C2C12 Cell Line is a Good Model to Explore the Effects of Herbal Extracts on GLUT4 Expression and Translocation

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Abstract

Objective: GLUT4 is a type of glucose transporter and plays a central role in whole-body metabolism of carbohydrates. The muscle is the major site of GLUT4 and cell line models, to explore GLUT4 behaviors under new therapeutic approach, such as herbal components, should be evaluated. Here, C2C12 cell line is evaluated for GLUT4 translocation from intracellular compartment into the cell membrane.

Materials and Methods: C2C12 cell lines were cultured and differentiated into myotubes. Cinnamon/Turmeric-water soluble extract (CWE and TWE) were prepared and differentiated myotubes were exposed to the 100, 1000 μg/ml CWE, 100 μg/ml TWE or dimethylsulfoxide for 3 hours. Intracellular/cytoplasmic membrane compartments were separated using ultracentrifugation. GLUT4 percentages quantities were measured using western blotting. Data analysis of 3 sample sizes was done by comparing mean±SD of GLUT4 quantities and independent samples t-test.

Results: CWE enhances GLUT4 translocation from intracellular compartment into the cytoplasmic membrane; its effect is also dose-dependent meaning that 1000 μg/ml concentration has a more potent effect than 100 μg/ml ($p<0.05$). However, 100 μg/ml TWE had a reverse effect ($p<0.05$).

Conclusion: Here we have shown that C2C12 is a good model for exploring GLUT4 changes under the effect of herbal extract. Induction or blockade of GLUT4 maybe under control of different signals transduction pathways. Furthermore, although turmeric ingredients are declared to have somewhat anti-diabetic effects, here we have shown that such effect is not applied via TWE effects on GLUT4 intracellular compartments movement into the cell membrane.

Keywords: Cinnamon, Turmeric, GLUT4, Diabetes, Herbal ingredient

Introduction

Cytoplasmic cell membrane lipid bilayers are nonpolar structures which make an impermeable barrier to polar molecules such as glucose and hexoses. Glucose transporters (GLUTs), a type of trans-membrane glycoproteins, do transport of

glucose and hexoses via facilitative transfusion mechanism (1-4). Different isoforms of GLUTs have been discovered in different tissues (5). Insulin resistant, the main cause of diabetes type 2 is associated with disturbed GLUTs metabolism (6,7). As the main regulator of glucose metabolism, i.e. insulin, acts via GLUT4, the main insulin-regulated glucose transporter (2,3,6,8-10), researchers explore a cell line model to investigate anti-diabetic agents such as herbs and spices to describe unknown aspects of cellular, molecular and biochemical mechanisms of their action (11-13).

Adipose and muscular tissues have the most potency for glucose expenditure. Also, these tissues are the main targets of insulin where it regulates GLUT4 metabolism, gene induction, cell trafficking and movement (1-3,5,6,9,10,14-26). Generally, cell culture models for GLUT4 variations under different interventional or lab trial studies have been limited mostly to the 3T3-L1, L6 and C2C12-derived cell lines from mouse (15,23,25,27-29). However, in human body, more than 75% of glucose is metabolized by muscular tissue (29) and adipocytes have a little role in this context (1,10,18). Most of the molecular studies on GLUT4 metabolism have been done using L6 (10,17,29-34) and C2C12 (5,10,19,22,24,25,35-40) cell lines. As there are a few studies on cellular and molecular aspects of herbs and spices, in the present study we have studied if C2C12 cell line maybe a candidate for exploring GLUT4 changes in cell compartments under cinnamon and turmeric water-soluble extracts, as two selected spices.

