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Insulin acutely increases glucose transporter 1 on plasma membranes and glucose uptake in an AKT-dependent manner in chicken adipocytes

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ABSTRACT

Avian glucose transporters (GLUT) responsible for insulin-responsive glucose uptake into adipocytes remain poorly characterized. We aimed to identify the insulin-responsive GLUT using primary culture of chicken adipocytes. Acute stimulation with 1 μM insulin for 20 min increased 2-deoxyglucose uptake, AKT protein phosphorylation, and GLUT1 protein levels on the plasma membrane of the chicken adipocytes, whereas pretreatment with 10 μM triciribine, an AKT inhibitor, canceled these effects. Furthermore, the insulin stimulation did not affect GLUT12 protein levels on the plasma membrane of the chicken adipocytes. Our results suggest that GLUT1 is an insulin-responsive GLUT in chicken adipocytes.

1. Introduction

Glucose is an essential metabolic substrate that is transported into animal cells by the glucose transporter (GLUT) family proteins. In mammals, insulin stimulates glucose uptake into adipose tissue and skeletal muscle (Leto and Saltiel, 2012). The molecular mechanisms of insulin-responsive glucose uptake have been studied using cultured mammalian adipocytes (Kohn, et al., 1996; Chen et al., 1983; Kozka et al., 1991; Holman et al., 1990; Lee et al., 2000; Yang and Holman, 1993). In mammals, GLUT4 is well known as a major insulin-responsive transporter and is distributed between the plasma membrane and intracellular storage vesicles (Leto and Saltiel, 2012). Insulin stimulation enhances the exocytosis of GLUT4 from intracellular vesicles via the phosphorylation of AKT, a Ser/Thr kinase, and consequently increases glucose uptake in adipocytes (Kraegen et al., 1993; Kohn et al., 1996; Malide et al., 2000; Watson and Pessin, 2001).

Chickens maintain higher blood glucose levels than mammals and other vertebrates (Hazelwood and Lorenz, 1959; Belo et al., 1976; Akiba et al., 1999; Tokushima et al., 2005). It has been reported that chickens lack a homolog of the *GLUT4* gene, which encodes a major insulin-responsive transporter in mammals (Seki et al., 2003). Nevertheless, the injection of insulin into chickens acutely decreases their plasma glucose levels (Akiba et al., 1999; Tokushima et al., 2005). In addition, Tokushima et al. (2005) reported that insulin stimulation increased glucose uptake into skeletal muscles and liver. Although the glucose uptake into adipose tissue was lower than that into skeletal muscles and liver (Tokushima et al., 2005), Rudas and Scanes (1983) reported that insulin stimulation increased glucose uptake into explant culture of adipose tissue after 2–15 min. Therefore, insulin-responsive GLUT isoforms involved in such acute glucose uptake in adipose tissue of chicken are of interest, but they have not been fully characterized. Furthermore, the relationship among insulin stimulation, AKT phosphorylation, GLUT translocation, and glucose uptake has not yet been reported in both adipose tissue and in other tissues of chicken.

In cultured primary chicken adipocytes, insulin successfully increased glucose uptake (Gomez-Capilla and Langslow, 1977); therefore, in this study, we employed primary chicken adipocytes as a model to study the molecular mechanisms of insulin-responsive glucose uptake in chicken. We show here that, in primary chicken adipocytes, stimulation with 1 μM insulin acutely increased AKT phosphorylation and 2-deoxyglucose (2DG) uptake 20 min after stimulation. From studies in the skeletal muscle of chickens, among GLUT isoforms, GLUT1 and GLUT12 have been suggested to be insulin-responsive GLUTs (Kono et al., 2006; Zhao et al., 2012; Coudert et al., 2015). Therefore, in this

Abbreviations: 2DG, 2-deoxyglucose; BSA, bovine serum albumin; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT, glucose transporter; KRPH, Krebs–Ringer phosphate–HEPES; Na^+/K^+ ATPase β, sodium/potassium ATPase beta 1; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; SEM, standard error of the mean

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study, we examined the impact of acute insulin stimulation on GLUT1 and GLUT12 protein levels on the surface of primary chicken adipocytes to identify the insulin-responsive glucose transporter(s).

