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## INTRODUCTION

The steroid hormone 20-Hydroxyecdysone (20E) plays a key role in insect development through nuclear ecdysone receptors (EcRs) and at least one membrane receptor (DopEcR). Although mammals lack EcR, 20E displays pharmacological effects on mammals: for example, it stimulates protein synthesis and is marketed as a physical performance enhancer. Based on 20E physiological effects on muscle mass, a 20E based-drug termed BIO101 has been designed for the treatment/prevention of muscle pathologies, e.g. sarcopenia. However, the mechanism of action on mammals has not been elucidated. Thus, the goal of this study was to identify a receptor involved in 20E effects.

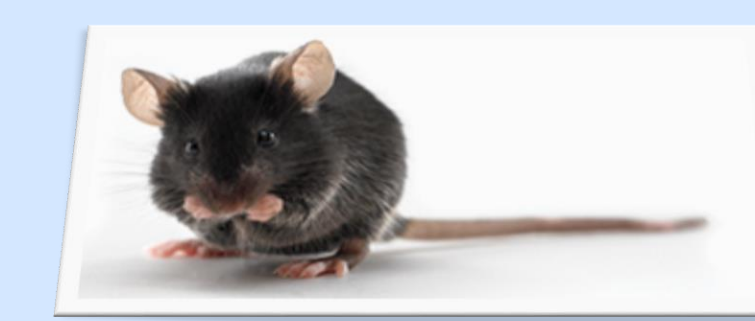
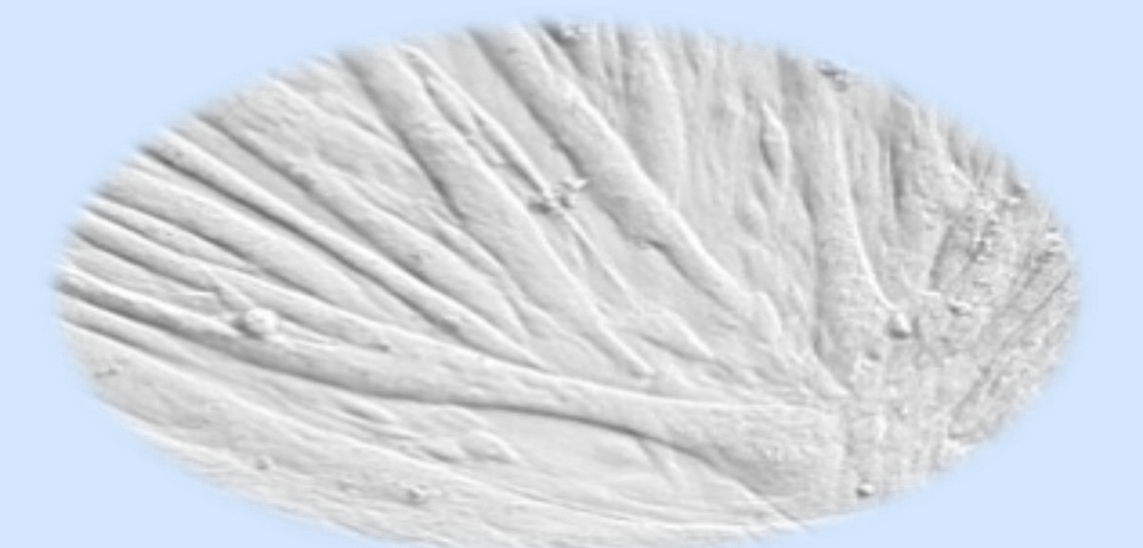
## MATERIAL & METHODS

**Cell line:** C2C12 cells were induced into differentiation by decreasing FBS in culture media for 5 days. Appropriate treatment was applied during 6h (for gene expression analysis) or 4 days (for immunofluorescence).

