Insulin stimulates glucose transporter 1 (GLUT1) and hexokinase II (HK II) gene expression in chicken skeletal muscle

T. KONO*, Y. NISHIKI, Y. SEKI, K. SATO and Y. AKIBA

Animal Nutrition, Life Science, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

***Corresponding author: kono@bios.tohoku.ac.jp**

Chickens have a blood glucose level that is twice as high as that in most mammals and are regarded as an insulin resistant animal. We previously reported that the major insulin responsive glucose transporter gene, GLUT4, is deficient in broiler chickens, therefore insulin regulation of blood glucose level in chickens is not well understood. In the present study, we characterized gene expressions of GLUT, hexokinase (HK) and glycogen synthase (GS) in insulin-stimulated state in chicken skeletal muscles and cultured chicken myotubes. In a study in vivo, 3-week-old male chickens were injected with insulin (400μg/kg body weight) or 0.9% of NaCl. Skeletal muscles were collected at 1 and 3 h after insulin injection. Expressions of GLUT1, GLUT3, GLUT8, HK I, HK II, GS mRNA and 18S rRNA were determined by Real-Time PCR. GLUT1, GLUT3 and HK II mRNA expression were significantly increased at 3 h after insulin injection in skeletal muscle but no significant increase in GLUT8 and HKI mRNA expression was observed. In a study in vitro, myotubes derived from muscle satellite cells were incubated with serum-free medium for 1 or 3 h in presence or absence of insulin (0, 0.2, 1, 5 μg/ml). GLUT1 and HK II mRNA expression of cultured chicken myotubes at both 1 and 3 h after the incubation were lineally increased with an increase of insulin added to cultures. GLUT3 mRNA expression showed significantly increase at 3 h after incubation with insulin. No significant increase by insulin was observed in expression of HK1 mRNA. Although GLUT1 is not regarded as an insulin-responsive GLUT in mammals, our in vivo and in vitro studies show that insulin stimulates GLUT1 and HK II mRNA expressions in chicken muscles, suggesting that glucose uptake and glucose phosphorylation is regulated in species-specific manner in chicken skeletal muscles.

Keywords: broiler chickens; skeletal muscle; insulin; GLUT; hexokinase

Introduction

Glucose transport across the plasma membrane mediated by glucose transporter proteins (GLUTs) plays a crucial role in whole body glucose homeostasis. To date, twelve isoforms (GLUT1-12) of the GLUT family have been identified in mammals, with these proteins expressed in a tissue-specific

manner (Wood and Trayhurn, 2003). Considering glucose homeostasis in human postprandial state, more than 70 % of glucose is cleared by skeletal muscles (DeFronzo *et al*., 1992). Once inside skeletal muscle cells, glucose is immediately converted to glucose-6-phosphate (G6P) by hexokinase (HK). In mammals, four hexokinase isoforms (HKI, HKII, HKIII and HKIV) have been identified on the biochemical characteristics and genetic basis (Kanno, 2000).

Studies on insulin regulated gene transcription in muscle biopsies obtained during hyperinsulinemic-euglycemic clamp conditions have been undertaken in humans and mammalian animal models in vivo using a candidate gene approach (RT-PCR). These studies have reported that GLUT4, HK II and glycogen synthase (GS), a key enzyme in glycogen synthesis, are induced by insulin (Laville *et al*., 1996; Ducluzeau *et al*., 2001; Huang *et al*., 2000). In addition, it was shown that induction of HK II, GLUT4 and GS was impaired in type 2 diabetes, comprising one reason for muscle insulin resistant in this disease.

Compared with mammals, chickens have higher blood glucose concentration, even in the fasting state, and are markedly resistant to the injection of high concentrations of insulin (Akiba *et al*., 1999). Our previous study showed insulin injection in vivo stimulated glucose uptake by 2-fold in chicken muscle (Tokushima *et al*., 2005), while broiler chickens lack GLUT4 homologous gene (Seki *et al*., 2003). Thus, to date insulin responsible GLUT is not identified. Moreover, insulin-regulated gene transcription in skeletal muscle and the regulation of blood glucose level are not well understood in chickens. In the present study, we characterized gene expressions of GLUT, HK and GS in the insulin-stimulated state in chicken skeletal muscles and cultured chicken myotubes.

