

Receptor Interacting Protein 140 (RIP140) silencing via RNAi interferes with fatty acid metabolism but not with glucose uptake in L6 muscle cells

Silvana Constantinescu and Lorraine P. Turcotte

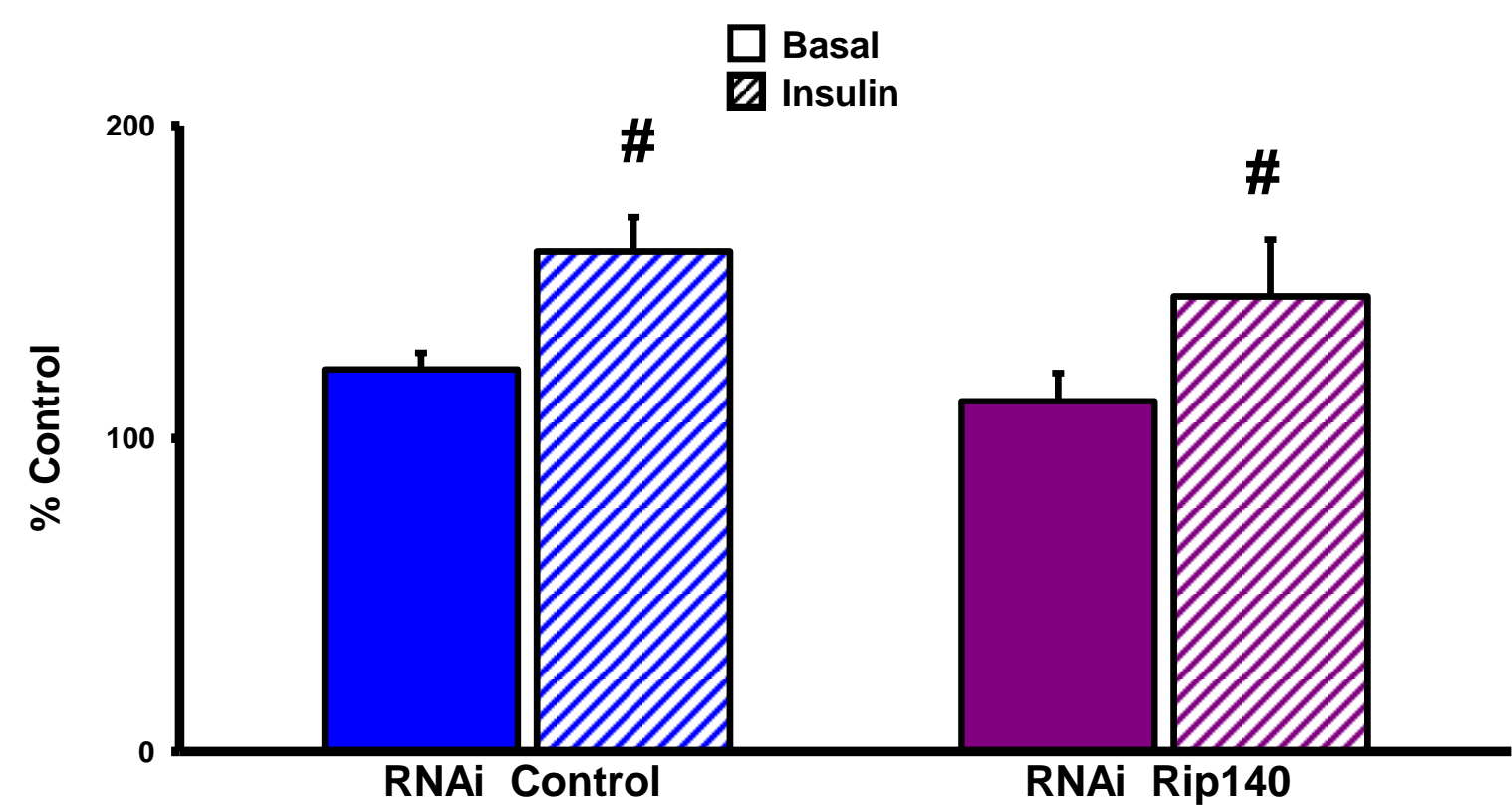
Department of Biological Sciences and Department of Kinesiology,
University of Southern California, Los Angeles, CA

