Effect of Lithium on the Mechanism of Glucose Transport in Skeletal Muscles

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Summary While lithium is known to stimulate glucose transport into skeletal muscle, the mechanisms of the increased glucose transport by lithium in skeletal muscle are not well defined yet. We excised epitrochlearis muscles from male Wistar rats and measured the transport rates of a glucose analog into lithium-, insulin-, and muscular contraction-stimulated skeletal muscle tissue and we also analyzed the levels of cell surface glucose transport using a photolabeling and multicolor immunofluorescence method. In addition, we generated a cell line that stably expresses myc-tagged GLUT4 to measure the rates of GLUT4 internalization and externalization. Lithium significantly increased 2-DG glucose transport rate in skeletal muscles; however, it was significantly lower than the stimulation induced by the maximum concentration of insulin or tetanic contraction. But co-treatment of lithium with insulin or tetanic contraction increased glucose transport rate by ~200% more than lithium alone. When skeletal muscle tissues were treated with lithium, insulin, and muscular contraction, the levels of cell surface GLUT4 protein contents were increased similarly by ~6-fold compared with the basal levels. When insulin or lithium stimuli were maintained, the rate of GLUT4myc internalization was significantly lower, and lithium was found to suppress the internalization of GLUT4myc more strongly. The lithium-induced increase in glucose uptake of skeletal muscles appears to increase in cell surface GLUT4 levels caused by decreased internalization of GLUT4. It is concluded that co-treatment of lithium with insulin and muscular contraction had a synergistic effect on glucose transport rate in skeletal muscle.

Key Words lithium, glucose transport, GLUT4myc, epitrochlearis muscles

Obesity causes a defective functioning of insulin signaling pathways in skeletal muscles, thereby decreasing GLUT4 translocation: this leads to insulin resistance in skeletal muscles, which further acts as the main cause of hypertension, hyperlipidemia, cardiac diseases, and diabetes (1, 2). In contrast, since the level of glucose transport stimulated by muscular contraction/exercise is maintained at normal levels even in obesity, regular exercise can be one of the best ways to improve blood glucose levels and decrease the occurrence of complications in patients with diabetes and impaired glucose tolerance. However, when considering the low exercise participation and limited physical activity of Korean adults, a more practical and effective way to treat and prevent diabetes is needed.

Lithium is an alkali metal element which increases glucose transport and shows an insulin-like effect in metabolic tissues such as skeletal muscle and adipose tissues (3–7). Currently, the molecular mechanisms regarding the insulin-mimetic activity of lithium are unknown. However, Rossetti (8) reported that lithium treatment completely restored insulin sensitivity to normal in diabetic rats and this effect may be due to reversing the defect in the glycogen pathway in skeletal muscle. In vitro studies showed that lithium increased glucose transport in insulin-sensitive skeletal muscle regardless of the presence of insulin (9–12) and caused an increase in both glucose transport and glycogen synthesis even in insulin-resistant skeletal muscle (13).

Glucose transport in human skeletal muscle occurs primarily by facilitated diffusion, utilizing glucose transporter carrier proteins (glucose transporter, GLUT). Both muscle contraction and insulin increases glucose uptake in skeletal muscle are through the translocation of glucose transporter proteins from an intracellular compart-

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Abbreviations: AEBSF, aminoethyl benzenesulfonyl fluoride hydrochloride; ATB-BMPA, 4-(1-azi-2,2,2-trifluoroethyl)-benzoyl-1,3-bis(d-mannos-4-yloxy)-2-propylamine; 2-DG, 2-deoxy-D-glucose; EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum; GLUT4, glucose transporter 4; GLUT4myc, myc-tagged GLUT4; GS, goat serum; HRP, horseradish peroxidase; KHB, Krebs-Henseleit buffer; α-MEM, minimum essential medium; OCT, optical coherence tomography; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PM, plasma membrane; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SE, standard error.
ment to the surface of the cell (14). Muscle contraction and insulin utilize different signaling pathways, both of which lead to the activation of glucose transport (14, 15). Although a number of studies have been carried out on the mechanism of lithium glucose uptake (10, 16–21), the mechanism is still unclear. Based on our preliminary data, lithium increases glucose transport activity in skeletal muscle but the transport activity is significantly lower compared with maximum stimulation of insulin or tetanic contraction. However, when skeletal muscles were pre-treated with lithium 1 h before the submaximal stimulation of insulin and twitch muscle contraction, the muscle glucose transport rate was dramatically increased as high as maximum stimulation of insulin or tetanic contraction. Currently, it is not known how these combined effects of co-treatment of lithium with insulin or muscular contraction affect the elevated glucose transport in skeletal muscle function.

