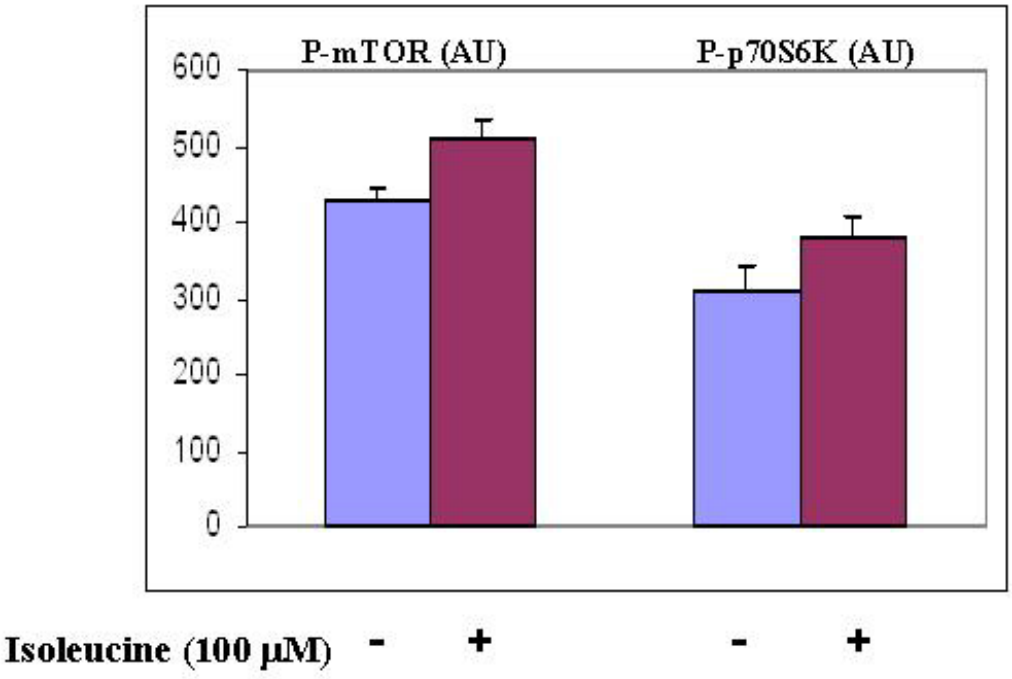
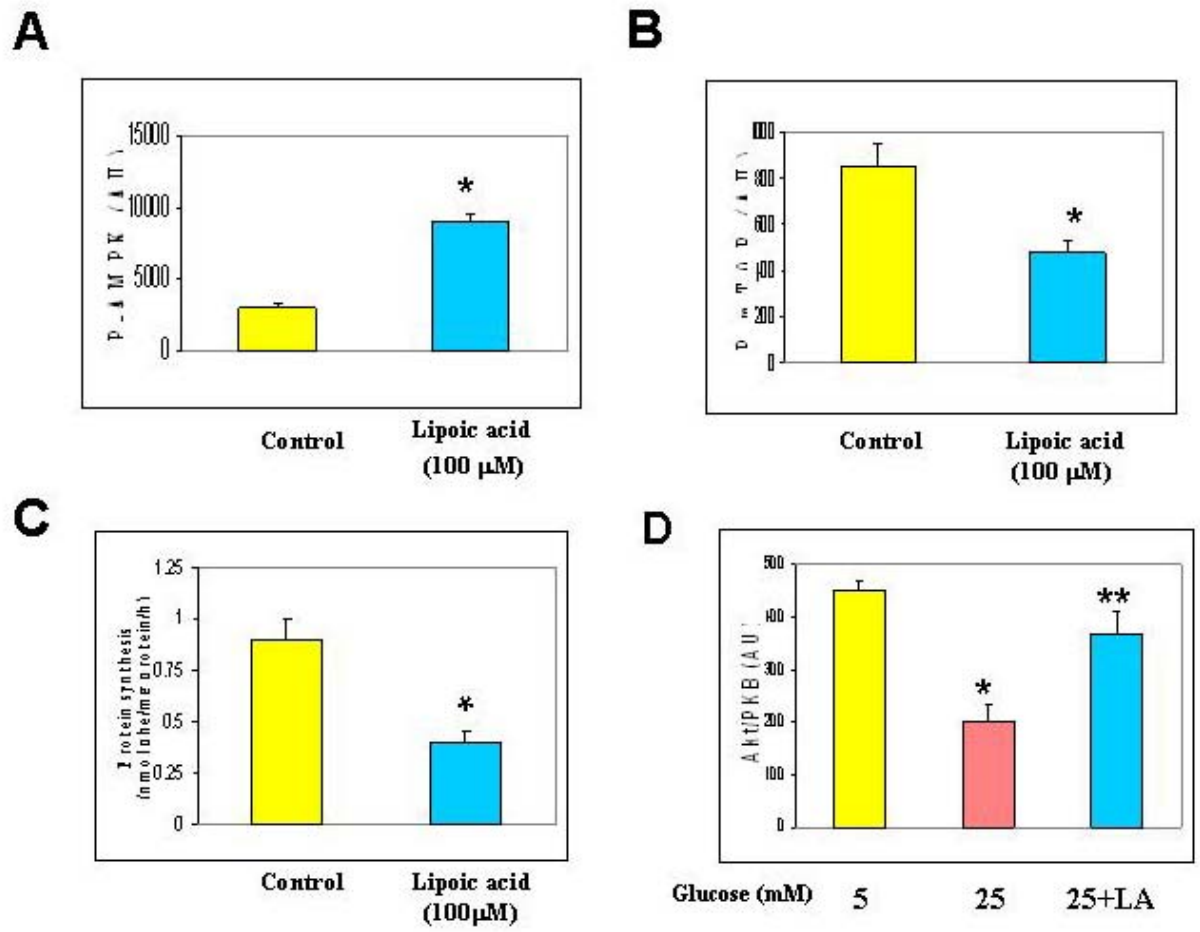
Online appendix - Supplemental material