Materials and Methods

C2C12 cell line (code No. ATCC :CRL-1772) was purchased from National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. Cinnamon and turmeric powder were purchased from herbal markets routinely importing them into Iran from china and India, respectively. Acetic acid, absolute ethanol, Tris, Sodium Dodecyl Sulfate (SDS), NaCl and Na_2EDTA all were from (Merck Inc. Frankfurter Straße Darmstadt, Germany). Dimethylsulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM) cell culture media, phenylmethylsulfonyl fluoride (PMSF) and sucrose were from Sigma-Aldrich Manufacturing, LLC (US, Saint Louis). Foetal bovine serum (FBS) and heat inactivated horse serum (H.S) were from Gibco. (Life Technologies Corporation, US). Bradford reagent and $NaHCO_3$ were from Sigma-Aldrich Manufacturing, LLC (US, Saint Louis). Penicillin G and streptomycin were, HEPES, Nonidet P-40, sodium deoxycholate were from AppliChem, LLC (Darmstadt, Germany). Nitrocellulose membrane was from Millipore (UK, Croxley Green Business Park Watford). ECL Advance Western immunoblotting kit was from Amersham Inc. (UK, Stanley Hill, Buckinghamshire, Amersham). Mouse anti-GLUT4 antibody 1F8 and goat anti-mouse IgG-HRP were from Santa Cruz Biotech (US, California, Santa Cruz). Mammography films were from Kodak (US, New-York, Eastman Kodak Company, Rochester).

Cinnamon Water-soluble Extract (CWE)

It was prepared according to the method described by Anderson et al (41) with modifications; 200 grams of cinnamon powder dissolved in 500 ml of 0.1N acetic acid, boiled for 20 minutes and centrifuged. One part of the 2000rpm supernatant mixed with 4 parts of absolute ethanol and kept in 4ºC overnight; then the mixture was filtered using Whatman filter paper and dried in a 60ºC oven; resulted powder dissolved in dimethylsulfoxide (DMSO) with a concentration of 100 mg/ml as stock solution.

Turmeric Water-soluble Extract (TWE)

To compare the effect of turmeric and cinnamon on GLUT4 contents in cell compartments we prepared a water-soluble extract of turmeric as same as CWE. We prepared a 1000 µg stock solution in DMSO of curcumin as described for CWE.

C2C12 primary culture and differentiation

About 8×10^4 C2C12 myoblasts were seeded in DMEM containing 4 mM/l glutamine, 0.025 mol/l glucose, 1mmol/l sodium pyruvate, 0.018 mol/l NaHCO3, 100 U/ml Penicillin G, 100 µg/ml streptomycin and 10% foetal bovine serum in 250 ml tissue culture flasks. Cell culture medium was exchanged each day until reaching near confluent conditions. In all steps, cell culture flasks were kept in a humidified incubator with 5% CO_2 and 37ºC. Myoblasts differentiation to myotubes induced by addition of DMEM supplemented with 2% heat inactivated horse serum (H.S) and polynucleated myotubes formation monitored microscopically until day 5 of differentiation induction. Differentiation medium was replenished each 48 hours.

Affecting CWE and TWE on myotubes and subcellular fractionation

For CWE, two different concentrations of 100mg/ml and 1000mg/ml were prepared in DMEM+2% H.S. Also a 100µg concentration of TWE in DMEM+2% H.S was prepared to assess if CWE and TWE have any effect on GLUT4 content of cytoplasmic membrane (CM) and nuclear/endoplasmic reticulum (N/ER). Also, a control group was included without any additive except 1% DMSO in DMEM+2% H.S (DDH). Ultimately CWEs, TWE and DDH were added to culture flasks containing differentiated myotubes on day 6 post-differentiation. For each category 3 flasks were considered. As Cao et al have shown the best results for GLUT4 enhancement in 3T3-L1 under CWE treatment on time exposure of 3 hours (15), we also kept myotubes in such exposure time status. After 3 hours exposure to CWEs, TWE or DMSO, myotubes were washed 3 times with ice cold phosphate buffer (pH=7.4) and then detached from flasks with scraper and homogenized with tissue homogenizer for 3 minutes in HES buffer, pH 7.4; 225 mM sucrose; 4 mM Na_2EDTA, 20 mM HEPES; 1 mM PSMF and 1 tablet/dl anti-protease cocktail. Cell homogenate was centrifuged according to the instruction presented in Tortorella et al published paper (23); the first spin performed at 19000g for 20 minutes; To crude plasma membrane or N/ER of myotubes resulted pellet was homogenized in HES buffer and layered on a high sucrose buffer (HSB) composed of 1.12 M sucrose in 20 mM HEPES and 1 mM Na_2EDTA buffer and centrifuged further at 100000g for 1 hour. Interphase portion of this step was contained plasma membrane particles that were floated on HSB and resulted pellet included of N/ER particles. Interphase particles were aspirated and centrifuged at 40000g for 20 minutes to be pelleted; the pellet was re-suspended in PBS, pH 7.4, containing protease inhibitors. N/ER particles were re-suspended in a buffer containing 50 mM NaCl, 2% Nonidet P-40, 0.5% sodium deoxycholate, 0.2% SDS, 20 mM Tris (pH 7.4) and protease inhibitor cocktail. All centrifugation steps were done at 4ºC with Beckman coulter ultracentrifuge and Type 90 Ti rotor. Also g force and round per minute (RPM) values for each step of centrifugation were calculated by the Beckman coulter ultracentrifuge calculating software which is available online.
(www.beckmancoulter.com).