Therefore, in order to elucidate the mechanism of lithium-stimulated glucose transport, we excised the skeletal muscles from rats and measured the lithium-, insulin-, and muscular contraction-stimulated glucose analog (2-deoxy-d-glucose; 2-DG) transport activities; moreover, using photolabeling and multicolor immunofluorescence, we also comparatively analyzed levels of GLUT4 in sarcolemma under each stimulus. Furthermore, in order to investigate whether the change in GLUT4 levels in sarcolemma was caused by activation of GLUT4 translocation or by differences in internalization of cell surface GLUT4, we produced and analyzed cells that stably expressed myc-tagged GLUT4 (22) through the aforementioned steps, and thereby sought to elucidate the mechanism of lithium-induced glucose uptake in skeletal muscles.

MATERIALS AND METHODS

Animals for experiment and excision of tissues. Thirty-six-week-old male Wistar rats were obtained, and the rats were allowed to adapt to the new environment for 1 wk. After the adaptation period, the rats were fasted for 12 h. Then, they were anesthetized with pentobarbital sodium (5 mg/100 g body mass), and the epitrochlearis muscles were excised and measured the lithium-, insulin-, and muscular contraction-stimulated glucose uptake rates. Epitrochlearis muscles, which are small thin muscles in forelimbs (15% type I, 20% type IIa, 65% type IIb), are most appropriate for in vitro measurement of glucose transport rate (23, 24). We obtained the approval of the Animal Experiment Ethics Committee of Daegutechnopark Biohealth Center (BHCC-IACUC-2015-08).

Glucose uptake rate in skeletal muscles.

Glucose uptake rate in skeletal muscles induced by maximum insulin, tetanic contraction, and lithium stimuli: Excised epitrochlearis muscles were recovered for 60 min in Krebs-Henseleit buffer (KHB). Then, the muscles were placed in new KHB with the same composition, and were stimulated with 10 mm lithium (lithium chloride, Sigma, St. Louis, MO), insulin (porc insulin, Eli Lilly, Indianapolis, IN), or muscle contraction (first incubation). Lithium and insulin were mixed into buffers, and the muscles were incubated in the solutions for 60 min (35°C). For insulin stimulation, the maximum insulin concentrations (2 mU/mL) were used (25, 26); for muscular contraction, tetanic contractions (100 Hz, 0.2-ms pulses for 10 s, 1 contraction/1 min) were applied for 10 min with Grass stimulators (27, 28). The concentration of lithium was maintained at 10 mm, which was the concentration that resulted in the maximum glucose transport rate according to the result of previous studies (12). In order to remove glucose in extracellular tissues after incubation, muscle samples were washed for 10 min with KHB containing 40 mm mannitol (30°C). After being washed, the samples were incubated at 30°C for 20 min (second incubation) in KHB containing 4 mm $^{2}$[H]$^{12}$DG (1.5 μCi/mL, American Radiolabeled Chemicals, St. Louis, MO) and 36 mm [14C]mannitol (0.2 μCi/mL, ICN Radiochemicals, Laval, Canada). The same stimuli as in the first incubation were maintained during the washing and second incubation. The samples were compress-frozen and were stored at −80°C until analysis. The intracellular and extracellular concentrations of $^{3}$H and $^{14}$C were measured with scintillation counters (29). All liquid culture media were saturated with 95% O₂ : 5% CO₂ gas, and the media were continuously shaken with Dubnoff incubators in order to increase contact with the gas.

Glucose transport rate in skeletal muscles induced by pre-processing with lithium and short-term combined stimulation with insulin and tetanic contraction: The epitrochlearis muscles were recovered in KHB for 60 min. Then, the muscles were placed in new KHB with the same composition and were stimulated with 10 mm lithium for 60 min. Then, 2 mU/mL insulin was added for 10 min into the same KHB, or tetanic contractions (100 Hz, 0.2-ms pulses for 10 s, 1 contraction/1 min) were applied for 1 min (35°C). The wash and second incubation conducted in this experiment were the same as those used in the previous experiment.