Online appendix Figure S1. Effect of leucine on p-mTOR, P-p70S6K

EDL were incubated in Krebs-Henseleit solution containing 0, and 100 μM of leucine for 1hr. Phosphorylation of mTOR and p70 S6 kinase were done by immunoblot and densitometric analysis. Results are means ± SE, (n = 5).

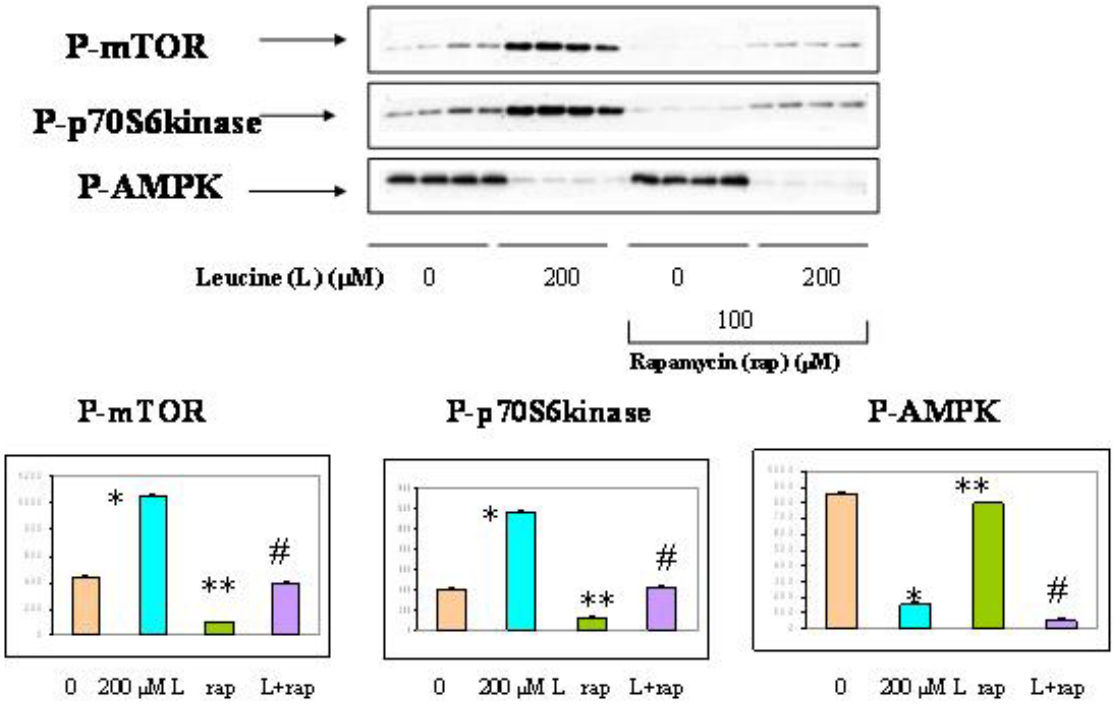


Online appendix Figure S2. ***Effect of lipoic acid (ALA) on AMPK (A) and mTOR phosphorylation (B), protein synthesis(C) and insulin resistance (D).***
EDL were preincubated with Krebs-Henseleit solution containing 5.5 mM glucose for 20 min and then additional 1 h in the presence of ALA (100 μM). For insulin resistance, EDL muscle were preincubated with glucose (25 mM) for 1 hour and then ALA for 1hr following 10 min incubation with