AZP-3404, a 9-Amino Acid Peptide Analog of Insulin-Like Growth Factor-Binding Protein 2, Reverses Insulin Resistance in Leptin-Deficient ob/ob Mice

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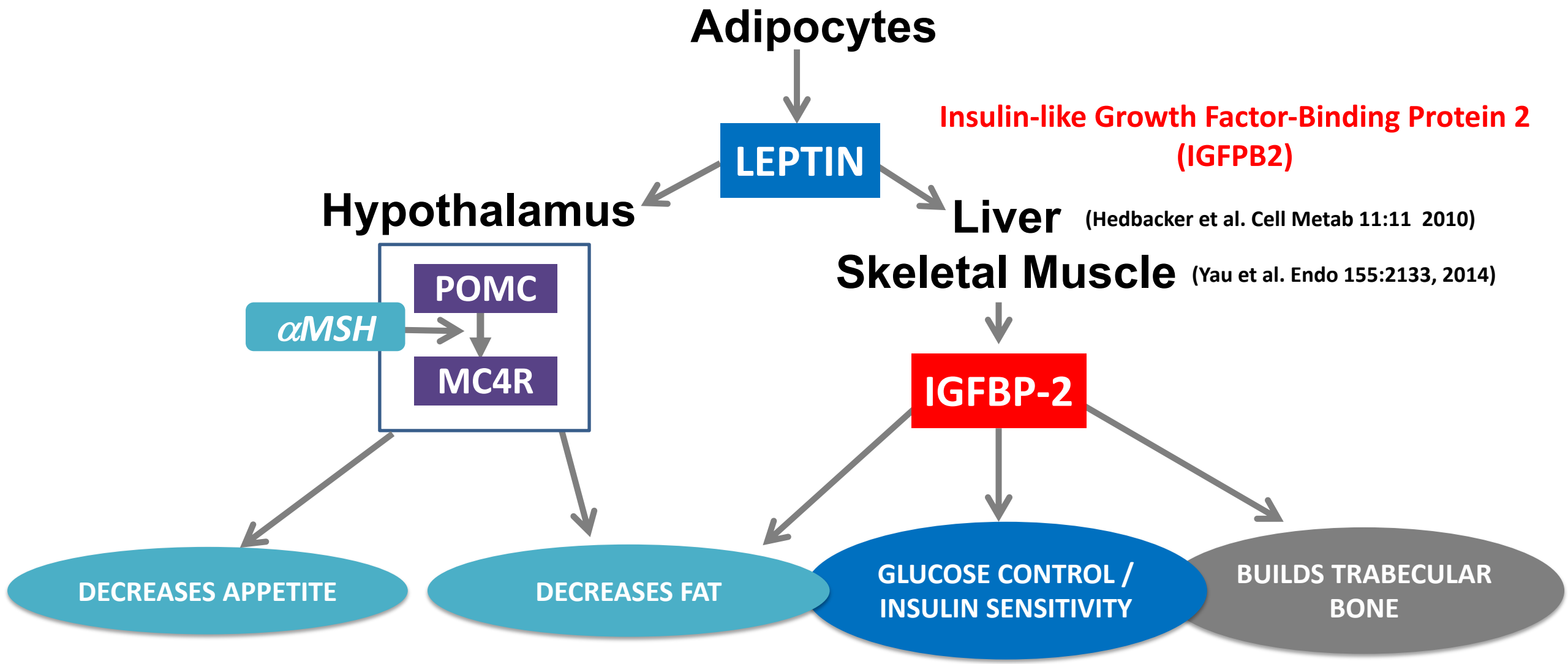
OR05: Diabetes: From Genetics to Novel Therapeutic Targets

Disclosures

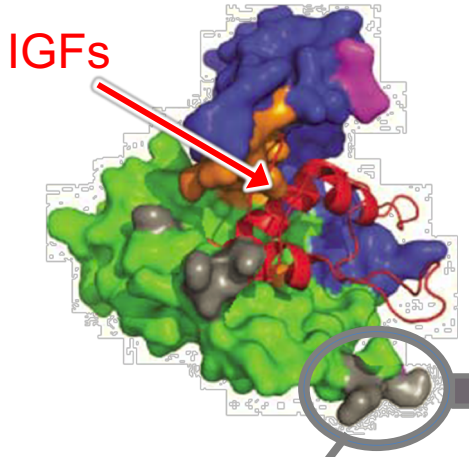
None

The Metabolic Effects of Leptin



IGF-1-Independent Actions of IGFBP-2

IGFBP-2
Insulin-like Growth Factor Binding Protein-2



Endocrine reservoir of IGFs

- In circulation, one of the 6 known binding proteins for IGFs

Direct tissue effects

- Improvement in glucose control and insulin sensitivity
- Regulation of fat and bone metabolism

Reversal of Glucose Dysregulation:

- ob/ob
- Diet-induced obese
- Ay diabetic
- Streptozotocin T1D

Hedbacker et al.
Cell Metab 11:11 2010

Unique Heparin Binding Domain (HBD1) - 13 Amino Acids

AZP-3404

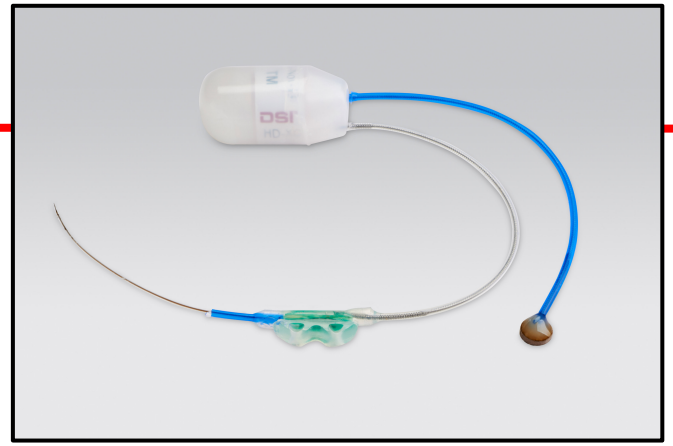
- 9 amino acid peptide
- Resistant to degradation in human plasma – 37°
- Enhanced PK – Suitable for 1x/day administration