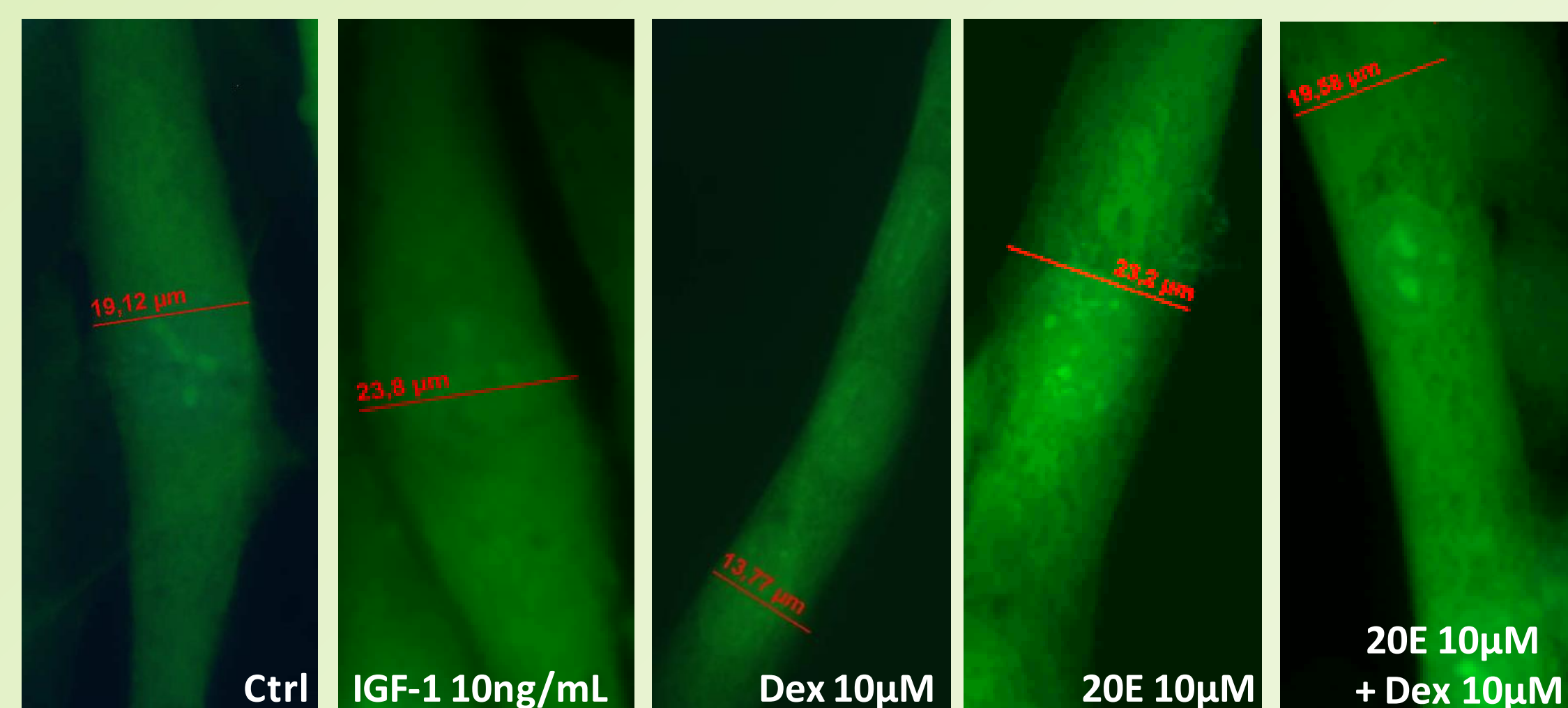
**Gene expression:** RNAs were extracted and purified using the phenol/chloroform method. Subsequently, RNAs were converted into cDNAs and gene expression was analyzed by q-PCRs.  $\beta$ -Actin was used as housekeeping gene.

**Immunofluorescence:** Cells were grown, differentiated and treated in 8 well chamber. At the end of incubation, cells are fixed in a solution of glutaraldehyde 2.5%/triton 0.1% for 1h at room temperature and covered by a mounting media. After 24h in a dark, cells were observed under microscope