Sodium Dodecyl Sulphate Polyacrylamid Gel Electrophoresis (SDS-PAGE) and Western blotting

Total protein concentrations were analyzed using Bradford reagent and proteins were separated by a 10% SDS-PAGE according to the Laemmli instruction (24). Briefly 100 µg protein was loaded to SDS-PAGE wells and separated with 30 mAmp for 3 hours. Separated proteins were transferred to the nitrocellulose membrane at 4ºC for 400 mAmp overnight. Blotted membranes were blocked using 2% blocking agent included in immunoblotting kit and was prepared in Tweenated phosphate buffer saline containing 1% Polysorbaat-20 (TBS), pH 7.5, for 3 hours with gently shaking. After 3×15 min washing in TBS, blotted paper immersed in 2% blocking agent containing mouse anti-GLUT4 antibody 1F8 with a dilution of 1:200 for 4

hours. After repeating wash steps, goat anti-mouse IgG-HRP was added with a concentration of 1:1000 in 2% blocking agent for 5 hours with gently shaking. After rewashing the membrane, chemiluminescent detection reagents were added according to the kit instruction and in a dark room. GLUT4 bands were visualized using mammography films and its related reagents.

Quantifications of GLUT4 bands and data analysis

Resulted bands from western blotting were quantified by scanning and comparing with together by Photo EP software. Data were a percent that divided between intervention group and its equal vehicle. Comparative histograms plotted in Excel and data were analyzed by SPSS software ver. 11.5 with two independent samples t-test.

Results

To study the effects of water-soluble extracts and also to examine whether each of those concentrations affects the GLUT4 expression, we investigated the effects of two concentrations (100 and 1000 mg/ml) of CWE and TWE on CM and N/ER, separately, using chemiluminescent technique. Chemiluminescent detection reagents were chosen for this study to determine whether the inducible or inhibitory effects of herbal extracts are dependent to concentration or not. Quantification and paired comparison of immunoblotting bands are presented in Table 1 and Figures 1 and 2. As it is stated in interpretation column of Table 1, GLUT4 contents were higher in N/ER fractions of Quantification and paired comparison of immunoblotting bands are presented in Table 1 and Figures 1 and 2. As it is stated in interpretation column of Table 1, GLUT4 contents were higher in N/ER fractions of control categories than CWE-exposed myotubes; also, 100µg/ml CWE-exposed myotubes had higher GLUT4 contents than 1000µg/ml CWE-exposed. However, CM fractions of control category had lower GLUT4 contents in comparison to the CWE-exposed myotubes; moreover, 100µg/ml

Table 1. GLUT4 contents of intervention and control categories determined using immunoblotting method

Fraction	Category	GLUT4 (Mean±SE)	p-value*	Interpretation
N/ER[1]	Vehicle	58.60±1.1	0.001	GLUT4 contents were reduced in N/ER but increased in CM
	100 µg/ml CWE	41.40±1.4		
CM[2]	Vehicle	45.59±0.45	0.000	
	100 µg/ml CWE	54.41±0.48		
N/ER	Vehicle	55.54±1.01	0.001	GLUT4 contents were reduced in N/ER but increased in CM
	1000 µg/ml CWE	44.46±1.01		
CM	Vehicle	36.08±1.9	0.000	
	1000 µg/ml CWE	62.92±3.25		
N/ER	100 µg/ml CWE	59.46±2.56	0.006	GLUT4 contents were lower in N/ER but higher in CM of category exposed to 1000 µg/ml CWE in comparison to100 µg/ml CWE
	1000 µg/ml CWE	40.54±2.56		
CM	100 µg/ml CWE	46.13	0.000	
	1000 µg/ml CWE	53.87		
N/ER	Vehicle	43.83±0.8	0.000	GLUT4 contents were reduced in CM but increased in N/ER
	100 µg/ml TWE	56.17±0.8		
CM	Vehicle	62.8	0.000	
	100 µg/ml TWE	37.2		