Testing Ability of AZP-3404 to Correct Glucose Dysregulation - Study Design

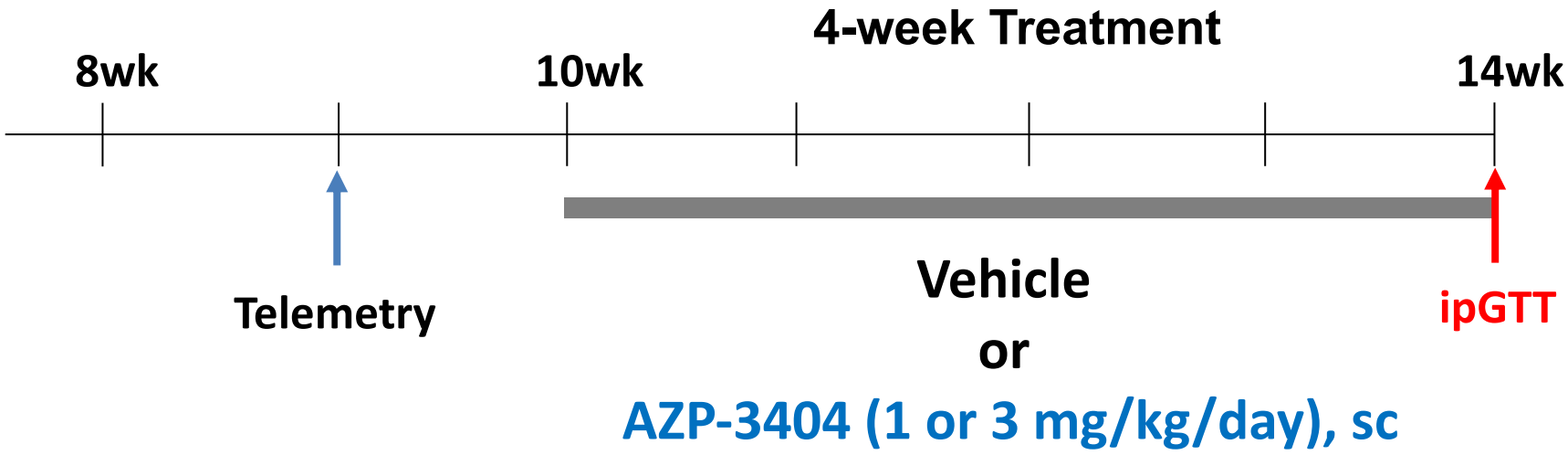
Leptin-deficient ob/ob mice

Obese, Severely Insulin-Resistant, Diabetic

HD-XG Telemetric Device
(Data Sciences International)



Conscious, unrestrained, continuous, wireless monitoring of carotid blood glucose

