Leucine stimulates PPAR signaling promoting mitochondrial biogenesis and GLUT4 expression in skeletal muscle

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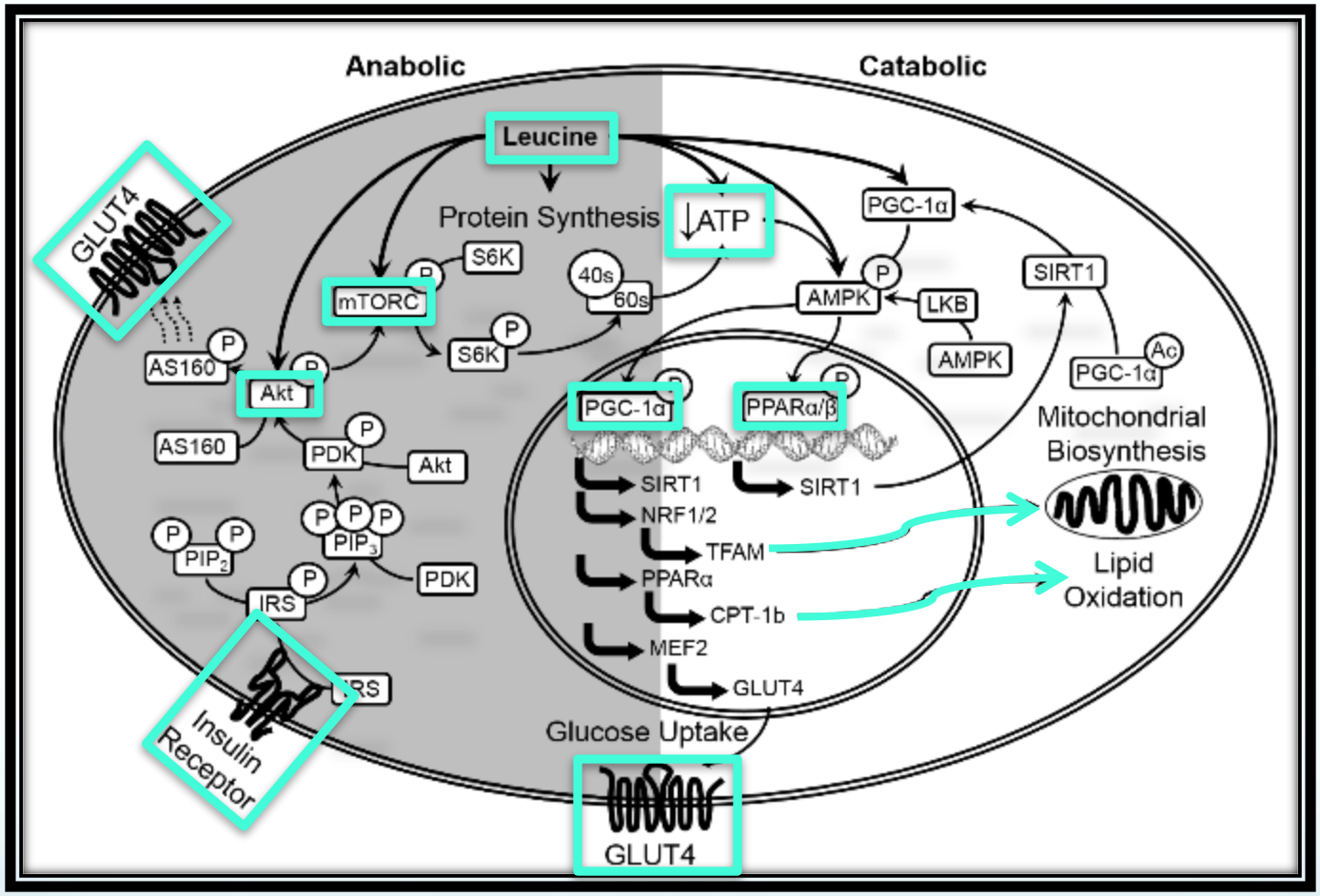
Background

- Leucine is a common constituent of many dietary supplements
- Potential anabolic and catabolic effects:
  - Increases muscle protein synthesis via mTOR activation
  - Increases fatty acid oxidation and mitochondrial content
- Peroxisome Proliferator-Activated Receptor (PPAR) superfamily regulates cellular energetics and substrate utilization
  - PPARα and PPARβ/δ stimulate oxidative metabolism
  - PPARγ promotes fat synthesis and storage
  - PGC-1α increases mitochondria through NRF and TFAM expression

Proposed mechanisms of leucine



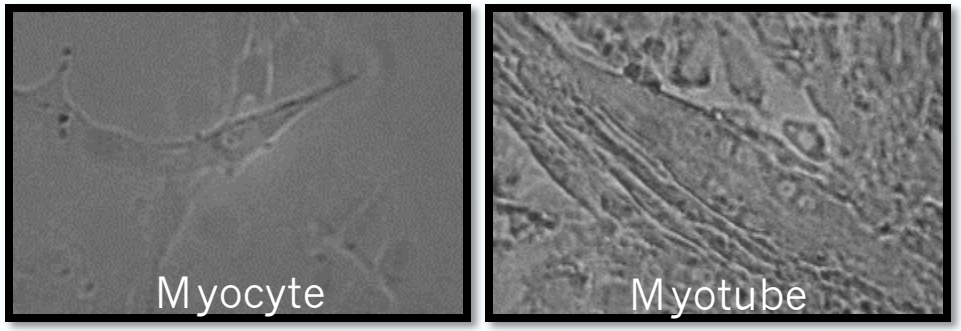
Gannon NP and Vaughan RA. Leucine-induced anabolic-catabolism: two sides of the same coin. *Amino Acids* 2015, Epub ahead of print. doi: 10.1007/s00726-015-2109-8

Purpose

To characterize the effects of leucine treatment on myotube expression of the PPAR superfamily as well as related downstream targets that regulate cellular energetics and inflammation.

