Combined effects of BIO101 on anabolism and mitochondrial function in skeletal muscle cells

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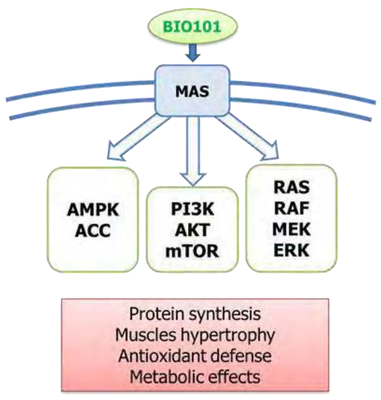
Introduction

- Muscle wasting disorders, including cachexia and sarcopenia, are multifactorial diseases which contribute to overall physical frailty. They represent a worldwide health challenge with limited therapeutic options. Many cellular factors have been identified which maintain normal muscle function. Cell metabolism is influenced by AKT/mTOR and mitochondrial biogenesis in parallel with physical activity and contributes to maintaining muscle mass and strength. Age-related deregulation of these mechanisms leads to muscle wasting.
- Ecdysteroids are natural compounds found in insects and plants which increase protein synthesis in mammals, improving skeletal muscle strength and endurance.
- The drug candidate BIO101 is a pharmaceutical-grade preparation of the ecdysteroid 20-hydroxyecdysone purified from *Stemmacantha carthamoides*. In animals receiving chronic oral doses of BIO101, skeletal muscles exhibited higher protein content and significantly reduced myostatin gene expression.
- BIO101 is the API of Sarconeos currently being tested in a clinical IIb trial in sarcopenic patients.
- The aim of this study was notably to characterize the effects of BIO101 on energy metabolism in C2C12 myocytes and in primary human muscle cells.

Hypothetical mechanism of action



Methods

Cell lines: C2C12 murine myoblasts and AB1167C20FL primary human muscle cells were induced to differentiate for 6 days. Appropriate treatment was administered for the final 3 days.

Immunofluorescence: Cells were grown, differentiated and treated on 8-well chamber slides. Then, the cells were fixed with 2.5% glutaraldehyde/0.1% Triton X-100, covered by DAPI-containing mounting medium. MHC, Mitotracker and DAPI staining were performed using standard procedures. After 24h in the dark, myotubes were observed under a fluorescent microscope. Immunofluorescence intensity was calculated using ImageJ software.

Western blot: Cells were lysed, equal amounts of proteins were electrophoresed on 4-12% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% non-fat milk and incubated with specific antibodies overnight. Immunostaining was visualized using ECL. Band intensity was quantified using ImageJ software.

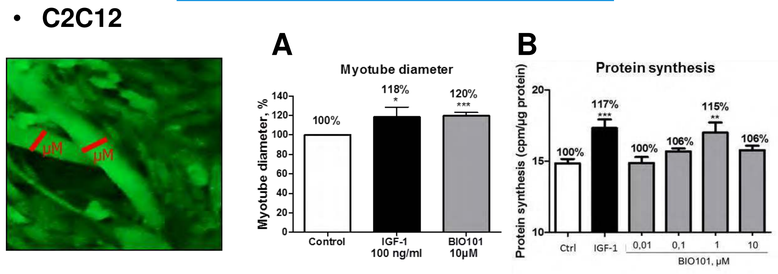
OCR measurements: Oxygen consumption was recorded using a Seahorse XF24 Analyzer.

***In vivo* studies.** Old (22-month) C57Bl6/J female mice were treated orally for 14 weeks with either vehicle or BIO101 (50 mg/kg). After treatment, the animals were tested for functional activity *in toto* (Vmax). Muscles were collected after sacrifice for further analysis

