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Glucose Transporter Type 4 Redistribution on the Membrane Induced by Insulin through Akt in Hydrocortisone Treatment in Rat Skeletal Muscles

Chien-Min Chen[1, 2, 3], Lian Chiu[4], Hung-Chi Chen[5], Chun-Yuan Cheng[3], Woei-Cherng Shyu[6, 7], Chii-Wen Chou[8], Cheng-You Lu[6, 7], and Chung-Tien Lin[1, 9]

[1]*Department and Graduate Institute of Veterinary Medicine, School of Veterinary Medicine National Taiwan University, Taipei 10617*
[2]*School of Medicine, Kaohsiung Medical University, Kaohsiung 80708*
[3]*Division of Neurosurgery, Department of Surgery, Changhua Christian Hospital, Changhua 50006*
[4]*Department of Nursing, College of Medicine and Nursing, Hungkuang University, Taichung 43302*
[5]*Department of Plastic and Reconstructive Surgery, China Medical University and China Medical University Hospital, Taichung 40447*
[6]*Graduate Institute of Immunology, China Medical University, Taichung 40402*
[7]*Center for Neuropsychiatry, China Medical University Hospital, Taichung 40402*
[8]*Division of Neurosurgery, Tungs' Taichung Metroharbor Hospital, Taichung 43503*
and
[9]*Institute of Veterinary Clinical Science, School of Veterinary Medicine, National Taiwan University Taipei 10617, Taiwan, Republic of China*

Abstract

Hydrocortisone is a growth hormone frequently used in the treatment of low back pain. Hydrocortisone treatment has an anti-inflammation effect, which also inactivates glucose transporter type 4 (GLUT4) by p38 mitogen-activated protein kinase (MAPK) inhibition. Translocation of GLUT4 regulates body glucose homeostasis and muscle repair and is induced by insulin. In this study, 56 SD rats were divided into seven groups, and were treated with insulin or hydrocortisone in sedentary or exercise training groups. The muscle proteins and biochemical blood parameters were analyzed after 7 days of treatments. The results showed that the serum glucose increased in hydrocortisone treatment accompanied by GLUT4 inactivation in both the sedentary and exercise training rats. In the exercise training groups, GLUT4 was redistributed on the plasma membrane on co-treatment with insulin and hydrocortisone through Akt phosphorylation. Insulin treatment exerted a compensatory feedback effect on the GLUT4 translocation on hydrocortisone co-treatment, which was the cause of GLUT4 inactivation.

Key Words: Akt phoshphorylation, GLUT4, hydrocortisone, insulin, skeletal muscle

Introduction

Some patients suffer from chronic low back pain after strenuous exercise, and hydrocortisone injection usually provides instant cure affection (4, 18, 29). There were several regiments using hydrocortisone

Corresponding authors: [1] Cheng-You Lu, Ph.D., Center for Neuropsychiatry, China Medical University Hospital, No. 6, Xueshi Road, Northdist., Taichung 40402, Taiwan, R.O.C. Tel: +886-4-22052121 ext. 7811, Fax: +886-4-22052121 ext. 7810, E-mail: ymt150@gmail.com; and [2] Chung-Tien Lin, Ph.D., Institute of Veterinary Clinical Science, School of Veterinary Medicine, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan, R.O.C. Tel: +886-2-27396828 ext. 2010, Fax: +886-2-23661475, E-mail: L02041984@mail.cmu.edu.tw
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for anti-inflammation or pain relief after surgery (7, 11, 17, 26). However, the optimal treatments or adjuvant therapies seem unavailable, and relatively little is known about the involved molecular mechanisms of the hydrocortisone action. Such patients continue to suffer from chronic low back pain in a vicious circle of the same etiology.

The use of hormone preparations, such as anabolic steroids, growth hormone and insulin, appear to be increasing and their use is widespread in sport, herbal medicines and nutritional supplements (2, 8, 13). Despite of some adverse effects due to overdose or abuse, it is currently legal and its use by athletes is not considered as doping (6). The so-called "natural products", "health foods", "dietary supplements" and the like often contain such hormone preparations, which have been clarified by broad-based investigations of the international nutritional supplement market (10). It is now critical to understand the mechanisms of the action of muscle remodeling and pain relief by hydrocortisone to facilitate proper use of these nutritional supplements.

Hydrocortisone is a steroid hormone produced by the adrenal gland in the zona fasciculate and the main function of hydrocortisone is pro-inflammation cytokine release inhibition (5). Particularly, the hydroortisone can inhibit p38 mitogen-activated protein kinase (MAPK) activation in cytokine simulation (28). Moreover, a study pointed out that 2 mM hydrocortisone could improve the charcoal-treated fetal calf serum and restored 75% of the lost mitogenicity in Schwann cells (21). This may help the synaptic coupling cells and reduces pain.

The skeletal muscle cells are remodeled through glucose transporter type 4 (GLUT4) activation and translocation to the plasma membrane (16). Although AMPK can cause GLUT4 translocation, p38 MAPK is a stronger inducer (20, 27). Hydrocortisone may block skeletal muscle proliferation by its p38 MAPK suppression effect. Recently, insulin was reported to possess the GLUT4 directive activation inducer through AKT rather than the AMPK or p38 MAPK pathway (22, 31).