Methods

- **Cell Culture**
  - Mouse myotubes (C2C12) were treated with leucine or valine (isonitrogenous control) at 2mM for 24 hours



- **Experimental Protocol**
  - mRNA expression quantified via qRT-PCR
  - Protein expression quantified via western blot
  - O_2 Consumption Assay (MitoXpress)®
  - Glucose Uptake Assay (2-NBDG)

Leucine stimulates oxidative metabolism (Figure 1)

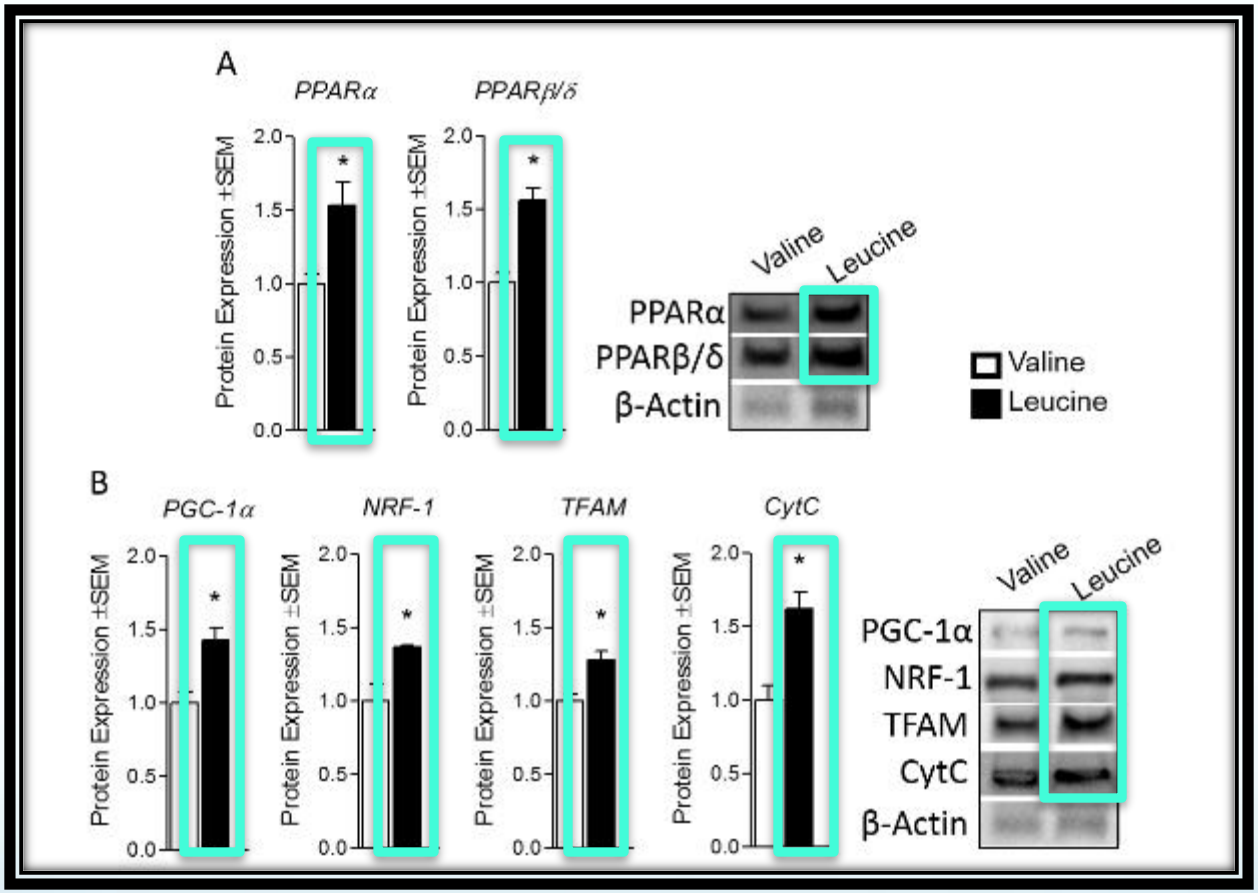


Fig 1. Leucine stimulates PPAR expression and mitochondrial biogenesis in cultured myotubes. (A) Peroxisome proliferator-activated receptor α (PPARα) and PPARβ/δ protein expression following treatment of C2C12 myotubes with either isonitrogenous control valine or leucine at 2mM for 24 hours. (B) Peroxisome proliferator-activated receptor γ co-activator 1 (PGC-1α), nuclear respiratory factor 1 (NRF-1), mitochondrial transcription factor A (TFAM), and cytochrome c (CytC) protein expression following treatment of C2C12 myotubes as described above. *Notes: Target protein expression was normalized actin as housekeeping loading control. * indicates $p < 0.05$*

Leucine stimulates oxidative metabolism (Figure 1cont.)