insulin Values are means ± SE from 3 independent measurements *, $p<0.01$ vs control (0 μM ALA). *, p<0.01 vs 5 mM glucose and **, p<0.05 vs 25 mM glucose.

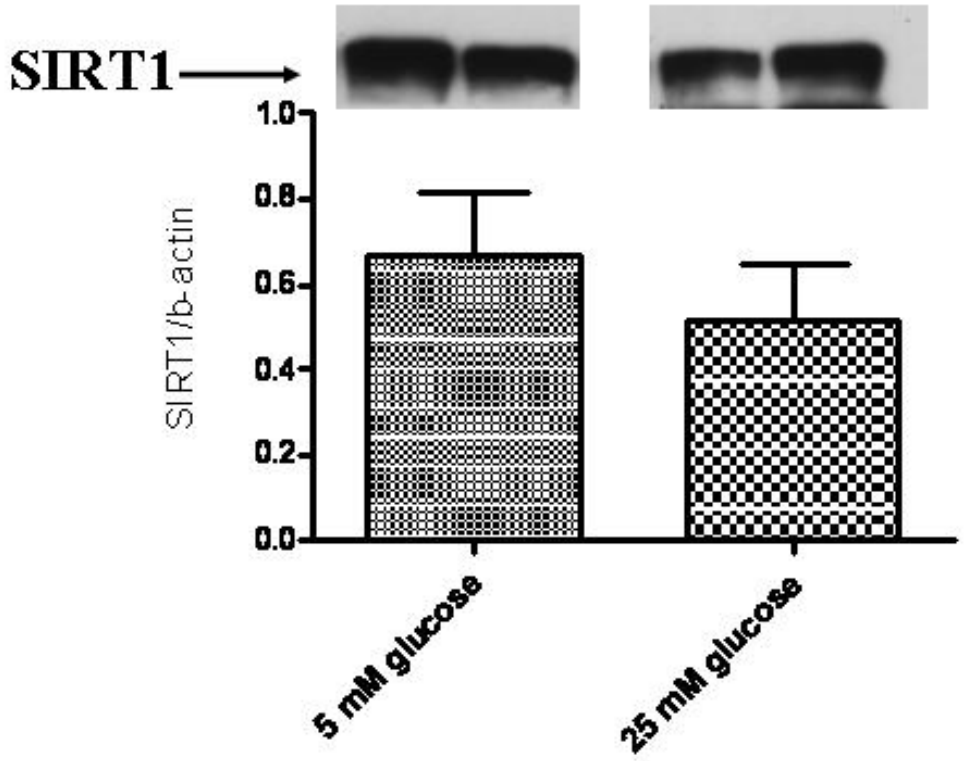


Online appendix Figure S3. Inhibition of leucine induced mTOR/p70S6K phosphorylation does not suppress AMPK phosphorylation.

EDL muscle were preincubated in the presence of rapamycin (100 μM) for 30 min and then with or without leucine (200 μM) for 1hr. Muscle lysates were analyzed for P-mTOR, P-p70S6K and P-AMPK using SDS-PAGE (Upper panel). The quantification of western blot (lower panel). Results are means ± SE (n = 5). *, p<0.01 relative to values for muscles with no leucine added to medium. **, p<0.01 vs when compared to 200 μM leucine, #, p<0.01 vs rapamycin.

