Myostatin deficiency in skeletal muscle alters the lipids composition of mitochondrial membranes

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Background

Myostatin (Mstn) is a negative regulator of skeletal muscle growth

Natural mutation or targeted inhibition in mstn gene

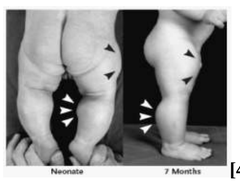
⇒ *results in twofold increase in skeletal muscle mass (**hypertrophic phenotype**) in some species*

⇒ *is considered as a promising treatment for various muscle-wasting disorders.*


D'après Grobet et al. 1997 [1]


D'après Mosher et al. 2007 [2]


D'après McPherron et al. 1997 [3]



[4]

Beyond muscle hypertrophy, mstn knock-out mice (KO) showed

- **Increased muscle Fatigability [5]**
- **Decreased in number of mitochondria and mitochondrial respiratory function [6]**
- **A loss of fat mass [7]**

In skeletal mucles

Muscles Membranes (Maintain architecture of the muscle fiber) **and** Mitochondrial Membranes (Contain respiratory chain) { **Consisted mainly lipids and phospholipids**

"Does exist an alteration of the lipid composition of muscle and mitochondrial membranes in KO mstn mice that could participate in the metabolic and contractile alterations observed in this model ?"

Aim of the study

To Characterize the lipid composition and the lipid metabolism in muscle and mitochondrial membranes in KO mstn mice.

Lack of Mstn led to decline in Fat membrane transporter in KO mstn muscle

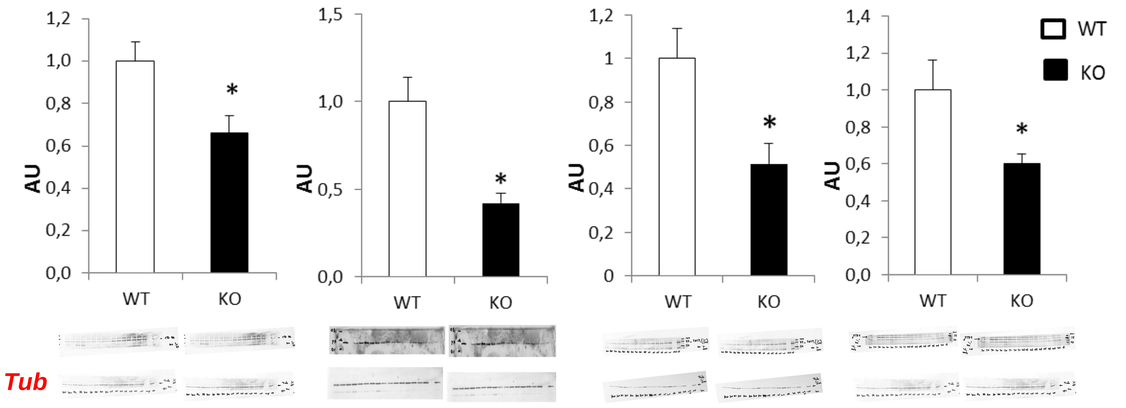


Figure1: *Reduce of FAT/CD36, FABP3, FATP1 and FATP4 evaluated by Western blots in KO mstn Gastrocnemius referenced to Tubulin (n=8 for each protein) compared to Wild type mice (n=7 for each protein). Values are shown as means ± SEM. P-Values were calculated using the parametric test T-student (*p< 0.05).*