*** Obtained p-values are calculated for independent samples t-tests with α=0.05.**
[1] Nuclear/Endoplasmic Reticulum
[2] Cytoplasmic membrane

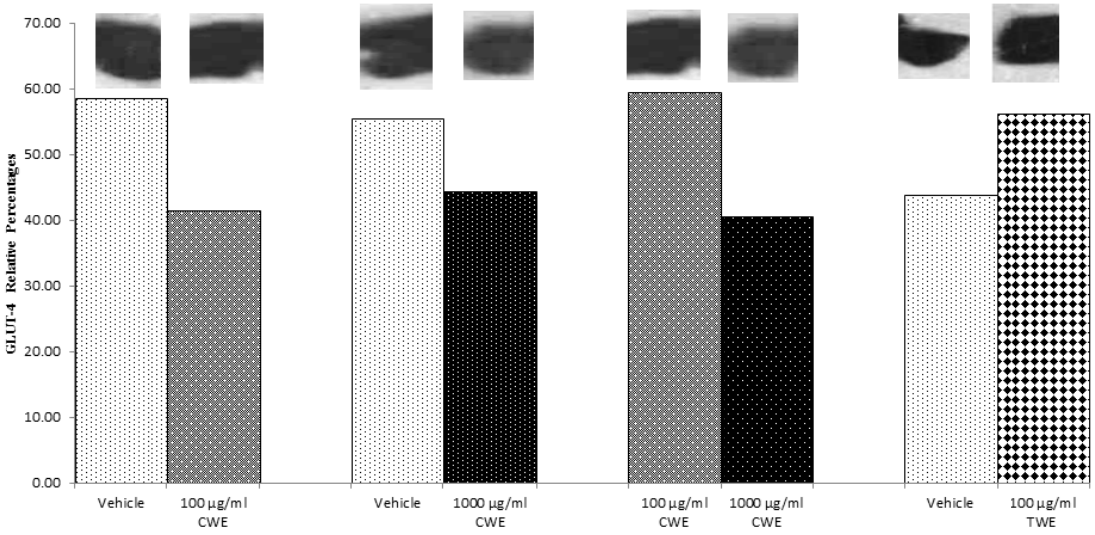

Figure 1. Quantification and paired comparisons of GLUT4 relative percentages obtained from Nuclear/Endoplasmic Reticulum (N/ER) fractions of myotubes between categories 100 and 1000µg/ml Water-soluble Cinnamon Extract (CWE), 100µg/ml Turmeric water-soluble extract (TWE) treated and their concurrent vehicle (1% DMSO) myotubes. Data were gathered from western-blot band scans. In all cases, Mean±SD were compared using independent samples t-test.

CWE-exposed myotubes had lower GLUT4 contents than 1000µg/ml CWE-exposed. But results for 100 µg/ml TWE-treated were in contrast to the results obtained for CWE. In all cases, all comparisons were significant at 95% confidence interval as showed in Table 1.