**In vivo model:** c57Bl/6J under high fat diet treated with cyclodextrin (control) or 20E (5-50mg/kg) for 6 weeks.



## ANABOLIC EFFECT OF 20E ON C2C12 MYOTUBE SIZE

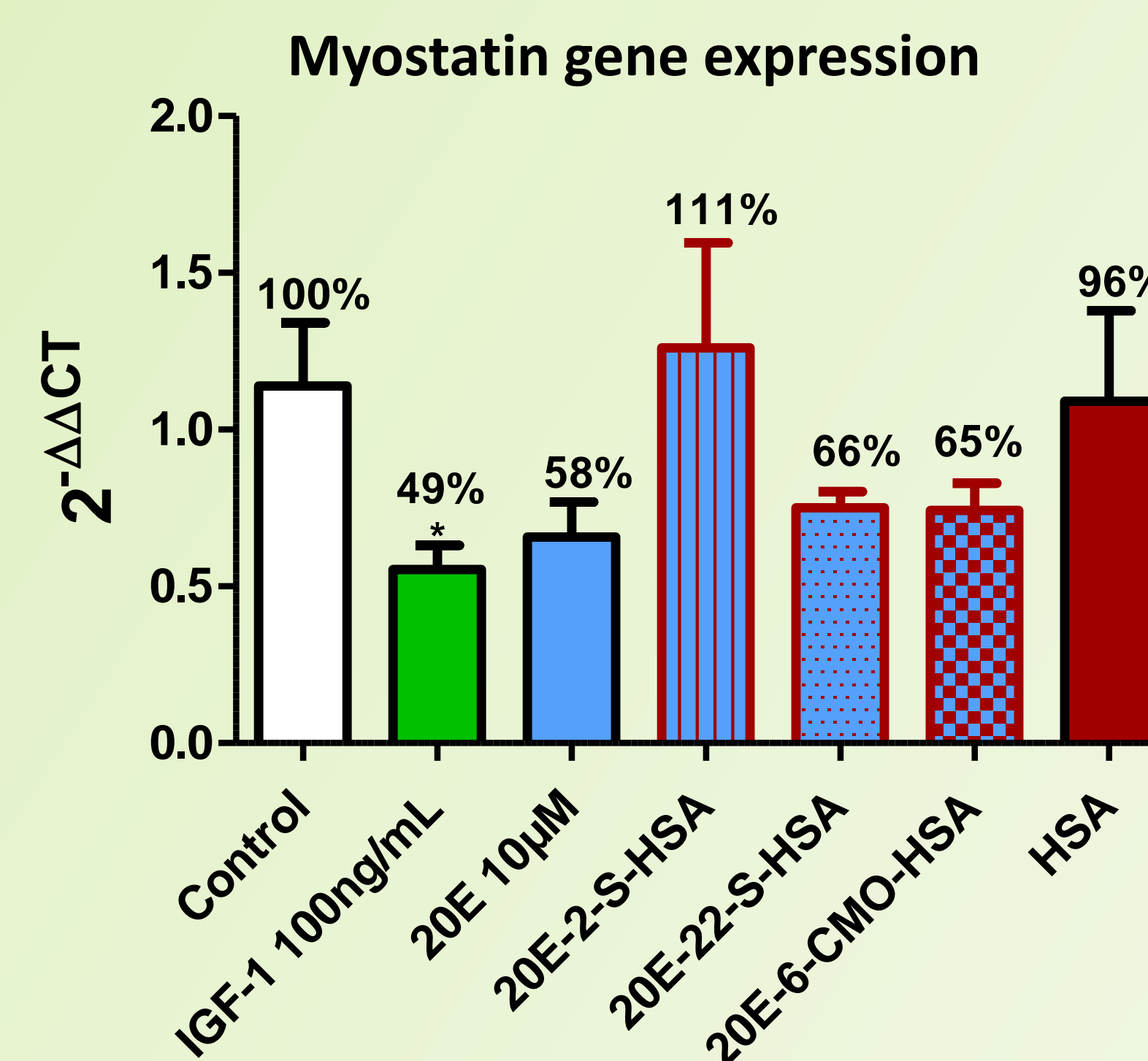


•10ng/mL IGF-1 treatment induced a significant enlargement of myotube diameter of C2C12 (+27%, p<0.001) and dexamethasone induced a significant decrease of myotube diameter (-25%, p<0.001) as expected and described in the literature.  
•20E 10µM, induced a significant enlargement of fiber diameter of C2C12 (+24%, p<0.001) and was able to counteract the dexamethasone effect.

KW & Dunn's test, \* p<0.05; \*\* p<0.01; \*\*\* p<0.001

## EVIDENCE FOR INVOLVEMENT OF A MEMBRANE RECEPTOR

We previously documented a dose-dependent inhibition of myostatin gene expression after treatment with 20E. In order to determine if this effect was mediated by a membrane receptor, we compared the effects of three 20E-protein conjugates respectively involving positions 2, 6 and 22 of the molecule on myostatin gene expression.