Lack of Mstn led to decreased oxidative pathway of lipids in KO mstn muscle

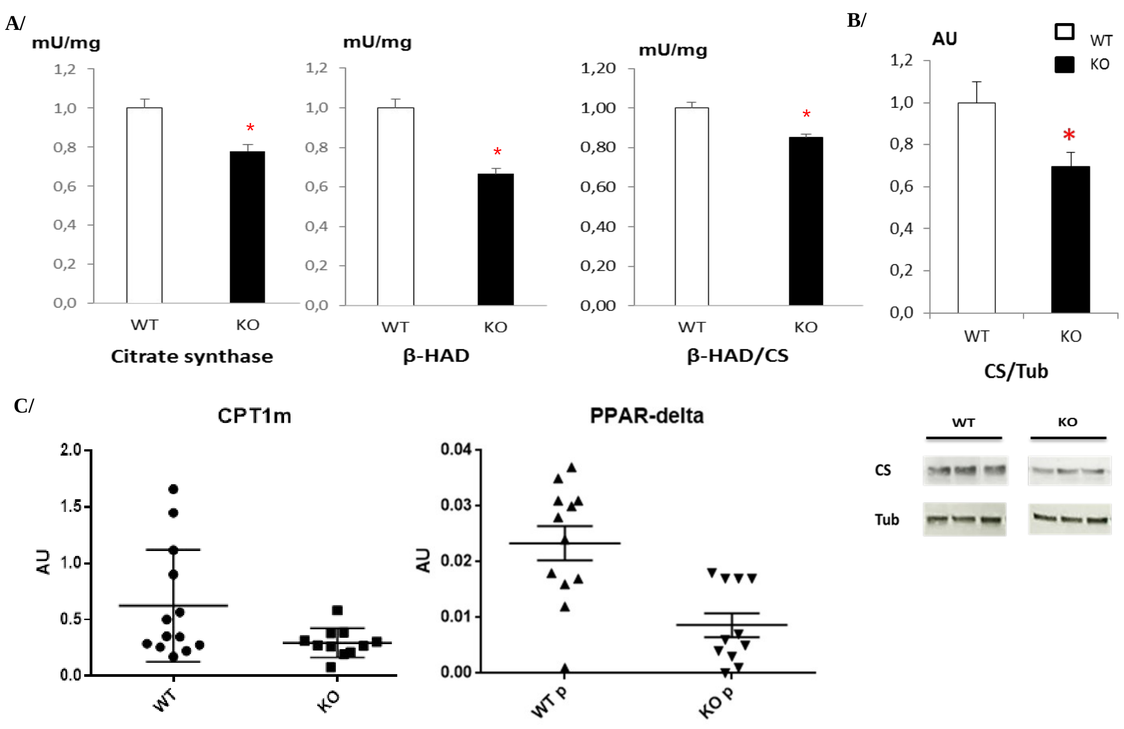


Figure2: *Decline of oxidative pathway of lipids in KO mstn Gastrocnemius*
A/ *Reduce of Citrate synthase and beta-HAD activities evaluated by spectrophotometry in KO mstn muscle (n=7) compared to wild type muscle (n=7)* ***B/*** *Citrate synthase quantity evaluated by Western blot declined in KO mstn muscle compared to Wild type muscle.* ***C/*** *CPT1m and PPAR-delta gene expression evaluated by RT-PCR in KO mstn muscle and wild type muscle. Values are shown as means ± SEM. P-Values were calculated using the parametric test T-student (*p< 0.05).*

1 Grobet et al., 1997, Nat Gene ,17(1):71-4.
2 Mosher et al., 2007, PLoS Genet. 2007;3(5):79
3Mcpherron et al.,1997, Nature, 387, 83-90.
4Shuelke et al., 2004, N Engl J Med. ;350(26):2682-8.
5 Amthor et al.,2007, PNAS, 104(6) 1835–1840.
6 Ploquin et al.,2012, American physiological society, 302(8), 1000-1008.
7 Zhang C et al., 2012, Diabetologia, 55(1):183-93.
8 Paradies et al, 2014, Biochim Biophys Acta; 1837(2014):408-17