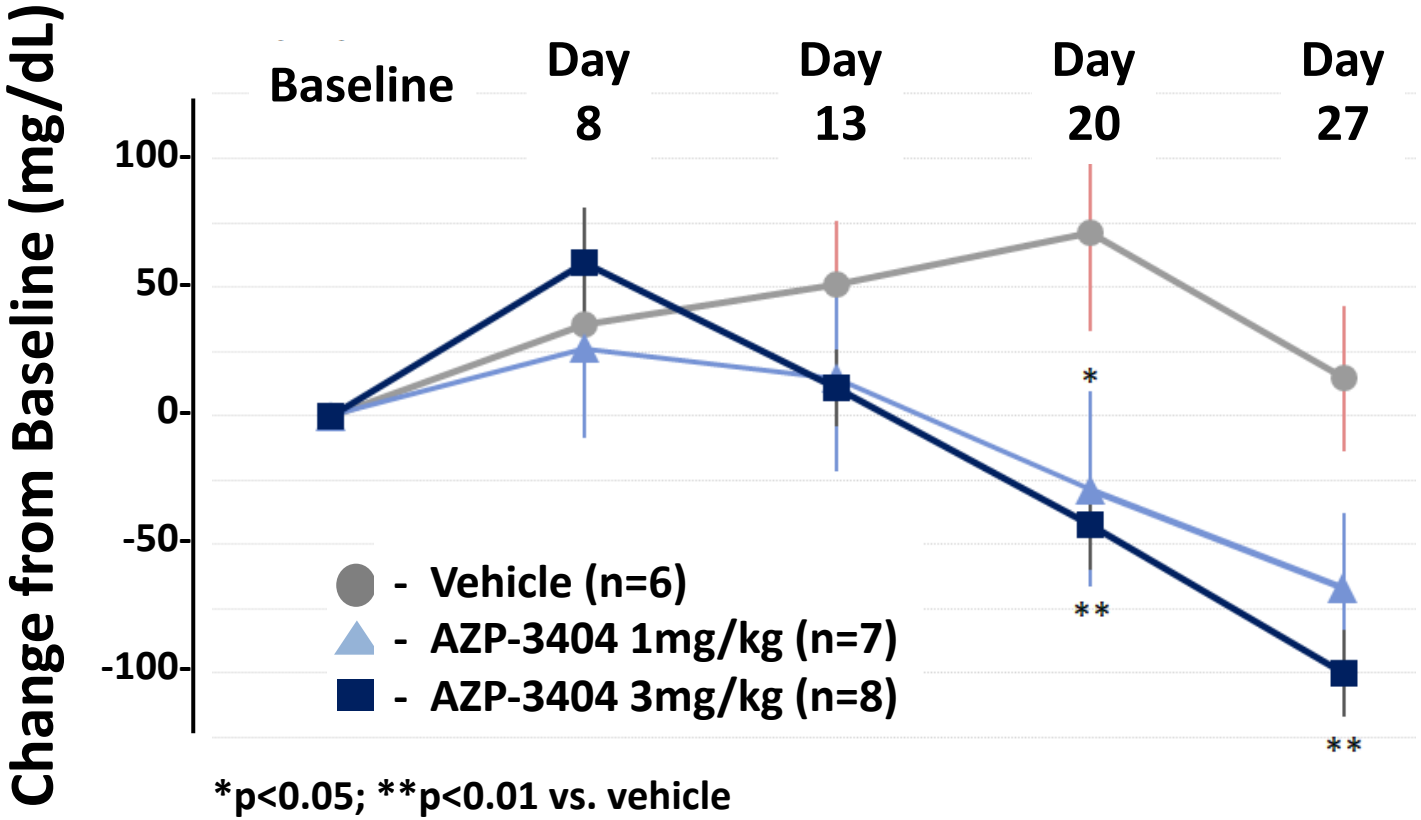


AZP-3404 Reduces Daily Average Blood Glucose in ob/ob Mice

Basal Glucose - Change from Baseline

Mean 16H Blood Glucose Levels

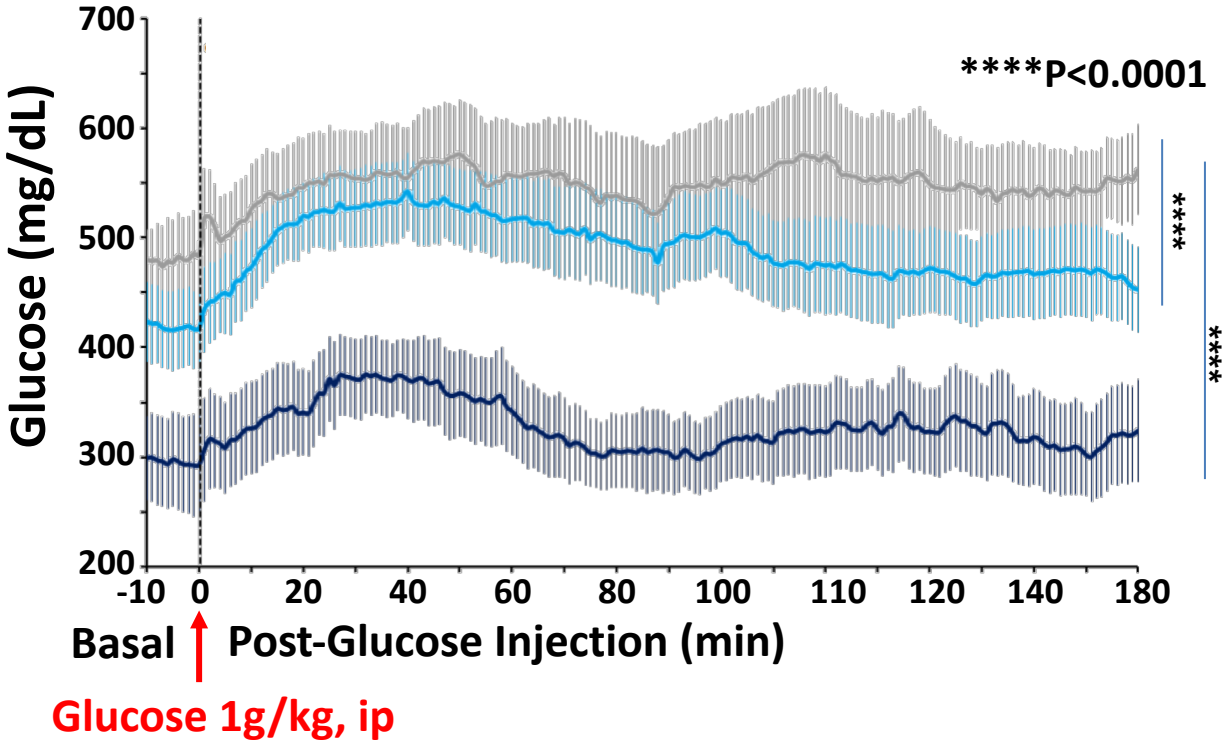
Mean Pretreatment Glucose = 507 ± 102mg/dl



