The Effects of Piperine on Rodent Sarcomere Muscle Contraction Using Synchrotron X-Ray Diffraction

Malik Adi, Lindsay Aceremo, Ash Argueta, Harrison Brandt, Abdulmonem Akili, Marcel Duda, Andrea Midnight, Erhan Osmani Christopher Repa, Weikang Ma, Thomas C. Irving

ESRP Team

Oak Lawn Community High School, Oak Lawn, IL 60453, U.S.A.

Abstract

Piperine is the alkaloid responsible for the pungency of black pepper and long pepper. Despite its use in some forms of traditional medicine, Piperine is not classified as a drug, and has yet to be FDA approved. Piperine has been shown to destabilize the super-relaxed state (SRX) in rabbit fast-twitch fibers but not slow muscles. In other words, it serves as an activator to the muscle fibers resulting in a state of moving disorder. Myosin, a fiber released simultaneously with Actin, which indicates muscular movement, uses very little ATP in the SRX state. If it is released to a disordered relaxed state (DRX), which is the turnover state of myosin in the absence of Actin, Piperine uses much more ATP. For this reason, it may be a possible treatment for obesity by increasing energy use in muscles, thus resulting in the burning of excess fat.

Introduction

Transitions between SRX and DRX have been proposed to be associated with changes in the position of myosin heads from a sequestered state close to the thick filament- where they cannot interact with the thin filaments- to positions close to the thin filament which allow contraction. These structural changes can be detected using X-ray diffraction and related to force output by the muscle. This synchrotron radiation illustrates molecular scale structures, allowing us to derive information and diffraction patterns of muscles, allowing the effects of piperine to be analyzed. Furthemore, the x-ray microbeam elucidates the distance of lattice spacing between thick and thin filaments, allowing the rate and extent of contraction to be evaluated.

Motivation

Piperine is not approved by the FDA as a drug, but has been used historically in traditional medicine. This study examines the effects of piperine on the mobility of myosin molecules in resting muscle, which if increased, can be effective as a treatment for obesity.

Methods

In this investigation, rodent Extensor Digitorum Longus (EDL) skeletal muscle samples were prepared by beamline staff at Argonne National Laboratory. Muscle samples were exposed to different concentrations of piperine in order to assess its effects on sarcomere structure in slow and fast muscle. The small-angle X-ray fiber diffraction instrument on the BioCAT Beamline 18ID at the Advanced Photon Source, Argonne National Laboratory was used to perform this experiment. X-ray diffraction patterns were analyzed using the “Equator” routine of the MuscleX software package developed by BioCAT

Discussion

We examined the effects of Piperine on Extensor Digitorum Longus (EDL)–a fast twitch muscle. According to the data from our experiment, there was a significant decrease in the lattice spacing between filaments (d10), as well as an increase in the equatorial intensity ratio (I11/I10) with increasing Piperine concentration. This information is important because intensity ratios increase when myosin heads reach out and bind to actin, converting ATP into force and motion. The decrease in spacing between thick filaments, consisting primarily of myosin, and thin filaments, consisting primarily of actin, suggest that myosin heads are binding to actin when Piperine is introduced.

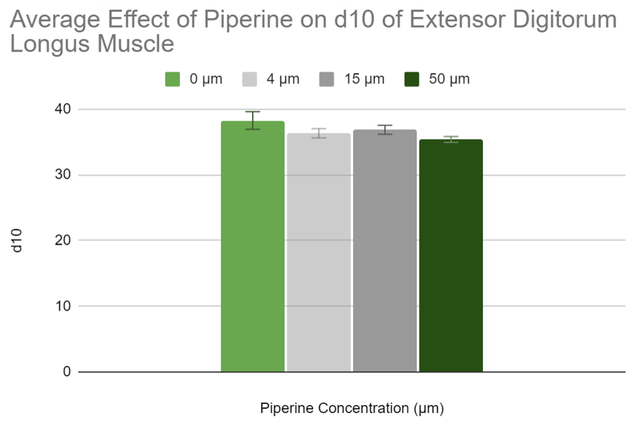

Figure 1: d10 decay with the administration of Piperine illustrates a decrease in lattice spacing between Actin and Myosin filaments, indicating a shift from SRX to DRX.

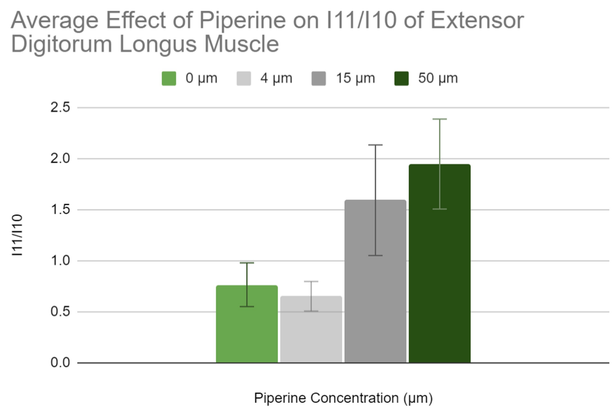

Figure 2: X-Ray Synchrotron Radiation reveals Piperine’s directly proportional relationship to I11/I10 fibers during muscle contraction-showing increase dynamic muscular oscillation of protein binding between Actin and Myosin filaments-indicating proficiency in muscular contractions.