Lack of Mstn induced a decreased of lipogenesis in KO mstn muscle

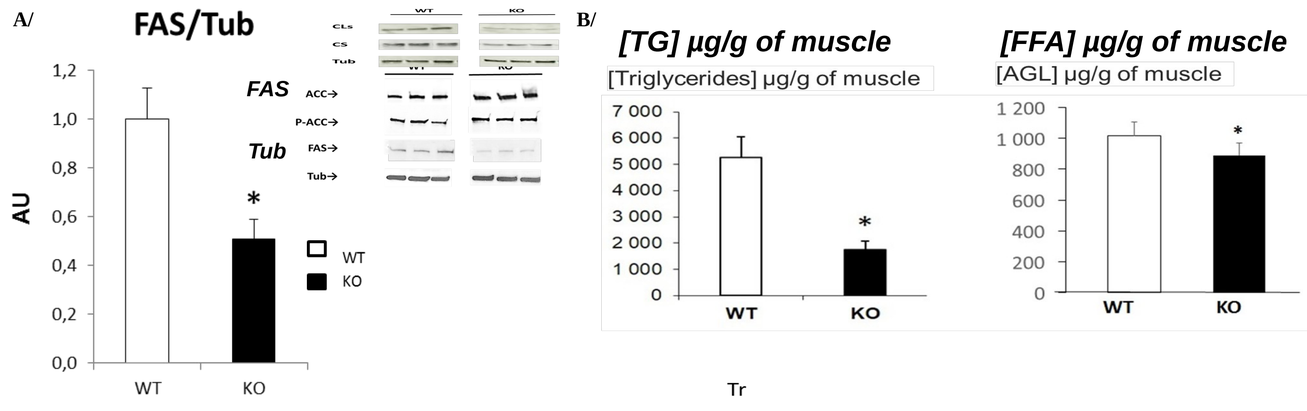


Figure 3: *Decrease on lipogenesis pathway in KO mstn muscle.* ***A)*** *Fatty acid synthase (FAS) quantity evaluated with western blot declined in KO mstn (n=8) gastrocnemius compared with Wild type (n=8).* ***B)*** *Triglyceride (TG) and free fatty acids (FFA) quantity evaluated with thin layer chromatography decreased in KO mstn muscle (n=7) compared with Wild type (n=7). Values are shown as means ± SEM. P-Values were calculated using the parametric test T-student (*p< 0.05).*

Modification of lipids composition of muscle and mitochondrial membrane in KO mstn muscle

Fatty acids composition of muscle:

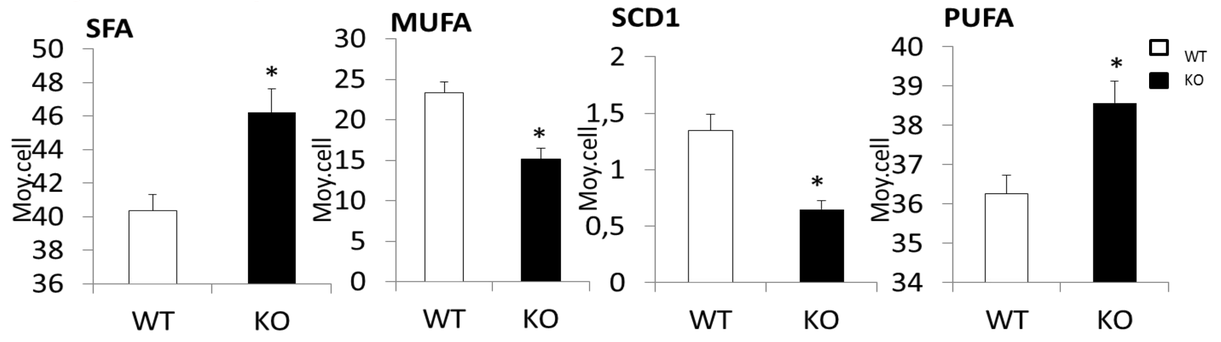
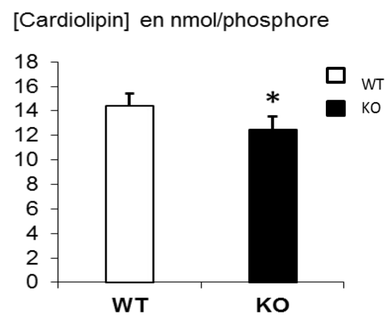


Figure 4: *Fatty acids distribution in Wild type and KO mstn muscle evaluated by gas chromatography. Increase of SFA,(Saturated Fatty Acids) decrease of MUFA (Mono unsaturated Fatty acids) associated with a decrease of SCD1 (Delta9 desaturase) index and a increase of PUFA in KO mstn muscle (n=7) compared with wild type (n=8). Values are shown as means ± SEM. P-Values were calculated using the parametric test T (*p< 0.05).*

Phospholipids composition of mitochondrial membrane :



No change in the concentrations of major phospholipids PC (phosphatidylcholine) and PE (phosphatidylethanolamine).