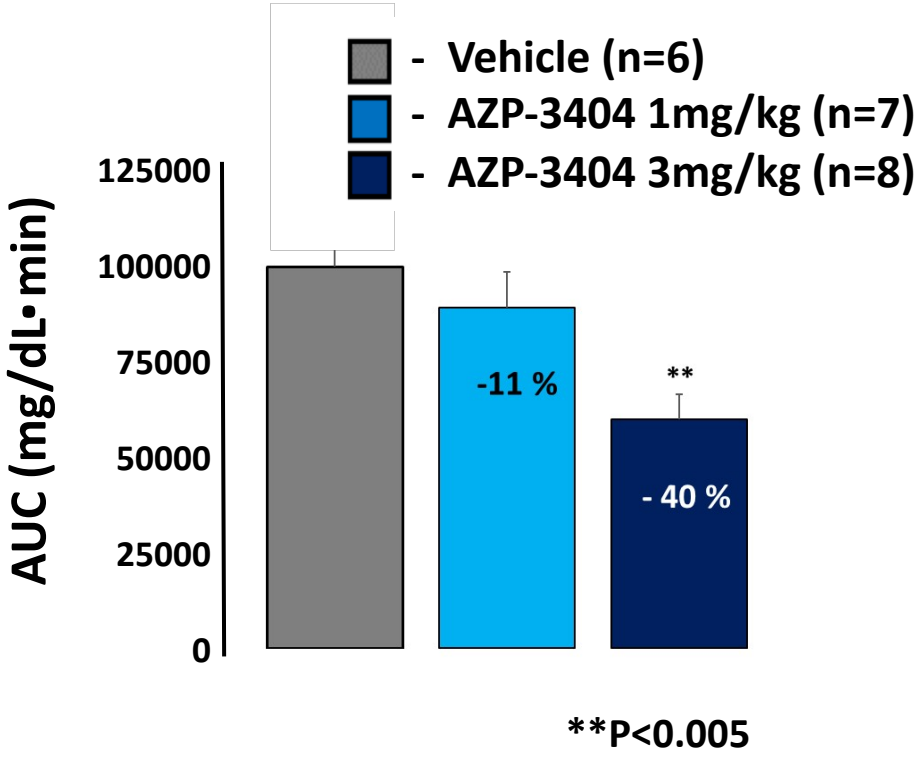
*p<0.05; **p<0.01 vs. vehicle

AZP-3404 Reduces Fasting Glucose and Improves Glucose Tolerance in ob/ob Mice

Basal Fasting Glucose Levels and ipGTT



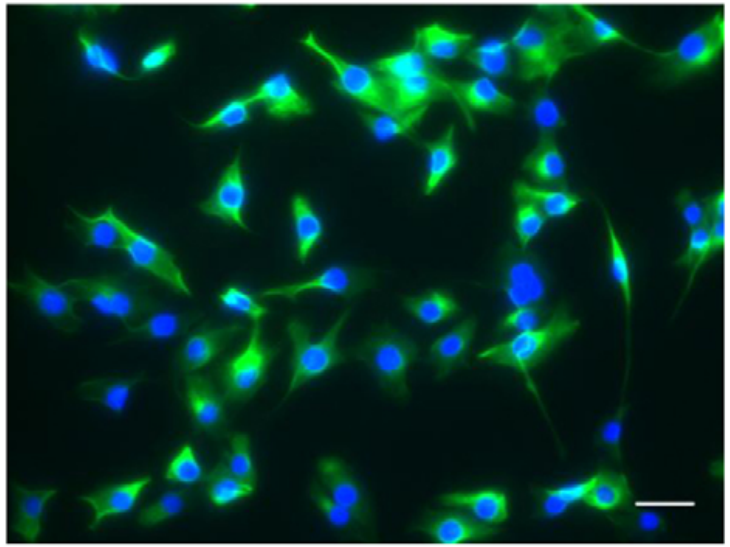
Total Glucose Post-ipGTT



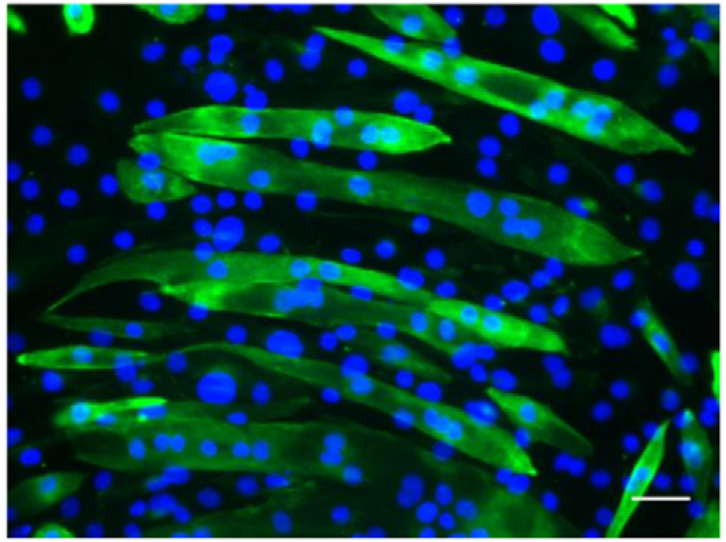
In Vitro Mechanism Studies

Test System: Fully Differentiated C2C12 Mouse Skeletal Muscle Myotubes

C2C12 myoblasts



C2C12 myotubes



Differentiation Medium - 6 Days

Glucose Uptake

^{3}H-[2-deoxyglucose] (0.5 µCi/well)

Treatment → Extracted → Counted

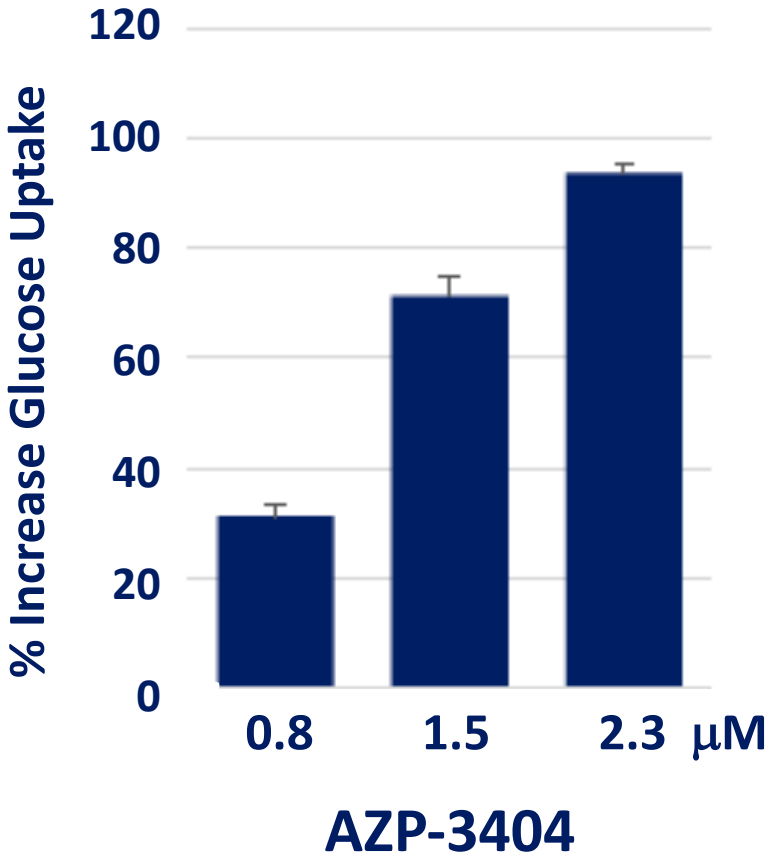
Treatment → Extracted → SDS PAGE → Immunoblot