Photolabeling of muscle cell surface GLUT4. The epitrochlearis muscles were treated as described above, but instead of measurement of 2-DG transport, the muscles were incubated in KHB buffer containing 0.5 mm biotinylated ATB-BMPA (w/or w/o 10 mm lithium) for 8 min at 18°C in the dark. Muscles were then irradiated for 2 min using a mercury vapor lamp (450 W; Conrad-Hanovia, Fairfield, NJ); the muscles were then turned, and the other side was radiated for 2 min. After irradiation, the muscles were homogenized in 255 mm sucrose containing 1 mm EDTA, 20 mm HEPES, 1 μg/mL each of antipain, aprotinin, pepstatin, and leupeptin, and 100 μM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF). The homogenates were centrifuged at 165,000 × g at 4°C for 90 min. The pellet was solubilized in Thesit detergent buffer containing (wt/g) 2% Thesit, 5 mm sodium phosphate, and 5 mm EDTA, pH 7.2, 1.0 mg/mL each of antipain, aprotinin, pepstatin, and leupeptin, and 100 μM AEBSF. The pellets were solubilized for 60 min with rotation for 60 min at 4°C followed by centrifugation at 20,000 × g for 20 min at 4°C. The supernatants were treated with streptavi-
The cells were cultured in confluence in low serum, differentiate into myotubes. GLUT4myc myoblasts, upon reaching for GLUT4myc expression and the ability to differentiate lin, and 100 \( \frac{\text{g/mL}}{\text{H}9262} \) was maintained in 2 \( \frac{\text{g/mL}}{\text{H}9251} \) plasmid, and selected with blasticidin-HCl. Glass clon- 31 and pSV2-bsr, a blasticidin S deaminase expression cotransfection of L6 myoblasts with pCX2-GLUT4myc L6 stably expressing GLUT4myc were prepared by (31). L6-GLUT4myc cells were described previously (30). The epitrochlearis muscle was embedded in Tissue Tek OCT compound and immediately frozen in liquid nitrogen cooled isopentane (Sigma Aldrich, St. Louis, MO). The sample was then transferred to an aluminium cryotube for storage at −80°C. For immunofluorescence staining, frozen muscle samples were cryosectioned using a microtome within a cryostat (Thermo Electric, Totowa, NJ) to a thickness of 10 \( \mu \text{m} \) onto uncoated glass microscope slides. The sections were dried for 30 min at room temperature and blocked with goat serum diluted to 10% with PBS for 1 h. Subsequently sections were washed 3 times for 5 min in phosphate-buffered saline. GLUT4 antibody (Abcam) was applied to the sections for 2 h at room temperature. GLUT4 primary antibody was combined with either dystrophin (Sigma Aldrich) antibody. Following primary antibody incubation, sections were washed 3 times for 5 min in PBS. Secondary antibodies were applied to sections for 30 min at room temperature. GLUT4 antibody was targeted with goat anti-rabbit IgG 488 (Invitrogen, Waltham, MA), dystrophin with goat anti-mouse IgG2b 594 (Invitrogen). After secondary antibody incubation sections were washed 3 times for 5 min in PBS and cov- erslips were mounted with prolong gold antifade reagent (ThermoFisher, Waltham, MA). The slide was visualized with use Nikon eclipse te2000-u (Nikon, Melville, NY) and X-Cite 120 PC (Excitellas, Covina, CA). The micro- scope is equipped with a red (excitation: BP 545/25 nm; emission: BP 560/70 nm) and green (excitation: BP 470/ 40 nm; emission: BP 525/50 nm) filters. The image was captured with MetaMorph software (Molecular Devices, Sunnyvale, CA).

**L6-GLUT4myc cells, cell culture, and incubations.** L6 muscle cells stably expressing myc-tagged GLUT4 (L6-GLUT4myc cells) were described previously (31). L6 stably expressing GLUT4myc were prepared by cotransfection of L6 myoblasts with pCX2-GLUT4myc and pSV2-bsr, a blastocidin S deaminase expression plasmid, and selected with blastocidin-HCl. Glass cloning cylinders (Bellco Glass, Vineland, NJ) were used to select individual colonies for expansion. Each clonal line was maintained in 2 \( \mu \text{g/mL} \) blastocidin-HCl and tested for GLUT4myc expression and the ability to differentiate into myotubes. GLUT4myc myoblasts, upon reaching confluence in low serum, differentiate into myotubes. The cells were cultured in α-MEM supplemented with 10% FBS (fetal bovine serum), 100 units/mL penicillin, and 100 \( \mu \text{g/mL} \) streptomycin and were grown at 37°C in 95% humidified air with 5% CO₂. Cells at 80% confluence were re-suspended and plated until reaching 80% confluence; after that, they were cultured for 6 d in α-MEM and 2% FBS to induce the L6 differentiation.