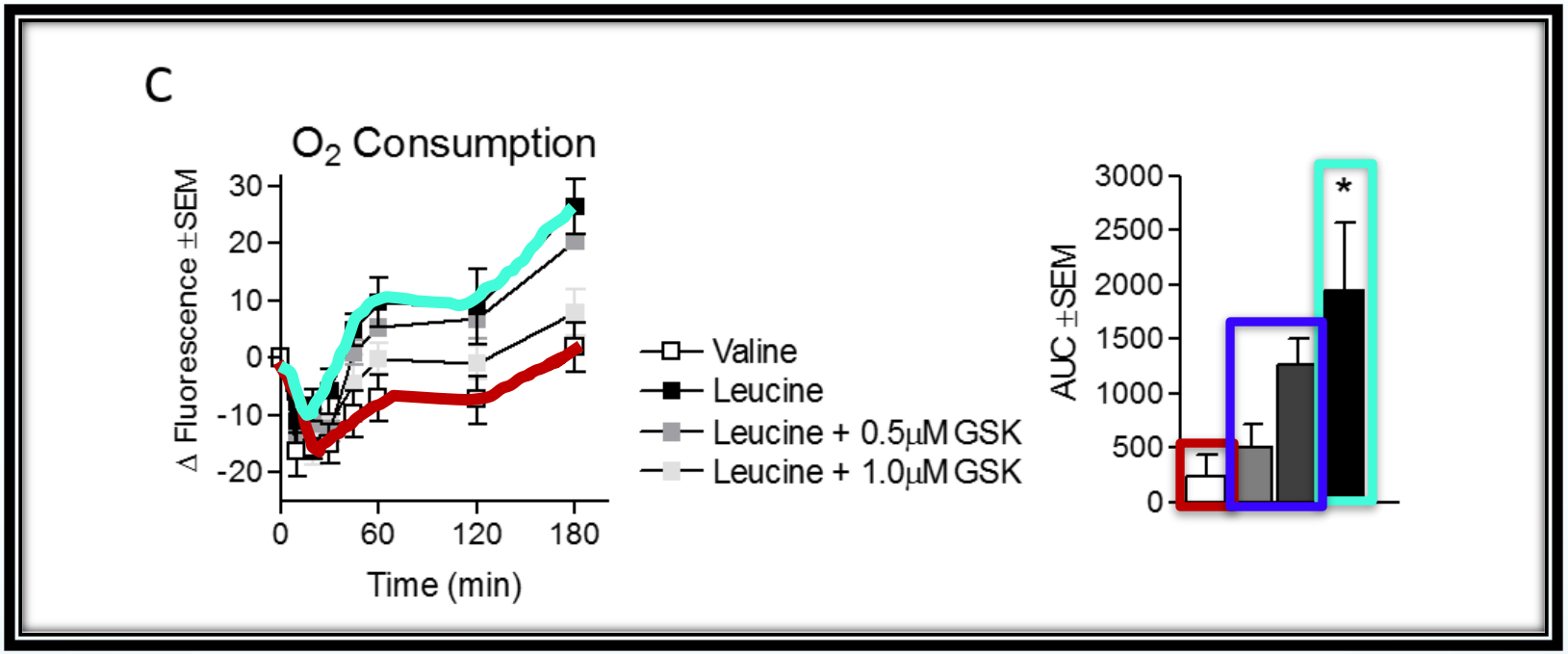


Fig 1 cont. Leucine stimulates PPAR expression and mitochondrial biogenesis in cultured myotubes (C) Oxygen consumption following treatment described above with leucine treated cells with and without the selective PPARβ/δ inhibitor GSK3787 (GSK) with time trial (left) and area-under-the-curve (AUC) (right). *Notes: Target protein expression was normalized to β–actin as loading control. * indicates $p < 0.05$ compared with valine control.*

Leucine stimulates GLUT4 via Akt (Figure 2)