In order to define the benefits of insulin co-treatment in hydrocortisone treatment, the exercise-training animal model was used in this work. p38 MAPK and GLUT4 translocation were shown to be recovered by insulin in hydrocortisone treatments. The results might explain the benefit of insulin and hydrocortisone co-treatment in therapeutic effects of clinical muscle pain.

Materials and Methods

Animal Model

There were 56 SD rats (250 g, aged 6 weeks) purchased from BioLASCO Taiwan Co., Ltd., which were divided into 7 groups (n = 8 each). Groups designed were set as control, hydrocortisone treatment only (2 mg/kg/day), insulin treatment only (2 IU/kg/day), exercise training only, exercise training co-treated with hydrocortisone (2 mg/kg/day), exercise training co-treated with insulin (2 IU/kg/day), exercise training co-treated with insulin (2 IU/kg/day) and hydrocortisone (2 mg/kg/day). All the rats were treated once as indicated and were then fasted overnight for blood glucose administration before sacrificed. The exercise training was 30 min swimming in 25°C warm water. Insulin glargine (3.64 mg/ml, 100 IU/ml) was purchased from Sanofi-Aventis (Paris, France), and was diluted to 2 IU/ml in phosphate-buffered saline (PBS) pH 4.0, as the stock solution. Hydrocortisone was purchased from Sigma-Aldrich (Missouri, USA), and was dissolved in PBS at 2 mg/ml as the stock solution. Hydrocortisone and insulin treatments were applied by subcutaneously injection into the rats for 4 h before the exercise training or sedentary sessions. All procedures involving laboratory animal use were in accordance with the guidelines of the Instituted Animal Care and Use Committee of Chung-Shan Medical University Experimental Animal Center (IACUC approval No. 844) for the care and use of laboratory animals.

Biochemical Blood Parameters

Blood samples were taken from the jugular veins then centrifuged at 2500 *g* (4°C, 20 min) for serum isolation. Assessments were analyzed on a Beckman Synchron CX9 clinical system, and serum parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), urea, uric acid (UA) and glucose (GLU).

Protein Extraction

All skeletal muscle tissue extracts of 8 rats in each group were obtained by homogenizing in a lysis buffer (0.05 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA) at a ratio of 100 mg tissue/ml lysis buffer. The homogenates were placed on ice and then centrifuged at 13,000 rpm for 40 min. The supernatants were collected and stored at -80°C for further experiments. For GLUT4 translocation analysis, the protein samples used were homogenized in a buffer with 10 mM Tris-HCl, 1 mM EDTA, 250 mM sucrose. All samples were then subjected to differential centrifugations and store at -80°C until analysis.

Immunoblotting

Protein concentrations of skeletal muscle tissue

Table 1. The serum biochemistry markers analysis

	Sedentary			Exercise			Hydrocortisone +
	Control	Hydrocortisone	Insulin	Control	Hydrocortisone	Insulin	Insulin
CK	933.74 ± 97.86	868.01 ± 113.50	921.39 ± 107.44	1498.67 ± 189.97*	1471.15 ± 163.24*	1659.36 ± 199.59**	1314.78 ± 109.66
ALT	26.73 ± 2.30	25.64 ± 2.15	24.95 ± 2.54	27.65 ± 3.16	26.89 ± 2.95	27.12 ± 2.56	27.89 ± 3.21
AST	127.32 ± 9.76	126.55 ± 10.20	125.19 ± 9.99	134.42 ± 11.28	133.41 ± 10.13	129.87 ± 2.62	126.95 ± 9.67
Urea	23.17 ± 1.99	23.45 ± 2.03	23.21 ± 2.01	32.25 ± 2.61	30.77 ± 2.69	34.95 ± 3.74	30.15 ± 1.89
UA	0.70 ± 0.05	0.70 ± 0.04	0.71 ± 0.05	0.87 ± 0.06	0.87 ± 0.05	0.92 ± 0.06	0.70 ± 0.06
GLU	119.67 ± 5.66	154.59 ± 9.62***	99.81 ± 6.27*	101.28 ± 8.14	119.26 ± 7.53	92.11 ± 3.36$^{\#\#}$	102.79 ± 3.96$^{\#}$

All plasma samples (n = 8) were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), urea, uric acid (UA), and glucose (GLU). *P < 0.05, **P < 0.01, ***P < 0.001, compared with the sedentary control group; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01, compared with the exercise control group.

extracts were determined by the Lowry protein assay. Protein samples were separated in a 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) with a constant voltage of 75 V for 120 min. Proteins were then transferred to Hybond-C membranes (GE healthcare UK Ltd., Buckinghamshire, UK) using 50 V for 3 h. PVDF membranes were incubated in 3% bovine serum albumin (BSA) in TBS buffer. Primary antibodies, all purchased from Cell Signaling, MD, USA, including α-tubulin (3873S), Akt (9272S), p-Akt (9271S), GLUT4 (2213S), p-p38 (9211S), and p38 (9212S), were added into the membranes for recognizing the fitted proteins. Finally, horseradish peroxidase (HRP)-labeled antibodies were used and images were taken with Fujifilm LAS-3000 (GE healthcare UK limited). The band intensities were quantified by scanning and processed using the Image J v1.44 edition.