**GLUT4myc internalization.** The disappearance of GLUT4myc from the PM was measured as previously described (32). The serum-deprived (3 h) L6-GLUT4myc myoblasts (in 24-well plates) were blocked with ice-cold 5% GS (goat serum) in PBS for 10 min and then reacted with anti-myc antibody solution (1 : 300) in 5% GS at 4°C for 1 h. The cells were washed extensively in ice-cold PBS and incubated with α-MEM pre-warmed to 37°C for the indicated times in the presence or absence of 10 nm lithium and 2 mU/mL insulin. The cells were rapidly washed and fixed with 3% paraformaldehyde (PFA) in PBS (15 min), followed by quenching with 100 mm glycine in PBS (10 min) and blocking for 10 min with 5% goat serum (GS). GLUT4myc bound to anti-myc antibody remaining at the cell surface was detected with HRP-bound goat anti-rabbit secondary antibody (1 : 1,000) in 5% GS for 1 h at 4°C. Cells were washed six times with PBS and incubated with 1 mL/well of 0.4 mg/mL OPD and allowed to develop for 20–30 min at room temperature to obtain readings in the linear range. The reaction was stopped with 0.25 mL/well of 3 m HCl. Supernatant absorbance was measured at 492 nm. Background absorbance obtained from cells not incubated with anti-myc antibody was subtracted from all values. The results are expressed as fractions of the surface GLUT4myc level prior to internalization. Total cellular GLUT4myc was determined by incubating the cells with the anti-myc antibody following permeabilization with 0.1% (v/v) Triton X-100 in PBS at 4°C for 30 min and then proceeding with the secondary antibody as above. Percent internalization was calculated using the following equation.

\[
\% \text{Internalization} = \frac{\left(\text{total GLUT4myc} - \text{cell surface GLUT4myc}\right)}{\text{total GLUT4myc}} \times 100
\]

**Statistical analysis.** Results are presented as means±SE. Statistical analyses were carried out using SigmaPlot 12.0 software. Data sets with three or more groups were compared using one-way or two-way analy- sis of variance with Tukey’s post hoc analysis. A \( p \)-value of <0.05 was considered statistically significant.

**RESULTS**

**Glucose transport rate in skeletal muscles induced by lithium, insulin, and muscular contraction**

In order to assess the degree of glucose transport rate induced by lithium on its own, 10 mM lithium, maximum insulin (2 mU/mL), or tetanic contraction was applied to the excised epitrochlearis muscle samples. Although lithium significantly increased the glucose transport rate in comparison to the basal rate, the rate induced by lithium, which was approximately 1/4 of that induced by maximum insulin or muscular contraction, was significantly low (Fig. 1).

**Glucose transport rate in skeletal muscles induced by pre-processing with lithium and short-term combined stimula-
tion with insulin and tetanic contraction

After pre-processing the excised epitrochlearis muscle samples in 10 mM lithium for 60 min, 10-min-long stimulation with 2 mU/mL insulin or 1-min-long stimulation with tetanic contractions was applied to the samples. One-minute-long stimulation with 2 mU/mL insulin (0.9 μmol/mL/20 min) or tetanic contraction (0.8 μmol/mL/20 min) could not significantly increase the glucose transport rate in skeletal muscles in comparison to the basal levels (0.6 μmol/mL/20 min); however, when the same stimuli were applied after pre-processing the samples with lithium for 60 min, the rates of glucose transport in skeletal muscles showed substantial 6-fold increases (Fig. 2).

Cell surface GLUT4

In order to measure the cell surface GLUT4 levels under each stimulus, photolabeling and multicolor immunofluorescence were used in measuring the cell surface GLUT4 levels after stimulation with lithium, insulin, or muscular contraction. In comparison to the basal levels, the cell surface GLUT4 levels increased approximately 6-fold upon stimulation with 10 mM lithium, maximum insulin, and tetanic contraction (p<0.05); no significant difference in GLUT4 levels was observed among the three stimuli (Fig. 3a, 3b).