Mechanism of Action

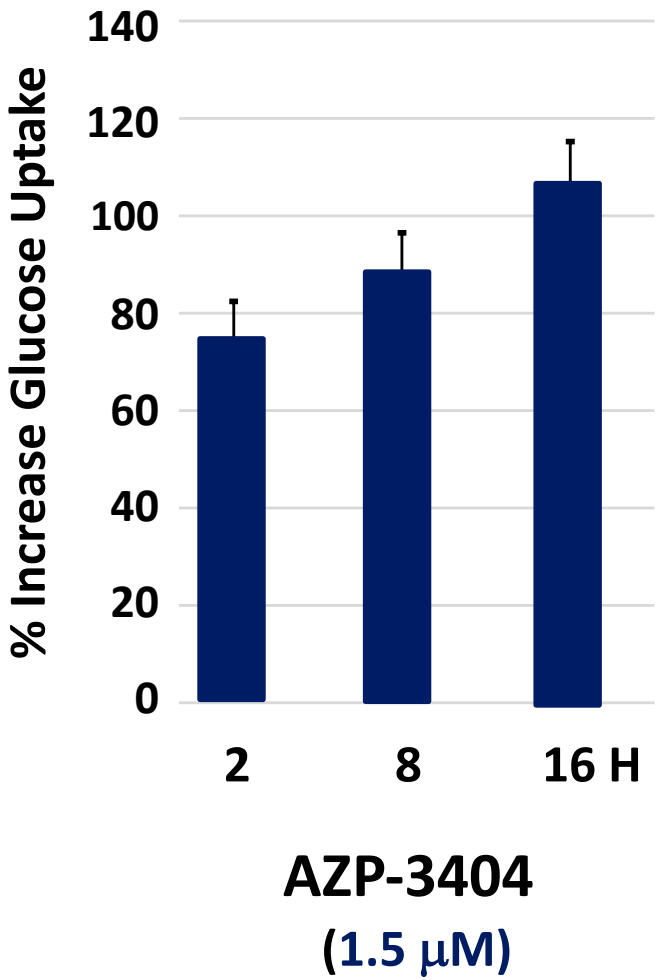
Images from: Wei et al. J Cell Sci 129:39 2016