This research was made possible through the Exemplary Student Research Program, supported by Argonne National Laboratory’s Educational Programs (CEPA), APS User Office, and supported by the Advanced Photon Source. Argonne National Laboratory is a U.S. Department of Energy laboratory managed by UChicago Argonne, LLC .

Conclusions

This study has elucidated the mechanism by which piperine stimulates muscular contractions, aiding in the elimination of obesity through ATP hydrolysis. Piperine has been shown to initiate the transition from the strain-induced martensitic phase (SRX) to the dynamic recrystallization phase (DRX), which enhances ATP hydrolysis and promotes muscle function. Our experimental findings indicate that a concentration between 15µm (P=0.054213) and 50µm (P=0.05288) will elicit a statistically significant effect on the lattice spacing between thick and thin filaments in the muscle fibers of rats. A one sample t-test was performed to compare I11/I10 per fiber under concentrations of 4µm, 15µm, and 50µm to a muscle with no piperine influence. The results with no treatment compared to the treatment of 15µm and treatment of 50µm demonstrated significantly better peak flow scores of 0.054 and 0.052 respectively. There was no significant change in D10 per fiber with 0µm concentration, P = 0.062, and 50µm concentration, P = 0.092, despite the mild difference in average D10 per fiber. This change in lattice spacing reflects the structural modifications induced by piperine, which ultimately result in improved muscle function. Therefore, it can be concluded that piperine is a potent agent for promoting muscular contractions and has promising therapeutic potential for managing obesity-related conditions.

Future Investigation

Piperine has been proven to be an effective anti-inflammatory agent as its presence, to an extent, inhibits tumor necrosis factor-a, P-glycoprotein, and cytochrome P4503A4, cytokines and enzymes responsible for chronic inflammation. This points to future scientific analysis of Piperine administration to patients with chronic hyper-inflammatory autoimmune diseases, replacing their reliance on biologics and corticosteroids that quiet the immune system, rather than strengthening it. Additionally, Piperine’s ability to manifest muscular contractions- thus resulting in free energy production - indicates an efficient energy transfer from contracting muscular cells to the dissociation of hyperlipidemia triglycerides and LDL cholesterol, which may be used as an agent of weight loss. Piperine has been studied to not only have physiological-corporeal effects, but psychological effects. It’s antidepressant and cognitive correcting abilities, observed through its status as an monoamine oxidase inhibitor stimulate the production of serotonin and norepinephrine

Literature Cited

TC;, M. W. I. (n.d.). Small angle X-ray diffraction as a tool for structural characterization of Muscle Disease. International journal of molecular sciences. Retrieved April 5, 2023, from https://pubmed.ncbi.nlm.nih.gov/35328477/

Bang, J. S., Oh, D. H., Choi, H. M., Sur, B.-J., Lim, S.-J., Kim, J. Y., Yang, H.-I., Yoo, M. C., Hahm, D.-H., & Kim, K. S. (2009). Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1beta-stimulated fibroblast-like synoviocytes and in rat arthritis models. Arthritis research & therapy. Retrieved April 5, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2688199/#:~:text=We%20found%20that%20piperine%20significantly,inflammatory%20effects%20in%20other%20systems

Kesarwani, K., Gupta, R., & Mukerjee, A. (2013, April). Bioavailability enhancers of herbal origin: An overview. Asian Pacific journal of tropical biomedicine. Retrieved April 5, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3634921/#:~:text=Piperine%20inhibits%20human%20P%2Dglycoprotein,CYP1B2%2C%20CYP2E1%2C%20CYP3A4%20etc.

Bhardwaj RK;Glaeser H;Becquemont L;Klotz U;Gupta SK;Fromm MF; (n.d.). Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. The Journal of pharmacology and experimental therapeutics. Retrieved April 5, 2023, from https://pubmed.ncbi.nlm.nih.gov/12130727/#:~:text=In%20summary%2C%20we%20showed%20that,major%20drug%2Dmetabolizing%20enzyme%20CYP3A4.

D;, G. N. D. (n.d.). Possible involvement of GABAergic and nitriergic systems for antianxiety-like activity of piperine in unstressed and stressed mice. Pharmacological reports : PR. Retrieved April 5, 2023, from https://pubmed.ncbi.nlm.nih.gov/25149996/#:~:text=Results%3A%20Piperine%20produced%20significant%20antianxiety,change%20in%20plasma%20nitrite%20levels.