GLUT4myc internalization

With the L6-cell line, which stably expresses GLUT4myc, we measured the GLUT4 internalization rate by measuring the proportion of cell surface GLUT4myc to the total amount of intracellular GLUT4myc after stimulation with lithium or insulin. To compare the inhibitory effect of GLUT4myc internalization on lithium and insulin, first stimulation with insulin or lithium was carried out for 1 h to stimulate GLUT4myc translocations to the cell surface. Then we measured the difference in internalization rate of GLUT4myc according to the type of the second stimulus. The results showed a high internalization rate of GLUT4myc in the first stimulation with insulin and lithium for 1 h and no stimulation in the second. However, when the second stimulation was given using insulin or lithium for 2 h, the internalization rate of GLUT4myc was significantly lower. In particular, internalization rate of GLUT4myc was lower than that of insulin when stimulated with lithium. In this study, we investigated whether there is a synergistic effect of lithium and insulin combination treatment on
transport increased approximately 6-fold in comparison to a single treatment of insulin or tetanic contraction. One can first hypothesize that the lithium treatment increased the insulin-responsive expression of GLUT4 or increased the intrinsic activity of GLUT4, leading to the aforementioned results. So far, a translocation of GLUT4 and a glucose transport in skeletal muscles (intrinsic activity of GLUT4) have been considered to be identical. However, according to multiple preceding studies, a translocation and an activity of GLUT4 are distinct from each other (34–39). When Furtado et al. (40) observed the movement of GLUT4 by overexpressing myc-tagged GLUT4 in cells, translocation of myc-tagged GLUT4 happened within 2.5 min of stimulation with maximum insulin. However, glucose uptake by cells happened after 6 min; therefore, since a translocation and an activity of GLUT4 did not agree with each other temporally, translocation and activity were reported to proceed separately. As a short-lived muscular contraction stimulus was applied for only 10 min in this study, it is difficult to assume that the expression of GLUT4 would be increased. Instead, the increased intrinsic activity of GLUT4 could be assumed to have increased the glucose uptake in skeletal muscles in the absence of any increase in the absolute quantity of intracellular GLUT4. However, according to the results of the present study, although lithium could increase the glucose transport on its own, the level of increase was much lower than that induced by insulin or muscular contraction. Moreover, as lithium resulted in substantial increase in glucose transport only when accompanied by stimulation with insulin or muscular contraction, lithium does not seem to influence the intrinsic activity of GLUT4. One of the reasons why the glucose transport capacity of lithium was not thought to be due to the presence of intrinsic activity is that the glucose uptake in tissues is about 40% of insulin stimulation, even though GLUT4 contents are increased more than insulin stimulation on the cell membrane surface after lithium stimulation. Ren et al. (41) reported that overexpression of human GLUT1 in skeletal muscle using transgenic mice resulted in a 7-fold increase in glucose transport during the basal state in skeletal muscle. In this study, we suggest that the explosive increase of cell membrane surface GLUT4 contents may increase glucose uptake, but further studies are needed to clarify the mechanism. Then, in order to explain the mechanism through which a lithium stimulates the glucose uptake without increasing the expression or intrinsic activity of GLUT4, the following hypotheses can be suggested: the cell surface GLUT4 levels could have increased due to lithium-stimulated increase in externalization of GLUT4 or due to lithium-stimulated decrease in internalization of GLUT4 that were translocated to the plasma membrane. GLUT4 cycles through the surface of cells in both the presence and absence of insulin or muscle contraction (42, 43). In the absence of stimulations, GLUT4 is efficiently internalized into early or recycling endosomes from where it traffics GLUT4-storage vesicles (43). This translocation and subsequent internalization of GLUT4 back into intracellular stores transits through numerous internalization rate of GLUT4myc; no synergistic effect was observed. In the basal state, approximately 10% of the GLUT4 is located at the cell surface and 90% in intracellular membrane compartments (33). However, in this study, the internalization rate of GLUT4myc at basal level was about 45%. The cause of this result is unclear, but is thought to be due to the specificity of the L6-GLUT4myc stable cell line. However, since there are not many studies using the L6-GLUT4myc stable cell line, it is necessary to clarify the cause of the disease by future studies (Fig. 4a, 4b).