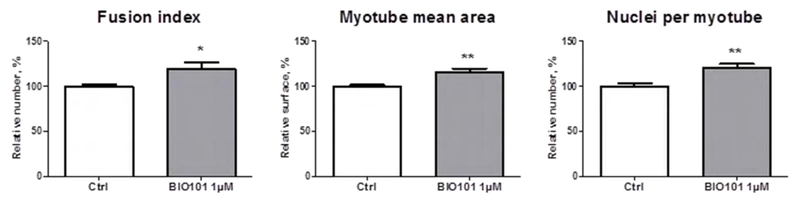
Results

Anabolic effects of BIO101

- **C2C12**



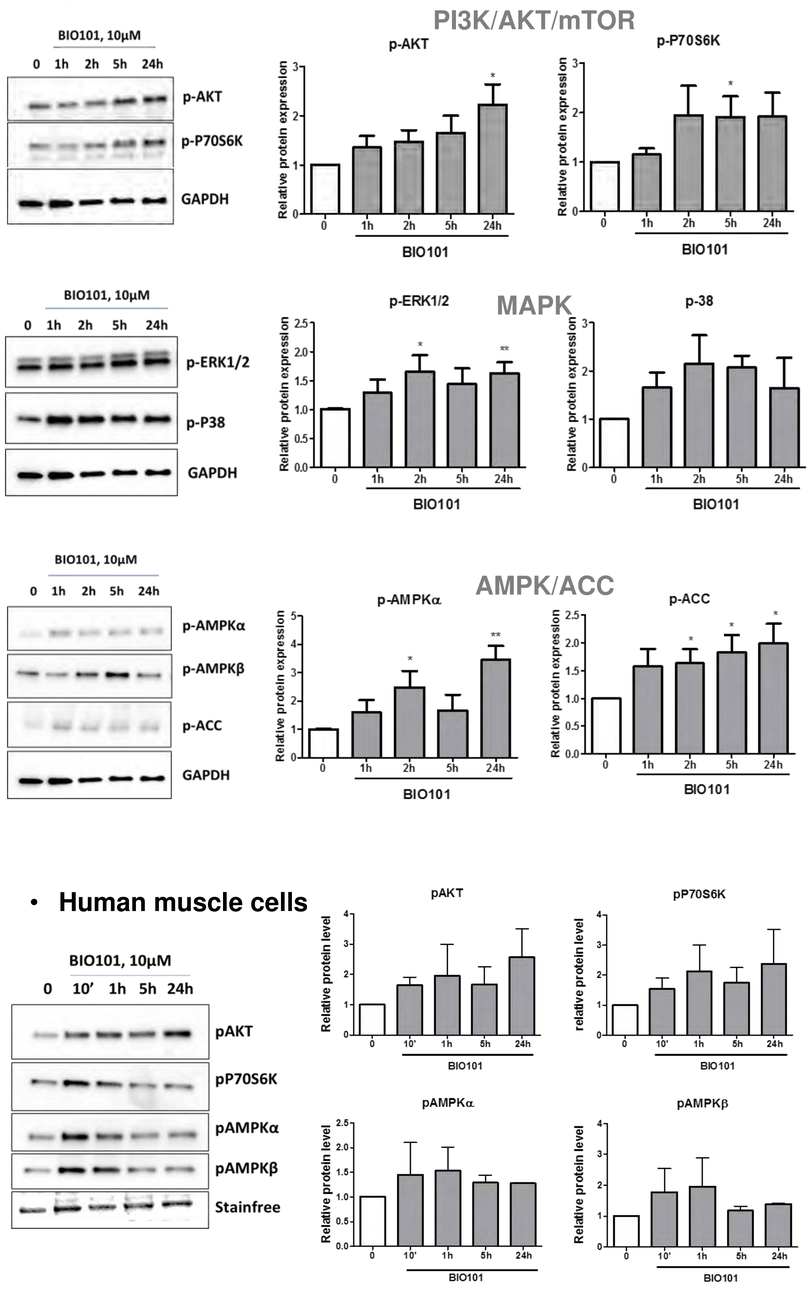
- **Human muscle cells**



→ *BIO101 increases protein synthesis consistently with an hypertrophy of myotubes*

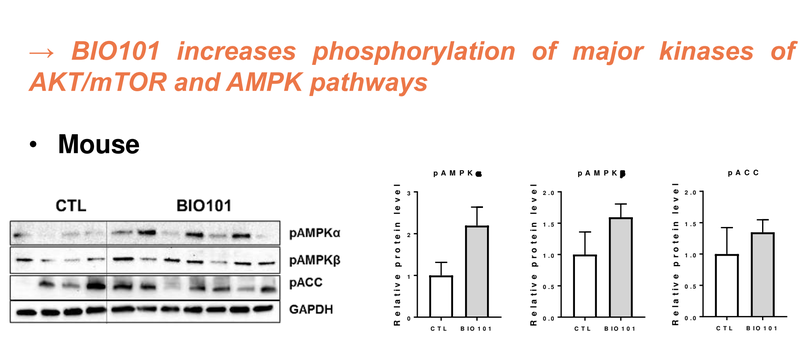
Effects of BIO101 on intracellular signaling

- **C2C12**



→ *BIO101 increases phosphorylation of major kinases of AKT/mTOR and AMPK pathways*

- **Mouse**

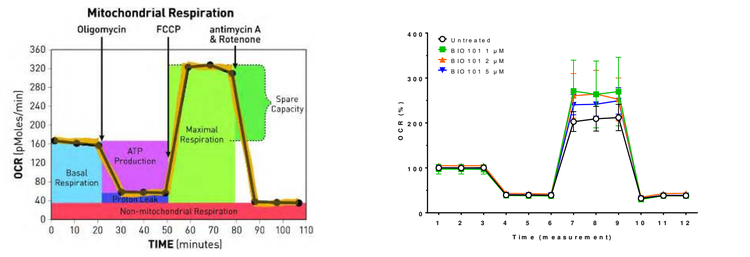


Chronic oral administration of BIO101 was responsible for a significant increase in animal physical performance when compared to old untreated animals.