Abstract

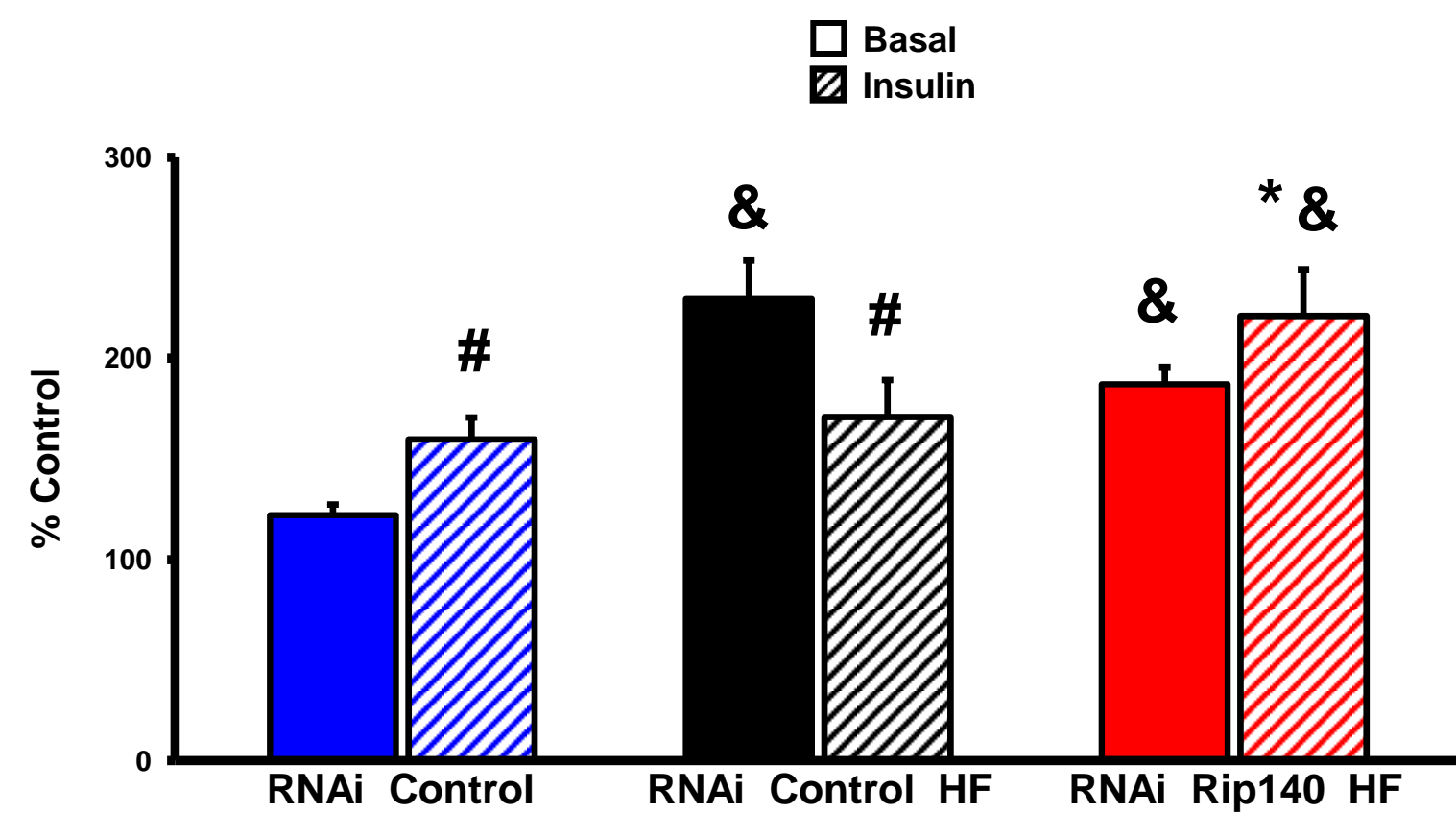
The role of RIP140 in the regulation of muscle metabolism has not been delineated. To investigate the role of RIP140 in the regulation of fatty acid (FA) uptake (FAU), oxidation (FAO) and glucose uptake (GU) in muscle cells, L6 myotubes were silenced using RNAi custom sequences for RIP140 (RIP) or a silencer negative (SN) using Lipofectamine as a transfection agent. Cells were incubated \pm insulin (I: 1000 nM) before measurement of glucose and FA kinetics. In SN cells, insulin ($P<0.05$) increased GU (31%) and FAU (20%) and decreased FAO (35%), demonstrating that the transfection protocol did not affect basal and insulin-mediated muscle metabolism. Basal and insulin-stimulated GU were not significant different ($P>0.05$) between RIP and SN cells. However, basal FAU and FAO were 20% higher and 38% lower in RIP cells when compared to SN cells. Furthermore, with RIP silencing no insulin-mediated increase in FAU and decrease in FAO were observed. Our data show that reducing RIP140 expression in muscle has no impact on the regulation of GU but leads to high rates of FAU and reduced sensitivity to insulin as it pertains to FA metabolism. Together, these cellular changes indicate that low RIP140 expression is associated with reduced metabolic flexibility.

