

Fetuin Inhibits insulin-stimulated glucose uptake and glycogen synthesis in L6-GLUT4myc skeletal muscle cells

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ABSTRACT

Background and Aims: Fetuin, also known as alpha2-HS glycoprotein (AHSG), is secreted by the liver into circulation, and known to inhibit insulin-stimulated autophosphorylation. Tyrosine kinase activity of insulin receptor (IR) (1, 4), and phosphorylation of IRS-1 & -2 (10). The *Ahsg* gene is located in human chromosome 3q27 that has been shown to be a sensitive locus for type 2 diabetes (6) and metabolic syndrome (7). The serum AHSG level in gestational diabetes is increased and correlated with insulin sensitivity (9). Recent studies have shown a polymorphism in the AHSG gene to be associated with type 2 diabetes in French Caucasians (9), and a common *Ahsg* gene variant to be associated with leanness in Swedish men (6). We have earlier shown that fetuin-null mice demonstrate improved insulin sensitivity, increased whole-body glucose utilization, and resistance to diet-induced obesity (2). Though earlier studies indicated a preferential inhibition of the mitogenic pathway of insulin signaling (3, 4, 11), **several lines of recent evidence** suggest the possibility that it may regulate glucose metabolism and insulin action *in vivo* (7). The present study aims to clarify the role of fetuin in the regulation of insulin-stimulated glucose uptake and glycogen synthesis in skeletal muscle cells.

Materials and Methods: Rat L6 skeletal muscle cells and mouse C2C12 myoblasts were differentiated into myotubes and treated with purified human AHSG (0.9 and 1.8 μM) in the presence of insulin. To assay GLUT4 translocation, glucose uptake, and glycogen synthesis, L6 myoblasts stably expressing myc-tagged GLUT4 (L6-GLUT4myc) (13) were treated with AHSG in the presence or absence of insulin. For the IR-TK assay, purified rat liver insulin receptors were treated with same conditions to other assays.

Results: AHSG inhibited insulin-stimulated Akt and GSK-3 phosphorylation in L6 skeletal muscle cells and C2C12 cells, suggesting that fetuin/AHSG may play a role in the regulation of glucose uptake and glycogen synthesis. Furthermore, AHSG, fetuin, and asialofetuin significantly inhibited insulin-stimulated GLUT4 translocation, glucose uptake, and glycogen synthesis in L6-GLUT4myc cells.

Conclusion: These studies establish a novel role for fetuin in the regulation of insulin's metabolic action and lend credence to previous findings for fetuin as a physiological regulator of insulin action.

Alpha2-Heremans Schmid Glycoprotein (AHSG)

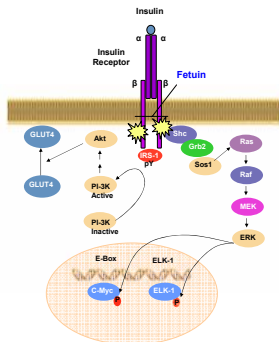


Fig. 2. Insulin signal transduction pathway molecules Insulin binding to the α-subunit of IR activates the autophosphorylation and TK activity of β-subunit of IR then the signal goes down through tyrosine phosphorylation of IRS-1 & 2 followed by two signaling pathways: mitogenic pathway via Shc, Grb2, Sos1, Ras, Raf, MEK, MAP kinase, and ERK. The metabolic pathway via PI-3 kinase, Akt, GSK3, IR, GLUT4, and GLUT4. AHSG blocks the primary steps in insulin signaling such as IR-TK and IRS-1 phosphorylation. It has been shown that AHSG inhibits only mitogenic pathway but not metabolic pathway in CHO-IR ovary cells, 3T3L1 adipocytes and L6 myotubes (11).

- AHSG must be phosphorylated either at Serine-120 or Serine-312 be active in mammals, and approximately 20% of circulating AHSG in human plasma pool is phosphorylated (10, 12).
- Recombinant AHSG is 100 times more active than AHSG isolated from plasma or hepatocytes. The recombinant AHSG was a fully phosphorylated single-chain polypeptide (11).
- AHSG blocks the primary steps in insulin signaling such as IR-TK and IRS-1 phosphorylation. It has been shown that AHSG inhibits only mitogenic pathway but not metabolic pathway in CHO-IR ovary cells, 3T3L1 adipocytes and L6 myotubes (11).

