Optimisation of specimen handling and mitochondrial analysis in patient skeletal muscle biopsies for nutritional and ageing research

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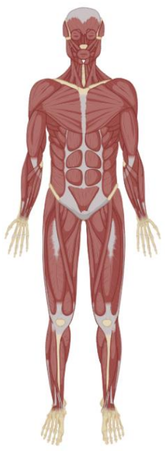
Background



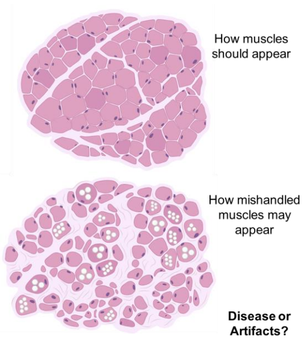
Skeletal muscles are crucial for mobility, metabolism, and quality of life. With increasing age, there is a decline in muscle mass and function.

Mitochondria play a fundamental role in skeletal muscle bioenergetics, making them vital to muscle function. With ageing, skeletal muscle bioenergetics undergo degenerative changes, attributed to mitochondrial dysfunction, and this can contribute to loss of muscle mass and performance.

Nutrition plays a key role in maintaining muscle health. Essential nutrients such as proteins, omega-3 fatty acids and polyphenols help mitigate age-related mitochondrial dysfunction and muscle decline.

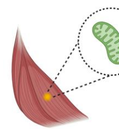
Traditional freezing methods often cause artefacts mistaken for disease, leading to incorrect diagnoses or misinterpretation of research findings. Proper handling of muscle biopsies is, therefore, critical for accurate histopathological and mitochondrial analysis. Additionally, the existing literature lacks comprehensive workflows for mitochondrial analysis in tissue samples.

Muscle health declines with age

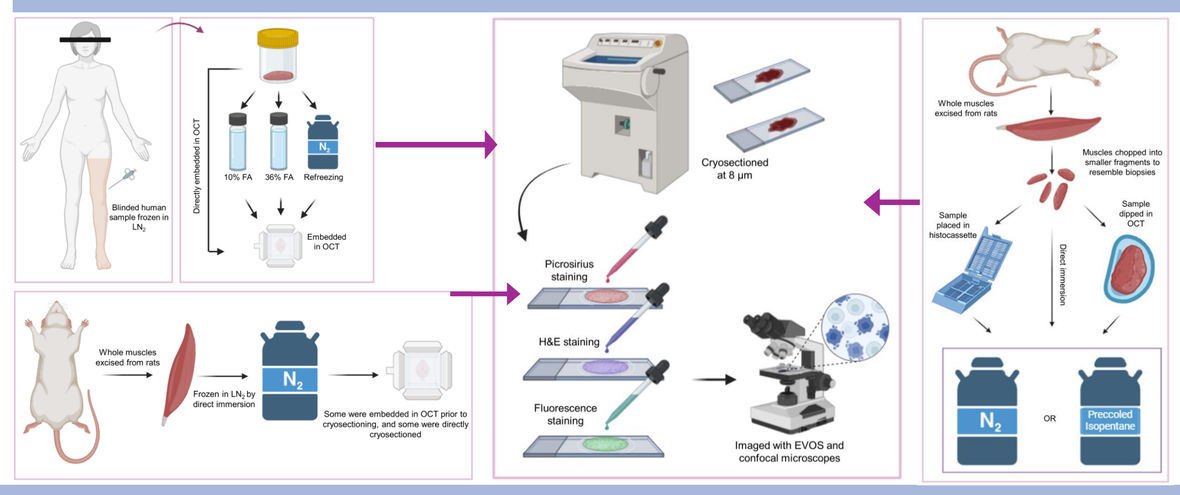


Aims

This study aimed to provide an effective methodological workflow to improve cryopreservation techniques for human and rodent muscle biopsies and create a reliable method for mitochondrial analysis in muscle tissues to aid in research on muscle health, nutrition and ageing

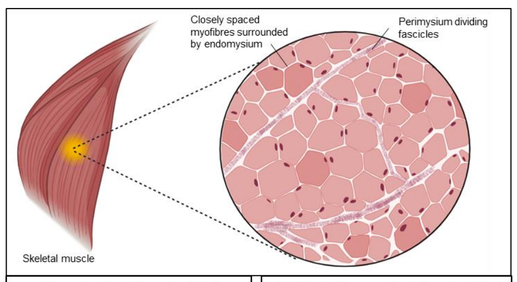


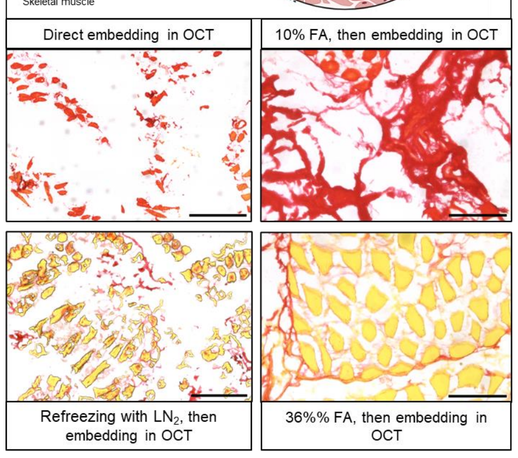
Methods



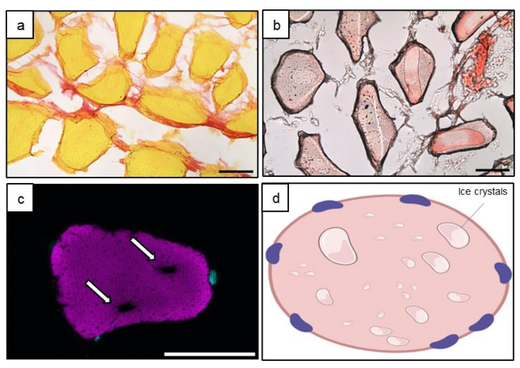
Results

Human skeletal muscle biopsies previously frozen in LN_2 only survive preservation with 36% formaldehyde. However, cells appear widely spaced