Results

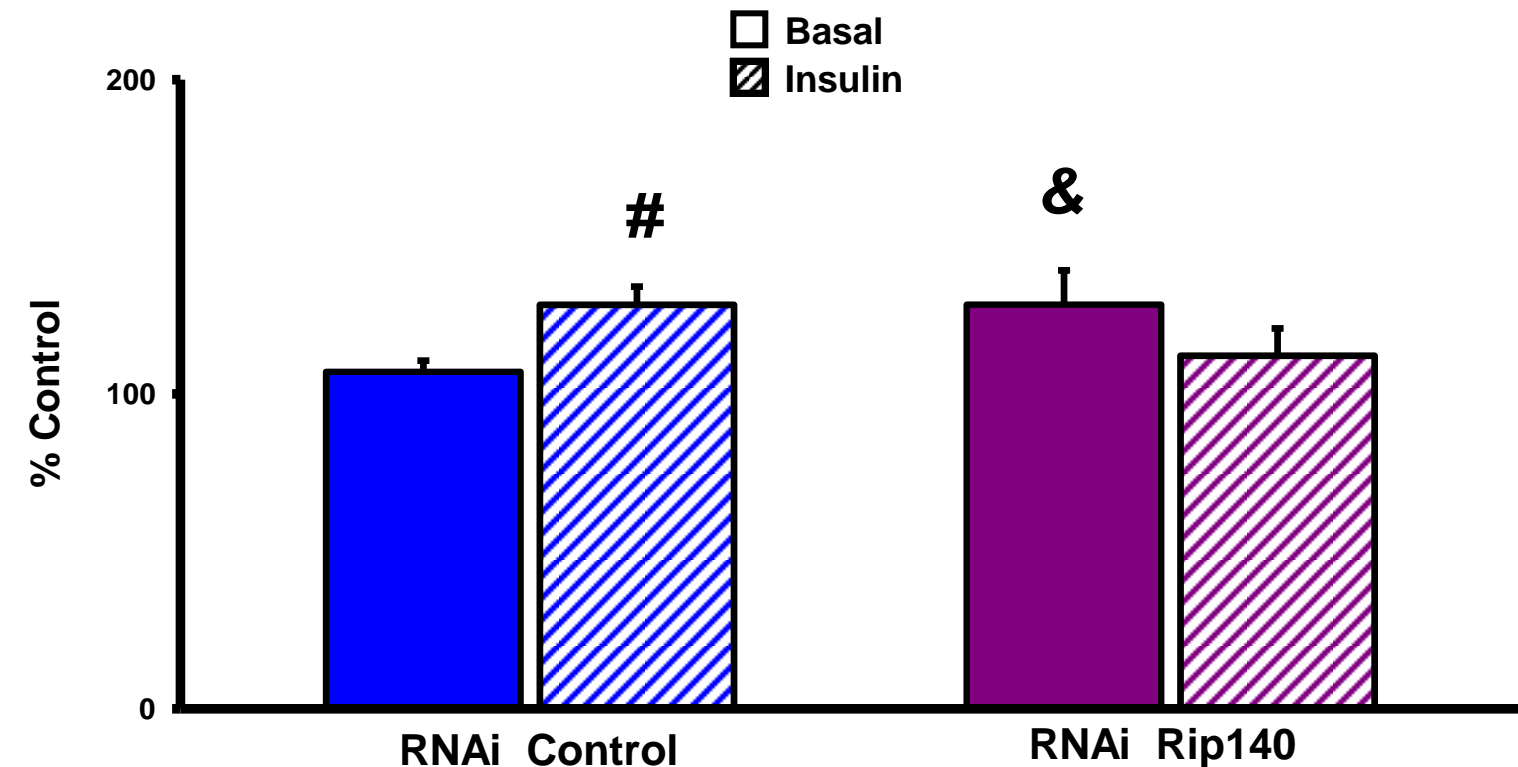
Glucose Uptake



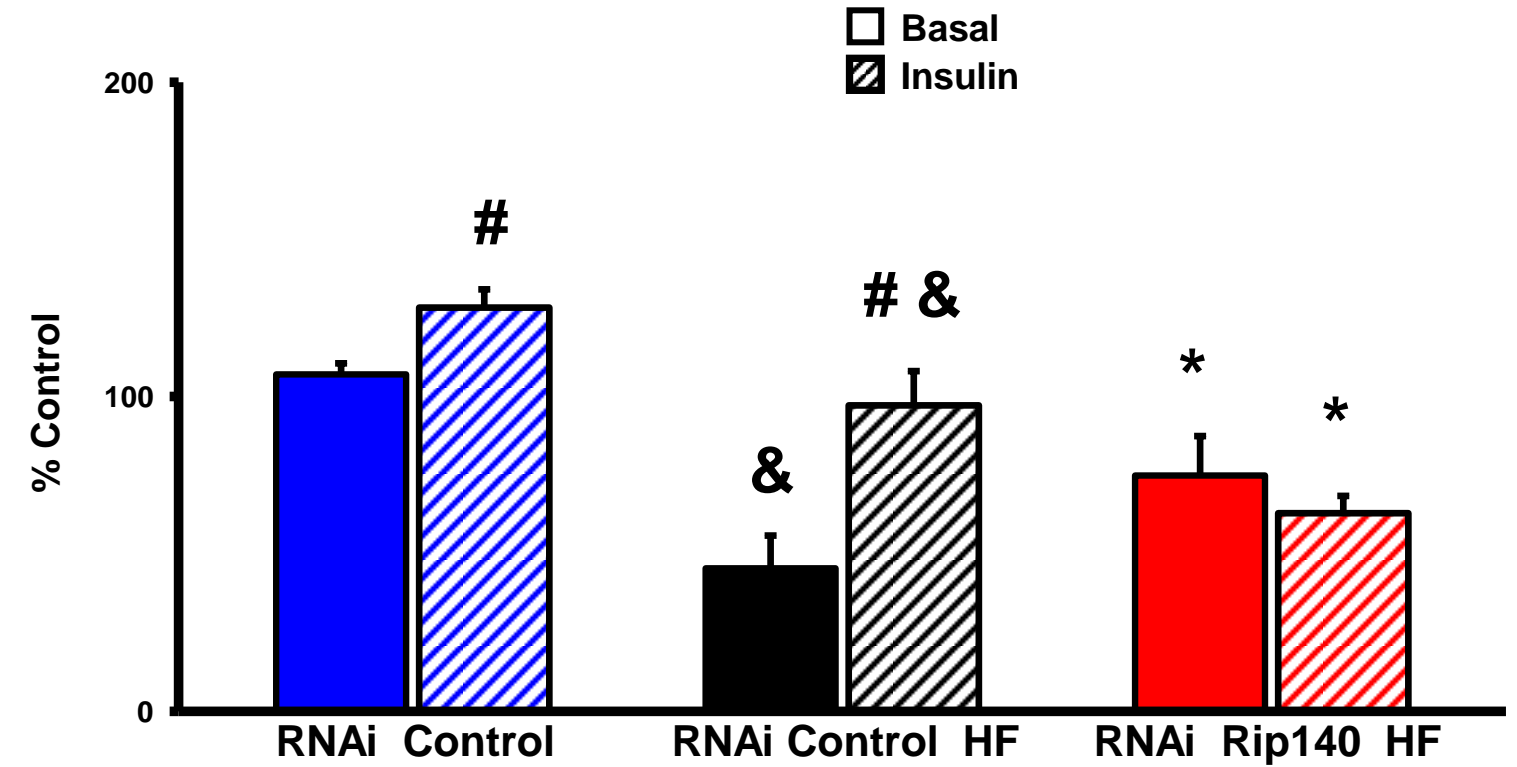
Glucose Uptake High Fat Diet



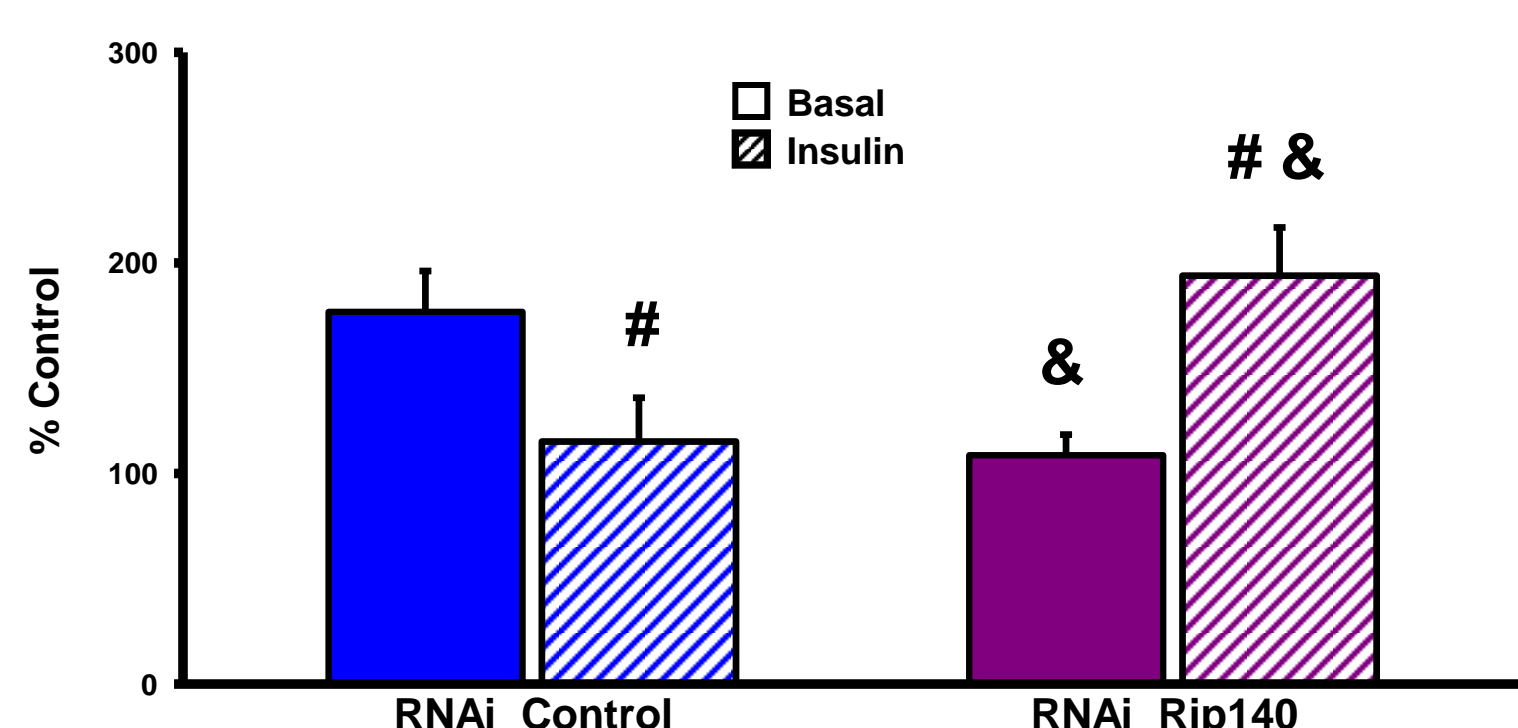
FA Uptake



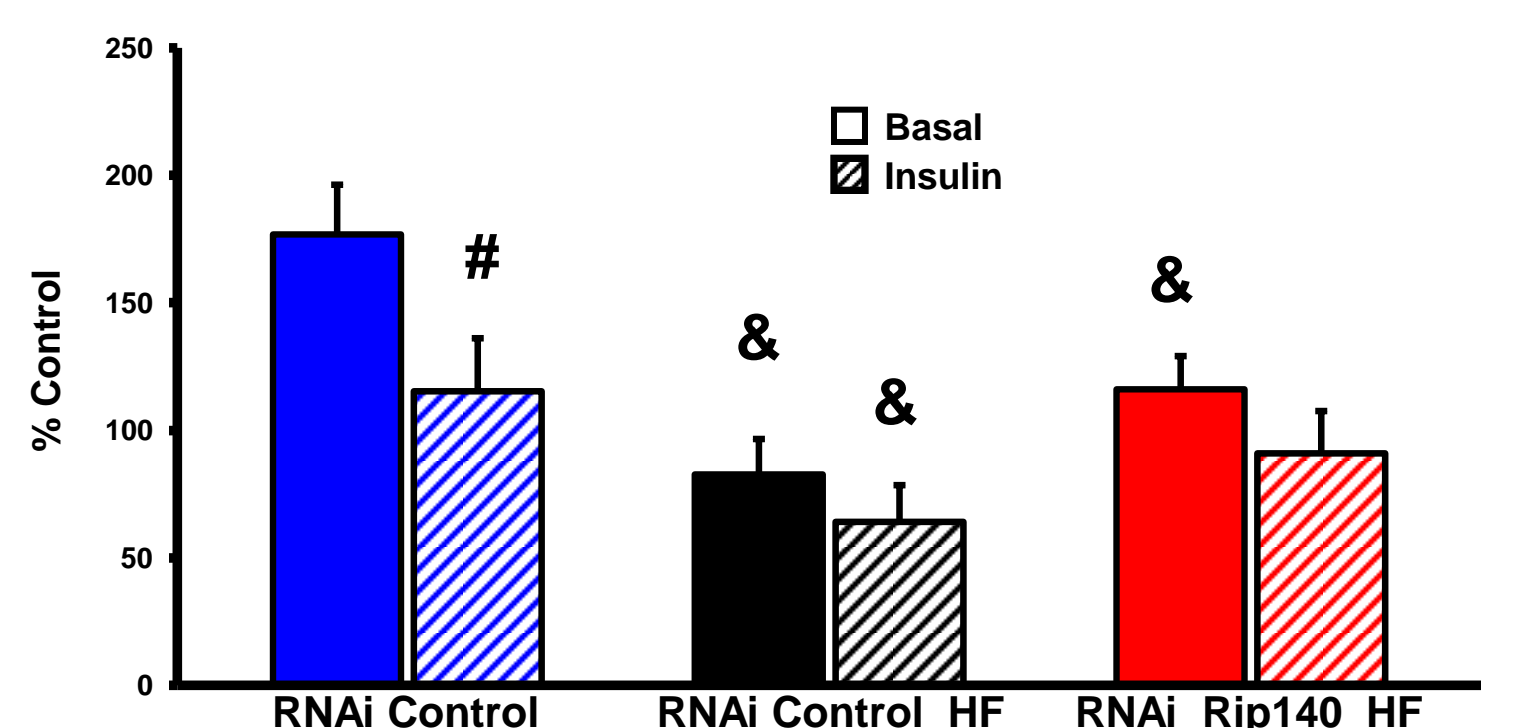
FA Uptake High Fat Diet