AHSG, a physiological inhibitor of IR tyrosine kinase

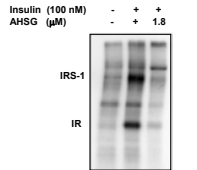


Fig. 1. AHSG inhibits insulin-stimulated IR autophosphorylation. HIRc B cells were treated with AHSG in the presence or absence of insulin for 15 min. Cells were washed three times with ice-cold PBS, lysed and immunoprecipitated with anti-AHSG antibody and immunoblotted with anti-insulin receptor antibody.

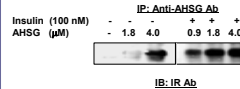


Fig. 2. AHSG interacts with the activated insulin receptor. HIRc B cells were treated with AHSG in the presence or absence of insulin for 15 min. Cells were washed three times with ice-cold PBS, lysed and immunoprecipitated with anti-AHSG antibody and immunoblotted with anti-insulin receptor antibody (11).

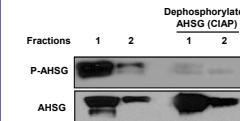
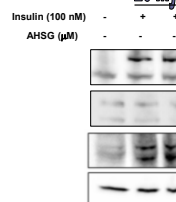


Fig. 3. Antibody has efficacy specific for 312-Ser residue of AHSG. AHSG purified from HepG2 cells was incubated with calf intestinal alkaline phosphatase (CIAP) for overnight then compared with non-treated one by Western blot analysis.

AHSG inhibits insulin signaling through Akt and GSK3 in skeletal muscle cells

L6 myoblasts



C2C12 myoblasts

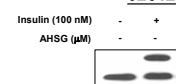


Figure 4. AHSG inhibits insulin stimulated activation of Akt and phosphorylation of GSK3. Serum-starved L6 skeletal muscle cells (myoblasts) were pre-treated with AHSG for 15 min before a 10 min insulin stimulation (100 nM). Cells were lysed and supernatants were separated by SDS-PAGE electrophoresis and immunoblotted with antibody against phospho-Akt (Ser-473; Cell Signaling #9272), Akt (Cell Signaling #9271), phospho-GSK3 (Ser-2), Cell Signaling #9337, GAPDH (Abcam #66245), or β-actin (Abcam #66245) for control. HRP-conjugated Secondary antibodies were from MP.

AHSG inhibits insulin-stimulated glucose uptake

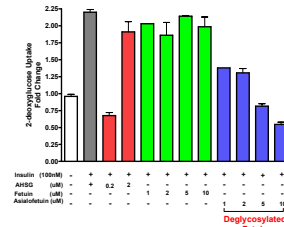


Fig. 5. AHSG inhibits insulin-stimulated glucose uptake. Serum & glucose depleted L6GLUT4myc cells were treated with different doses of AHSG, Fetuin, or Asialofetuin for 20mins then stimulated with insulin 100nM. The amounts of [³H] 2-deoxy-D-glucose up taken during 10 mins of each treatment were measured by scintillation counter for 2 mins. This bar-graph shows fold-change comparing basal level.

AHSG inhibits insulin-stimulated glycogen synthesis

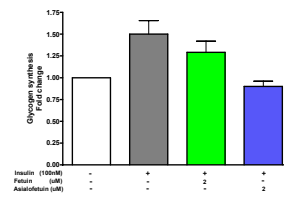


Fig. 6. Serum & glucose depleted L6GLUT4myc cells were treated with different doses of Fetuin, or Asialofetuin for 20mins then stimulated with insulin 100nM. The amounts of [¹⁴C]-Glucose incorporated into the synthesized cellular glycogen during 30mins of each treatment were measured by scintillation counter for 2 mins. This bar-graph shows fold-change comparing basal level.