Cell Culture

Mouse myoblast C2C12 cells were purchased from ATCC (Manassas, VA, USA) and were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum, 4.5 g/L glucose, in 5% CO_2 atmosphere at 37°C. After 2 days of incubation, the cultured medium was changed to DMEM with 2% horse serum for C2C12 myotube differentiation.

ELISA Assay

The mouse glucose transporter 4 ELISA kit (MyBioSource, Inc., CA, USA) was used to determine the GLUT4 activity of the cells. After drug treatments for 15 min, the cells were harvested and incubated together with GLUT4-HRP conjugate in pre-coated plates. After 1 h incubation, the wells were decanted and washed 5 times with a wash solution. The wells were then incubated with the substrate for the HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. A stop solution was next added to stop the reaction. The intensity of color was measured spectrophotometrically at 450 nm in a microplate reader.

Statistical Analysis

All the results were obtained from 8 rats in each experimental group and are presented as group mean ± SD in tables or in the text. One-way analysis of variance was used to indicate an overall statistical significance among the means of the seven experimental groups. For paired samples, the Student's t-test was applied. A P-value of less than 0.05 was considered as significant. Statistical analyses were performed using the SigmaPlot 11.0 edition.

Results

Data of the biochemical blood parameters analysis are shown in Table 1. The hydrocortisone treatment only in the sedentary group of rats did not affect the parameters other than the glucose concentration, which increased to 154.59 ± 9.62 mg/dl from 119.67 ± 5.66 mg/dl (control). In the insulin treatment only group, glucose levels decreased to 99.81 ± 6.72 mg/dl. Particularly, the serum glucose in the exercise training control group was slightly less than that of the sedentary control group. Although insulin treatment could reduce the serum glucose level, exercise training combined with insulin treatment could enhance more serum glucose reduction. There were no specific differences between the serum glucose levels in the exercise training co-treated with insulin and hydrocortisone group and the sedentary control group.

Moreover, the CK expression levels in all the exercise training groups were slightly higher than those in the sedentary groups (Table 1). But the CK expression levels were also slightly decreased in exercise training co-treated with hydrocortisone or co-treated with both hydrocortisone and insulin. The highest CK expression levels appeared in the sera of exercise training combined with insulin treatment group of rats.