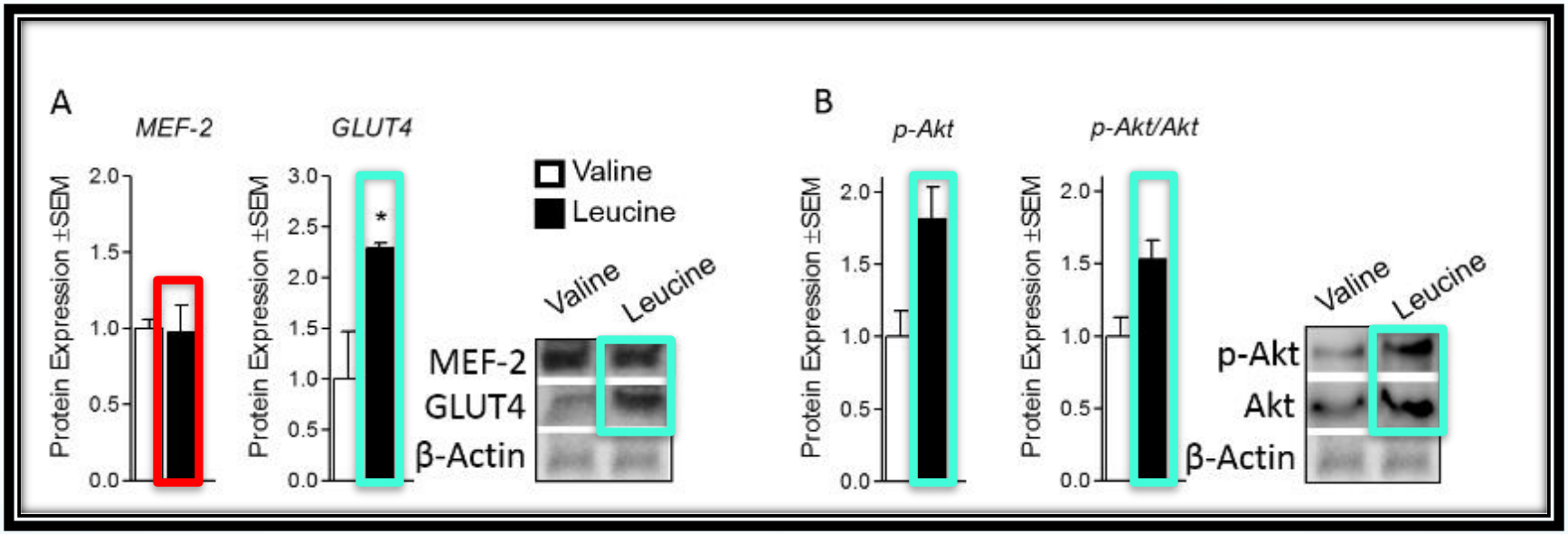


Fig 2. Leucine stimulates myotube GLUT4 expression through Akt activation independent of MEF-2. (A) Myocyte enhancer factor 2 (MEF-2) and glucose transporter 4 (GLUT4) protein expression following treatment of C2C12 myotubes with either isonitrogenous control valine or leucine at 2mM for 24 hours. (B) Phospho-Akt protein expression normalized to either β–actin (left) or total Akt expression (right) following treatment as described above. *Notes* indicates $p < 0.05$*

Leucine stimulates GLUT4 via Akt (Figure 2cont.)

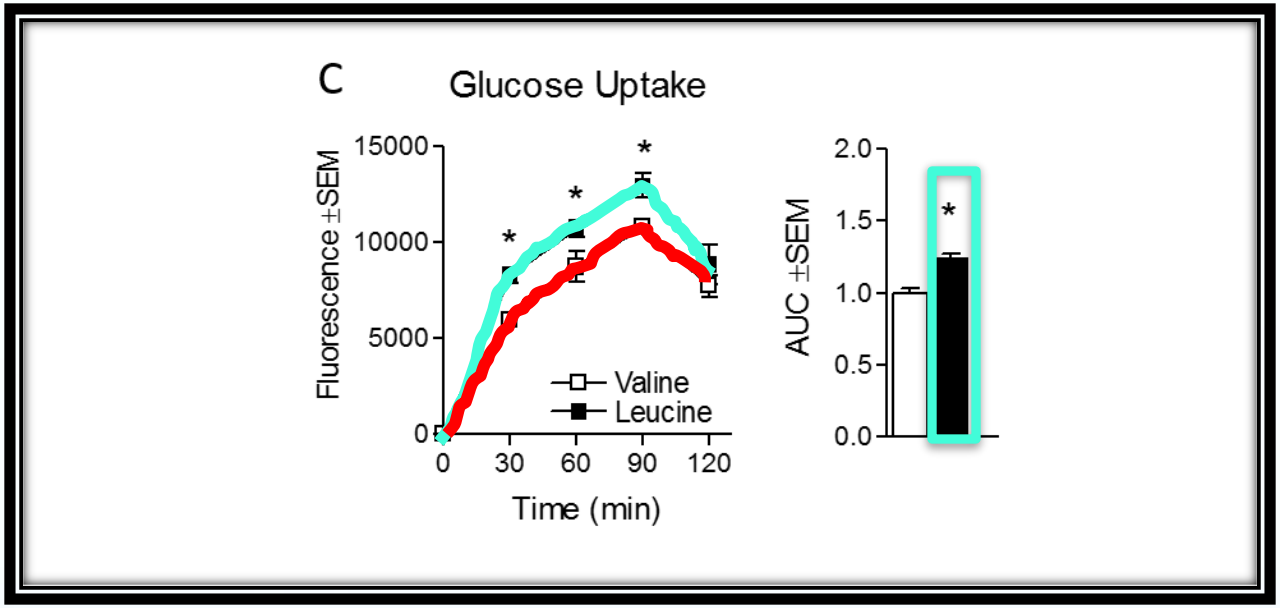


Fig 2 cont. Leucine stimulates myotube GLUT4 expression through Akt activation independent of MEF-2. . (C) Glucose uptake following treatment as described above with time trial (left) and area-under-the-curve (AUC) (right). *Notes* indicates $p < 0.05$*

Leucine stimulates FAS through PPARγ (Figure 3)