**DISCUSSION**

According to the results of this study, although lithium could increase the glucose uptake in skeletal muscles on its own, the level of increase was approximately 30–40% of the rate of glucose uptake induced by insulin in maximum concentration or by tetanic contraction. When insulin in maximum concentration or a tetanic contraction was applied after pre-processing the muscle samples with lithium for 1 h, the rate of glucose transport increased approximately 6-fold in comparison
small membrane-bound compartments, but the function of these different compartments is not clear (44). Through a photolabeling and multicolor immunofluorescence method, this study measured the cell surface GLUT4 levels upon stimulation with lithium, and tracked the intracellular movement of GLUT4 caused by the lithium treatment using a myc-tagged GLUT4 expressed stable cell line. Although the cell surface GLUT4 levels increased substantially after stimulation with lithium, insulin, and muscular contraction, no significant difference was observed between stimuli. Interestingly, we found that lithium significantly suppressed GLUT4 internalization when compared to insulin. Although the present study could not elucidate the mechanism through which lithium suppresses GLUT4 internalization, the decreased internalization of GLUT4 seems to have contributed to the lithium-induced increase in cell surface GLUT4. In other words, lithium is thought to increase the glucose uptake in skeletal muscles by significantly increasing the cell surface GLUT4 levels; such an increase in cell surface GLUT4 levels is thought to have been caused by decreased internalization of GLUT4. However, since lithium cannot increase the activity of GLUT4 on its own, the rate of glucose transport within the skeletal muscle tissue was not increased proportionally against the substantial increase in cell surface GLUT4 levels.

In conclusion, when lithium is administered with insulin or exercise, which can increase the activity of GLUT4, the small amounts of exercise or insulin will be able to increase the glucose transport in skeletal muscles in a significant manner. Since obesity-induced insulin resistance in type 2 diabetes patients is caused by defects in insulin signaling pathways and the resulting decreases in GLUT4 translocation, if GLUT4 translocation can be increased through lithium, low doses of insulin or light physical activity will be able to control the blood glucose levels in patients. Therefore, lithium can be expected to contribute to improving the insulin resistance in type 2 diabetes patients that cannot or are not willing to exercise; moreover, if possible, lithium should be administered in combination with insulin or exercise, which can increase the GLUT4 activity, in order to increase its effectiveness.