AZP-3404 Stimulates Increased Glucose Uptake by Mouse Skeletal Muscle

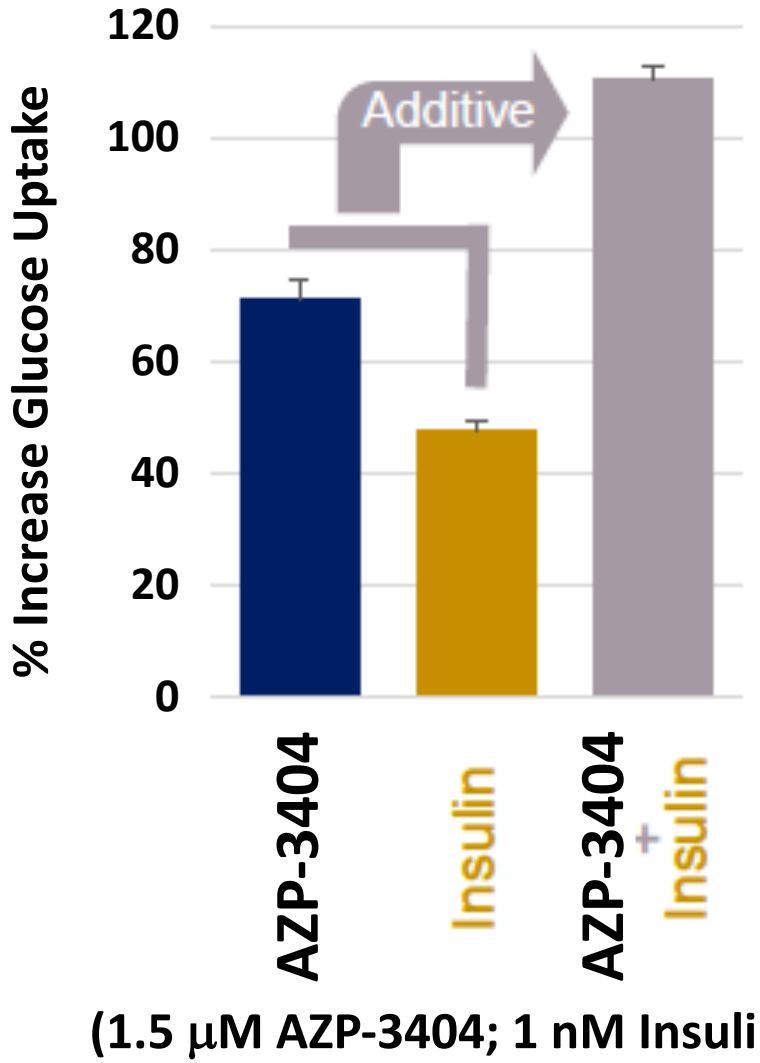
Dose-Related Increase



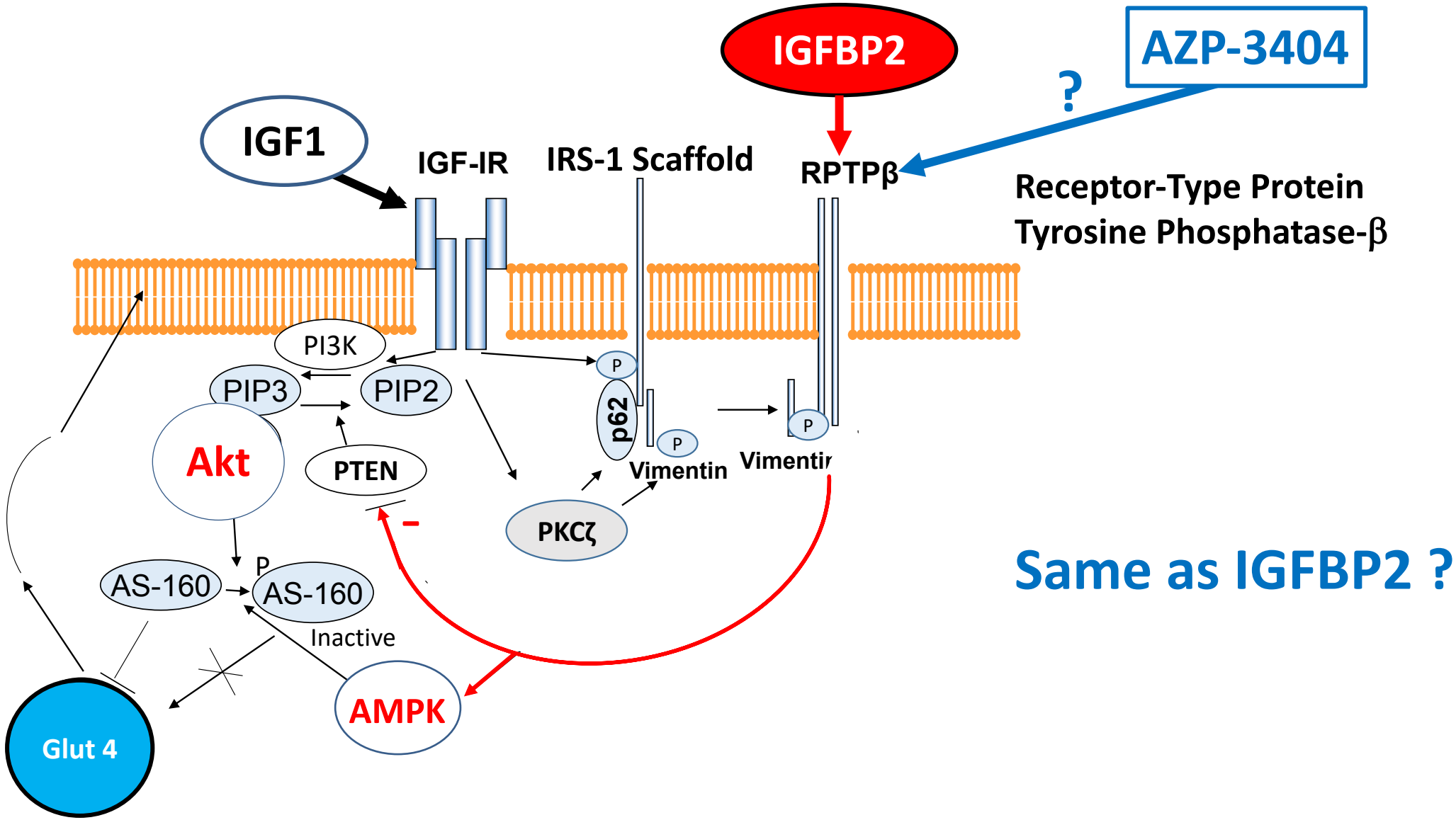
Time-Related Increase