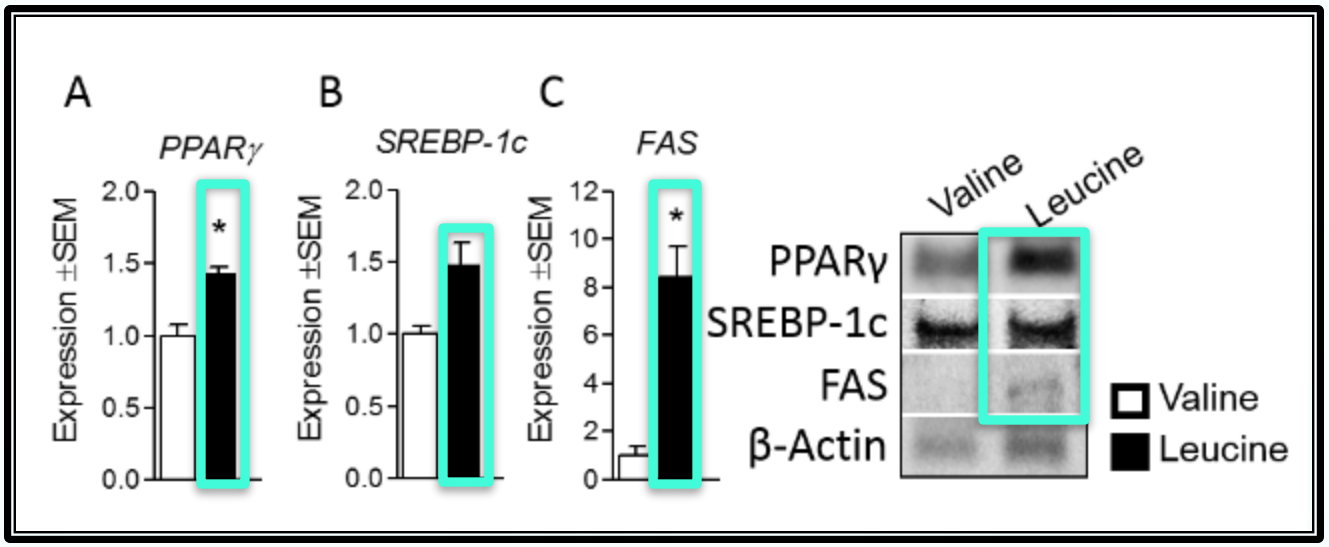
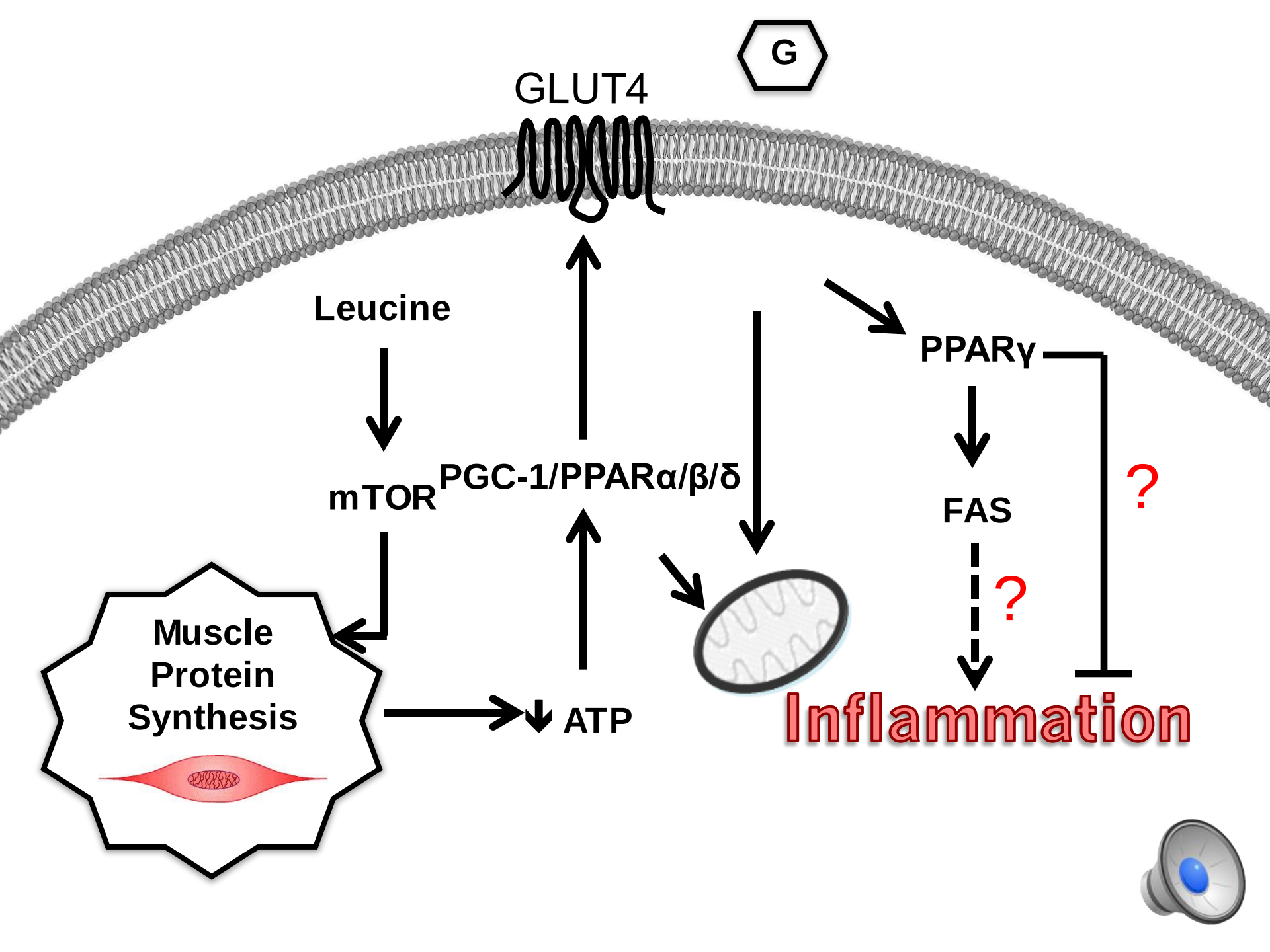