2. Materials and methods

2.1. Preparation of chicken adipocytes

All experimental protocols and procedures were reviewed and approved by the Animal Care and Use Committee of Kagoshima University (Kagoshima, Japan). Twenty newly hatched male broiler chicks (*Gallus gallus domesticus*) were supplied by a commercial hatchery (Kumiai Hina Center, Kagoshima, Japan). The chicks were housed in an electrically heated battery brooder maintained at 28–35 °C until they were 2–3 weeks old. All chicks were sacrificed by cervical dislocation under carbon dioxide anesthesia. Abdominal fat pad tissue obtained from the chicks was digested with collagenase. The resulting cell suspension was washed in phosphate-buffered saline (PBS) and the cells were counted and plated onto gelatin-coated six-well plates at a density of 2.0×10^5 cells/well. The cells were cultured in M199 medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and maintained at 37 °C in a 5% CO_2-enriched atmosphere of humidified air. The medium was replaced every other day until the cells were almost confluent. The cells were then differentiated in M199 medium containing 20% chicken serum and 1% penicillin/streptomycin for 5 days. The medium was replaced every other day. Oil red O staining was performed as described previously (Ramírez-Zacarías et al., 1992).

2.2. Determination of the insulin concentration required to phosphorylate AKT protein in differentiated chicken adipocytes

We determined the concentration of insulin that was most effective for the phosphorylation of AKT protein in differentiated chicken adipocytes. After differentiation, chicken adipocytes were incubated with insulin (0.1, 1, or 10 μM) or PBS in M199 medium with 0.5% bovine serum albumin (BSA). Twenty minutes after incubation, the medium was collected and the cell monolayer was washed three times with ice-cold PBS, detached by scraping with a rubber policeman using ISOGEN II (Nippon Gene, Tokyo, Japan), and stored at −80 °C until it was used for western blotting. Insulin was made up in PBS and subsequently added to the appropriate tissue culture wells (100 μL/mL). The vehicle control wells contained only PBS at the same volume as in the treatment groups. To study the effects of inhibition of AKT protein in chicken adipocytes, an inhibitor of AKT (triciribine, 10 μM) was used to block AKT phosphorylation.

2.3. Measurements of insulin-stimulated 2-deoxyglucose (2DG) uptake in chicken adipocytes

Although 2DG is readily transported into cells and is phosphorylated by hexokinase, it cannot be metabolized further and accumulates in the cell. To examine the amount of glucose uptake, we measured the uptake of 2DG in chicken adipocytes. The differentiated chicken adipocytes were divided into three groups (control, insulin, and triciribine + insulin). The cells were incubated in M199 medium with 0.5% BSA with (triciribine + insulin group) or without (control group and insulin group) 10 μM triciribine for 3 h. The cells were then incubated in Krebs–Ringer phosphate–HEPES (KRPH) buffer with (insulin group and triciribine + insulin group) or without (control group) 1 μM insulin. After incubation for 20 min, 2DG uptake was measured for another 20 min using a 2DG uptake measurement kit (CSR-OKP-PMG-K01T; Cosmo Bio Co., Ltd., Tokyo, Japan), in accordance with the manufacturer's instructions.

2.4. Antibodies

Anti-AKT (♯9272), anti-phospho-AKT (Thr308, ♯9275), and anti-phospho-AKT (Ser473, ♯9271) were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH, sc-20357, Lot F0706) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-GLUT1 (ab150299) and anti-GLUT12 (ab100993) were purchased from Abcam (Cambridge, UK). Anti-sodium/potassium ATPase beta 1 (Na^+/K^+ ATPase β; NB300-147ss) was purchased from NOVUS (Beverly, CO, USA).

2.5. Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis

The differentiated chicken adipocytes were divided into three groups (control, insulin, and triciribine + insulin). The cells were incubated in M199 medium with 0.5% BSA with (triciribine + insulin group) or without (control group and insulin group) 10 μM triciribine for 3 h. The cells were then incubated in the medium with (insulin group and triciribine + insulin group) or without (control group) 1 μM insulin. After incubation for 20 min, plasma membrane proteins were extracted using a plasma membrane protein extraction kit (101Bio, Palo Alto, CA, USA), in accordance with the manufacturer's instructions. The total protein concentration was estimated with a protein-dye binding assay using a commercial kit (500-0116; Bio-Rad, Hercules, CA, USA) with BSA as the standard.

Immunoblot analysis was performed as described previously (Shimamoto et al., 2016). In brief, equivalent amounts of protein from total cellular protein (20 μg) or cell membrane fractions (10 μg) were subjected to SDS-PAGE using 10% polyacrylamide gel and then transferred to a polyvinylidene difluoride membrane (Millipore Co., Billerica, MA, USA). The membrane was blocked with Bullet Blocking One for Western Blotting (13779-01; Nacalai Tesque, Kyoto, Japan) for 1 h at room temperature. Subsequently, the blocked membrane was incubated with primary antibody against AKT, phospho-AKT (Ser473), phosphor-AKT (Thr308), GLUT1, or GLUT12 in Can Get Signal I (Toyobo, Osaka, Japan) overnight at 4 °C (1:5000 dilution). Then, these membranes were incubated with secondary antibody in Can Get Signal II at 37 °C for 2 h (1:5000 dilution). The blots were detected using Western Blotting Detection Reagent (RPN2232; GE Healthcare, Madison, WI, USA), in accordance with the manufacturer's instructions. Relative band intensity was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The levels of AKT in total cellular protein were normalized to the levels of GAPDH. In addition, the levels of GLUT1 and GLUT12 of the plasma membrane fractions were normalized to the levels of Na^+/K^+ ATPase β subunit, respectively.