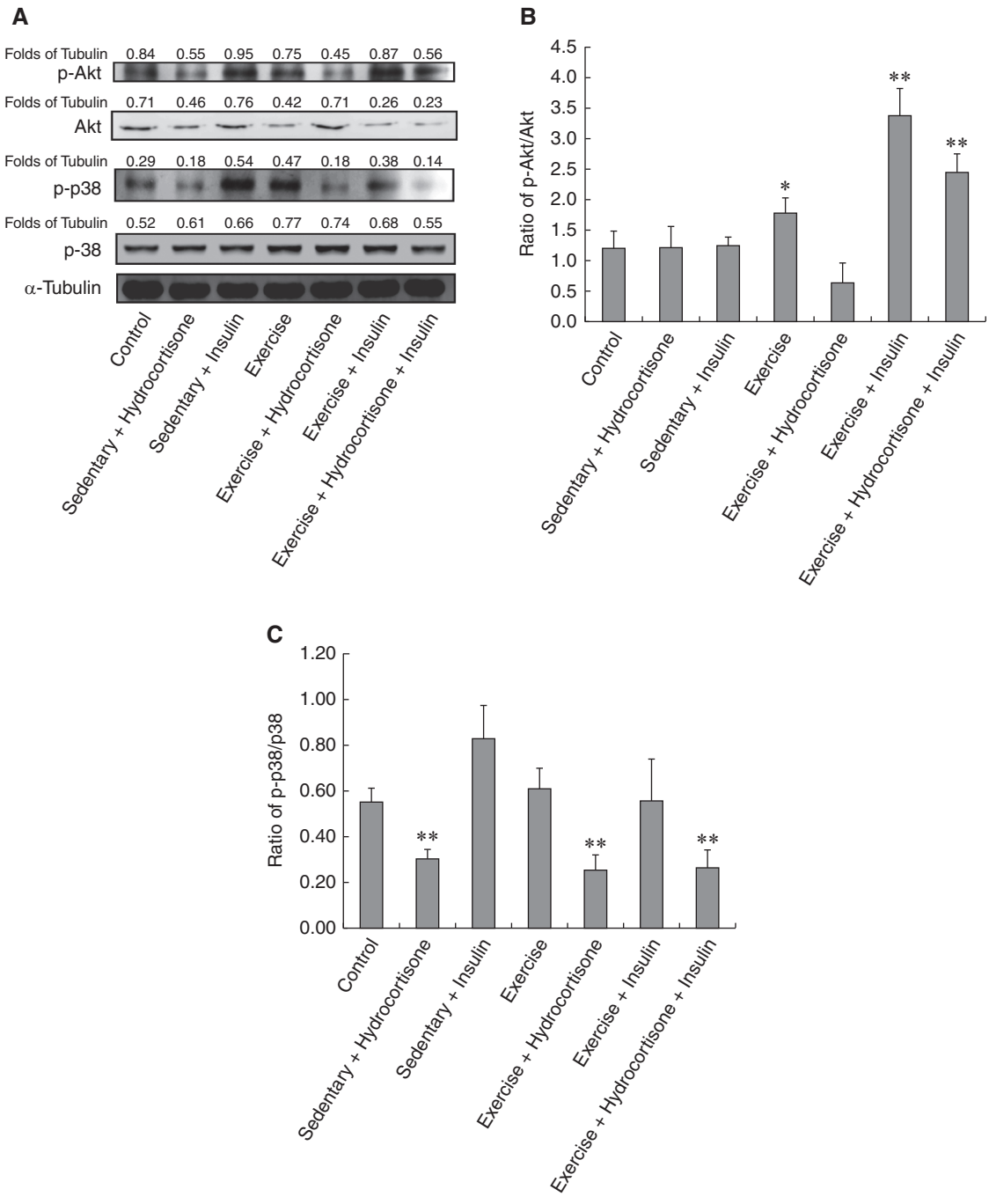

Fig. 1. Expression analysis of GLUT4 translocation upstream kinases. (A) After 7 days of the indicated treatments, the active portions of Akt and p38 MAPK were analysis by western immunoblotting assay. The folds of protein expression were normalized to that of α-tubulin. The ratio between phosphorylated kinases (active form) and native form are shown in (B) and (C). *$P < 0.05$, **$P < 0.01$, compared with the respective control group.

Hydrocortisone upstream activators are Akt and p38 MAPK. These active kinase proteins can be measured by the ratio between phosphorylated and native unphosporylated protein forms. The p-Akt expression levels in the sedentary groups were similar (Fig. 1). p-Akt expression was enhanced in the exercise training control group, and could be reduced after hydrocortisone treatment. Additionally, insulin treatment reversed the p-Akt expression ratio on co-treatment with hydrocortisone, or enhanced p-Akt expression in exercise training. Moreover, the p-p38 expression levels were inhibited in each of the hydrocortisone treatment groups, and exercise training or insulin treatment still failed to overcome the p38 MAPK inhibitory effects