Materials and methods

Experiment 1 (In vivo study)

Three-week-old male broiler chickens (Cobb) were starved for 12 h and then intraperitoneally injected with porcine insulin (400μg/kg body weight) or 0.9% of NaCl solution. Blood sample were collected at 0, 1 and 3 h after the insulin injection, and skeletal muscle (EDL: extensor digitorum longus; PS: pectoralis superficialis) were collected at 1 and 3 h after the insulin injection followed by rapid freezing of the tissues with dry ice.

Plasma glucose concentrations were measured by the glucose-assay kit (Wako Pure-Chemicals, Osaka, Japan). Total RNA was extracted using Trizol Reagent (Invitrogen) from collected skeletal muscles. The synthesis of first strand cDNA and quantitative PCR were performed as described in our previous report (Kono et al., 2005)

Experiment 2 (In vitro study)

Breast muscle (pectoralis superficialis) was collected aseptically from 1-day-old male chicks (Cobb). Satellite cells were obtained by enzyme digestion as previously described (Nishida *et al*., 1997) with some modification. Cells were seeded onto Φ60 mm collagen type I-coated dishes at a density of 15000 cells/cm^2 in growth medium [75% Dulbecco's modified Eagle medium (DMEM) and 25% Medium-199 supplemented with 100 U/ml penicillin, 100U/ml streptomycin, 10% FBS and 1% chicken embryo extract (CEE)] and cultured in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. After 48 h, culture medium was replaced with medium containing 2% FBS for 48h to induce the differentiation of myoblasts into myotubes After multinucleated myotubes were substantially observed, cultures were preincubated for 3 h in serum-free media followed by 1 or 3 h incubation in the presence of insulin (0, 0.2, 1, 5 μg/ml). After the incubation with insulin, cells were rinsed with

PBS and collected and subjected to the analysis of gene expressions.

Statistical analysis

Data were analyzed by the general linear model procedure of SAS.

Results

Experiment 1 (In vivo study)

To determine effects of insulin administration on mRNA expression of GLUT, HK and GS in chicken skeletal muscle, real-time quantitative PCR was performed. The plasma glucose concentrations in chickens were significantly decreased by the insulin injection to approximately 40 % that of control counterparts with no insulin. At 3 h after insulin injection, GLUT1, GLUT3, HK II and GS mRNA expressions in skeletal muscle were significantly increased to approximately 3.4, 2.0, 4.7 and 1.3-fold that of control, respectively. In contrast, no significant increase by insulin was observed in GLUT8 and HKI mRNA expressions (*Figure 1*).

Experiment 2 (In vitro study)

GLUT1 and HK II mRNA expression in cultured chicken myotubes was increased by both 1 and 3 h incubation with insulin, with the increase being in a dose dependent manner (*Figure 2*). GLUT3 mRNA expression showed significant increase at only 3 h after incubation with insulin but not after 1 h of incubation. No significant increase by insulin was observed in expression of HKI and GS mRNA (data not shown).