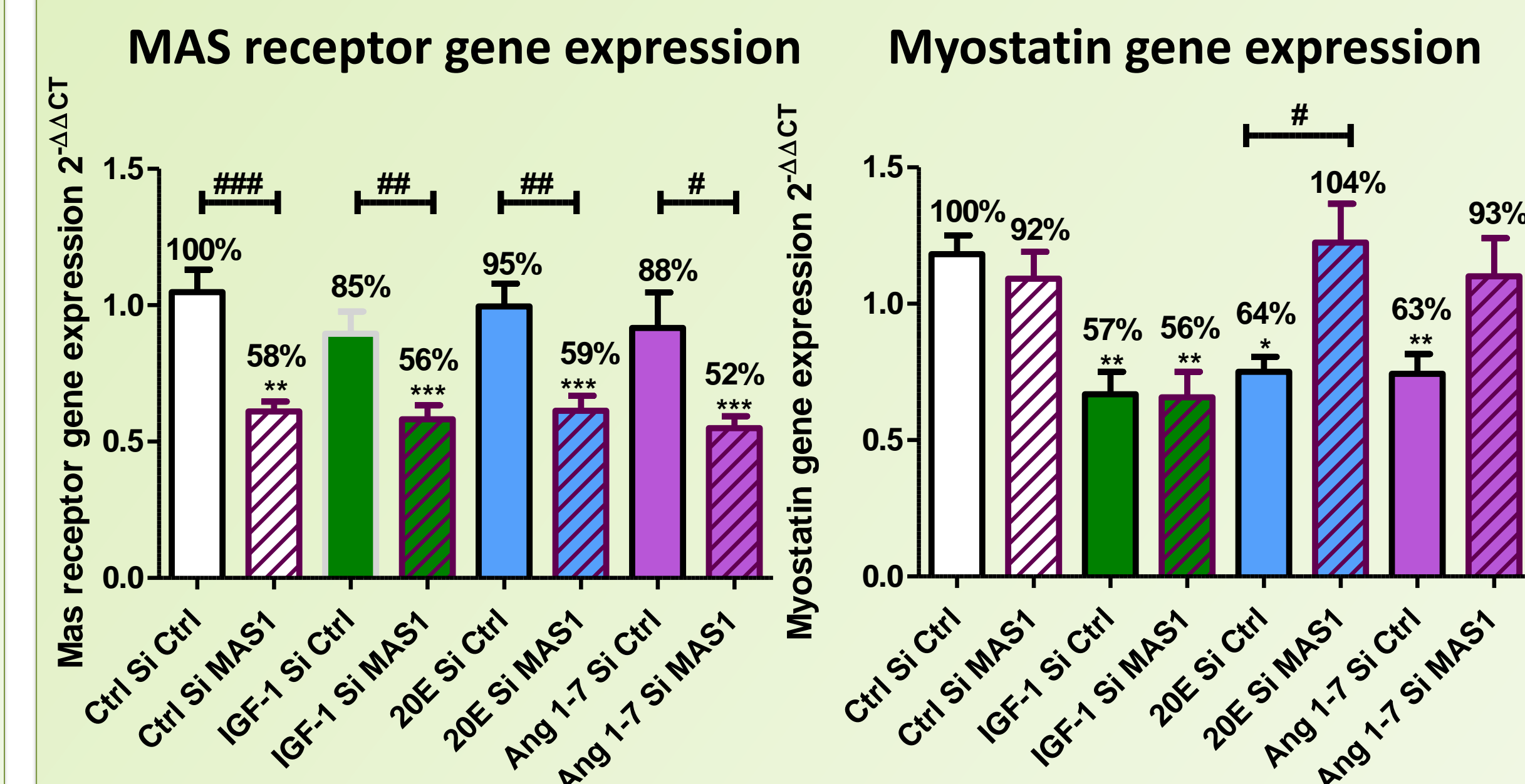


ANOVA & Dunnett's test, \* p<0.05; \*\* p<0.01; \*\*\* p<0.001

• We observed that two of them retained an activity close to that of free 20E, whereas the third one was inactive.  
• These results show that the presence of a bulky protein does not prevent 20E activity, provided that the A ring remains free.