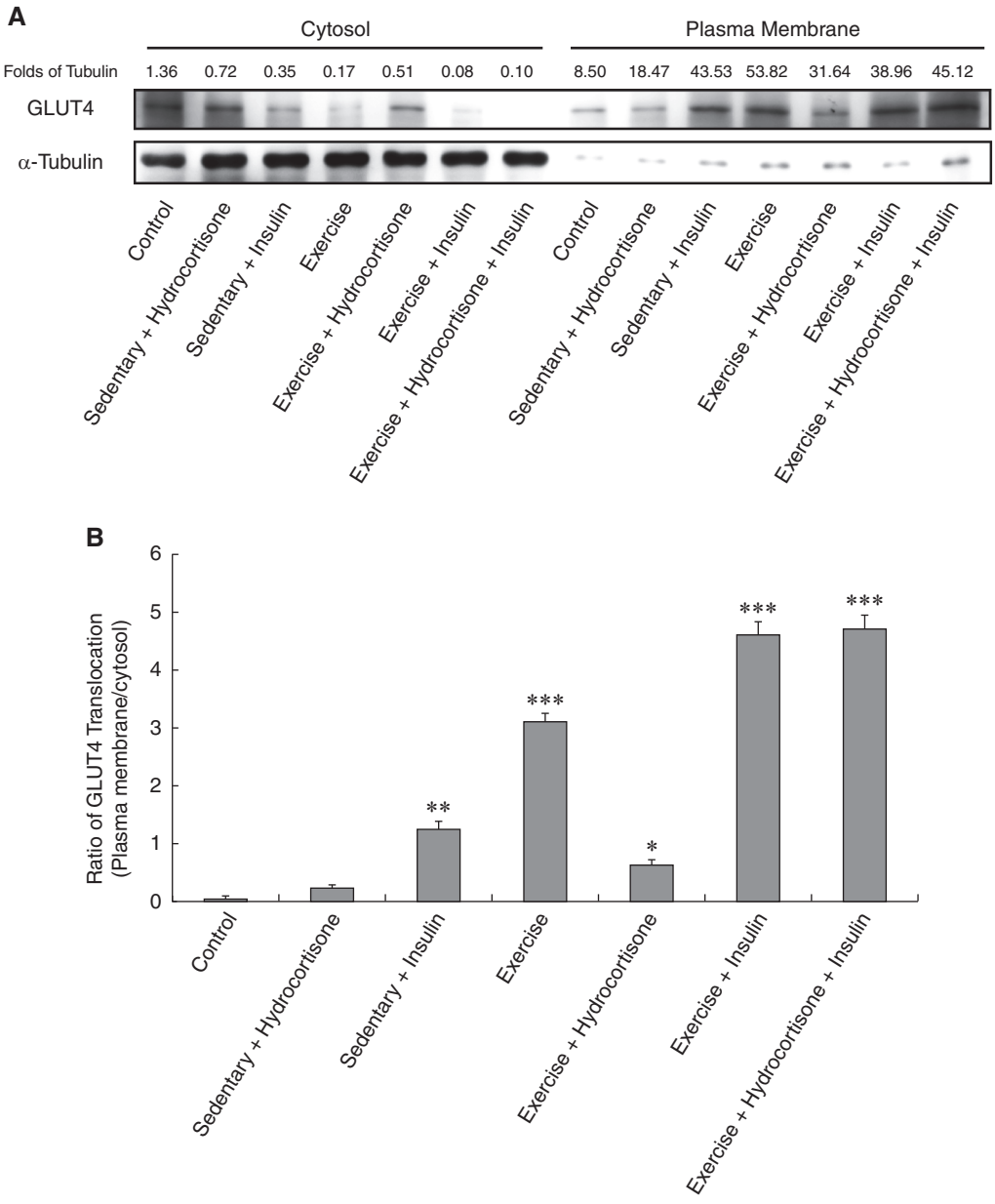

Fig. 2. Analysis of GLUT4 translocation. After 7 days of the indicated treatments, the active GLUT4 translocated to the plasma membrane from the cytosol. Analysis of the GLUT4 protein expression of the cytosol and plasma membrane are shown in (A). The ratio of GLUT4 translocation on the plasma membrane is shown in (B). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with the respective control group.

of hydrocortisone.

GLUT4 activation can be defined by its plasma membrane translocation. GLUT4 expression in the cytosol and the plasma membrane of each treatment group is shown in Fig. 2. In the sedentary groups, only the insulin treatment could increase the GLUT4 translocation to the plasma membrane. In the exercise groups, insulin treatment enhanced the GLUT4 translocation ratio from 3.16 fold up to 4.87 fold. Although the hydrocortisone could inhibit the GLUT4 translocation in the exercise training group, in which the ratio of GLUT4 translocation was 0.06. But GLUT4 translocation was compensatively reversed by insulin treatment, and the ratio of GLUT4 translocation was 4.51.

Additionally, the C2C12 myotubes were treated with insulin (10 mU/ml) and hydrocortisone (100 nM) for 15 min. The p-p38 and p-Akt expression levels were not enhanced on hydrocortisone treatment, but p-p38 and p-Akt were increased in the insulin treatment groups (Fig. 3A). These results were similar to the animal model results in this research. Furthermore,