2.6. RNA extraction, reverse transcription, and quantitative real-time polymerase chain reaction (qPCR)

The differentiated chicken adipocytes were incubated in medium containing 0.5% BSA with or without 1 μM insulin. After incubation for 20 min, the cells were homogenized in ISOGEN II (Nippon Gene, Tokyo, Japan) in accordance with the manufacturer's instructions. The purity and quantity of RNA were assessed using a NanoDrop™ Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The A260:A280 ratio ranged from 2.1 to 2.2. Sixty nanograms of total RNA from each sample was reverse-transcribed with the PrimeScriptRT Reagent Kit (RR036A; Takara, Kusatsu, Japan). Samples were incubated at 37 °C for 15 min, 85 °C for 5 s, and 4 °C for 5 min. Controls without reverse transcription (no-RT) were also prepared to check for non-specific amplification. cDNA samples were stored at −20 °C for subsequent use.

The amplifications by qPCR were carried out in a volume of 20 μL containing 2 μL of cDNA samples, 10 μL of SYBR Select Master Mix

(4472919; Thermo Fisher Scientific), and 400 nM of each primer using 96-well polypropylene PCR plates (3400-00; Ina Optika, Osaka, Japan). Thermal cycling conditions were as follows: an initial hold at 50 °C for 2 min, 95 °C for 2 min, and then 45 cycles at 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 1 min. A dissociation step was included for all reactions to confirm single specific PCR product amplification and define the Tm of each amplicon. PCR products were analyzed on 2% agarose gel to check the amplicon size. The following primers were used: *GLUT1*, 5′-GATGGCTTTGTCCTTTGAGATGC-3′ and 5′-CAAAGATGCTG GTGGAGTAGTAG-3′; *GLUT12*, 5′-TCTCTCTGGCCAGCCTACTT-3′ and 5′-TTCACTCAGGACGAGCCAAG-3′; and *GAPDH*, 5′-CCTCTCTGGCAAA GTCCAAG-3′ and 5′-CATCTGCCCATTTGATGTTG-3′. Reaction efficiency was determined for each purified and quantified PCR product by performing 10-fold serial dilution in five concentrations, in duplicate. Each sample was run in duplicate along with no-RT controls in each plate and R^2 was assessed. PCRs were highly specific ($0.96 < R2 < 1.00$) and reproducible, and all primer pairs had equivalent PCR efficiencies (from 98% to 100%). Melting curves were created and revealed a single peak for all primer pairs. Coefficients of variation were 7%–11%. Amplification, dissociation curves, and gene expression analysis were performed using Dissociation Curves software (Thermo Fisher Scientific). Because there were no significant differences of cycle threshold values of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) among the groups, the level of GAPDH was used as an internal standard. The gene expression results are shown as ratios to the values of the control adipocytes incubated without insulin.

2.7. Statistical analysis

The data are expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed using Student's *t*-test or Tukey's test. P values under 5% were considered to indicate statistical significance.

3. Results and discussion

Preadipocytes cultured in M199 medium containing 20% chicken serum showed a marked increase in lipid deposition compared with the control cells cultured in M199 medium containing 10% FBS (Fig. 1A, B). Although there were no lipid droplets in preadipocytes cultured in M199 medium containing 10% FBS, the presence of lipid droplets was confirmed in preadipocytes cultured in M199 containing 20% chicken serum by oil red O staining (Fig. 1C, D). These results indicated that the preadipocytes from the abdominal adipose tissue of 3-week-old chickens can differentiate into mature adipocytes.

