**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 were identified to maintain normal muscle function. Cell metabolism influenced by AKT/mTOR and mitochondrial biogenesis in parallel with physical activity contribute to maintaining of muscle mass and strength. Age-related deregulation of these mechanisms leads to muscle wasting.

**About ecdysteroids:** The steroid hormone (20E) plays a key role in insect development through nuclear ecdysone receptors (EcRs) and at least one membrane receptor (DopECR). 20E also displays pharmacological effects on mammals, where it stimulates protein synthesis although EcR were not found in mammals.

**About BIO103:** BIO103, ecdysteroid derivative, is the product of hemisyntesis associated with the screening of more than 100 derived molecules. This screening had the object of selecting a molecule with improved activity and bioavailability.

The aim of this study was to characterize a new small molecule, BIO103, *in vitro* on myocytes and *in vivo* on a mouse model designed to analyze the effects of aging and muscle disuse.

![Proposed mechanism of action](image)

**Methods**

**Cell line:** C2C12 murine myoblasts were induced for differentiation for 5 days. Appropriate treatment was administered for 5h (for gene expression analysis) or 4 days (for fluorescent microscopy).

**Gene expression:** Total mRNA was extracted and purified using Trizol method: mRNAs were reverse-transcribed into cDNAs and Myostatin gene expression was analyzed by quantitative RT-PCR. HPRT was used as housekeeping gene.

**Immunofluorescence:** Cells were grown, differentiated and treated on 8 well chamber slides. Then the cells were fixed with glutaraldehyde 2.5% /triton 0.1%, covered by DAPI-containing mounting medium. After 24h in the dark, myotubes were observed under fluorescent microscopy.

**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. Bands intensity was quantified using ImageJ software.

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

**In vivo studies:** Adult (12 months) and old (22 months) C57Bl6J female mice were used. The old animals were treated orally for 14 weeks with either vehicle or BIO103 (50 mg/kg/d). After 13 weeks of treatment, the animals were tested for functional activity in toto (*t*max). Plasma and muscles were collected after sacrifice for further analysis.

**Results**

- **In vitro**
  - **Effects of BIO103 on C2C12 myobute diameter**
  - **Effect of BIO103 on myostatin gene expression**
  - **Effects of BIO103 on intracellular signaling**
    - **MAPK**
    - **AMPK/ACC**

- **In vivo**
  - **BIO103 increases both basal and maximal oxygen consumption rates (OCR) in muscle C2C12 cells after 72 h exposure to differentiated cells**

**Conclusions**

- **BIO103 displayed in vitro and in vivo anabolic effects on myofibers. These effects were accompanied with Myostatin inhibition.**
- **Anabolic properties of BIO103 result from an activation of AKT/mTOR, MAPK and AMPK pathways.**
- **BIO103 warrant further studies towards its development as a drug candidate for the treatment of muscle wasting disorders.**