⇒ ***We showed a significant reduction (13%) of Cardiolipin in mstn KO mitochondria.***

This result could be associated with the alteration of mitochondrial function observed in KO mstn mice.. Indeed, cardiolipin plays a fundamental role in the stabilization of the respiratory chain and thus in mitochondria function. (8)

Figure 5: *Decrease of Cardiolipin content in mitochondrial membrane of Gastrocnemius muscle of WT muscle (n=8 for all phospholipids) and KO mstn muscle (n=8 for all phospholipids). Values are shown as means ± SEM. P-Values were calculated using the parametric test T-student (*p< 0.05).*

Synthetic Pathways of cardiolipin

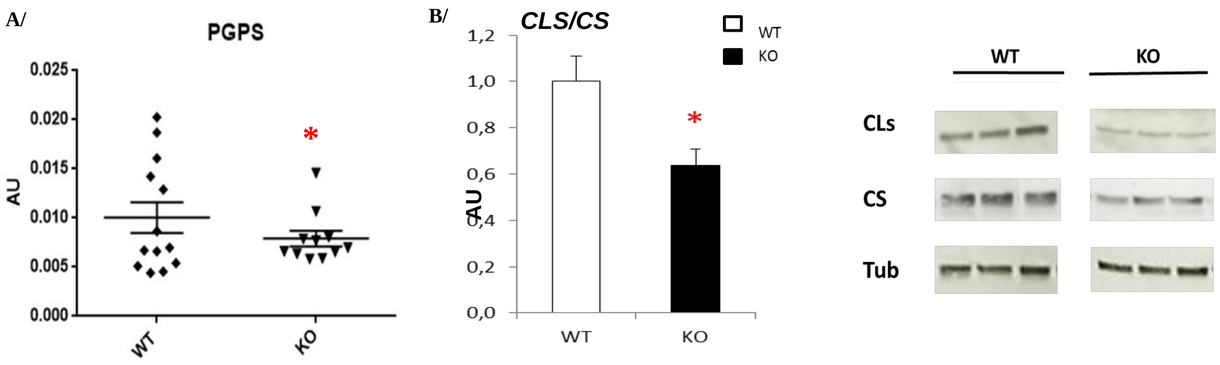
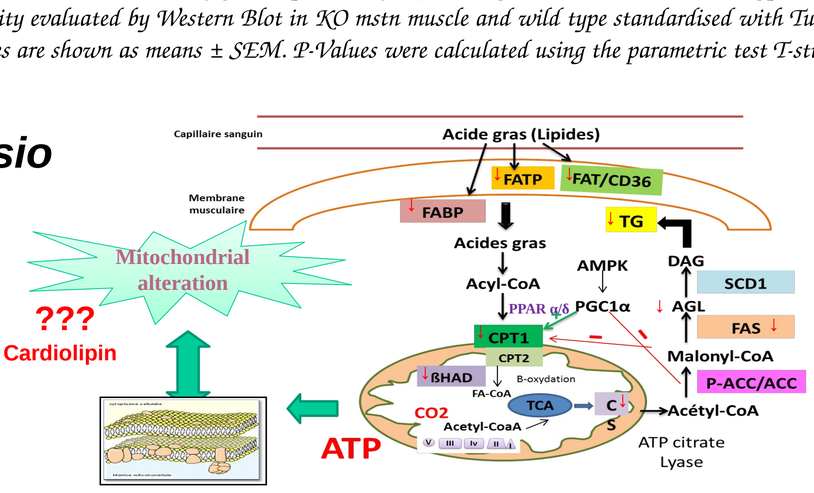


Figure 6: *Enzyme of cardiolipin synthetic route.* ***A/****Decline of gene expression of PGPS enzym in KO mstn and wild Type muscle.* ***B/****Decrease of Cardiolipin synthase quantity evaluated by Western Blot in KO mstn muscle and wild type standardised with Tubulin and citrate sythase in mitochondria. Values are shown as means ± SEM. P-Values were calculated using the parametric test T-student (*p< 0.05).*

Discussion/Conclusion



In this study, we demonstrated a decrease in mitochondrial cardiolipin content, in relation with a decrease in PGPS and cardiolipin synthase gene expressions. Overall, our results demonstrate that mstn deficiency reduced lipogenesis and lipid oxidation and alters the lipid composition of muscle and mitochondrial membranes, with a ***decrease in cardiolipin mitochondrial content.*** *This can be the cause of the mitochondrial function alteration observed in KO mstn muscle. In this regard, many research suggest the possibility to modulate the membrane phospholipids composition in mitochondria and muscle using physical training. Next study will be devoted to* ***modulate the phospholipids composition in KO mstn mitochondria using endurance training, and evaluate its consequences on mitochondrial function.***

ACKNOWLEDGEMENTS
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