REFERENCES

1) Etgen GJ Jr, Wilson CM, Jensen J, Cushman SW, Ivy JL. 1996. Glucose transport and cell surface GLUT-4 protein in skeletal muscle of the obese Zucker rat. Am J Physiol 271 (2 Pt 1): E294–E301.
2) King PA, Betts JJ, Horton ED, Horton ES. 1993. Exercise, unlike insulin, promotes glucose transporter translocation in obese Zucker rat muscle. Am J Physiol 265 (2 Pt 2): R447–R452.
3) Baldessarini RJ, Tondo L, Duviv P, Pompili M, Goodwin FK, Hennen J. 2006. Decreased risk of suicides and attempts during long-term lithium treatment: a meta-analytic review. Bipolar Disord 8 (5 Pt 2): 625–639.
4) Bhattacharya G. 1964. Influence of Li+ on glucose metabolism in rats and rabbits. Biochem Biophys Acta 93: 644–646.
5) Boppart MD, Asp S, Wojtaszewski JF, Fielding RA, Mohr T, Goodyear LJ. 2000. Marathon running transiently increases c-Jun NH2-terminal kinase and p38 activities in human skeletal muscle. J Physiol 526 (Pt 3): 663–669.
6) Freeman MP, Freeman SA. 2006. Lithium: clinical considerations in internal medicine. Am J Med 119: 478–481.
7) Haugaard ES, Mickel RA, Haugaard N. 1974. Actions of lithium ions and insulin on glucose utilization, glycogen synthesis, and glycogen synthase in the isolated rat diaphragm. Biochem Pharmacol 23: 1675–1685.
8) Rossetti L. 1989. Normalization of insulin sensitivity with lithium in diabetic rats. Diabetes 38: 648–652.
9) Fürnsinn C, Noe C, Herdlicka R, Roden M, Nowotny P, Leighton B, Waldhäusl W. 1997. More marked stimulation by lithium than insulin of the glycogenic pathway in rat skeletal muscle. Am J Physiol 273 (3 Pt 1): E514–E520.
10) Harrell NB, Teachey MK, Gifford NJ, Henriksen EJ. 2007. Essential role of p38 MAPK for activation of skeletal muscle glucose transport by lithium. Arch Physiol Biochem 113 (4-5): 221–227.
11) Henriksen EJ, Kinnick TR, Teachey MK, O’Keefe MP, Ring D, Johnson KW, Harrison SD. 2003. Modulation of muscle insulin resistance by selective inhibition of GSK-3 in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab 284: E892–E900.
12) Tabata I, Schluter J, Galve EA, Holloszy JO. 1994. Lithium increases susceptibility of muscle glucose transport by stimulation in various agents. Diabetes 43: 903–907.
13) Macko AR, Beneze AN, Teachey MK, Henriksen EJ. 2008. Roles of insulin signalling and p38 MAPK in the activation by lithium of glucose transport in insulin-resistant rat skeletal muscle. Arch Physiol Biochem 114: 331–339.
14) Goodyear LJ, Kahn BB. 1998. Exercise, glucose transport, and insulin sensitivity. Annu Rev Med 49: 235–261.
15) Hayashi T, Wojtaszewski JF, Goodyear LJ. 1999. Exercise regulation of glucose transport in skeletal muscle. Am J Physiol 273 (6 Pt 1): E1039–E1051.
16) Antonescu CN, Huang C, Niu W, Liu Z, Eyers PA, Heidenreich KA, Bilan PJ, Klip A. 2005. Reduction of insulin-stimulated glucose uptake in L6 myotubes by the protein kinase inhibitor SB203580 is independent of p38MAPK activity. Endocrinology 146: 3773–3781.
17) Fukushima T, Boppart M, Hirshman M, Goodyear L. 2001. Insulin does not increase p38 MAP kinase activity or phosphorylation in rat skeletal muscle. Diabetes 49: 61–69.
18) Hayashi T, Hirshman MF, Dufresne SD, Goodyear LJ. 1999. Skeletal muscle contractile activity in vitro stimulates mitogen-activated protein kinase signaling. Am J Physiol 277 (4 Pt 1): C701–C707.
19) Ho RC, Alcazar O, Fujii N, Hirshman MF, Goodyear LJ. 2004. p38gamma MAPK regulation of glucose transporter expression and glucose uptake in L6 myotubes and mouse skeletal muscle. Am J Physiol Regul Integr Physiol 286: R342–R349.
20) Ribe D, Yang J, Patel S, Koumanov F, Cushman SW, Holman GD. 2005. Endocelial competitive inhibition of glucose transporter-4 intrinsic activity by the mitogen-activated protein kinase inhibitor SB203580. Endocrinology 146: 1713–1717.
21) Somwar R, Koterski S, Sweeney G, Scioiti R, Djuric S,
Berg C, Trevillyan J, Scherer PE, Rondinone CM, Klip A. 2002. A dominant-negative p38 MAPK mutant and novel selective inhibitors of p38 MAPK reduce insulin-stimulated glucose uptake in 3T3-L1 adipocytes without affecting GLUT4 translocation. *J Biol Chem* **277**: 50386–50395.

Ishikura S, Antonescu CN, Klip A. 2010. Documenting GLUT4 exocytosis and endocytosis in muscle cell monolayers. *Curr Protoc Cell Biol* Chapter 15 Unit 15:15.

Nesher R, Karl IE, Kipnis DM. 1980. Epitrochlearis muscle. II. Metabolic effects of contraction and catecholamines. *Am J Physiol* **239**: E461–E467.

Young DA, Uhl JJ, Cartee GD, Holloszy JO. 1986. Activation of glucose transport in muscle by prolonged exposure to insulin: effects of glucose and insulin concentration. *J Biol Chem* **261**: 16049–16053.

Han DH, Nolte LA, Ju JS, Coleman T, Holloszy JO, Semenkov CF. 2004. UCP-mediated energy depletion in skeletal muscle increases glucose transport despite lipid accumulation and mitochondrial dysfunction. *Am J Physiol* **286**: E347–E353.

Hancock CR, Han DH, Chen M, Terada S, Yasuda T, Wright DC, Holloszy JO. 2008. High fat diets cause insulin resistance despite an increase in muscle mitochondria. *Proc Natl Acad Sci USA* **105**: 7815–7820.

