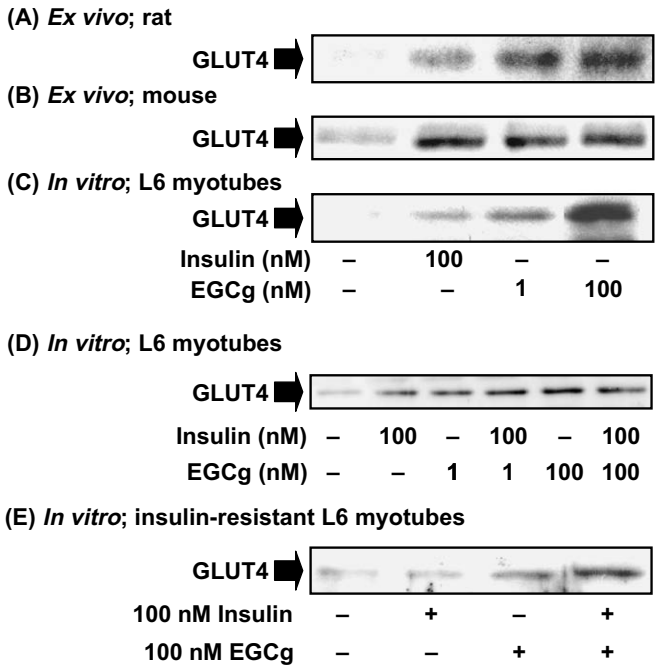

Fig. 3. Effect of EGCg on GLUT4 translocation in normal and insulin-resistant skeletal muscle cells. (A), L6 myotubes and skeletal muscle of (B) rat and (C) mouse were treated with 1 or 100 nM EGCg or 100 nM insulin for 15 min. (D), the cells were treated simultaneously with 1 or 100 nM EGCg and/or 100 nM insulin for 15 min. (E), insulin-resistant L6 cells were incubated with 100 nM EGCg and/or 100 nM insulin for 15 min.

study, we assume that EGCg acts through an insulin-independent pathway, because it promoted the translocation in insulin-resistant L6 myotubes and had neither a synergistic nor an additive effect with insulin. Moreover, genistein inhibited insulin-increased glucose uptake but not EGCg-increased one, while wortmannin completely inhibited both insulin- and EGCg-increased uptake (Fig. 4). These results suggest that the mechanism of EGCg was partially different from that of insulin. GLUT4 translocation was promoted by not only insulin but also exercise and contraction through the activation of 5′-AMP-activated protein kinase (AMPK) in skeletal muscle [21]. In adipose tissue, 100 μM EGCg promoted the phosphorylation of AMPK, IR_β and IRS-1 [22]. In hepatocytes, 1 μM EGCg activated AMPK [23]. It is, therefore, necessary to investigate the molecular mechanism of EGCg in skeletal muscles including the activation of AMPK. In conclusion, EGCg induces GLUT4 translocation in normal and insulin-resistant muscle cells at the physiological concentration. Thus, EGCg has the potential to reduce the postprandial blood glucose level and to improve hyperglycemia in type-2 diabetes mellitus.

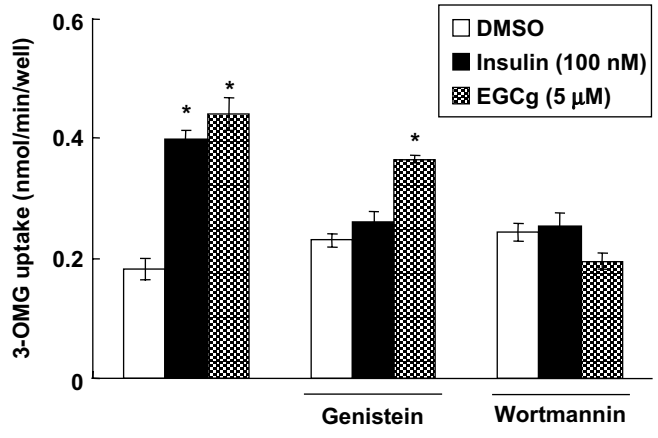

Fig. 4. Effects of genistein and wortmannin on the EGCg-induced glucose uptake activity in L6 myotubes. Serum-starved L6 myotubes were incubated with 10 μM genistein or 1 μM wortmannin for 15 min, and treated with 5 μM EGCg or 100 nM insulin for another 15 min. DMSO and 100 nM insulin were used for a negative and positive control, respectively. Data are expressed as the means ± SE. *Significant difference from control, $p < 0.05$ by Student's *t*-test.