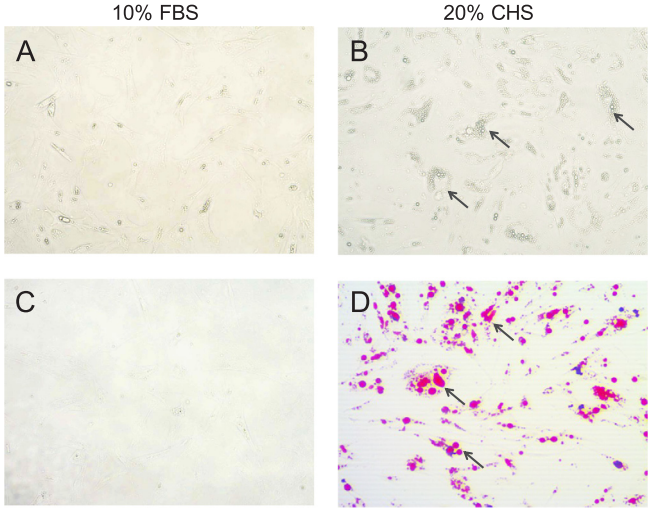

Fig. 1. Oil red O staining of chicken adipocytes. A phase contrast image of cells cultured in M199 medium containing 10% FBS (A) and 20% chicken serum (B). (C) Oil red O staining of cells cultured in M199 medium containing 10% FBS (C) and 20% chicken serum (D). Arrows denote lipid droplets.

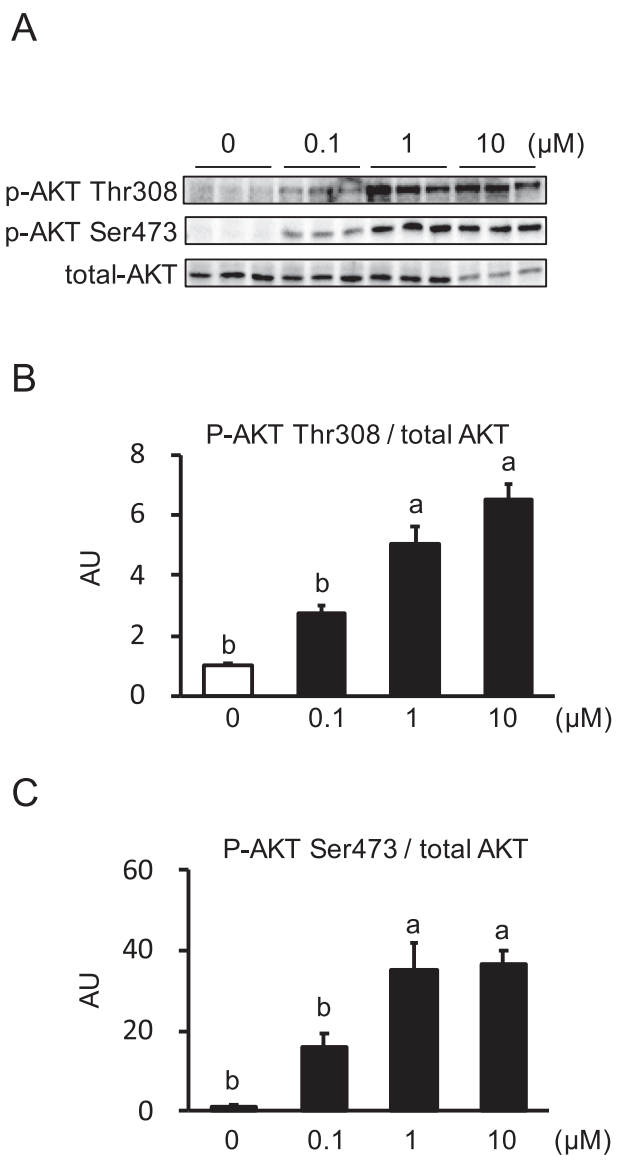

Fig. 2. Determination of the insulin concentration most effective for phosphorylating AKT protein in chicken adipocytes. After differentiation in M-199 medium containing 20% chicken serum for 5 days, the chicken adipocytes were incubated with insulin (0.1, 1, or 10 μM) or PBS in M199 medium with 0.5% BSA. Total and phosphorylated AKT were detected by immunoblotting (upper panel). The ratios of phosphorylated to total AKT were quantified (lower panel). Values are expressed as mean ± SEM (n = 6). Different letters indicate significant differences at $P < 0.05$.

Next, we determined the optimal concentration of insulin for stimulating the differentiated chicken adipocytes. Insulin stimulation (0.01–0.1 μM) has been reported to increase the phosphorylation and kinase activity of AKT protein in rat adipocytes (Holman et al., 1990) and 3T3L1 adipocytes (Kozka et al., 1991). In agreement with this, differentiated chicken adipocytes stimulated with insulin showed concentration-dependent increases in the phosphorylation levels of both Thr308 and Ser473 of AKT protein (Fig. 2), suggesting that chicken adipocytes are insulin-responsive, like mammalian adipocytes. Because insulin stimulation at a concentration of 1 μM was sufficient to induce AKT protein phosphorylation, this concentration was used in all subsequent experiments.