## INVOLVEMENT OF MAS RECEPTOR IN MEDIATING 20E EFFECT

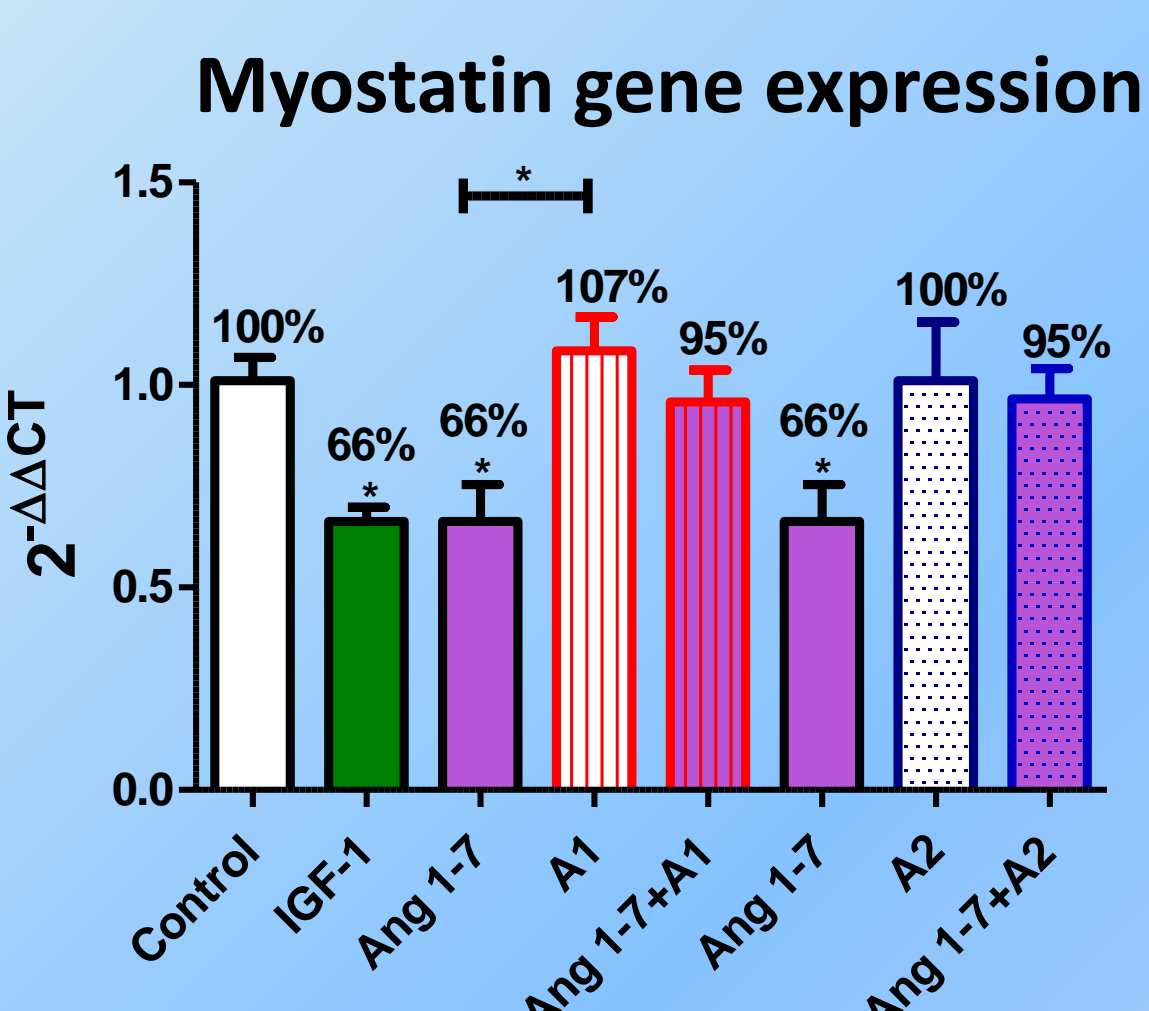
There are plenty of GPC receptors, thus to reduce our field of investigation, we performed a wide literature survey focusing on control of muscle cells, insulin sensitivity and fat mass gain. A set of 9 receptors was thus selected, and the only receptor which gave positive data was *Mas*, the receptor of angiotensin-(1-7)