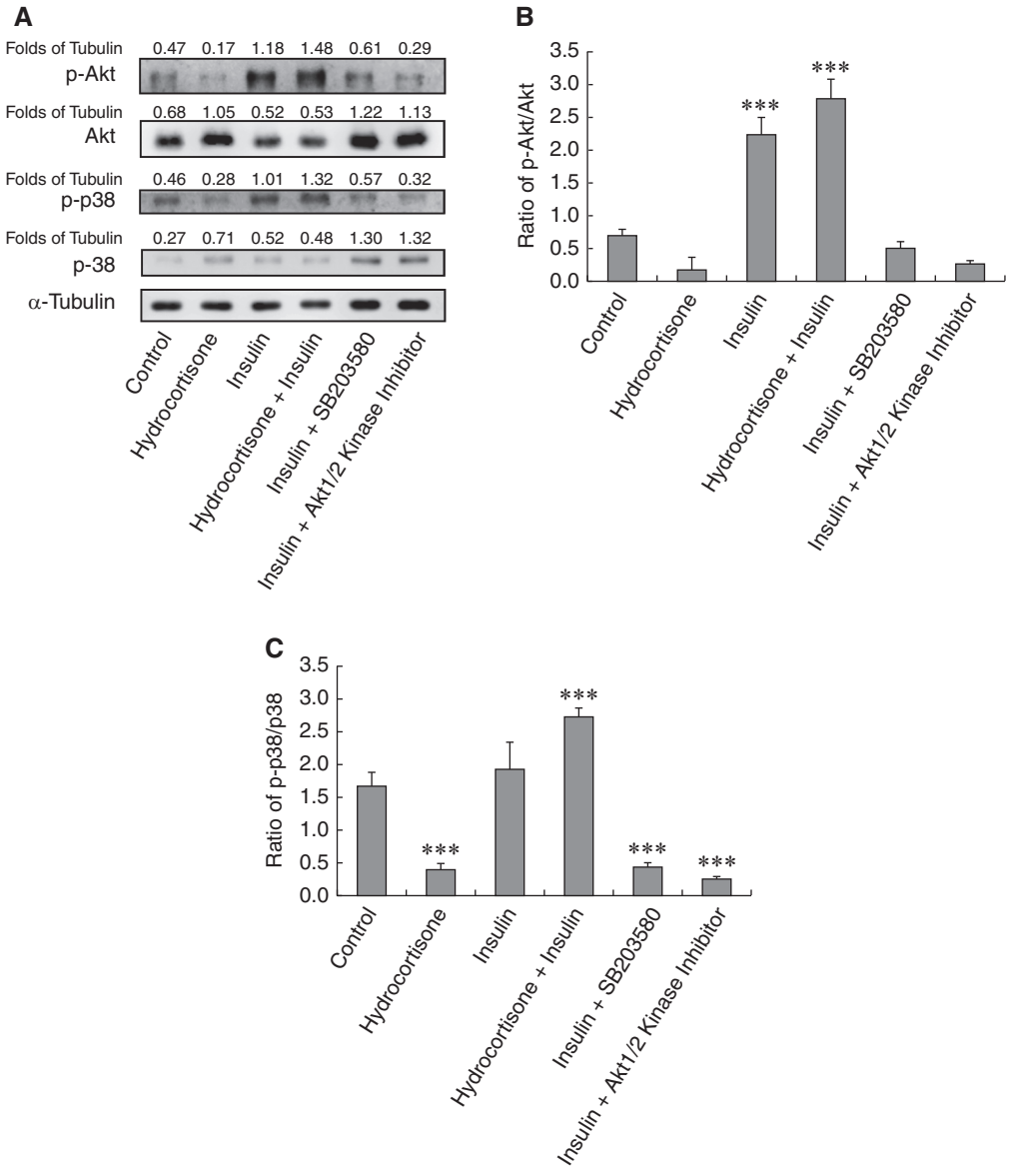

Fig. 3. Expression analysis of GLUT4 translocation upstream kinases in C2C12 myotubes. (A) After 6 h treatment with p38 inhibiter (SB203580, 10 nM) and Akt1/2 kinase inhibitor (50 nM), insulin (10 mU/ml) was cotreated with the C2C12 myotubes for an additional 15 min. The proteins of these cells were analyzed compared to the control and the insulin- or hydrocortisone (100 nM) only treatment groups for 15 min. (B) The ratio between phosphorylated kinases (active form) and the unphosporylated form are shown in (B) and (C). ***$P < 0.001$, compared with the respectively control group.

the p38 and Akt kinase activation were blocked by the p38 and Akt1/2 kinase inhibitor pretreatment in C2C12 myotubes (Figs. 3B & 3C). The GLUT4 activity of C2C12 myotubes was analyzed by the same indicated treatments. The hydrocortisone treatment inhibited the GLUT4 translocation activity and insulin enhanced it (Fig. 4). Further, the GLUT4 translocation activities enhanced by insulin were blocked by both p38 and Akt1/2 kinase inhibitor pretreatments in C2C12 myotubes.

Discussion

An exercise training-induced muscle damage is regulated to oxidative stress in impaired insulin signaling pathway and releases the CK into serum; hence, CK elevations indicate muscle damage and inflammation (1, 23). Hydrocortisone is an efficient anti-inflammation drug owing to its TGF-β inhibitory properties, and is applied in muscle pain treatments and muscle recovery (14, 15). In this research, the CK expression levels were found to be high in exercise training groups, and hydrocortisone treatment, indeed, partially decreased CK elevations in the serum (Table 1).

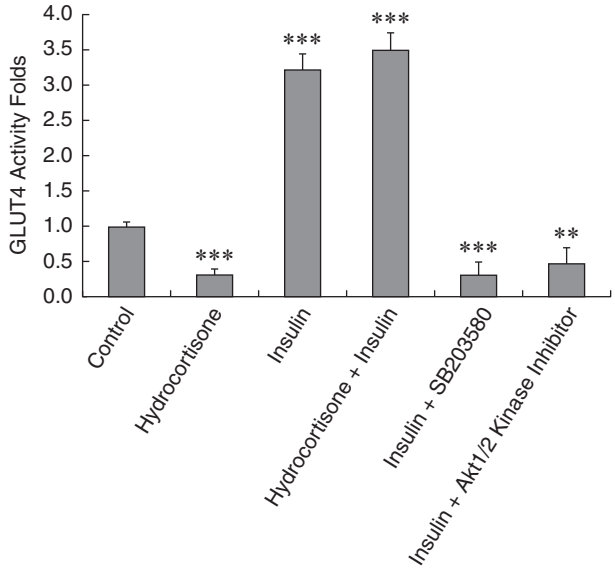

Fig. 4. Analysis of GLUT4 translocation expression in C2C12 myotubes. After 6 h of p38 inhibiter (SB203580, 10 nM) and Akt1/2 kinase inhibitor (50 nM) treatment, insulin (10 mU/ml) was cotreated with the C2C12 myotubes for an additional 15 min. The GLUT4 translocation activities were analyzed by ELISA assays compared to the control and insulin (10 mU/ml) or hydrocortisone (100 nM) only treatment for 15 min. $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the respective control group

When skeletal muscles contract, AMPK is activated, with a concurrent increase in free AMP concentration (12, 25). The principal glucose transporter protein GLUT4 plays a key role in regulating whole-body glucose homeostasis (11). Moreover, p38 MAPK is a strong inducer of GLUT4 and hydrocortisone can act as an inhibitor of p38 MAPK (20, 28), which causes a problem in hydrocortisone application in muscle repairing processes. Here, the GLUT4 translocation ratio was shown to increase in the muscles of the exercise training groups of rats, and this effect was blocked by hydrocortisone treatment (Fig. 2). In fact p38 MAPK expression was all inhibited in the muscle of each hydrocortisone rat.