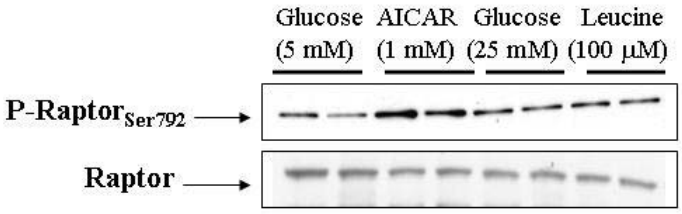


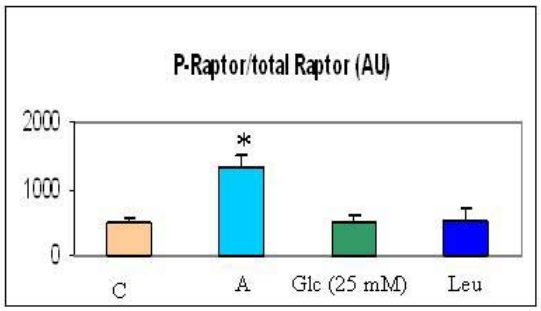
Online appendix Figure S4. ***Effect of high glucose on SIRT1 protein abundance.***
EDL were incubated in 5.5 or 25 mM glucose for 1 h. Western blot analysis and quantification of representative blot shown. Results are means+SE (n=6).

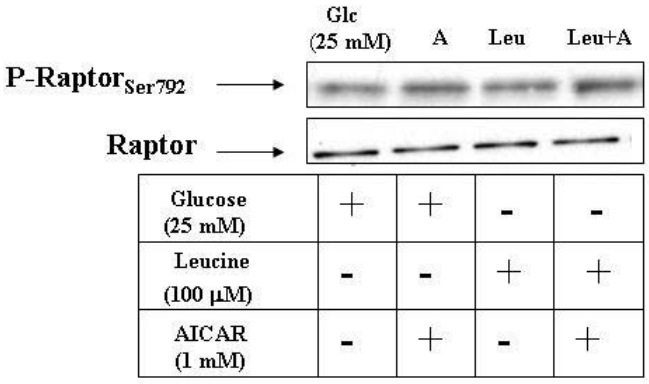


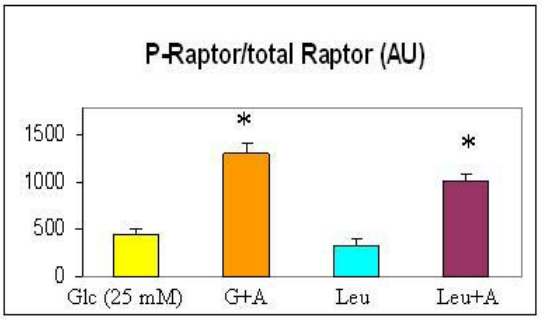
***Online appendix Figure S5. Phosphorylation of raptor in EDL muscle treated with* AICAR, glucose and leucine.**

EDL were incubated in Krebs-Henseleit solution containing 25 mM glucose and 100 μM of leucine for 1hr in the presence of 1 mM AICAR. Phosphorylation of raptor was done by immunoblot and densitometric analysis. Results are means ± SE, (n = 6).