Additive with Insulin

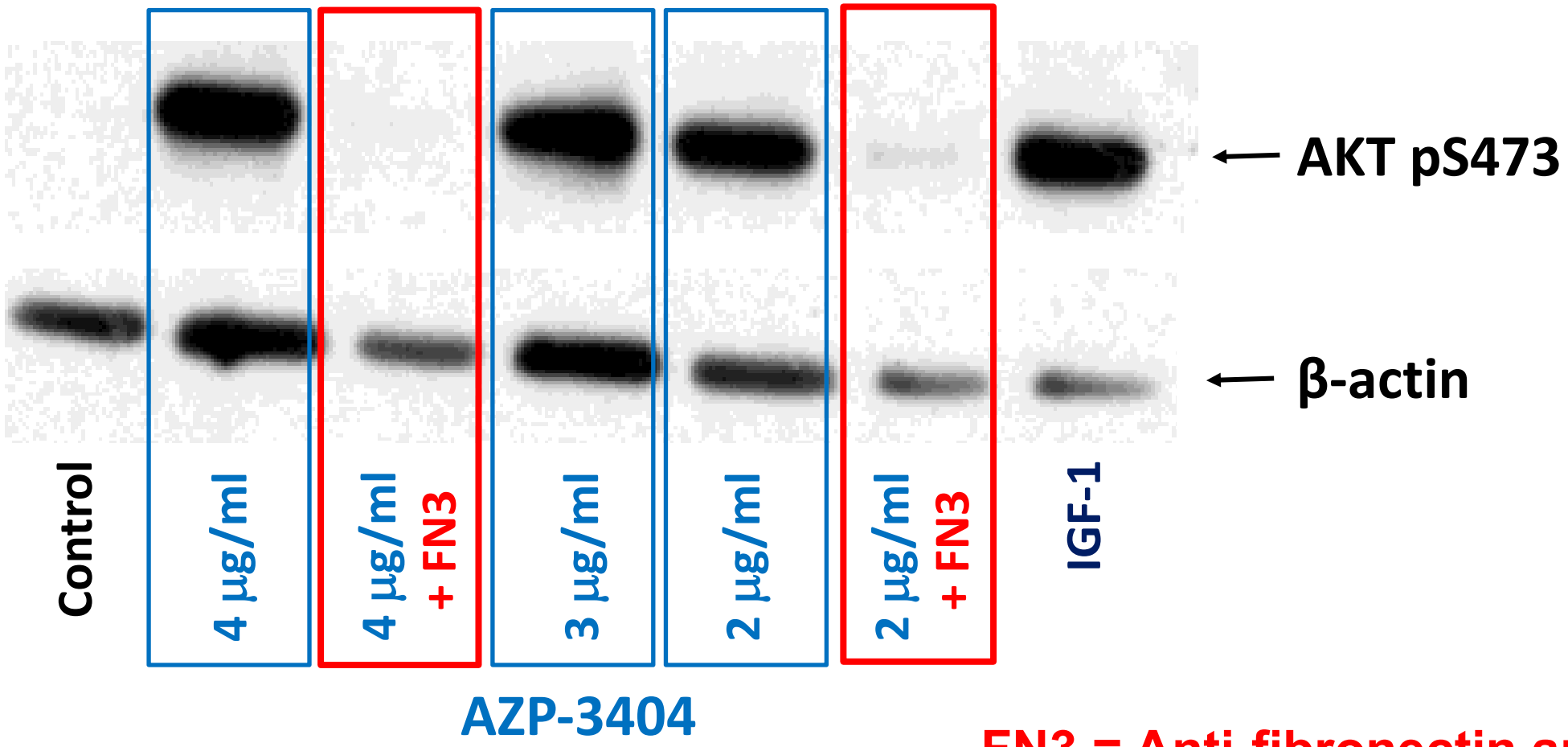


Mechanism of IGFBP2 Induction of Glut 4 Translocation and Glucose Uptake



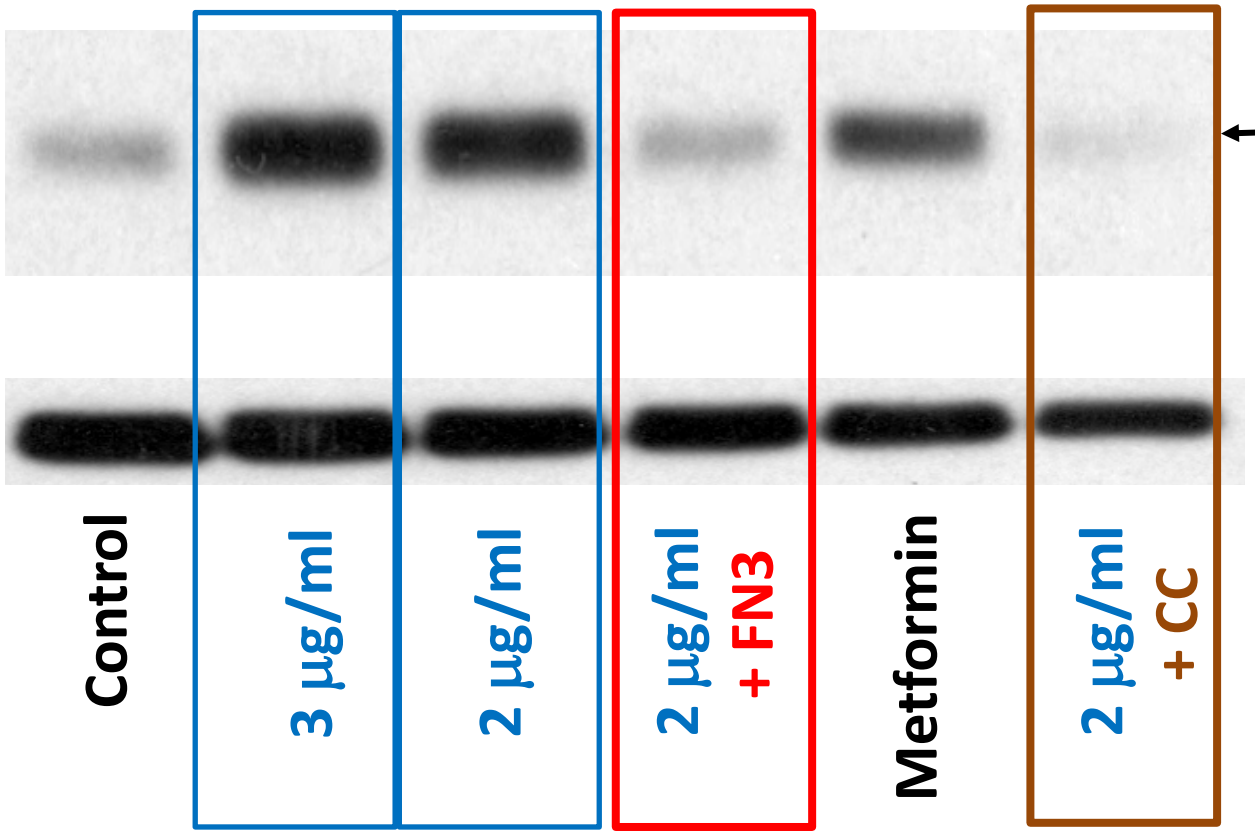
Same as IGFBP2 ?