Furthermore, an important role for Akt in the stimulation of glucose transport through GLUT4 translocation by insulin in muscle has been reported (19, 30). The insulin signaling pathway is triggered by the activation of the insulin receptor (IR) and downstream tyrosine kinase, leading to tyrosine phoshphorylation of insulin receptor substrate proteins (IRS) and recruitment of PI3K. Subsequently, the phoshphorylated PI3K activates the Akt and further induces GLUT4 translocation to the membrane (3).

In this work, the ratios of phoshphorylated Akt were increased in each insulin treatment group (Fig. 1), and downstream GLUT4 translocation was not inhibited. Interestingly, the original GLUT4 translocation inhibition by hydrocortisone treatment was also reversed to the activated form (24). The GLUT4 translocation activities enhanced by insulin were blocked by both p38 and Akt1/2 kinase inhibitor pretreatment in C2C12 myotubes (Fig. 4). These results suggested that activation of the p38 and Akt kinase plays the key role in insulin-dependent GLUT4 translocation activity in C2C12 myotubes. This experimental evidence supported that GLUT4 translocation was controlled by dual Akt and p38 MAPK pathways, and Akt is a dominator although p38 MAPK is still a strong inducer of GLUT4. Insulin co-treatment has a compensatory feedback effect on the GLUT4 translocation on hydrocortisone treatment that causes GLUT4 inactivation.

References

1. Aoi, W., Naito, Y. and Yoshikawa, T. Role of oxidative stress in impaired insulin signaling associated with exercise-induced muscle damage. *Free Radic. Biol. Med.* 65: 1265-1672, 2013.
2. Baker, J.S., Graham, M.R. and Davies, B. Steroid and prescription medicine abuse in the health and fitness community: a regional study. *Eur. J. Intern. Med.* 17: 479-484, 2006.
3. Bryant, N.J., Govers, R. and James, D.E. Regulated transport of the glucose transporter GLUT4. *Nat. Rev. Mol. Cell Biol.* 3: 267-277, 2002.
4. Chatzitheodorou, D., Mavromoustakos, S. and Milioti, S. The effect of exercise on adrenocortical responsiveness of patients with chronic low back pain, controlled for psychological strain. *Clin. Rehabil.* 22: 319-328, 2008.
5. Davidson, D., Patel, H., Degoy, A.C., Gershkovich, I., Vancurova, I. and Miskolci, V. Differential effect of exogenous interleukin-10 versus glucocorticoids on gene expression and pro-inflammatory cytokine release by polymorphonuclear leukocytes and monocytes of the newly born. *Am. J. Transl. Res*. 5: 103-115, 2013.
6. De Cock, K.J.S., Delbeke, F.T., Van Eenoo, P., Desmet, N., Roels, K. and De Backer, P. Detection and determination of anabolic steroids in nutritional supplements. *J. Pharm. Biomed. Anal.* 25: 843-852, 2001.
7. Epstein, W.L. and Kligman, A.M. Suppression of local allergic reactions with hydrocortisone. *J. Allergy* 27: 395-397, 1956.
8. Ernst, E. Adulteration of Chinese herbal medicines with synthetic drugs: a systematic review. *J. Intern. Med.* 252: 107-113, 2002.
9. Feffer, H.L. Treatment of low-back and sciatic pain by the injection of hydrocortisone into degenerated intervertebral discs. *J. Bone Joint Surg. Am.* 38-A: 585-592, 1956.
10. Geyer, H., Parr, M.K., Mareck, U., Reinhart, U., Schrader, Y. and Schänzer, W. Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroids - results of an international study. *Int. J. Sports Med.* 25: 124-129, 2004.
11. Huang, S. and Czech, M.P. The GLUT4 glucose transporter. *Cell Metab*. 5: 237-252, 2007.
12. Hutber, C.A., Hardie, D.G. and Winder, W.W. Electrical stimulation inactivates muscle acetyl-CoA carboxylase and increases AMP-activated protein kinase. *Am. J. Physiol.* 272: E262-E266, 1997.
13. Kafrouni, M.I., Anders, R.A. and Verma, S. Hepatotoxicity associated with dietary supplements containing anabolic steroids. *Clin. Gastroenterol. Hepatol.* 5: 809-812, 2007.
14. Kaminsky, P., Walker, P.M., Deibener, J., Barbe, F., Jeannesson, E., Escanye, J.M., Dousset, B. and Klein, M. Growth hormone