FA Oxidation

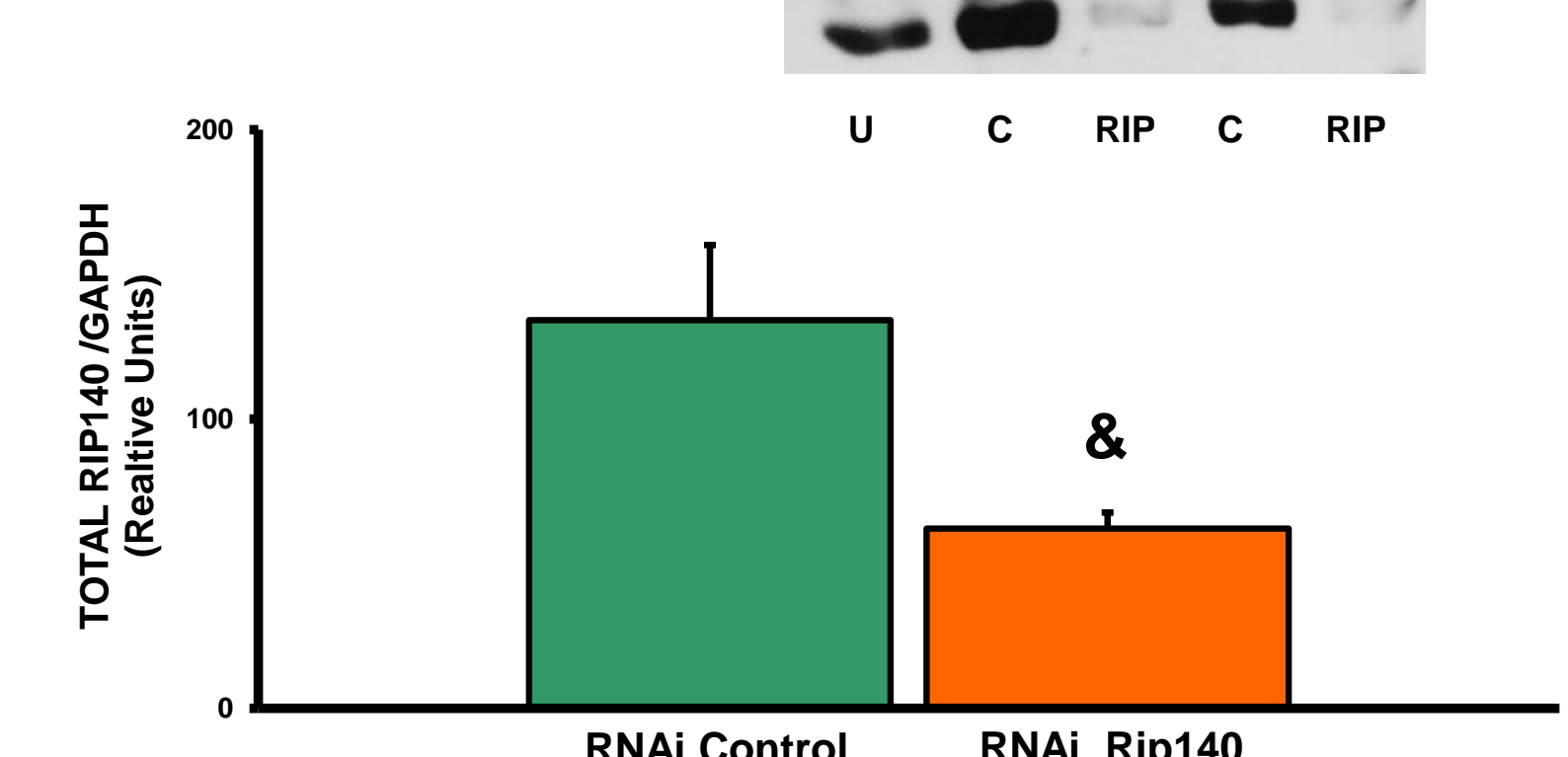


FA Oxidation High Fat Diet

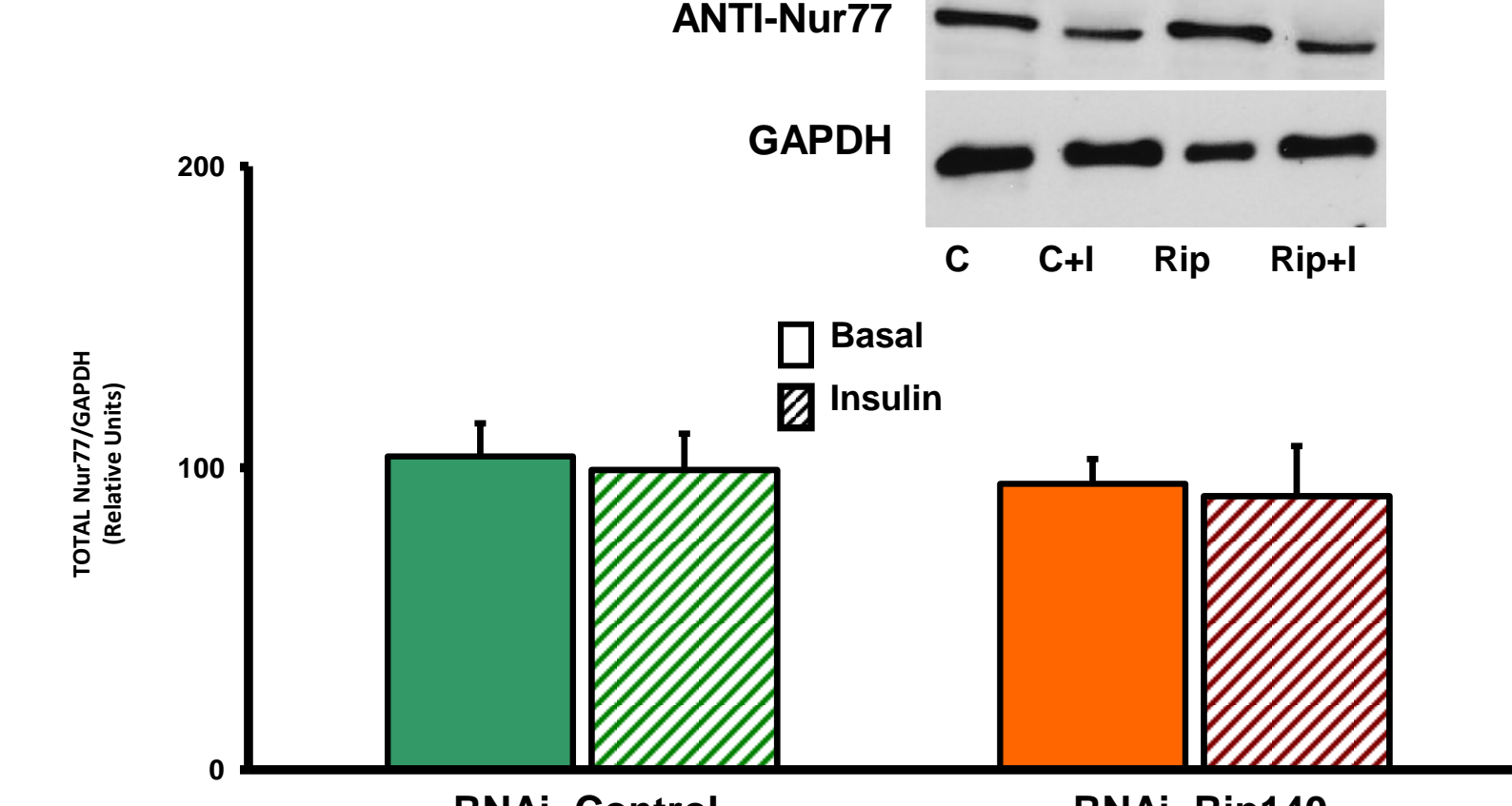


Effect of Low RIP140

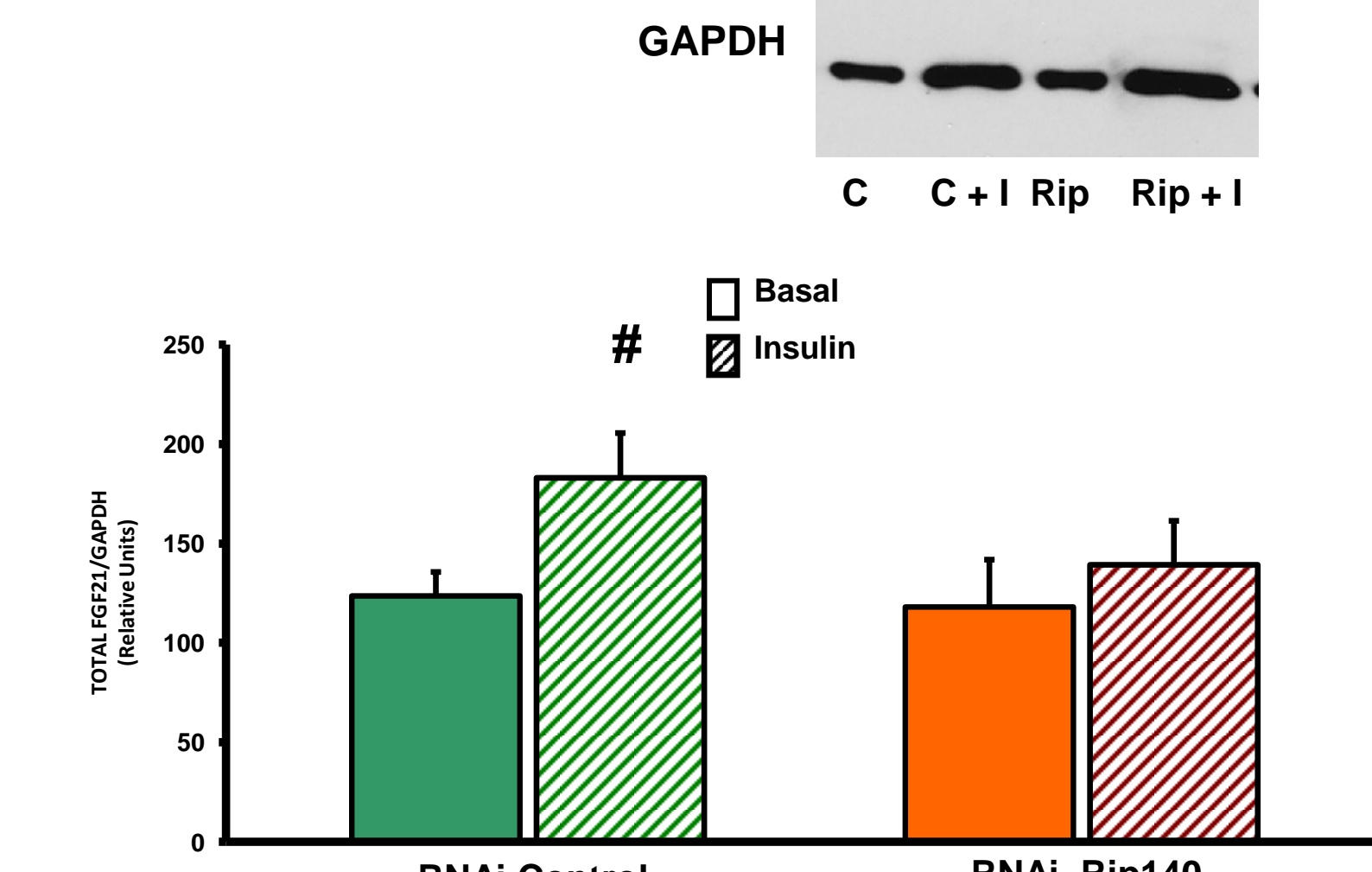
Total RIP140



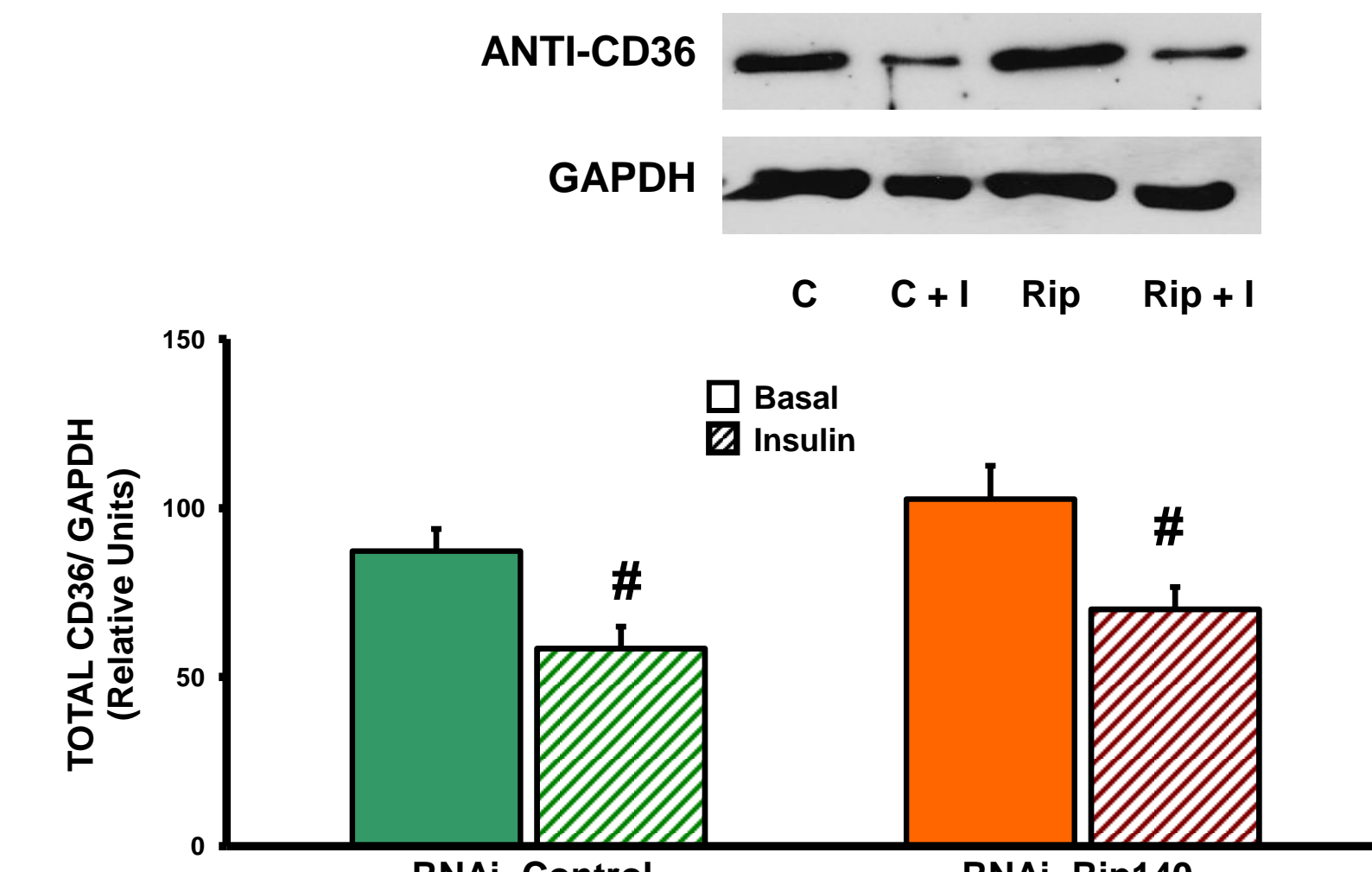
Total Nur77