Henriksen EJ, Bourey RE, Rodnick KJ, Koranyi L, Permutt MA, Holloszy JO. 1990. Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *Am J Physiol* **259**: E593–E598.

Wright DC, Geiger PC, Han DH, Holloszy JO. 2006. Are tyrosine kinases involved in mediating contraction-stimulated muscle glucose transport? *Am J Physiol Endocrinol Metab* **290**: E123–E128.

Hansen PA, Wang W, Marshall BA, Holloszy JO, Mueckler M. 1998. Dissociation of GLUT4 translocation and insulin-stimulated glucose transport in transgenic mice overexpressing GLUT1 in skeletal muscle. *J Biol Chem* **273**: 18173–18179.

Bradley H, Shaw CS, Worthington PL, Cocks M, Wagenmakers AJ. 2014. Quantitative immunofluorescence microscopy of subcellular GLUT4 distribution in human skeletal muscle: effects of endurance and sprint interval training. *Physiol Rep* **2**: e12085.

Ueyama A, Yaworsky KL, Wang Q, Ebina Y, Klip A. 1999. GLUT-4myc ectopic expression in L6 myoblasts generates a GLUT-4-specific pool conferring insulin sensitivity. *Am J Physiol* **277** (3 Pt 1): E572–E578.

Antonescu CN, Randhawa VK, Klip A. 2008. Dissecting GLUT4 traffic components in L6 myocytes by fluorescence-based, single-cell assays. *Methods Mol Biol* **457**: 367–378.

Zhou X, Shentu P, Xu Y. 2017. Spatiotemporal regulators for insulin-stimulated GLUT4 vesicle exocytosis. *J Diabetes Res* **2017**: 1683678.

Brozinick JT Jr, Egen GJ Jr, Yaspelkis BB, Ivy JL. 1994. Glucose uptake and GLUT-4 protein distribution in skeletal muscle of the obese Zucker rat. *Am J Physiol* **267**: R236–R243.

Goodyear LJ, Hirshman MF, Horton ES. 1991. Exercise-induced translocation of skeletal muscle glucose transporters. *Am J Physiol* **261** (6 Pt 1): E795–E799.

Guma A, Zierath JR, Wallberg-Henriksson H, Klip A. 1995. Insulin induces translocation of GLUT-4 glucose transporters in human skeletal muscle. *Am J Physiol* **268**: E613–E622.

King PA, Horton ED, Hirshman MF, Horton ES. 1992. Insulin resistance in obese Zucker rat (fa/fta) skeletal muscle is associated with a failure of glucose transporter translocation. *J Clin Invest* **90**: 1568–1575.

Klip A, Ramilal T, Bilan PJ, Cartee GD, Gulve EA, Holloszy JO. 1990. Recruitment of GLUT-4 glucose transporters by insulin in diabetic rat skeletal muscle. *Biochem Biophys Res Commun* **172**: 728–736.

Zhao R, Qiu B, Li Q, Zhang T, Zhao H, Chen Z, Cai Y, Ruan H, Ge W, Zheng X. 2014. LBP-4a improves insulin resistance via translocation and activation of GLUT4 in OLETF rats. *Food Funct* **5**: 811–820.

Furtado LM, Poon V, Klip A. 2003. GLUT4 activation: thoughts on possible mechanisms. *Acta Physiol Scand* **178**: 287–296.

Ren JM, Marshall BA, Gulve EA, Gao J, Johnson DW, Holloszy JO, Mueckler M. 1993. Evidence from transgenic mice that glucose transport is rate-limiting for glycogen deposition and glycolysis in skeletal muscle. *J Biol Chem* **268**: 16113–16118.

Bryant NJ, Govers R, James DE. 2002. Regulated transport of the glucose transporter GLUT4. *Nat Rev Mol Cell Biol* **3**: 267–277.

Kandror KV, Pilch PF. 2011. The sugar is sIR Ved: sorting GLUT4 and its fellow travelers. *Methods Mol Biol* **457**: 367–378.

Kloumourtzoglou D, Pryor PR, Gould GW, Bryant NJ. 2015. Alternative routes to the cell surface underpin insulin-regulated membrane trafficking of GLUT4. *J Cell Sci* **128**: 2423–2429.