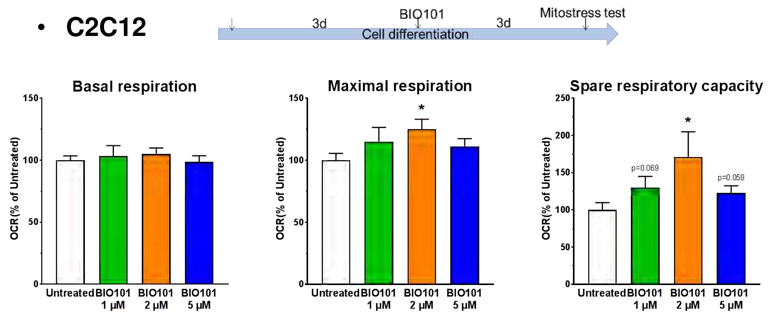
→ *In gastrocnemius muscles of animals treated with BIO101 AMPK pathway activity tends to be increased*

Results

Effects of BIO101 on oxygen consumption

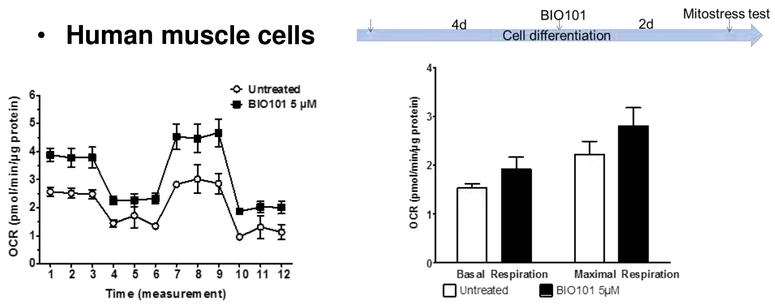


- **C2C12**



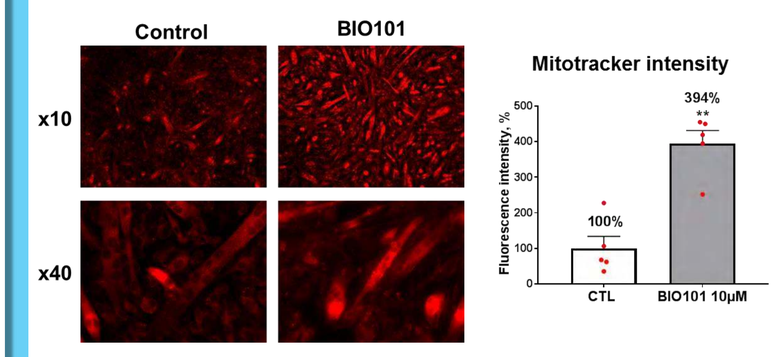
→ *BIO101 treatment for 72 hours during differentiation increases mitochondrial respiration of C2C12 cells*

- **Human muscle cells**



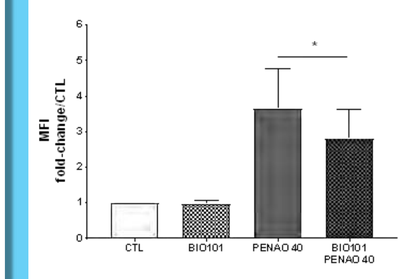
→ *BIO101 treatment for 48 hours during differentiation increases mitochondrial respiration of AB1167C20FL human muscle cells*

BIO101 effects on mitochondrial biogenesis



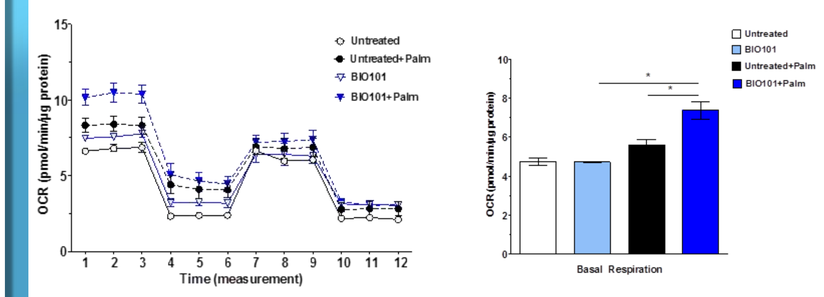
→ *BIO101 treatment for 72 hours during differentiation increases mitochondrial content of C2C12*

Antioxidant properties of BIO101



→ *BIO101 treatment lowers reactive oxygen species (ROS) levels in cells subjected to oxidative stress with PENAO*

BIO101-induced metabolic switch



→ *BIO101 facilitates the use of fatty acids as an energy supply when glucose is limited. These data suggest that metabolic flexibility is improved by BIO101 in muscle cells*

Conclusions

Overall, the beneficial properties of BIO101 on muscle function rely on both anabolic and mitochondrial effects:

- **Hypertrophy of myofibers in mouse and human muscle cells associated with activation of AKT/mTOR and AMPK pathways**
- **Increase in mitochondrial biogenesis and respiration**
- **Improvement of energy metabolism flexibility in favor of FA consumption**
- **Antioxidant capacity**

These results support the clinical development of BIO101 in patients with sarcopenia