AZP-3404 Stimulates Increased Akt in Mouse Skeletal Muscle Cells – Effect is RPTPβ-Dependent



FN3 = Anti-fibronectin antibody
- Blocks RPTPβ

AZP-3404 Stimulates Increased AMPK in Mouse Skeletal Muscle Cells – Effect is RPTPβ-Dependent

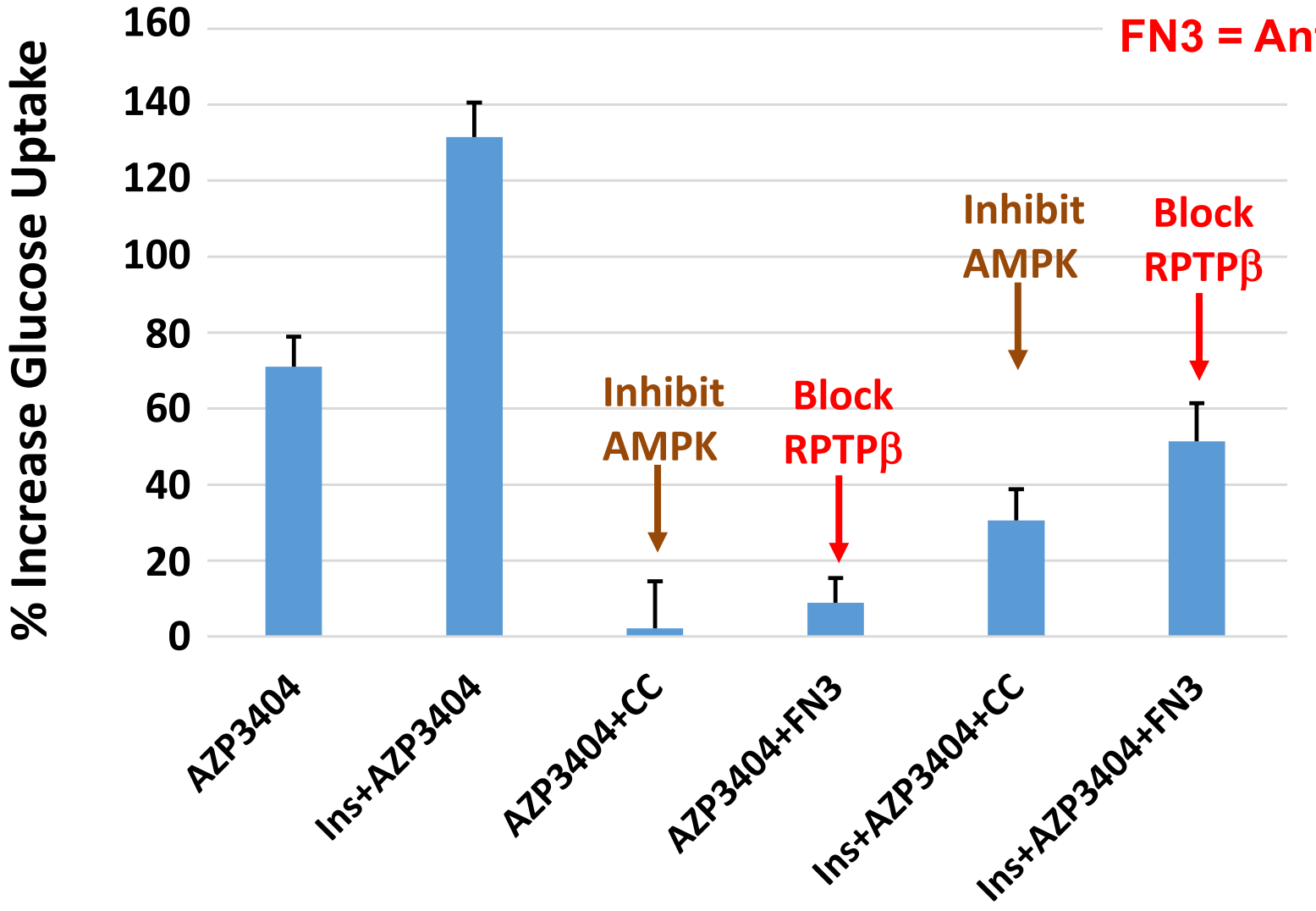


FN3 = Anti-fibronectin antibody
- Blocks RPTPβ

CC = Compound C
- AMPK Inhibitor

Blocking RPTPβ or Inhibiting AMPK Prevents AZP-3404-Stimulated Glucose Uptake

CC = Compound C
FN3 = Anti-fibronectin antibody



1.5 μM AZP-3404
10 nM Insulin

Summary and Conclusions

- IGFBP2 is a mediator of the peripheral action of leptin on the liver and skeletal muscle, and it acts independently of IGF-1
- IGFBP2 contains a unique heparin-binding domain (HBD1) that binds to the cell surface RPTPβ receptor and stimulates glucose uptake
- AZP-3404 is an enzymatically stable, 9-amino acid analog derived from the HBD1 of IGFBP2

The present results demonstrate that:

- AZP-3404 restores glucose homeostasis in the leptin-deficient ob/ob mouse
- AZP-3404 stimulates glucose uptake in mouse skeletal muscle through binding to RPTPβ, resulting in:
    - stimulation of Akt (enhances IGF-1 and insulin action)
    - stimulation of AMPK (IGF-1 and insulin independent)
- AZP-3404 reproduces the glucose-modulating action of IGFBP2, and has therapeutic potential in syndromes of severe insulin resistance

Thank you!