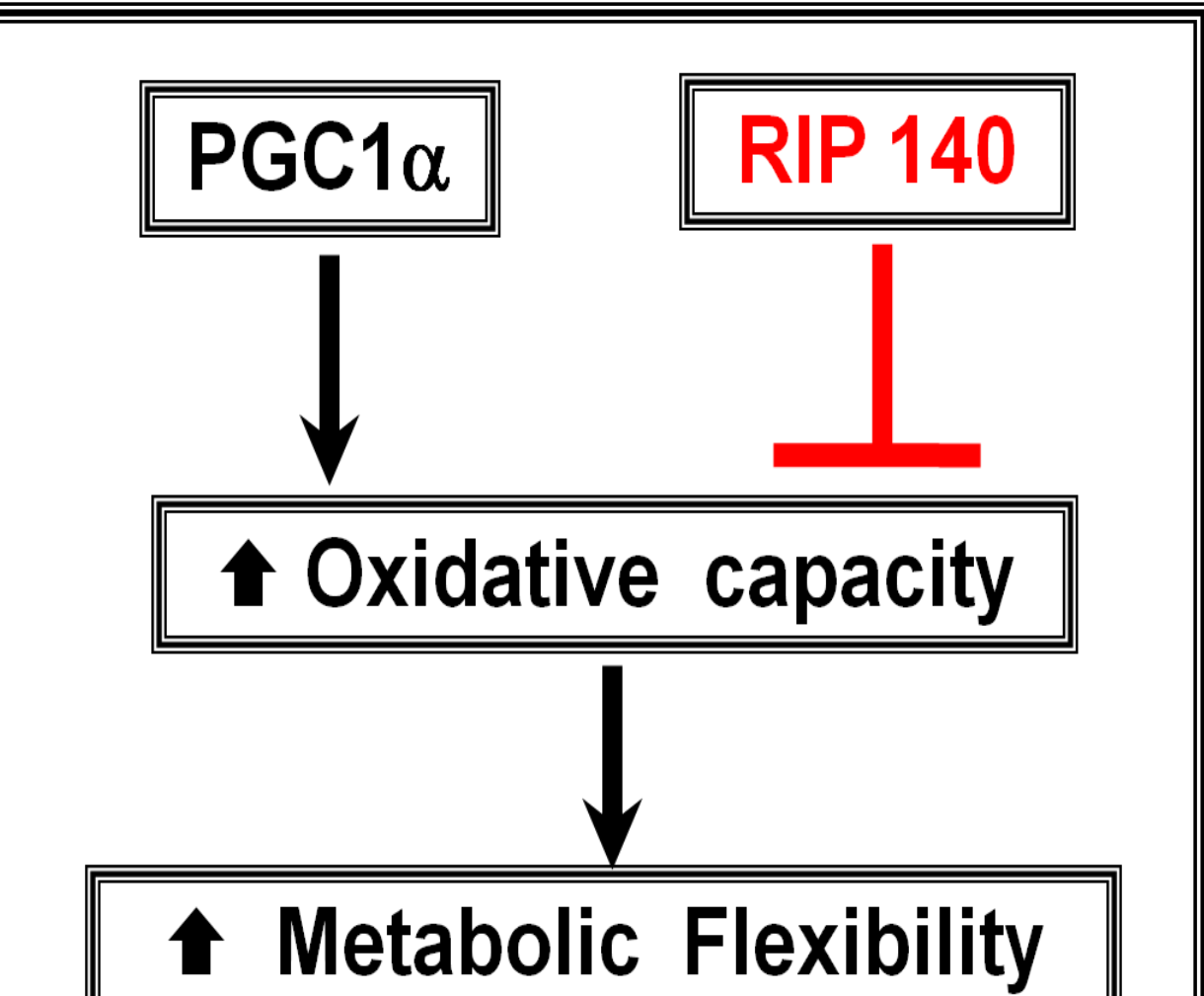
Total FGF21



Total CD36



Introduction



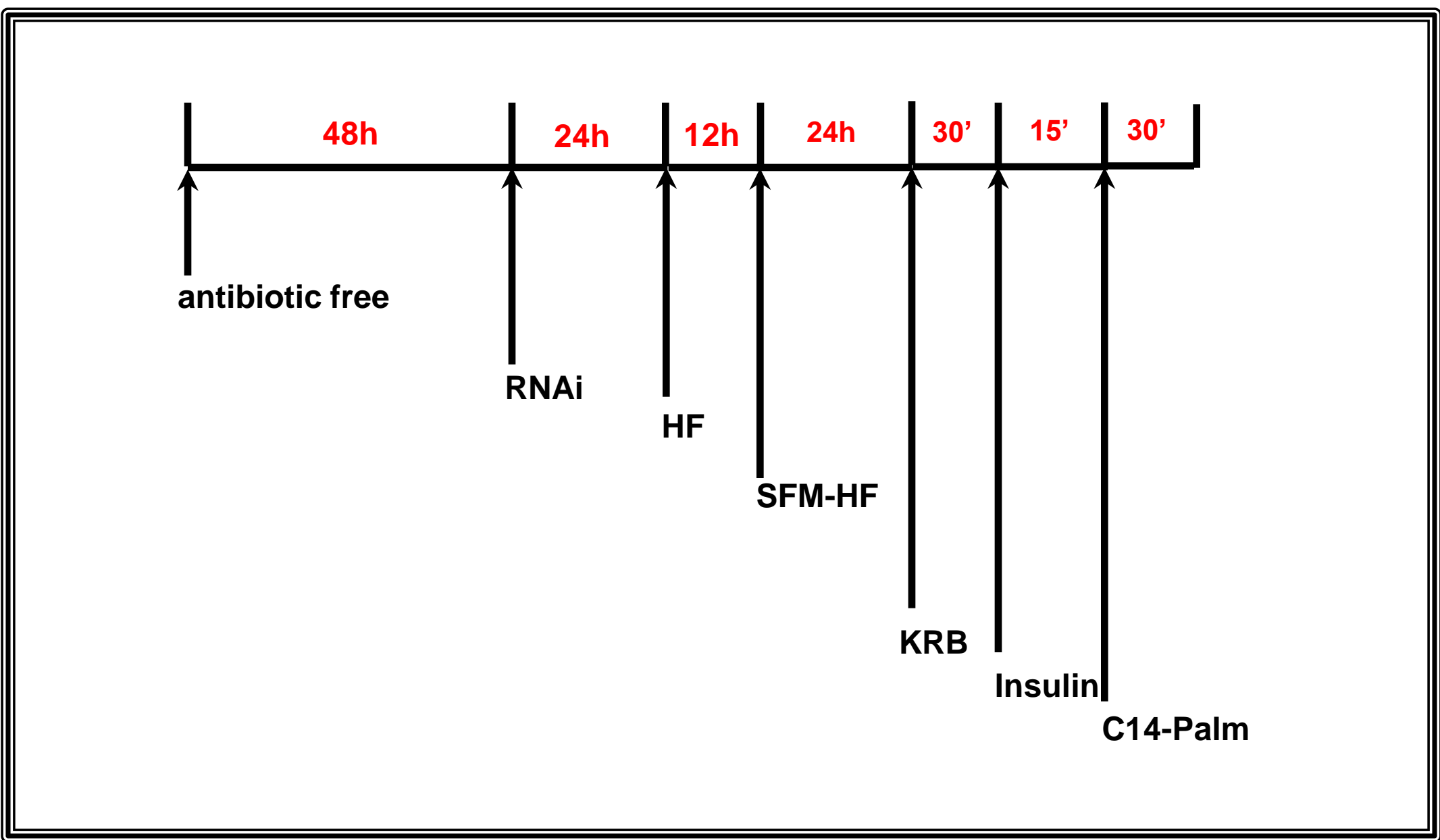
Purpose

To determine whether low RIP140 expression would improve insulin-sensitive muscle metabolism.

Hypothesis

Low Rip140 expression will improve insulin stimulated glucose uptake (GU), fatty acid uptake (FAU) and oxidation (FAO).

Experimental Design



Statistics

vs. respective Basal, $P<0.05$
& vs. respective RNAi Control, $P<0.05$
* vs. respective RNAi Control HF, $P<0.05$

		Basal	Insulin-Stimulated
		No Effect	No Effect
GU	Low Rip 140	No Effect	No Effect
	HF	Higher	Reversed Insulin Effect
FAU	Low Rip 140	No Effect	Restored Insulin Effect
	HF	Higher	No Insulin Effect
FAO	Low Rip 140	Lower	Reversed Insulin Effect
	HF	Lower	No Insulin Effect
FAO	Low Rip 140	Lower	Reversed Insulin Effect
	HF	Lower	No Insulin Effect

Low RIP140	Basal	Insulin-Stimulated
Total Nur77	No Change	No Change
FGF21	No Change	Lower
CD36	No Change	No Change

Acknowledgements

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