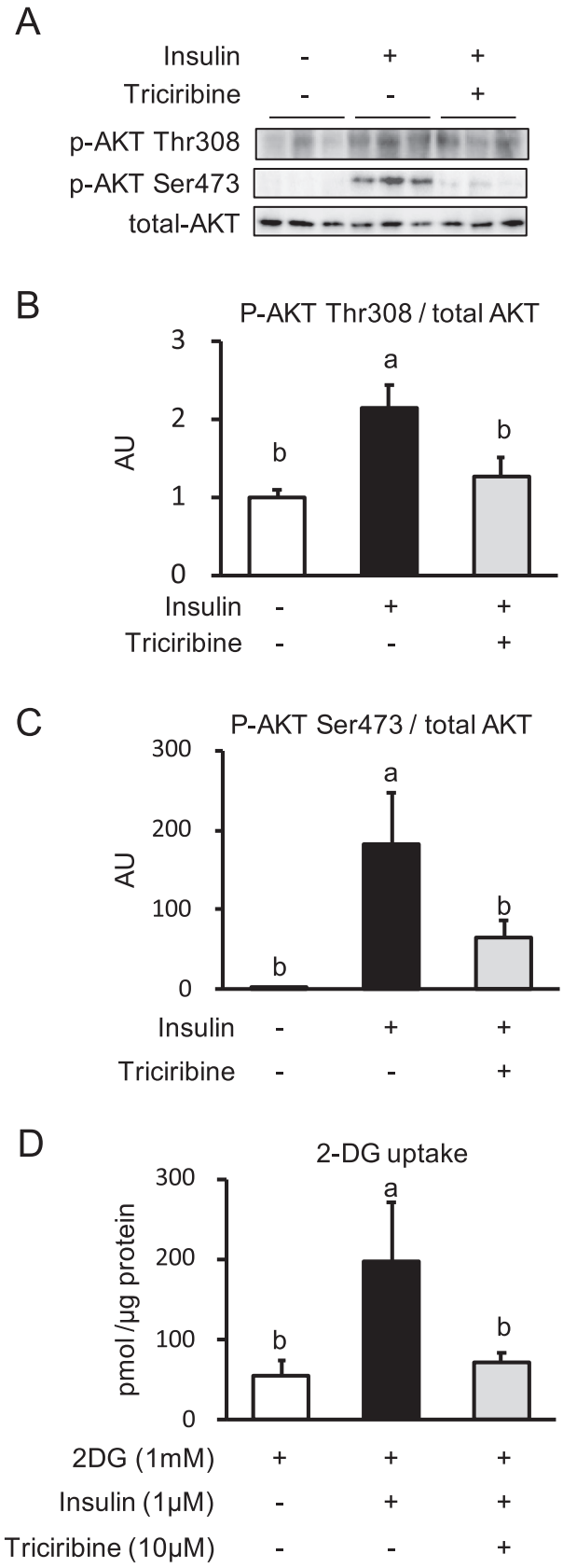

Fig. 3. Effects of insulin on AKT phosphorylation and 2DG uptake in chicken adipocytes. After differentiation, the chicken adipocytes were incubated with (triciribine + insulin group) or without (control group and insulin group) 10 μM triciribine for 3 h. The cells were then incubated with (insulin group and triciribine + insulin group) or without (control group) 1 μM insulin. Total and phosphorylated AKT (Thr308 and Ser473) were detected by immunoblotting (A). The ratios of phosphorylated AKT (Thr308 and Ser473) to total AKT were quantified (B, C). 2DG uptake was measured for 20 min (D). Values are expressed as mean ± SEM (n = 5–6). Different letters indicate significant differences at $P < 0.05$.

In mammalian 3T3L1 adipocytes, expression of a constitutively active variant of AKT resulted in constitutive glucose uptake (Kozka et al., 1991). In chickens, insulin stimulation increased glucose uptake into both explant culture of adipose tissue (Rudas and Scanes, 1983) and cultured adipocytes (Gomez-Capilla and Langslow, 1977). Therefore, we performed 2DG uptake assays to examine the potential impact of insulin on glucose uptake in the differentiated chicken adipocytes, which depends on AKT phosphorylation. In response to insulin stimulation, chicken adipocytes increased the 2DG uptake accompanied by increased phosphorylation of both Thr308 and Ser473 of AKT protein (Fig. 3A–C). However, pretreatment of differentiated chicken adipocytes with 10 μM triciribine, an AKT inhibitor, canceled out both the insulin-induced AKT phosphorylation and the 2DG uptake (Fig. 3A–D), suggesting that insulin enhances glucose uptake through AKT phosphorylation in differentiated chicken adipocytes.