Fig 3. Leucine stimulates PPARγ and fatty acid synthase expression in cultured myotubes. (A) Peroxisome proliferator-activated receptor γ (PPARγ) protein expression following treatment of C2C12 myotubes with either isonitrogenous control valine or leucine at 2mM for 24 hours. (B) Sterol response element binding protein-1c (SREBP-1c) protein expression following treatment of C2C12 myotubes as described above. (C) Fatty acid synthase (FAS) protein expression following treatment of C2C12 myotubes as described above. *Notes: Target protein expression was normalized to β–actin as housekeeping loading control. * indicates $p < 0.05$*


G
GLUT4
Leucine
mTOR
PGC-1/PPARα/β/δ
PPARγ
FAS
?
?
Muscle
Protein
Synthesis
ATP
Inflammation

Leucine reduces inflammation (Figure 4)

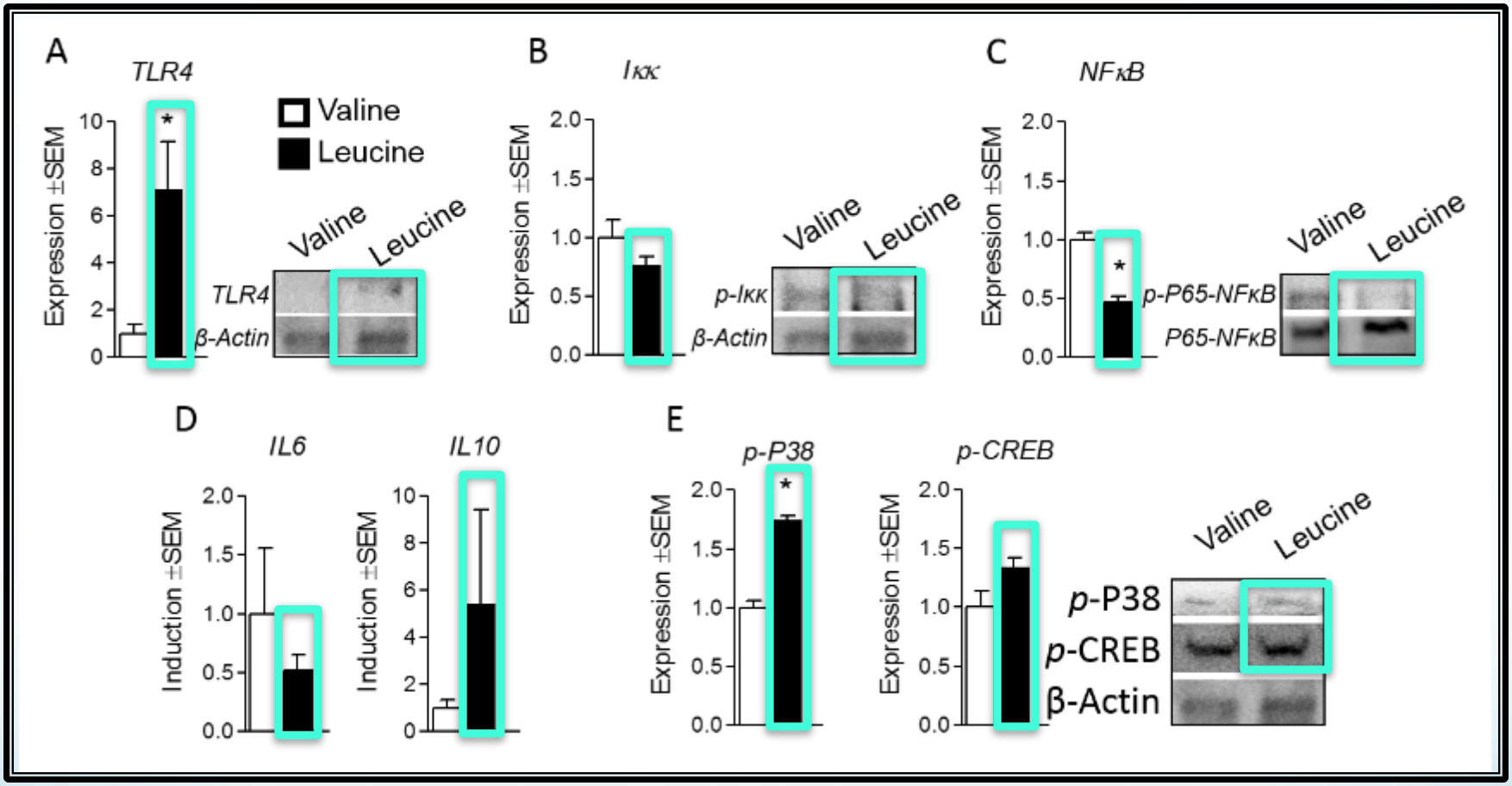
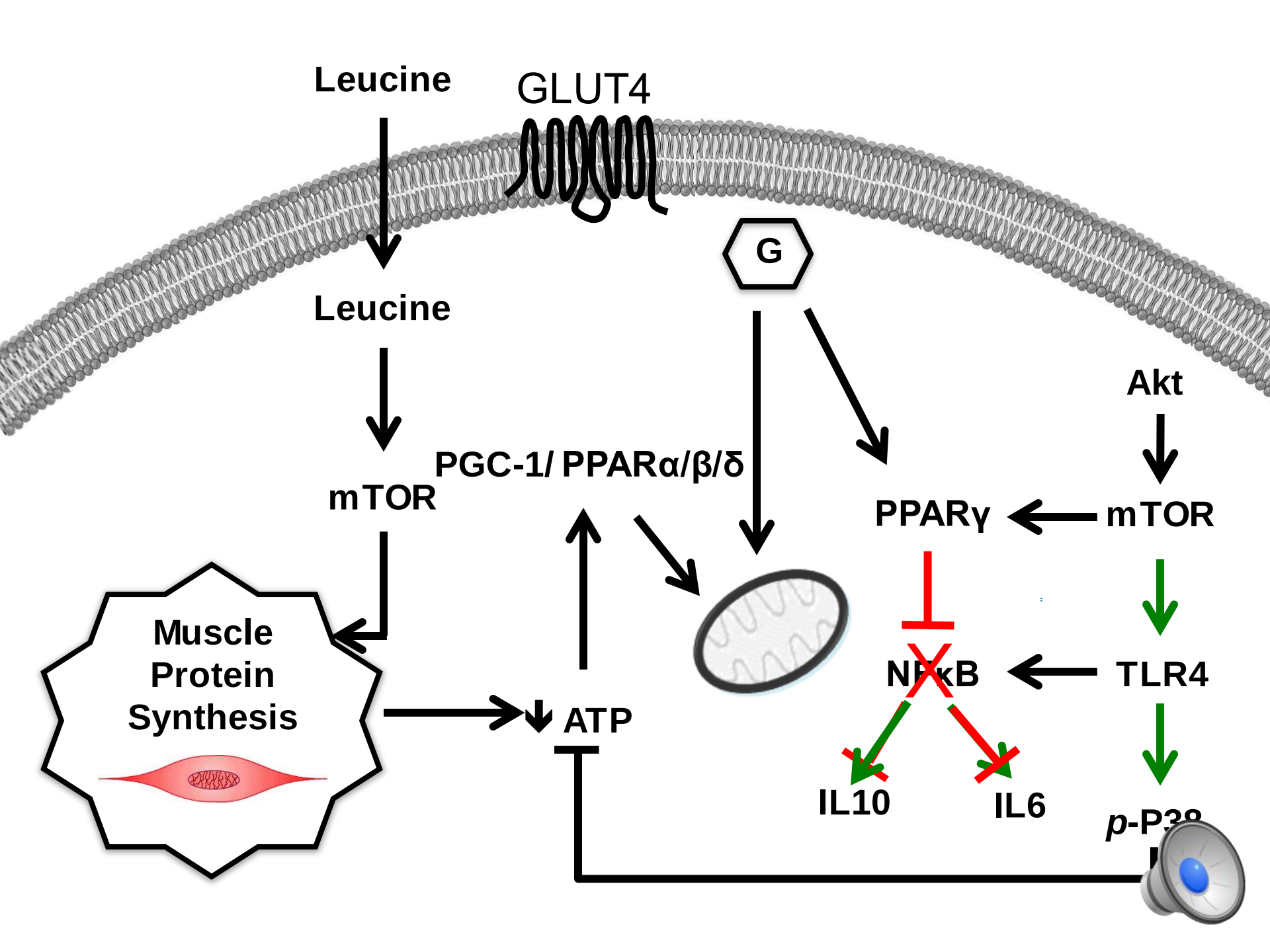