potentiates thyroid hormone effects on post-exercise phosphocreatine recovery in skeletal muscle. *Growth Horm. IGF Res.* 22: 240-244, 2012.
15. Kimura, H., Li, X., Torii, K., Okada, T., Kamiyama, K., Mikami, D., Kasuno, K., Takahashi, N. and Yoshida, H. Glucocorticoid enhances hypoxia- and/or transforming growth factor-β-induced plasminogen activator inhibitor-1 production in human proximal renal tubular cells. *Clin. Exp. Nephrol.* 15: 34-40, 2011.
16. Kurth-Kraczek, E.J., Hirshman, M.F., Goodyear, L.J. and Winder, W.W. 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes* 48: 1667-1671, 1999.
17. Langmayr, J.J., Obwegeser, A.A., Schwarz, A.B., Laimer, I., Ulmer, H. and Ortler, M. Intrathecal steroids to reduce pain after lumbar disc surgery: a double-blind, placebo-controlled prospective study. *Pain* 62: 357-361, 1995.
18. Lentjes, E.G.W.M., Griep, E.N., Boersma, J.W., Romijn, F.P.T.H.M. and de Kloet, E.R. Glucocorticoid receptors, fibromyalgia and low back pain. *Psychoneuroendocrinology* 22: 603-614, 1997.
19. Lin, C.H., Ho, C.Y., Liu, C.S., Lin, W.Y., Li, C.I., Yang, C.W., Bau, D.T., Li, T.C. and Lin, C.C. Influence of adiponectin gene polymorphisms on adiponectin serum level and insulin resistance index in taiwanese metabolic syndrome patients. *Chinese J. Physiol.* 55: 405-411, 2012.
20. Montessuit, C., Rosenblatt-Velin, N., Papageorgiou, I., Campos, L., Pellieux, C., Palma, T. and Lerch, R. Regulation of glucose transporter expression in cardiac myocytes: p38 MAPK is a strong inducer of GLUT4. *Cardiovasc. Res.* 64: 94-104, 2004.
21. Neuberger, T.J., Kalimi, O., Regelson, W., Kalimi, M. and De Vries, G.H. Glucocorticoids enhance the potency of Schwann cell mitogens. *J. Neurosci. Res.* 38: 300-313, 1994.
22. Nozaki, S., Takeda, T., Kitaura, T., Takenaka, N., Kataoka, T. and Satoh, T. Akt2 regulates Rac1 activity in the insulin-dependent signaling pathway leading to GLUT4 translocation to the plasma membrane in skeletal muscle cells. *Cell. Signal.* 25: 1361-1371, 2013.
23. O'Fallon, K.S., Kaushik, D., Michniak-Kohn, B., Dunne, C.P., Zambraski, E.J. and Clarkson, P.M. Effects of quercetin supplementation on markers of muscle damage and inflammation after eccentric exercise. *Int. J. Sport Nutr. Exerc. Metab.* 22: 430-437, 2012.
24. Piroli, G.G., Grillo, C.A., Reznikov, L.R., Adams, S., McEwen, B.S., Charron, M.J. and Reagan, L.P. Corticosterone impairs insulin-stimulated translocation of GLUT4 in the rat hippocampus. *Neuroendocrinology* 85: 71-80, 2007.
25. Ponticos, M., Lu, Q.L., Morgan, J.E., Hardie, D.G., Partridge, T.A. and Carling, D. Dual regulation of the AMP-activated protein kinase provides a novel mechanism for the control of creatine kinase in skeletal muscle. *EMBO J.* 17: 1688-1699, 1998.
26. Sarvestani, A.S., Amini, S., Kalhor, M., Roshanravan, R., Mohammadi, M. and Lebaschi, A.H. Intraperitoneal hydrocortisone for pain relief after laparoscopic cholecystectomy. *Saudi J. Anaesth.* 7: 14-17, 2013.
27. Shen, Y., Zhao, Y., Zheng, D., Chang, X., Ju, S. and Guo, L. Effects of orexin A on GLUT4 expression and lipid content *via* MAPK signaling in 3T3-L1 adipocytes. *J. Steroid Biochem. Mol. Biol.* 138: 376-383, 2013.
28. Stuhlmeier, K.M. and Pollaschek, C. Glucocorticoids inhibit induced and non-induced mRNA accumulation of genes encoding hyaluronan synthases (HAS): hydrocortisone inhibits HAS1 activation by blocking the p38 mitogen-activated protein kinase signalling pathway. *Rheumatology* 43: 164-169, 2004.
29. Sudhaus, S., Mollenberg, T., Plaas, H., Willburger, R., Schmieder, K. and Hasenbring, M. Cortisol awakening response and pain-related fear-avoidance versus endurance in patients six months after lumbar disc surgery. *Appl. Psychophysiol. Biofeedback* 37: 121-130, 2012.
30. Wang, Q., Somwar, R., Bilan, P.J., Liu, Z., Jin, J., Woodgett, J.R. and Klip, A. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Mol. Cell. Biol.* 19: 4008-4018, 1999.
31. Yang, J. Role of clusters in insulin-regulated GLUT4 trafficking in adipose cells: a new paradigm? *Int. J. Biol. Sci.* 6: 716-718, 2010.