

Acylated and Unacylated Ghrelin Impact on Protein synthesis and Signaling Pathways of L6 Myotubes

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ABSTRACT

Ghrelin has recently become a hormone of interest in the fight against type two diabetes. Ghrelin is found in two forms (acylated vs. unacylated), with the acylated form of ghrelin being cited as diabetogenic purportedly due to its interaction with growth hormone secretion. Both the Acylated Ghrelin (AG) and Unacylated Ghrelin (UAG) forms are reported to have metabolic functions within skeletal muscle, despite having little expression of the only known ghrelin receptor known as the growth hormone secretagogue receptor 1a (ghsr-1a). The investigation into AG and UAG on skeletal muscle in type 2 diabetes (T2D) shows promise; however, the measures and dosing used to establish the impact of AG vs. UAG has varied widely, resulting in varied and sometimes contradictory results. **PURPOSE:** The purpose of this study was to establish the impact of AG or UAG on cultured myotubes in-vitro (100nM). **METHODS:** Differentiated, cultured L6 myotubes were treated for 48 hours with or without either AG or UAG. In the second 24-hour window, media was changed and enriched with 4% deuterium. Cells were harvested from each treatment group at 24 hours post deuterium enrichment and processed for protein synthesis and western blot protein analyses. **RESULTS:** Cells incubated with either AG or UAG doubled the content of phosphorylated AKT at Ser 473 (109% ↑ and 97% ↑, respectively; $p < 0.05$), implicating an increase in mTORC2 activity. 100nM AG or UAG also increased phosphorylation of GSK3 β (83% ↑ and 54% ↑, respectively; $p < 0.05$). However, AG had increased Phosphorylation of 4EBP1 when compared to control (80% ↑) while UAG did not, suggesting that mTORC1 was the predominate complex under that condition. Physiologically there were no differences of fractional synthesis rates among Control, 100nM AG or 100nM UAG. Interestingly, while protein synthesis data were similar among groups, the differences between mTORC1 vs. mTORC2 signaling may implicate that the impact of ghrelin might direct the types of proteins being manufactured. **CONCLUSION:** Results from this study indicate that 100nM of Ghrelin is sufficient to impact healthy skeletal muscle albeit by a yet to be defined mechanism. Further, our data suggest that both AG and UAG upregulate both mTOR pathways but that AG appears to favor mTORC1 as indicated by the hyperphosphorylation of 4EBP1. This increase in mTORC1 activity supports previous literature indicating that AG may be a diabetogenic hormone, and furthers the understanding of Ghrelin's impact on skeletal muscle metabolism and its involvement in development of diabetes.