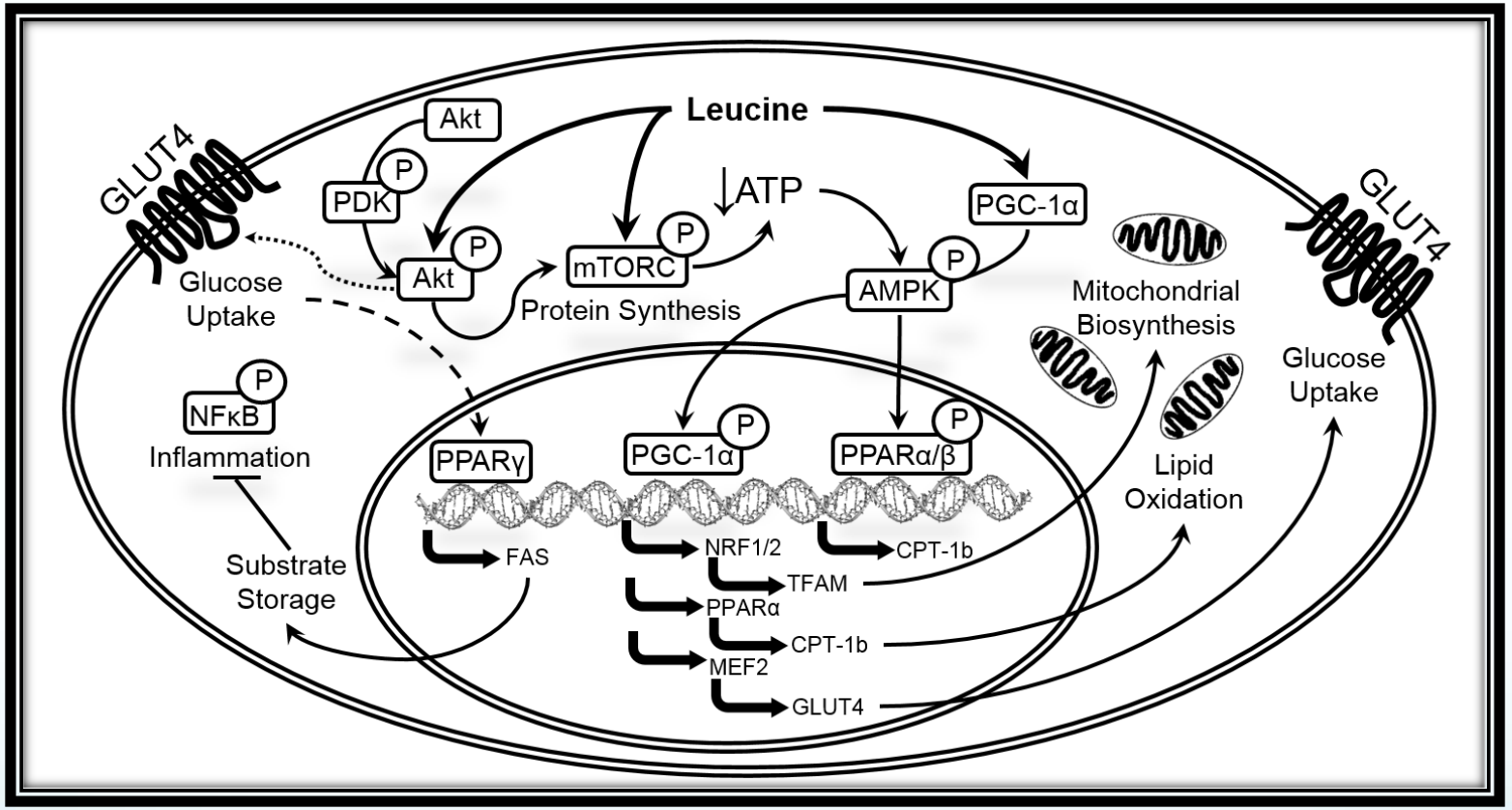
Fig 4. Leucine stimulates TLR4 expression but suppresses inflammatory signaling in cultured myotubes. (A) Toll-like receptor 4 (TLR4) expression of myotubes treated with either leucine or isonitrogenous control valine at 2mM for 24 hours. (B) Ikκ expression of myotubes treated as described above. (C) Nuclear factor κ B (NFκB) activity indicated by phospho-P65 expression (normalized to total P65 content) in myotubes treated as described above. (D) mRNA expression of interleukin 6 (IL6) and interleukin 10 (IL10) following treatment as described above. (E) Phospho-P38 MAPK (p-P38) and phospho-cAMP Response Element (p-CREB) protein expression following treatment *Notes: Target protein expression was normalized to β actin as housekeeping loading control. Target mRNA expression was normalized to housekeeping gene TATA Binding Pro (TBP). * indicates p < 0.05*