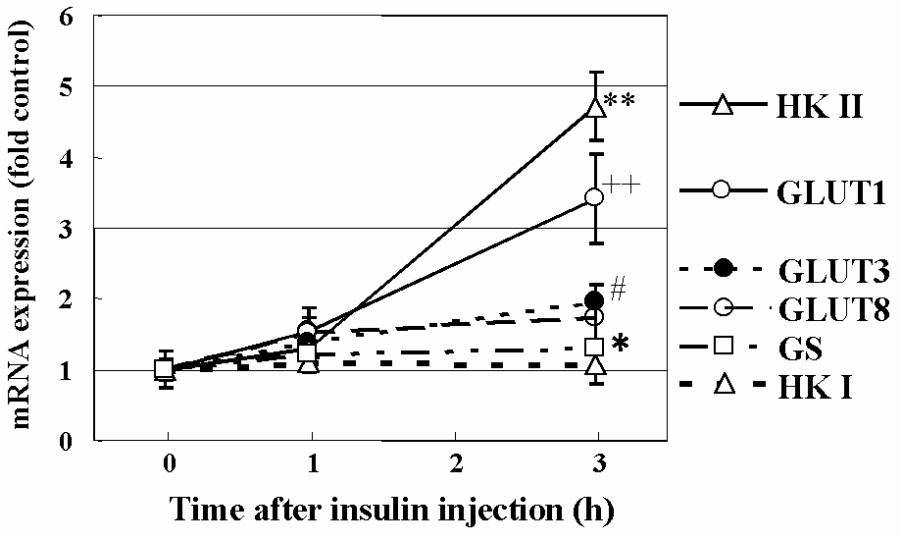

Figure 1 Expressions of GLUT, HK and GS mRNA in insulin stimulated state in chicken skeletal muscles. Chickens were intraperitoneally injected with insulin (400 μg/kg body weight), and the tissues were collected at 0, 1 and 3 h after the insulin injection. Real-Time PCR was performed using specific primers of chicken GLUT1, GLUT3, GLUT8, HKI, HK II and GS. All mRNA levels, represented in fold control, were normalized to 18S rRNA levels. ($^{*,\#}$ P<0.05 and $^{**,++}$ P<0.01 compared to each control)

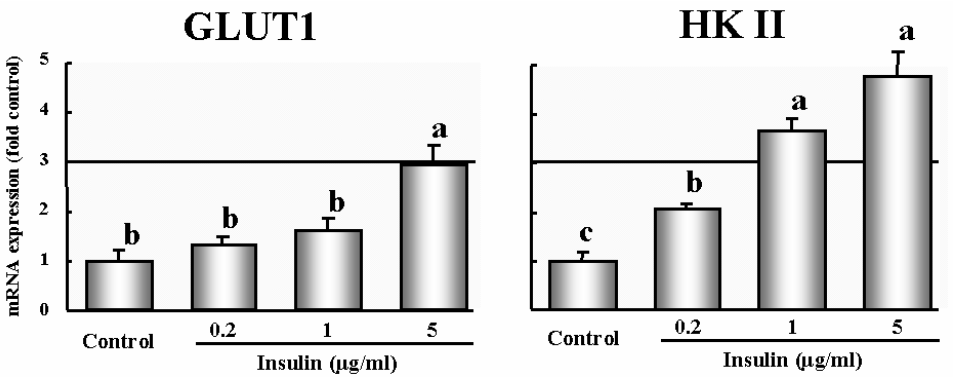

Figure 2 Expressions of GLUT1 and HK2 mRNA in chicken myotubes cultured with insulin. Chicken myotubes were treated for 3h with insulin at the indicate concentrations. Real-Time PCR was performed using specific primers of chicken GLUT1 and HK II. All mRNA levels, represented in fold control, were normalized to 18S rRNA levels. Values with different superscripts are significantly different ($P < 0.05$).

Discussion

Glucose uptake in skeletal muscles is dependent on specific GLUTs and HK, whereas glucose storage in muscle cells being regulated by GS. Studies on insulin regulated gene transcription of muscle biopsies in mammals showed that expressions of GLUT4, HK II and GS were induced by insulin (Laville *et al*., 1996; Ducluzeau *et al*., 2001; Huang *et al*., 2000).

Chickens are characterized by hyperglycemia and insulin resistance compared to mammals. Even though a major insulin-responsive transporter, GLUT4, is deficient in chickens (Seki *et al*., 2003), previous study showed that insulin administration increased [^{3}H]2DG uptake by 2-fold in skeletal muscle in vivo (Tokushima *et al*, 2005) and by 43 % in cultured myotubes in vitro (Duclos *et al*., 1993). Therefore, it is hypothesized that some alternative mechanism of insulin-responsive glucose transport exists in chicken skeletal muscles.

In the present study, induction of GLUT1 and GLUT3 mRNA expressions by insulin was observed both in vivo and in vitro, with the increase being exaggerated in GLUT1 while upregulation of GLUT8 was observed only in vitro. Although GLUT1 is not regarded as insulin-responsive GLUT in mammals, it is tempting to propose that in chickens intrinsically deficient with GLUT4, GLUT1 mediates, at least in part, insulin-responsive glucose transport in skeletal muscles.

The present data also showed significant increase in muscle HK II mRNA expression by insulin in both in vivo and in vitro experiments, in contrast to no significant changes in HK I expressions by insulin. These observations in insulin response in HK I and II expressions were similar to the previous study in skeletal muscle cell lines of mammals (Osawa *et al*., 1996). Because HK II gene expression generally conforms to the enzyme activity in mammals, it is conceivable that glucose phosphorylation in insulin-stimulated state in chickens may be mediated by HK II as similar to mammals. It is further suggested that chicken HK I is involved, at least, in the glucose phosphorylation in the basal state with no insulin stimulation. On the other hand, since in contrast to mammals very little increase in the GS expression by insulin in chicken skeletal muscle in vivo or no significant increase in cultured myotubes in vitro were observed, insulin modulation of GS gene expression may not be existed in chickens. In the mechanism by which insulin modulates GLUT and HK, whether insulin-signaling pathway (i.e. Akt/PKB kinase) in chicken skeletal muscle is involved in the activation of GLUT1 and

HK II gene expression remains to be determined in vivo and in vitro.