Dephosphorylated AHSG is devoid of inhibitory activity

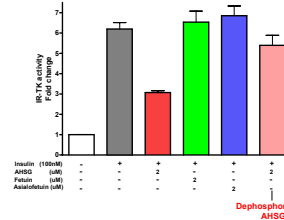


Fig. 7. AHSG inhibits Insulin Receptor Tyrosine Kinase (IR-TK) activity. Insulin receptors purified from rat liver were incubated with AHSG, Fetuin, or Asialofetuin for 10mins and stimulated insulin for 10mins then added poly(Glu-Tyr) substrate (Sigma #P0275) with [³²P]-ATP. The incorporated [³²P]-ATP into poly(Glu-Tyr) was measured by scintillation counter. This bar-graph shows fold-change comparing basal level.

AHSG inhibits insulin stimulated GLUT4 translocation

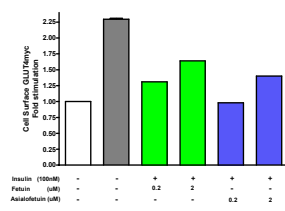


Fig. 8 AHSG inhibits GLUT4 translocation We need to repeat the experiment!

SUMMARY

- AHSG interacts with the activated IR, and inhibits insulin-stimulated IR autophosphorylation.
- AHSG inhibits insulin signaling through Akt and GSK3 in skeletal muscle cells.
- AHSG does inhibit insulin-stimulated glucose uptake, glycogen synthesis, and GLUT4 translocation.
- The Ser-312 phosphorylation of AHSG is crucial for the inhibition effect.
- The glycan structure of AHSG also influences the inhibition effect.

REFERENCES

- Mathews S. T., Chellam N., Srinivas P. R., Cinton V. J., Leon M. A., Goustin A. S., and Grunberger G. *Mol. Cell Endocrinol.* 2000;164:87-98.
- Mathews S. T., Singh G. P., Ranzollato M., Cinton V. J., Dong X., Goustin A. S., Jen C. K., Charron M. J., Johnson-Ochenet W., and George Grunberger. *Diabetes.* 2002;51:2450-2458.
- Chen H., Srinivas P. R., Cong L., Li Y., Grunberger G., and Quon M. J. *Endocrinol.* 1998;139:4147-4154.
- Srinivas P. R., Wagner A. S., Reddy L. V., Deutsch D. D., Leon M. A., Goustin A. S., and Grunberger G. *Mol. Endocrinol.* 1993;7:1445-1450.
- Siddiqi A., Leprette F., Herreberg S., Froguel P., and Gibson F. *Diabetes.* 2005;54:2477-2481.
- Vornet N., Hani EH., Dupont S., Galina S., Francke S., Dotte S., De Maizos F., Durand E., Leprette F., Lecocq C., Gallia P., Zekiri L., Dina C., and Froguel P. *Am. J. Hum. Genet.* 2003; 67:1470-1485.
- Kissbah A. H., Sonnenberg G. E., Mjellebaust J., Goldstein M., Brozman K., James R. G., Marks J. A., Kralover G. R., Jacob H. J., Weber J., Martin L., Bhangoo J., and Comuzzie A. G. *Proc. Natl. Acad. Sci. U.S.A.* 2000;97:14478-14483.
- Lavebrat C., Wahajvis S., Nordfors L., Hoffstedt J., and Arner P. *Hum. Genet.* 2005;117:54-60.
- Katabay L., Coak K., Paik A., Bandy E., Coakley G. M., Metzger Z., Speer G., Kovacs M., Siler G., Karadi L., and Winkler G. *Eur. J. Endocrinol.* 2002;147:243-248.
- Katabay L., Mathur S., Bobin S., and Arnaud P. *Endocrinophores.* 1996;17:529-532.
- Srinivas P. R., Deutsch D. D., Mathews S. T., Goustin A. S., Leon M. A., and Grunberger G. *Cell Signaling.* 1996;8:567-573.
- Haglund A. C., Ek B., and Nil P. *Biochem. J.* 2001;357:439-445.
- Somwar R., Niu W., Kim D. Y., Sweetney G., Randsava V. K., Huang C., Ramtal T., and Kip A. J. *Biol. Chem.* 2001;276:46079-46087.