Because chickens lack a homolog of the *GLUT4* gene, which encodes a major insulin-responsive transporter in mammals (Seki et al., 2003), the GLUT isoforms that are responsible for insulin-responsive glucose uptake in adipocytes remain unclear. In this study, we found that differentiated chicken adipocytes treated with insulin stimulation for 20 min showed an increase in GLUT1 among the enriched membrane proteins compared with that in control adipocytes treated with PBS (Fig. 4A, B). In birds, intraperitoneal insulin injection was reported to induce GLUT12 translocation to the cell surface 1 h after injection in the skeletal muscle of fasted chickens (Coudert et al., 2015). However, we found that acute insulin stimulation did not affect GLUT12 expression among the enriched membrane proteins of differentiated chicken adipocytes (Fig. 4A, C). Even though *GLUT1* mRNA expression increased in both chicken myoblasts (Zhao et al., 2012) and chicken skeletal muscle (Kono et al., 2006) in response to insulin stimulation, we found that acute insulin stimulation affected neither *GLUT1* nor *GLUT12* mRNA expression (Fig. 4D). These results suggested that the increased GLUT1 protein level on the plasma membrane was involved in acute insulin-induced glucose uptake in differentiated chicken adipocytes. Furthermore, pretreatment of the differentiated chicken adipocytes with 10 μM triciribine canceled out the insulin-induced increase in GLUT1 protein levels on the enriched membrane protein (Fig. 4A, B). Conversely, neither insulin nor insulin with triciribine stimulation changed the GLUT12 protein level on the plasma membrane (Fig. 4A, C). These results suggested that AKT phosphorylation is involved in the insulin-induced recruitment of GLUT1 protein to the plasma membrane of chicken adipocytes. Moreover, in chickens, *GLUT1* mRNA expression in adipose tissue is much higher than that in skeletal muscle, whereas *GLUT12* mRNA expression shows the opposite pattern (Kono et al., 2005; Coudert et al., 2015). These results suggest that GLUT1 is a more active insulin-responsive GLUT than GLUT12 in chicken adipocytes.

However, the molecular mechanism underlying the insulin-induced GLUT1 recruitment on the plasma membrane is unclear. One possible explanation for this is a change in the endocytosis rate of GLUT1. In mammalian 3T3L1 adipocytes, insulin stimulation was reported to increase both GLUT4 and GLUT1 protein levels on the plasma membrane (Yang and Holman, 1993), in which GLUT4 was more responsive than GLUT1 in response to acute insulin stimulation (Lee et al., 2000). Insulin modulates the GLUT4 trafficking at multiple steps in rat adipocytes, not only increasing exocytosis but also decreasing endocytosis