Discussion

Glucose is transported into the adipose and muscular tissues mainly via a GLUT4-dependent facilitated transport (10,18). In the other hand, about 75% of total glucose metabolism of the body is done by muscle after insulin secretion. However, adipose tissue has a slight role in glucose expenditure (31,29,26). So the most suitable tissue for exploring glucose metabolism, especially for determination of molecular mechanisms, is a muscle-derived ex vivo-based systems. Lauritzen et al. have stated that it is difficult to clarify GLUT4 measurement or relocation regulatory mechanisms due to the muscular tissue complexities and t-tubulin structures. Also they have declared that currently there is not a suitable cell line model to explore GLUT4 relocation in muscular tissue. Also Michael and coworkers have certified that almost all cell culture models of muscular tissues do not have the machineries for synthesis of GLUT4 or such machineries maybe in low quantities (13-15,27-29). Most studies on GLUT4 metabolism in cell line models under the effect of natural or pharmaceutical agents have been focused mainly on 3T3-L1, an adipose tissue-derived cell line. But this cell line is not a good model to survey of glucose metabolism regulating agents such as GLUT4 mainly because of two important aspects: firstly, the most glucose utilizing tissue in the human body is muscular tissue; then, 3T3-L1 could not be a good candidate for the mentioned purpose; and secondly, GLUT1 which its synthesis and metabolism regulation is not regulated by positive effectors such as insulin, are enhanced, despite GLUT4 down-regulation, under long-term exposure to insulin (10,35-40).

This was particularly that the whole-body GLUT4 itself may be less informative due to up-regulation of compensatory mechanisms that may promote survival of the animal. Thus,

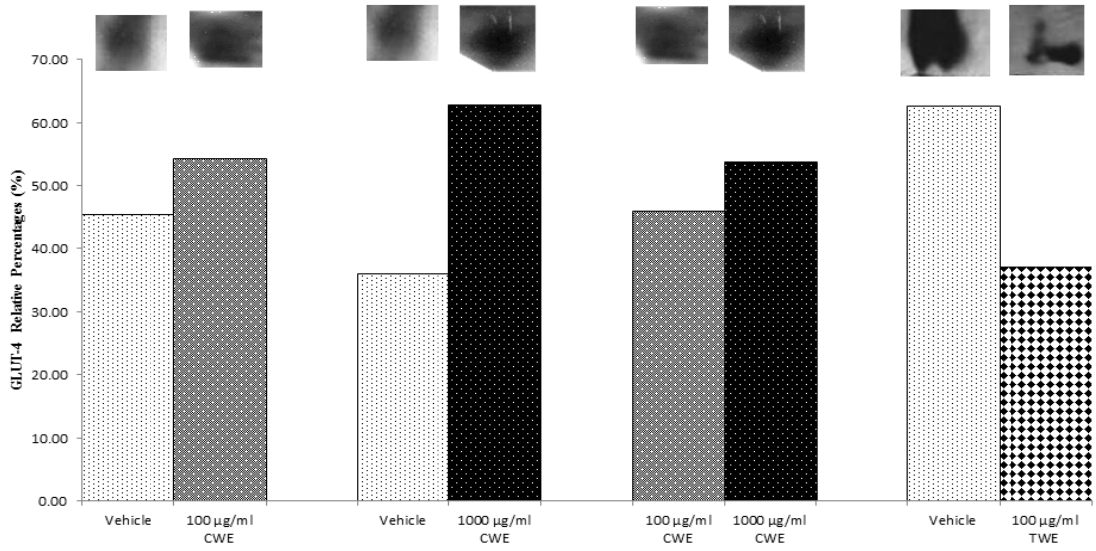

Figure 2. Quantification and paired comparisons of GLUT4 relative percentages obtained from cytoplasmic membrane (CM) fractions of myotubes between categories 100 and 1000µg/ml Cinnamon Water-Soluble Extract (CWE), 100 µg/ml Turmeric water-soluble extract (TWE) treated and their concurrent vehicle (1% DMSO) myotubes. Data were gathered from western-blot band scans. In all cases, Mean±SD were compared using independent samples t-test.

GLUT4 in muscle and adipose tissue is indispensable for normal global glucose homeostasis, while insulin receptor in these tissues appears much less critical. However, adipose tissue accounts for only a small fraction of total body glucose disposal and also lack of the lipogenic and antilipolytic actions of insulin in adipocytes, which make muscle cells more favorable in vitro model for glucose uptake studies. Interestingly, decrement and overexpression of GLUT4 protein in muscle tissue show the expected insulin resistance and propensity toward diabetes or normalizes insulin sensitivity and glucose tolerance, respectively that is consistent with a major role of GLUT4 in glucose disposal. So, researchers are looking for a cell line that could be a good representative for muscular tissue with the ability to synthesize GLUT4 and its relocating machinery (27-34).

