**From Molecules to Muscles: Computational and Cellular Approaches to Fulvic Acid’s Enhancement of Creatine Kinase and Mitochondrial Biogenesis Involved Muscle Mass Regeneration**

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**Background and aims.** Muscle mass regeneration relies on intricate energy metabolism, mitochondrial dynamics, and cellular repair. Fulvic acid, a bioactive natural compound, may help regulate cellular homeostasis and muscle adaptation, though its molecular effects are not fully understood. This study aims to investigate fulvic acid’s impact on creatine kinase activity and mitochondrial function using computational modelling and cellular assays, aiming to uncover new therapeutic strategies for muscle mass degeneration.

**Methods.** A combined *in silico* and *in vitro* approach was used to study fulvic acid’s effects on muscle energy metabolism and mitochondrial biogenesis. Molecular docking predicted fulvic acid’s binding to creatine kinase and mitochondrial regulators. L6 myotubes were treated to assess creatine kinase activity, mitochondrial DNA content, and PGC-1α expression. Cell viability, serum creatine kinase activity, and muscle histopathology were evaluated by *in vivo* techniques to determine cellular health and structural integrity.

**Results.** Computational analyses revealed strong interactions between fulvic acid and creatine kinase, indicating that fulvic acid may influence this enzyme’s function in muscle energy metabolism. *In vitro*, fulvic acid treatment of L6 myotubes reduced creatine kinase levels, increased mitochondrial DNA, and upregulated mitochondrial biogenesis genes like PGC-1α. *In vivo* studies showed reduced serum creatine kinase, improved muscle tissue morphology, highlighting fulvic acid’s potential benefits for muscle health.

**Conclusion/Discussion.** The findings showed that fulvic acid aids muscle recovery by preventing creatine kinase leakage, preserving muscle fibre integrity, and promoting mitochondrial biogenesis at molecular and cellular levels. These benefits are likely due to its direct enzyme interactions, modulation of genes related to energy metabolism, and its antioxidant properties, all of which reduce muscle damage and speed recovery, supporting fulvic acid’s use as a supplement for improving muscle mass degeneration.

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**References:**

1. Leduc-Gaudet, Jean-Philippe, et al. "Mitochondrial dynamics and mitophagy in skeletal muscle health and aging." *International Journal of Molecular Sciences* 22.15 (2021): 8179.
2. Romanello, Vanina, and Marco Sandri. "The connection between the dynamic remodelling of the mitochondrial network and the regulation of muscle mass." *Cellular and Molecular Life Sciences* 78.4 (2021): 1305-1328.
3. Winkler, John, and Sanjoy Ghosh. "Therapeutic potential of fulvic acid in chronic inflammatory diseases and diabetes." *Journal of diabetes research* 2018.1 (2018): 5391014.
4. Issac, Praveen Kumar, et al. "Insulin signalling pathway assessment by enhancing antioxidant activity due to morin using *in vitro* rat skeletal muscle L6 myotubes cells." *Molecular Biology Reports* 48 (2021): 5857-5872.
5. Xie, Wen‐qing, et al. "Mouse models of sarcopenia: classification and evaluation." *Journal of cachexia, sarcopenia and muscle* 12.3 (2021): 538-554.