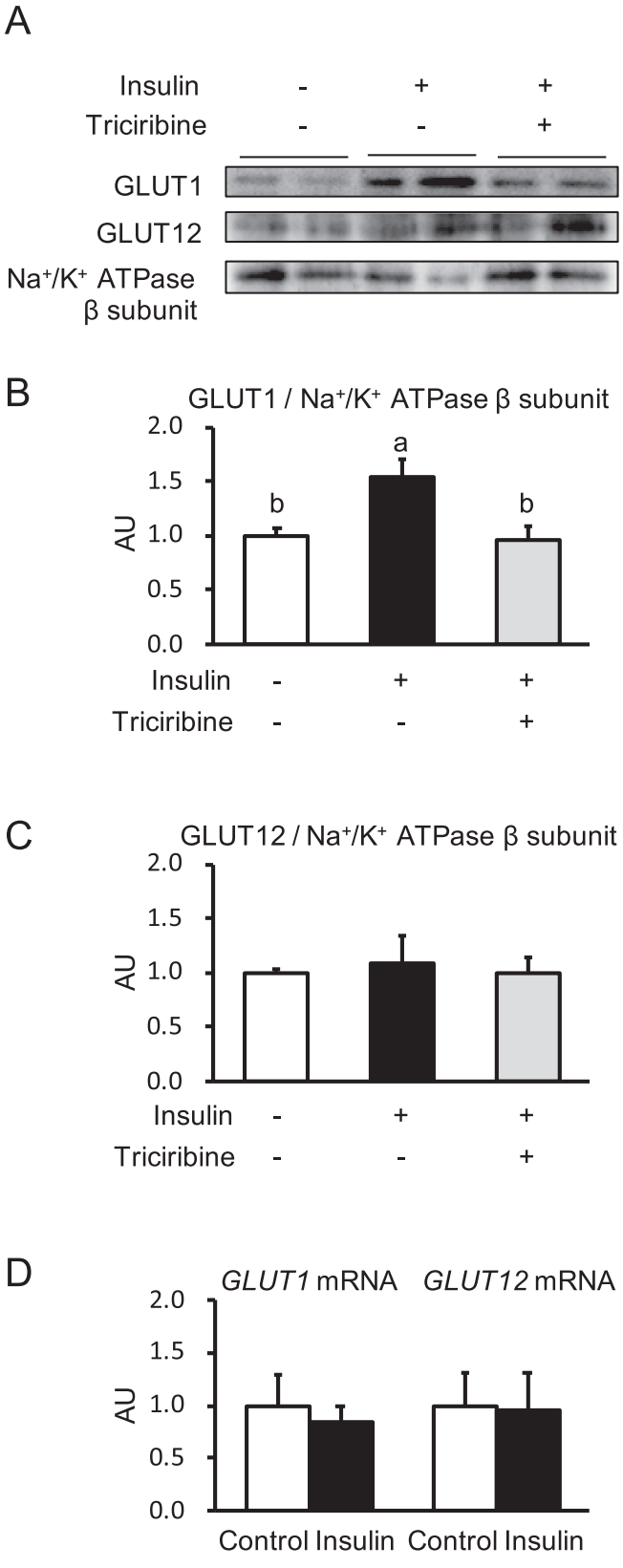

Fig. 4. Effects of insulin on GLUT1 and GLUT12 protein expression on plasma membranes and *GLUT1* and *GLUT12* mRNA expression in chicken adipocytes. After differentiation, the chicken adipocytes were incubated with (triciribine + insulin group) or without (control group and insulin group) 10 μM triciribine for 3 h. The cells were then incubated with (insulin group and triciribine + insulin group) or without (control group) 1 μM insulin. Representative immunoblot data showing GLUT1, GLUT12, and Na^+/K^+ ATPase β subunit in enriched plasma membrane protein fractions from chicken adipocytes (A). Protein levels of (B) GLUT1 and (C) GLUT12 were quantified. Protein levels of GLUT1 and GLUT12 are normalized to the levels of Na^+/K^+ ATPase β subunit, respectively. Values are expressed as mean ± SEM (n = 6). Different letters indicate significant differences at $P < 0.05$. After differentiation, the chicken adipocytes were divided into two groups (control and insulin). Quantitative real-time PCR analysis of *GLUT1* and *GLUT12* (D). Results are normalized to the levels of *GAPDH* and expressed relative to the mean value for the control group. * $P < 0.05$ (versus control). Values are expressed as mean ± SEM (n = 6).

(Lee et al., 2000; Yang and Holman, 1993). However, GLUT1 cycling depends on an insulin-sensitive endocytosis step in common with the GLUT4 system, but the exocytosis step of GLUT1 is insensitive to insulin (Rudas and Scanes, 1983). Indeed, insulin decreased the endocytosis rate of GLUT1 by 30% compared with the basal state in rat adipocytes (Yang and Holman, 1993). These results allow us to postulate that insulin might inhibit the rate of GLUT1 endocytosis in differentiated chicken adipocytes. Therefore, their glucose uptake might be increased despite the lack of a homolog of the *GLUT4* gene.

4. Conclusion

Acute insulin stimulation of chicken adipocytes increased GLUT1 protein levels on the plasma membrane accompanied by increased glucose uptake in an AKT-phosphorylation-dependent manner, suggesting that GLUT1 is a major insulin-responsive transporter in these cells.

Author contributions

Saki Shimamoto, Kazuki Nakashima, Akira Ohtsuka, and Daichi Ijiri conceived and designed the experiments. Saki Shimamoto, Ryo Kamimura, Rukana Kohrogi, Hiroki Inoue, and Nao Nishikoba performed the experiments and contributed reagents/materials/analysis tools. Saki Shimamoto, Kazuki Nakashima, and Daichi Ijiri wrote the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.113232.

References

Akiba, Y., Chiba, Y., Takahashi, T., Ohtomo, Y., Sato, K., Takahashi, K., 1999. Persistent hypoglycemia induced by continuous insulin infusion in broiler chickens. Br. Poult. Sci. 40, 701–705.