In other side, other works have demonstrated that chemicals such as 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) that is an AMPK activator, Indinavir that is a protease inhibitor of human immunodeficiency virus (HIV), and Wortmannin, a phosphatidylinositol-3 kinase (PI3K) inhibitor, affect glucose uptake and some AMPK pathway components (39-41).

The current study showed differentiated C2C12 cells are a good model to explore GLUT4 changes under herbal extracts exposure. Cinnamon hydro-alcoholic extract-treated cells had more GLUT4 contents in CM compartments and lower contents in intracellular membranes, and N/ER, in comparison to their concurrent DMSO-treated controls. These results propose that GLUT4 is translocated from intracellular compartments in to the cell surface or cytoplasmic membranes. Also, comparison of two different concentrations of cinnamon hydro-alcoholic extracts shows that myotubes treated by higher concentrations of CWE had more GLUT4 contents in cytoplasmic membrane and lower contents in their intracellular compartments. This means that GLUT4 containing vesicles which transfer GLUT4 molecules from intracellular environments to the cell surface of C2C12-drived myotubes in response to herbal extracts in a dose-dependent manner. In the other hand, TWE-exposed myotubes did

not transfer GLUT4-containing vesicles from intracellular into the cell surface compartments in comparison to their concurrent DMSO-treated control myotubes. Importantly, DMSO-treated myotubes had more contents of GLUT4 in their cytoplasmic membrane compartments. This observation may be due to the effect of turmeric ingredients, especially curcumin, which may induce invagination and reverse movement of GLUT4 vesicles strictly in contrast to the effects seen for cinnamon. Acute hyperglycemia provides an insulin-independent inducer for GLUT4 translocation in C2C12 myotubes and rat skeletal muscle. In addition, our study showed that in concurrent control categories of both cinnamon-treated myotubes, GLUT4 contents were mostly present in nuclear or endoplasmic reticulum membranes in contrast to the cinnamon-treated cells.

Our results are in agreement with other studies. For example; Ralston and Ploug have shown that GLUT4 is mostly localized in the core of the unstimulated C2 myotubes (20). Electron micrographs had been revealed that GLUT4 containing vesicles are mainly localized in tubulovesicular structures of unstimulated C2 myotubes (35).

Considering results for extracts of two different herbs both of which are candidates for diabetes treatment, it could be discussed that reverse and dose-dependent effects, due to herbal ingredients were seen for both extracts on GLUT4 intracellular or cytoplasmic membrane contents of C2C12-derived myotubes. So, differentiated C2C12 cells could be a good model for ex vivo investigation of GLUT4 variations and metabolism under the effect of herbal extracts. In other words, C2C12-drieved myotubes had enough sensitivity for such investigations.

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References

1. Shepherd PR, Kahn BB. Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. The New England journal of medicine. 1999 Jul 22;341(4):248-57.
2. Martin S, Slot JW, James DE. GLUT4 trafficking in insulin-sensitive cells. A morphological review. Cell biochemistry and biophysics. 1999;30(1):89-113.
3. Han J, Park S, Lee W. Cadmium induces impaired glucose tolerance in rat by - Regulating GLUT4 expression in adipocytes. Diabetes. 2003 Jun;52:A532-A.
4. Medina RA, Owen GI. Glucose transporters: expression, regulation and cancer. Biological Research. 2002;35(1):9-26.
5. Richardson JM, Pessin JE. Identification of a Skeletal Muscle-Specific Regulatory Domain in the Rat Glut4 Muscle-Fat Gene. Journal of Biological Chemistry. 1993 Oct 5;268(28):21021-7.
6. Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, et al. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. New England Journal of Medicine. 1999 Jul 22;341(4):240-6.
7. Kim W, Khil LY, Clark R, Bok SH, Kim EE, Lee S, et al. Naphthalenemethyl ester derivative of dihydroxyhydrocinnamic acid, a component of cinnamon, increases glucose disposal by enhancing translocation of glucose transporter 4. Diabetologia. 2006 Oct;49(10):2437-48.
8. Pessin JE, Thurmond DC, Elmendorf JS, Coker KJ, Okada S. Molecular Basis of Insulin-stimulated GLUT4 Vesicle Trafficking Location! Journal of Biological Chemistry. 1999;274(5):2593-6.
9. Miura T, Suzuki W, Ishihara E, Arai I, Ishida H, Seino Y, et al. Impairment of insulin-stimulated GLUT4 translocation in skeletal muscle and adipose tissue in the Tsumura Suzuki obese diabetic mouse: a new genetic animal model of type 2 diabetes. European journal of endocrinology / European Federation of Endocrine Societies. 2001 Dec;145(6):785-90.
10. Nedachi T, Kanzaki M. Regulation of glucose transporters by insulin and extracellular glucose in