In summary, although GLUT1 is not regarded as insulin responsive GLUT in mammals, our in vivo and in vitro studies firstly demonstrated that insulin stimulates GLUT1 and HK II mRNA expressions in chicken skeletal muscles, suggested that insulin regulation in glucose uptake and glucose phosphorylation occurs in species-specific manner in chicken skeletal muscles intrinsically deficient with GLUT4.

References

AKIBA, Y., CHIDA, Y., TAKAHASI, T., OHTOMO, Y., SATO, K. and TAKAHASHI, K. (1999) Persistent hypoglycemia induced by continuous insulin infusion in broiler chickens. *Br Poult Sci*, **40**: 701-705.

DEFRONZO, R.A., BONADONNA R.C. and FERRANNINI E. (1992) Pathogenesis of NIDDM. *Diabetes Care*, **15**: 318-368.

DUCLOS, M.J., CHEVALIER, B., LE MARCHAND-BRUSTEL, Y., TANTI, J.F., GODDARD, C. and SIMON, J. (1993) Insulin-like growth factor-I-stimulated glucose transport in myotubes derived from chicken muscle satellite cells. *J Endocrinol*, **137**(3): 465-472.

DUCLUZEAU, P.H., PERRETTI, N., LAVILLE, M., ANDREELLI, F., VEGA, N., RIOU, J.P. and VIDAL, H. (2001) Regulation by insulin of gene expression in human skeletal muscle and adipose tissue. Evidence for specific defects in type 2 diabetes. *Diabetes*, **50**(5): 1134-1142.

HUANG, X., VAAG, A., HANSSON, M., WENG, J., LAURILA, E. and GROOP, L. (2000) Impaired insulin- stimulated expression of the glycogen synthase gene in skeletal muscle of type 2 diabetic patients is acquired rather than inherited. *J Clin Endocrinol Metab*, **85**(4): 1584-1590.

KANNO, H. (2000) Hexokinase: gene structure and mutations. *Bailliere's Best Pract. Res., Clin. Haematol.* **13**: 83-88.

KONO, T., NISHIDA, M., NISHIKI, Y., SEKI, Y., SATO, K. and AKIBA, Y. (2005) Characterisation of glucose transporter (GLUT) gene expression in broiler chickens. *Br Poult Sci*, **46**(4): 510-515.

LAVILLE, M., AUBOEUF, D., KHALFALLAH, Y., VEGA, N., RIOU, J.P. and VIDAL, H. (1996) Acute regulation by insulin of phosphatidylinositol-3-kinase, Rad, Glut 4, and lipoprotein lipase mRNA levels in human muscle. *J Clin Invest*, **98**(1): 43-49.

NISHIDA, H., YAMAGUCHI, T., SATO, K., OHTSU H. and AKIBA, Y. (1997) Influenceof prostaglandin E_2 on chick myoblasts differentiation during the myogenesis. *Anim Sci Technol*, **68**(4): 381-384.

OSAWA, H., SUTHERLAND, C., ROBEY, R.B., PRINTZ, R.L. and GRANNER, D.K. (1996) Analysis of the signaling pathway involved in the regulation of hexokinase II gene transcription by insulin. *J Biol Chem*, **271**(28): 16690-16694.

SEKI, Y., SATO, K., KONO, T., ABE, H. and AKIBA, Y. (2003) Broiler chickens (Ross strain) lack insulin-responsive glucose transporter GLUT4 and have GLUT8 cDNA. *Gen Comp Endocrinol*, **133**(1): 80-87.

TOKUSHIMA, Y., TAKAHASHI, K., SATO, K. and AKIBA, Y. (2005) Glucose uptake in vivo in skeletal muscles of insulin-injected chicks. *Comp Biochem Physiol B Biochem Mol Biol*. **141**(1): 43-48.

WOOD, I.S. and TRAYHURN, P. (2003) Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *British Journal of Nutrition*, **89**: 3-9.