Leucine
GLUT4
Leucine
G
Akt
mTOR
PGC-1/ PPARα/β/δ
PPARγ
mTOR
Muscle
Protein
Synthesis
ATP
NFkB
TLR4
IL10
IL6
p-P38

Take Home Message

- Leucine treatment stimulated PPARα and PPARβ/δ as well as mitochondrial biogenesis
- Leucine treatment enhances GLUT4 content via Akt and independent of MEF-2 expression
- Leucine promotes simultaneous induction of PPARγ leading to elevated FAS expression
- Leucine suppresses markers of cellular inflammation through reduced NFκB and cytokine induction

Working Hypothesis



Working hypotheses of mechanisms governing leucine-induced metabolism in myotubes. Leucine stimulates Akt and protein synthesis resulting in increased ATP demands. Increased energy demands stimulate regulators of metabolism including AMPK, PPARβ/δ, and PGC-1α leading to increased oxidative metabolism, mitochondrial biogenesis, and GLUT4 content. Elevated GLUT4 content promotes increased glucose uptake to support rising energy needs. Increased energy uptake promotes substrate oxidation and storage (in part through increased PPARγ) leading to reduced cellul inflammation. Leucine may alter energy availability by stimulating substrate oxidation, substrate/lipid storage, a protein synthesis. Leucine may also reduce cellular inflammation by altering cell energetics (a hypothesis w requires further investigation).

Conclusion

Leucine induces PPAR expression, GLUT4 content, and mitochondrial biogenesis *in vitro*, suggesting leucine may increase substrate oxidation. Leucine-mediated enhanced glucose uptake may stimulate a compensatory response for cells to dissipate energy by (a) substrate oxidation (b) substrate/lipid storage, and (c) protein synthesis, thus providing a possible explanation for the simultaneous induction of the entire PPAR superfamily (anabolic and catabolic). Moreover, increased Akt and mTOR activity by leucine may promote TLR4 expression potentially sensitizing cells to inflammatory signals (such as cytokines), while concurrent increases in PPARγ may lead to reduced cellular inflammation (a hypothesis which requires further investigation).

Acknowledgements

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- Thank you to my corresponding author Dr. Roger Vaughan for overseeing the entire study and being an altruistic research mentor