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References

[1] H.G. Joost, G.I. Bell, J.D. Best, M.J. Birnbaum, M.J. Charron, Y.T. Chen, H. Doege, D.E. James, H.F. Lodish, K.H. Moley, J.F. Moley, M. Mueckler, S. Rogers, A. Schürmann, S. Seino, B. Thorens, Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators, Am. J. Physiol. Endocrinol. Metab. 282 (2002) 974–976.
[2] J.K. Kim, A. Zisman, J.J. Fillmore, O.D. Peroni, K. Kotani, P. Perret, H. Zong, J. Dong, C.R. Kahn, B.B. Kahn, G.I. Shulman, Glucose toxicity and the development of diabetes in mice with muscle-specific inactivation of GLUT4, J. Clin. Invest. 108 (2001) 153–160.
[3] E. Carvalho, K. Kotani, O.D. Peroni, B.B. Kahn, Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle, Am. J. Physiol. Endocrinol. Metab. 289 (2005) 551–561.
[4] L.Y. Wu, C.C. Juan, L.S. Hwang, Y.P. Hsu, P.H. Ho, L.T. Ho, Green tea supplementation ameliorates insulin resistance and increases glucose transporter IV content in a fructose-fed rat model, Eur. J. Nurt. 43 (2004) 116–124.
[5] S. Nishiumi, H. Ashida, Rapid preparation of a plasma membrane fraction from adipocytes and muscle cells: application to detection of translocated glucose transporter 4 on the plasma membrane, Biosci. Biotechnol. Biochem. 71 (2007) 2343–2346.
[6] R.A. DeFronzo, R.C. Bonadonna, E. Ferrannini, Pathogenesis of NIDDM-a balanced overview, Diabetes Care 15 (1992) 318–368.
[7] H. Asida, T. Furuyasiki, H. Nagayasu, H. Bessho, H. Sakakibara, T. Hasimoto, K. Kanazawa, Anti-obesity actions of green tea: Possible involvements in modulation of the glucose uptake system and suppression of the adipogenesis-related transcription factors, Biofactors 22 (2004) 135–140.
[8] C.S. Yang, Z.Y. Wang, Tea and cancer, J. Natl. Cancer Inst. 85 (1993) 1031–1049.
[9] C.S. Yang, L. Chen, M.J. Lee, D. Balentine, M.C. Kuo, S.P. Schantz, Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers, Cancer Epidemiol. Biomarkers Prev. 7 (1998) 351–354.
[10] G.L. Tipoe, T.M. Leung, M.W. Hung, M.L. Fung, Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection, Cardiovasc. Hematol. Disord. Drug Targets 7 (2007) 135–144.
[11] S. Klaus, S. Püeltz, C. Thöne-Reineke, S. Wolfram, Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation, Int. J. Obes. 29 (2005) 615–623.
[12] T. Furuyashiki, H. Nagayasu, Y. Aoki, H. Bessho, T. Hashimoto, K. Kanazawa, H. Ashida, Tea catechin suppresses adipocyte differentiation accompanied by down-regulation of PPARγ2 and C/EBPα in 3T3–L1 cells, Biosci. Biotechnol. Biochem. 68 (2004) 2353–2359.
[13] D. Dey, M. Mukherjee, D. Basu, M. Datta, S.S. Roy, A. Bandyopadhyay, S. Bhattacharya, Inhibition of insulin receptor gene expression and insulin signaling by fatty acid: interplay of PKC isoforms therein, Cell. Physiol. Biochem. 16 (2005) 217–228.
[14] Y.H. Kao, R.A. Hipakka, S. Liao, Modulation of endocrine systems and food intake by green tea epigallocatechin gallate, Endocrinology 141 (2000) 980–987.
[15] M.J. Lee, Z.Y. Wang, H. Li, L. Chen, Y. Sun, S. Gobbo, D.A. Balentine, C.S. Yang, Analysis of plasma and urinary tea polyphenols in human subjects, Cancer Epidemiol. Biomarkers Prev. 4 (1995) 393–399.
[16] L. Chen, M.J. Lee, H. Li, C.S. Yang, Absorption, distribution, elimination of tea polyphenols in rats, Drug Metab. Dispos. 25 (1997) 1045–1050.
[17] W. Kim, L.Y. Khil, R. Clark, S.H. Bok, E.E. Kim, S. Lee, H.S. Jun, J.W. Yoon, Napthalenemethyl ester derivative of dihydroxydrocinnamic acid, a component of cinnamon, increases glucose disposal by enhancing translocation of glucose transpoter 4, Diabetologia 49 (2006) 2437–2448.
[18] M.V. Vijiayakumar, S. Singh, R.R. Chhipa, M.K. Bhat, The hypoglycaemic activity of fenugreek seed extract is mediated through the stimulation of an insulin signaling pathway, Br. J. Pharmacol. 146 (2005) 41–48.
[19] B. Halliwell, Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies?, Arch. Biochem. Biophys. 476 (2008) 107–112.

[20] L.H. Long, M.V. Clement, B. Halliwell, Artifacts in cell culture: Rapid generation of hydrogen peroxide on addition of (−)-epigallocatechin, (−)-epigallocatechin gallate, (+)-catechin, and quercetin to commonly used cell culture media, Biochem. Biophys. Res. Commun. 273 (2000) 50–53.
[21] P. Misra, R. Chakrabarti, The role of AMP kinase in diabetes, Indian J. Med. Res. 125 (2007) 389–398.
[22] H.S. Moon, C.S. Chung, H.G. Lee, T.G. Kim, Y.J. Choi, C.S. Cho, Inhibitory effect of (−)-epigallocatechin-3-gallate on lipid accumulation of 3T3-L1 cells, Obesity 15 (2007) 2571–2582.
[23] Q.F. Collins, H.Y. Liu, J. Pi, Z. Liu, M.J. Quon, W. Cao, Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5′-AMP-activated protein kinase, J. Biol. Chem. 282 (2007) 30143–30149.