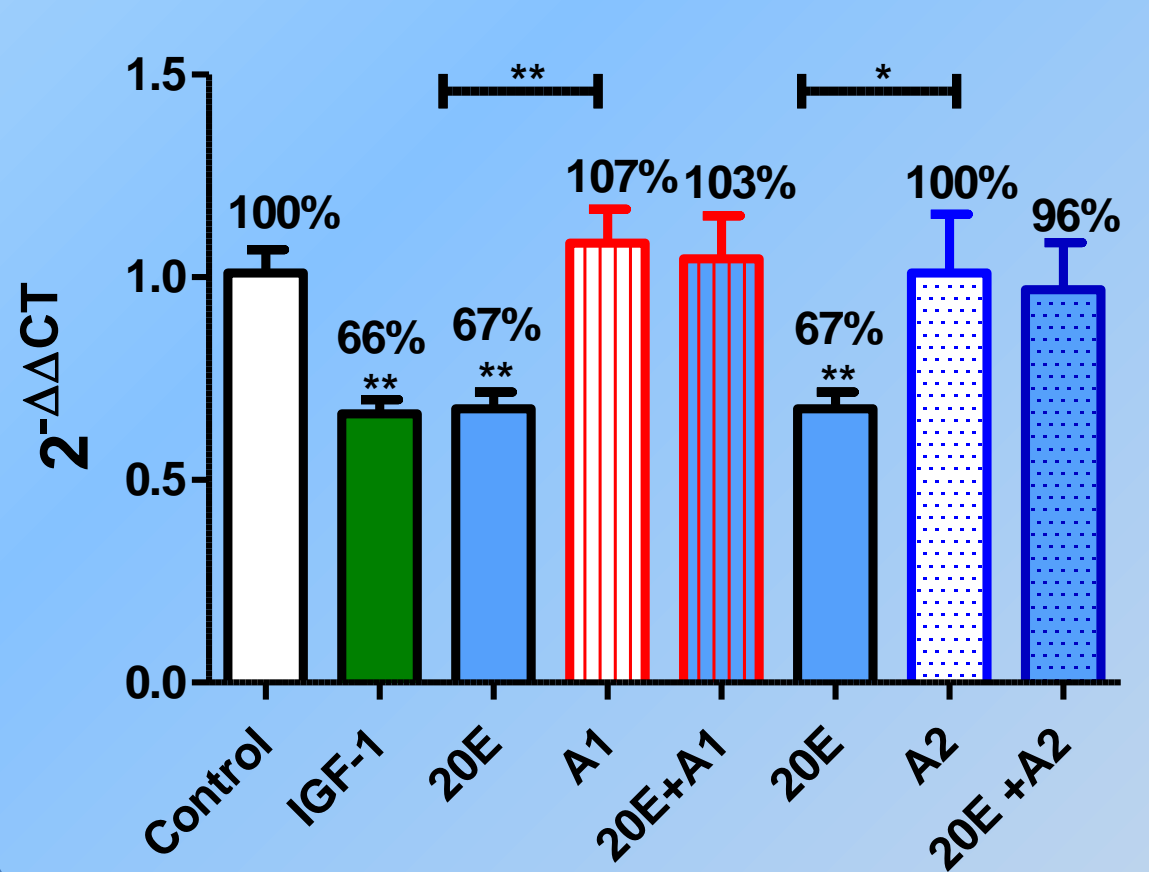
• Mas receptor down regulation by Si RNA led to significant decrease of MAS receptor gene expression in all transfected group by directed SiRNA versus scramble SiRNA.  
• IGF-1, 20E or Ang 1-7 inhibited significantly myostatin gene expression in cells transfected with scramble SiRNA.  
• Down-regulation of Mas receptor gene did not allow inhibition of myostatin gene after treatment with 20E or Ang 1-7.

*In vivo*, 20E oral treatment significantly increased protein content and decreased myostatin gene expression in *soleus* muscle, whereas several markers of myogenesis were enhanced.