Belo, P.S., Romsos, D.R., Leville, G.A., 1976. Blood metabolites and glucose metabolism in the fed and fasted chicken. J. Nutr. 106, 1135–1143.
Chen, D., Elmendorf, J.S., Olson, A.L., Li, X., Earp, H.S., Pessin, J.E., 1983. Osmotic shock stimulates GLUT4 translocation in 3T3L1 adipocytes by a novel tyrosine kinase pathway. J. Biol. Chem. 272, 27401–27410.
Coudert, E., Pascal, G., Dupont, J., Simon, J., Cailleau-Audouin, E., Crochet, S., Duclos, M.J., Tesseraud, S., Métayer-Coustard, S., 2015. Phylogenesis and biological characterization of a new glucose transporter in the chicken (*Gallus gallus*), GLUT12. PLoS One 10, e0139517.
Gomez-Capilla, J.A., Langslow, D.R., 1977. Insulin action on glucose utilisation by chicken adipocytes. Int. J. Biochem. 8, 417–420.
Hazelwood, R.L., Lorenz, F.W., 1959. Effects of fasting and insulin on carbohydrate metabolism of the domestic fowl. Am. J. Physiol. 197, 47–51.
Holman, G.D., Kozka, I.J., Clark, A.E., Flower, C.J., Saltis, J., Habberfield, A.D., Simpson, I.A., Cushman, S.W., 1990. Cell surface labeling of glucose transporter isoform GLUT4 by bis-mannose photolabel. Correlation with stimulation of glucose transport in rat adipose cells by insulin and phorbol ester. J. Biol. Chem. 265, 18172–18179.
Kohn, A.D., Summers, S.A., Birnbaum, M.J., Roth, R.A., 1996. Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J. Biol. Chem. 271, 31372–31378.
Kono, T., Nishida, M., Nishiki, Y., Seki, Y., Sato, K., Akiba, Y., 2005. Characterisation of glucose transporter (GLUT) gene expression in broiler chickens. Br. Poult. Sci. 46, 510–515.
Kono, T., Nishiki, Y., Seki, Y., Sato, K., Akiba, Y., 2006. Insulin stimulates glucose transporter 1 (GLUT1) and hexokinase II (HK II) gene expression in chicken skeletal muscle. EPC 2006 - 12th European Poultry Conference, Verona, Italy. 10-14 September, pp. paper 138.
Kozka, I.J., Clark, A.E., Holman, G.D., 1991. Chronic treatment with insulin selectively down-regulates cell-surface GLUT4 glucose transporters in 3T3-L1 adipocytes. J. Biol. Chem. 266, 11726–11731.
Kraegen, E.W., Sowden, J.A., Halstead, M.B., Clark, P.W., Rodnick, K.J., Chisholm, D.J., James, D.E., 1993. Glucose transporters and in vivo glucose uptake in skeletal and cardiac muscle: fasting, insulin stimulation and immunoisolation studies of GLUT1 and GLUT4. Biochem. J. 295, 287–293.
Lee, W.W., Ryu, J., Spangler, R.A., Jung, C.Y., 2000. Modulation of GLUT4 and GLUT1 recycling by insulin in rat adipocytes: kinetic analysis based on the involvement of multiple intracellular compartments. Biochemistry 39, 9358–9366.
Leto, D., Saltiel, A.R., 2012. Regulation of glucose transport by insulin: traffic control of GLUT4. Nat. Rev. Mol. Cell. Biol. 13, 383–396.
Malide, D., Ramm, G., Cushman, S.W., Slot, J.W., 2000. Immunoelectron microscopic evidence that GLUT4 translocation explains the stimulation of glucose transport in isolated rat white adipose cells. J. Cell Sci. 23, 4203–4210.
Ramírez-Zacarías, J.L., Castro-Muñozledo, F., Kuri-Harcuch, W., 1992. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry 97, 493–497.
Rudas, P., Scanes, C.G., 1983. Influences of growth hormone on glucose uptake by avian adipose tissue. Poult. Sci. 62, 1838–1845.
Seki, Y., Sato, K., Kono, T., Abe, H., Akiba, Y., 2003. Broiler chickens (Ross strain) lack insulin-responsive glucose transporter GLUT4 and have GLUT8 cDNA. Gen. Comp. Endocrinol. 133, 80–87.
Shimamoto, S., Ijiri, D., Nakashima, K., Kawaguchi, M., Ishimaru, Y., Furukawa, A., Ohtsuka, A., 2016. Clenbuterol changes phosphorylated FOXO1 localization and decreases protein degradation in the skeletal muscle of neonatal chicks. Biosci. Biotechnol. Biochem. 80, 1499–1504.
Tokushima, Y., Takahashi, K., Sato, K., Akiba, Y., 2005. Glucose uptake in vivo in skeletal muscles of insulin-injected chicks. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 141, 43–48.
Watson, R.T., Pessin, J.E., 2001. Intracellular organization of insulin signaling and GLUT4 translocation. Recent Prog. Horm. Res. 56, 175–193.
Yang, J., Holman, G.D., 1993. Comparison of GLUT4 and GLUT1 subcellular trafficking in basal and insulin-stimulated 3T3-L1 cells. J. Biol. Chem. 268, 4600–4603.
Zhao, J.P., Bao, J., Wang, X.J., Jiao, H.C., Song, Z.G., Lin, H., 2012. Altered gene and protein expression of glucose transporter1 underlies dexamethasone inhibition of insulin-stimulated glucose uptake in chicken muscles. J. Anim. Sci. 90, 904337–904345.