Evidence for autophagy attenuation during post-mortem maturation of hypertrophied muscle in myostatin deficient mice

Rim Nassar, Barbara Vernus, Gilles Fouret, Benedicte Goustard, François Casas, Lionel Tintignac, Isabelle Cassar-Malek, Brigitte Picard, Iban Seiliez, Arnaud Chatonnet, et al.

Evidence for autophagy attenuation during post-mortem maturation of hypertrophied muscle in myostatin deficient mice

Rim Nassar[1,2], Barbara Vernus[1], Gilles Fouret[1], Bénédicte Goustard[1], François Casas[1], Lionel Tintignac[3], Isabelle Cassar-Malek[4], Brigitte Picard[4], Iban Seliez[5], Arnaud Chatonnet[1], Aline Hamade[2], Fadia Najjar[2], Anne Bonnieu[1] et Béatrice Chabi[1]

1- INRA, UMR 866 Dynamique Musculaire et Métabolisme, Montpellier, France ; 2 - Laboratoire d'Innovation thérapeutique, Université Libanaise, Beyrouth, Liban; 3 - Département de Biomédecine, Université de Bâle, Bâle, Suisse; 4 - INRA, UMR1213 Herbivores, Saint-Genès-Champanelle, France; 5 - INRA, UMR1419 Nutrition, Métabolisme, Aquaculture, Saint Pée sur Nivelle, France.

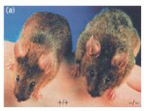
Introduction

The conversion of skeletal muscle into meat, i.e. maturation, is a complex process where muscle undergoes different biochemical and physiological changes (Ouali et al, 2006). In agronomic field, the study of these events is of particular interest, in order to improve the quality of the final product put on the market. If the characterization of the proteolytic mechanisms involved in skeletal muscle maturation is still ongoing, the participation of autophagy in this process is still under debate. The aim of the study was therefore to assess the involvement of autophagy during skeletal muscle maturation and to study the interaction between autophagy and myostatin, a negative regulator of skeletal muscle mass (Mc Pherron et al, 1997), within a 72h postmortem (PM) time frame in mice.

Methods and model validation

Animal model

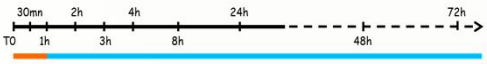
Healthy 6 month-old Mstn$^{+/+}$ (WT) and Mstn$^{-/-}$ (Mstn KO; Grobet et al, 2003) male mice (n=72 for each genotype) were used in this study.



WT KO Mstn

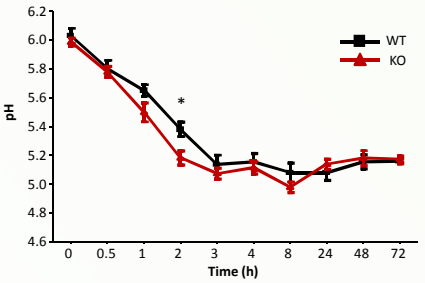
Muscle sampling

Mice were sacrificed using cervical dislocation. After decapitation and bleeding, mice were stored at room temperature (22±2°C) during 1h and then at 4°C until the dissection time.



Longissimus dorsi and gastrocnemius muscles were harvested and used for pH and western-blotting analyses respectively.

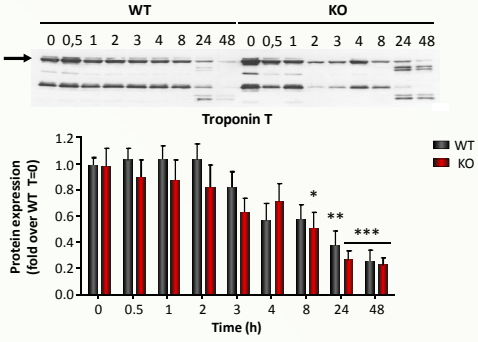
1) pH evolution in the Longissimus muscle



n=8 for each time point x genotype, two-way ANOVA, main effect * $p<0,05$ WT vs. KO

Postmortem muscle pH decreased faster in Mstn KO mice compared to WT mice.