## PHARMACOLOGICAL INHIBITION OF MAS RECEPTOR AND CONSEQUENCES ON 20E EFFECT



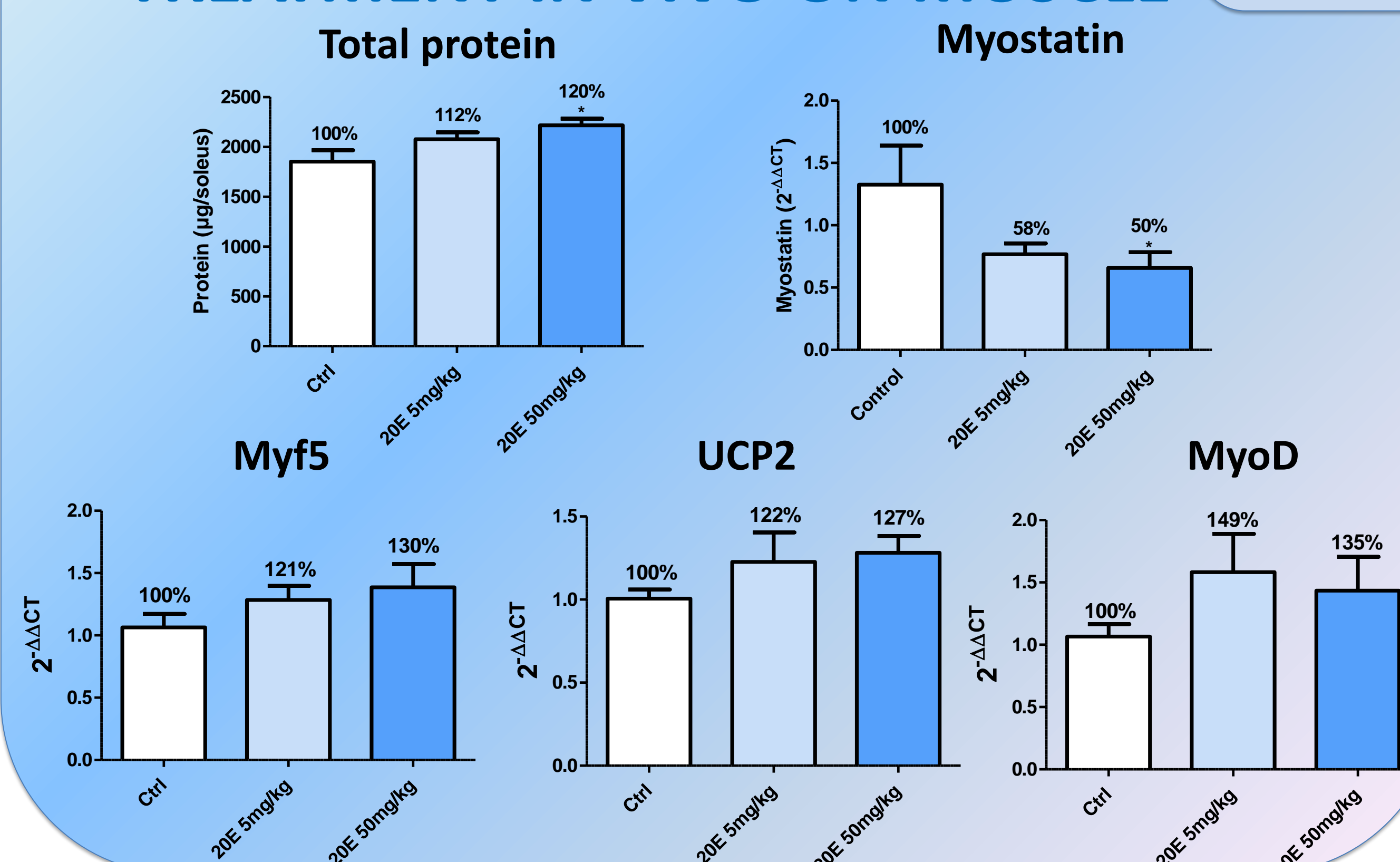
To confirm the importance of MAS receptor signaling in 20E mediating inhibition of myostatin gene expression, we performed pharmacological inhibition of this receptor.



• Angiotensin 1-7, as previously reported with 20E inhibited myostatin gene expression.  
• This inhibition was completely abolished when the molecules were incubated in the presence of Ang 1-7 antagonists (A1 or A2 10 µM).

ANOVA & Dunnett's test, \* p<0.05; \*\* p<0.01; \*\*\* p<0.001

## MOLECULAR EFFECT OF 20E TREATMENT IN VIVO ON MUSCLE



## CONCLUSION

20E was shown to enlarge fiber size *in vitro* and increase protein amounts *in vivo*. These effects are associated with the inhibition of myostatin gene expression. Cellular signaling studies showed that 20E involves the activation of MAS receptor. These results led us to design a clinical trial on obese sarcopenic patients with BIO101.