C2C12 myotubes. Am J Physiol Endocrinol Metab. 2006 Oct;291(4):817-28.
11. Liu F, Dallas-Yang Q, Castriota G, Fischer P, Santini F, Ferrer M, et al. Development of a novel GLUT4 translocation assay for identifying potential novel therapeutic targets for insulin sensitization. The Biochemical journal. 2009 Mar 1;418(2):413-20.
12. Mueckler M. Insulin resistance and the disruption of Glut4 trafficking in skeletal muscle. The Journal of clinical investigation. 2001 May;107(10):1211-3.
13. Michael LF, Wu Z, Cheatham RB, Puigserver P, Adelmant G, Lehman JJ, et al. Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. Proceedings of the National Academy of Sciences of the United States of America. 2001 Mar 27;98(7):3820-5.
14. Al-Achi A. Herbs that affect blood glucose levels. Women's Health in Primary Care 2005;8:325-30.
15. Cao H, Polansky MM, Anderson RA. Cinnamon extract and polyphenols affect the expression of tristetraprolin, insulin receptor, and glucose transporter 4 in mouse 3T3-L1 adipocytes. Arch Biochem Biophys. 2007 Mar 15;459(2):214-22.
16. Cao H, Urban JF, Jr., Anderson RA. Cinnamon polyphenol extract affects immune responses by regulating anti- and proinflammatory and glucose transporter gene expression in mouse macrophages. J Nutr. 2008 May;138(5):833-40.
17. Haney PM, Levy MA, Strube MS, Mueckler M. Insulin-Sensitive Targeting of the Glut4 Glucose-Transporter in L6 Myoblasts Is Conferred by Its Cooh-Terminal Cytoplasmic Tail. Journal of Cell Biology. 1995 May;129(3):641-58.
18. Huang S, Czech MP. The GLUT4 glucose transporter. Cell metabolism. 2007 Apr;5(4):237-52.
19. Kotliar N, Pilch PF. Expression of the Glucose Transporter Isoform Glut-4 Is Insufficient to Confer Insulin-Regulatable Hexose Uptake to Cultured Muscle-Cells. Molecular Endocrinology. 1992 Mar;6(3):337-45.
20. Ploug T, van Deurs B, Ai H, Cushman SW, Ralston E. Analysis of GLUT4 distribution in whole skeletal muscle fibers: Identification of distinct storage compartments that are recruited by insulin and muscle contractions. Journal of Cell Biology. 1998 Sep 21;142(6):1429-46.
21. Seraphim PM, Nunes MT, Giannocco G, Machado UF. Age related obesity-induced shortening of GLUT4 mRNA poly(A) tail length in rat gastrocnemius skeletal muscle. Molecular and cellular endocrinology. 2007 Sep 30;276(1-2):80-7.
22. Smith CW, Klaasmeyer JG, Edeal JB, Woods TL, Jones SJ. Effects of serum deprivation, insulin and dexamethasone on polysome percentages in C2C12 myoblasts and differentiating myoblasts. Tissue & Cell. 1999;31(4):451-8.
23. Sparling DP, Griesel BA, Olson AL. Hyperphosphorylation of MEF2A in primary adipocytes correlates with downregulation of human GLUT4 gene promoter activity. American Journal of Physiology-Endocrinology and Metabolism. 2007 Apr;292(4):1149-56.
24. Takazawa K, Noguchi T, Hosooka T, Yoshioka T, Tobimatsu K, Kasuga M. Insulin-induced GLUT4 movements in C2C12 myoblasts: evidence against a role of conventional kinesin motor proteins. The Kobe journal of medical sciences. 2008;54(1):14-22.
25. Tortorella LL, Pilch PF. C2C12 myocytes lack an insulin-responsive vesicular compartment despite dexamethasone-induced GLUT4 expression. Am J Physiol Endocrinol Metab. 2002;283(3):514-24.
26. Valverde AM, Navarro P, Teruel T, Conejo R, Benito M, Lorenzo M. Insulin and insulin-like growth factor I up-regulate GLUT4 gene expression in fetal brown adipocytes, in a phosphoinositide 3-kinase-dependent manner. The Biochemical journal. 1999 Feb 1;337(3):397-405.
27. Jarvill-Taylor KJ, Anderson RA, Graves DJ. A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. Journal of the American College of Nutrition. 2001;20(4):327-36.
28. Roffey B, Atwal A, Kubow S. Cinnamon water extracts increase glucose uptake but inhibit adiponectin secretion in 3T3-L1 adipose cells. Molecular nutrition & food research. 2006;50(8):739-45.
29. Sajan MP, Bandyopadhyay G, Kanoh Y, Standaert ML, Quon MJ, Reed BC, et al. Sorbitol activates atypical protein kinase C and GLUT4 glucose transporter translocation/glucose transport through proline-rich tyrosine kinase-2, the extracellular signal-regulated kinase pathway and phospholipase D. Biochemical Journal. 2002 Mar 15;362:665-74.
30. Kishi K, Muromoto N, Nakaya Y, Miyata I, Hagi A, Hayashi H, et al. Bradykinin directly triggers GLUT4 translocation via an insulin-independent pathway. Diabetes. 1998;47(4):550-8.
31. Lira VA, Soltow QA, Long JHD, Betters JL, Sellman JE, Criswell DS. Nitric oxide increases GLUT4 expression and regulates AMPK signaling in skeletal muscle. American Journal of Physiology-Endocrinology and Metabolism. 2007;293(4):1062-8.
32. Tong P, Khayat ZA, Huang C, Patel N, Ueyama A, Klip A. Insulin-induced cortical actin remodeling promotes GLUT4 insertion at muscle cell membrane ruffles. The Journal of clinical investigation. 2001;108(3):371-81.
33. Ueda M, Nishiumi S, Nagayasu H, Fukuda I, Yoshida K, Ashida H. Epigallocatechin gallate