2) Myofibrillar protein degradation



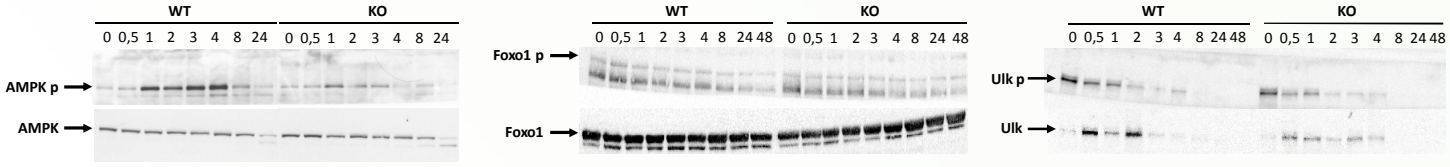
n=8 for each time point x genotype, two-way ANOVA, *$p<0,05$; ** $p<0,01$; *** $p<0,001$ vs. respective T=0

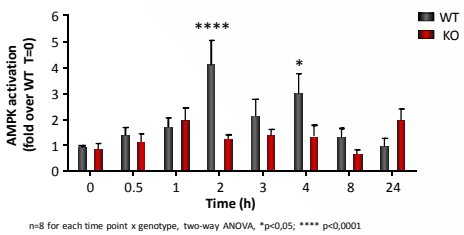
Myofibrillar proteins (e.g. Troponin T) showed a significant and progressive degradation over the 48h PM period and is more pronounced in gastrocnemius muscle of Mstn KO mice compared to WT mice.

=> 3) Following PM, autophagy was assessed in skeletal muscle from the two genotypes using signaling pathways and autophagic flux analyses.

Results

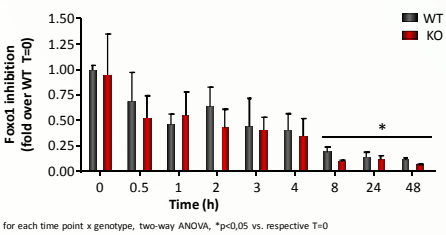
1) Phosphorylation state of proteins involved in autophagic signaling pathway (AMPK, FOXO and ULK)





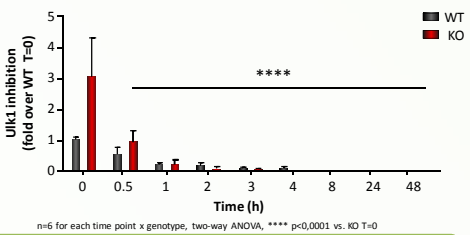
n=8 for each time point x genotype, two-way ANOVA, *p<0,05; **** p<0,0001

Activation of AMPK is greater in WT mice compared to Mstn KO mice Energetic stress or/and Autophagy ?



n=6 for each time point x genotype, two-way ANOVA, *p<0,05 vs. respective T=0

Inhibition of Foxo1 is relieved in the late PM interval (8h PM) in both genotypes => Activation of autophagy and Ub proteasome system

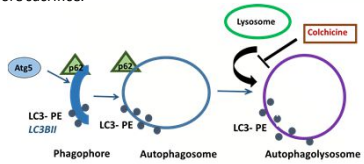


n=6 for each time point x genotype, two-way ANOVA, **** p<0,0001 vs. KO T=0

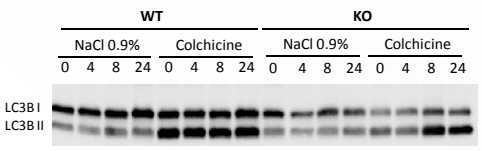
The decrease in ULK1 (Ser757) phosphorylation in Mstn KO mice suggests an early activation (1h PM) of autophagy

2) Autophagy flux measurement

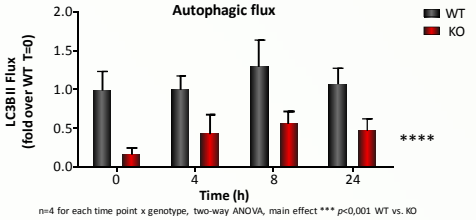
Autophagic flux was monitored using autophagy blockade with four colchicine injections (i.p., 0.1 mg/ml in NaCl 0.9%, every 12h) before sacrifice.



Autophagic flux is calculated as $LC3BII_{colchicine} - LC3BII_{NaCl\ 0.9\%}$ (*Jeong-Sun Jun et al, 2010*)



Colchicine administration resulted in LC3BII accumulation (NaCl 0.9% vs. colchicine)



n=4 for each time point x genotype, two-way ANOVA, main effect *** $p<0,001$ WT vs. KO

Basal and *postmortem* autophagic fluxes are greater in WT versus Mstn KO mice.

Conclusion

In conclusion, our data showed that autophagy is preserved during skeletal muscle PM maturation, but to a lower extent in Mstn-deficient mice, suggesting a relationship between myostatin and autophagy. This work will be completed with microscopic analyses of skeletal muscle tissue sections to visualize *in situ* autophagic events.

DMEM, UMR 866
2 Place Pierre Viala
34060 MONTPELLIER Cedex
http://www6.montpellier.inra.fr/dmem/

27-29 Novembre 2017 - Université Paris Descartes