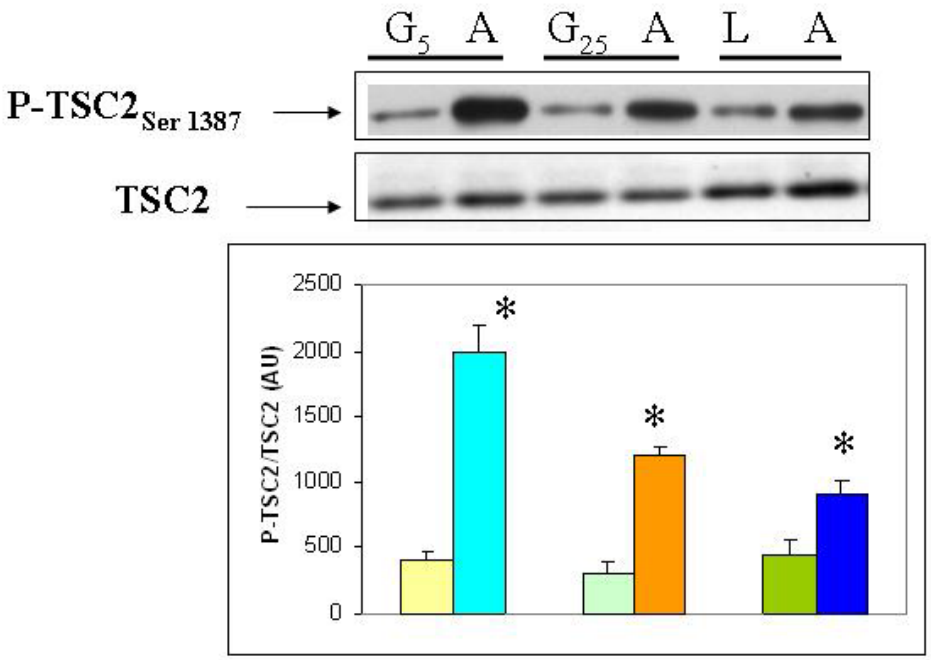






Online appendix Figure S6. ***TSC2 Phosphorylation of raptor in EDL muscle treated with*** **AICAR, glucose and leucine.**

EDL were incubated in Krebs-Henseleit solution containing 25 mM glucose and 100 μM of leucine for 1hr in the presence of 1 mM AICAR. Phosphorylation of TSC2 was done by immunoblot and densitometric analysis. Results are means ± SE, (n = 5).



Glucose (5 mM	+	+	-	-	+	-
AICAR (1 mM)	-	+	-	+	-	+
Glucose (25 mM)	-	-	+	+	-	-
Leucine (100 μM)	-	-	-	-	+	+

Online appendix Figure S7. Hypothetical mechanism by which leucine and glucose stimulate protein synthesis and cause insulin resistance in skeletal muscle.
Based on the evidence presented here they do so by decreasing AMPK activity, which in turn leads to an increase in mTOR/p70S6 kinase signaling. A logical assumption is that the insulin resistance is a normal physiological event and is readily reversible. The mechanism by which leucine and glucose decreases AMPK activity has yet to be determined.

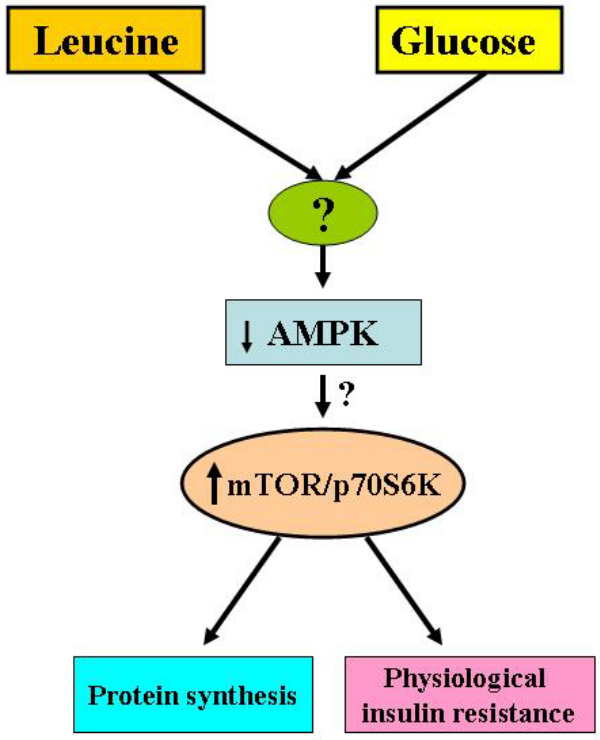


Table S1. Effects of compound C on P-AMPK, P-mTOR and protein synthesis in EDL incubated in presence of
5.5 mM glucose

	Glucose (5 mM)	Compound C (50 μM)
P-AMPK/AMPK (AU)	290±22	282±14
P-mTOR/mTOR (AU)	460±42	481±30
Protein synthesis (nmol Phe/mg protein/h)	0.9±0.16	1.0±0.1

EDL muscles were preincubated with Compund C (50 μM) for 30 min and additional 1 h in the presence of Krebs-Henseleit buffer containing 5.5 mM glucose. Results are means±SE of four experiments.