promotes GLUT4 translocation in skeletal muscle. Biochemical and biophysical research communications. 2008 Dec 5;377(1):286-90.
34. Wang Q, Khayat Z, Kishi K, Ebina Y, Klip A. GLUT4 translocation by insulin in intact muscle cells: detection by a fast and quantitative assay. FEBS letters. 1998 May 8;427(2):193-7.
35. Galante P, Mosthaf L, Kellerer M, Berti L, Tippmer S, Bossenmaier B, et al. Acute Hyperglycemia Provides an Insulin-Independent Inducer for Glut4 Translocation in C2c12 Myotubes and Rat Skeletal-Muscle. Diabetes. 1995;44(6):646-51.
36. Gazit V, Ben-Abraham R, Vofsi O, Katz Y. L-cysteine increases glucose uptake in mouse soleus muscle and SH-SY5Y cells. Metabolic Brain Disease. 2003;18(3):221-31.
37. Park CE, Kim MJ, Lee JH, Min BI, Bae H, Choe W, et al. Resveratrol stimulates glucose transport in C2C12 myotubes by activating AMP-activated protein kinase. Experimental and Molecular Medicine. 2007 Apr 30;39(2):222-9.
38. Schwartzenberg-Bar-Yoseph F, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. Cancer Research. 2004 Apr 1;64(7):2627-33.
39. Smith JL, Patil PB, Fisher JS. AICAR and hyperosmotic stress increase insulin-stimulated glucose transport. J Appl Physiol. 2005 Sep;99(3):877-83.
40. Tulipano G, Spano P, Cocchi D. Effects of olanzapine on glucose transport, proliferation and survival in C2C12 myoblasts. Molecular and cellular endocrinology. 2008 Sep 24;292(1-2):42-9.
41. Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, et al. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. Journal of agricultural and food chemistry. 2004 Jan 14;52(1):65-70.