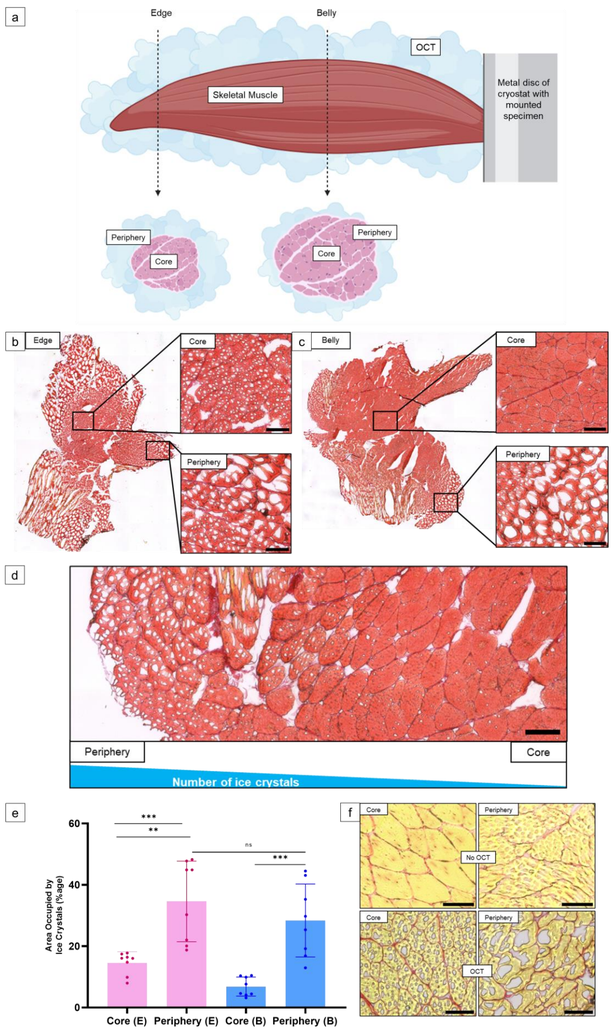


Ice crystals detected within myofibres upon PSR, ORO and fluorescence microscopy



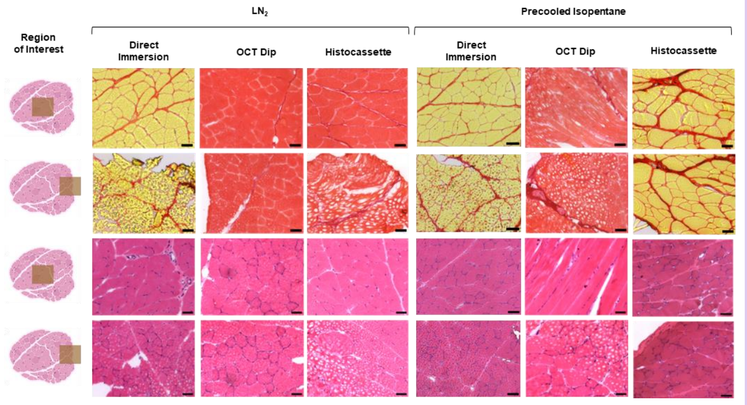
Ice crystals form with inadequate freezing and freeze-thaw cycles

Rat skeletal muscles frozen in LN_2 demonstrate variation in ice crystal content between edge and belly of the muscle, hinting towards an effect of OCT on freezing artefact formation



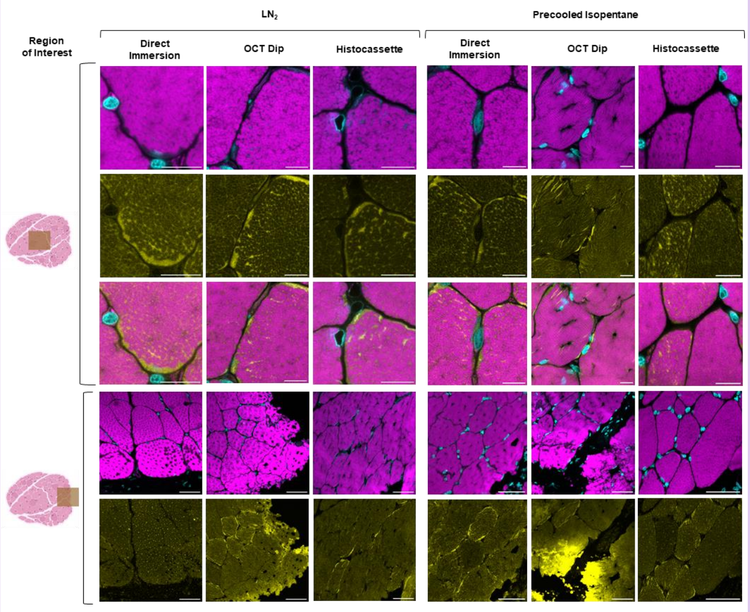
Cryosectioning without OCT showed fewer ice crystals and hence, use of OCT was abandoned for subsequent experiments

Rat muscle 'biopsies' frozen with six different cryopreservation techniques showed that isopentane/histocassette combination exhibits no ice crystal artefacts



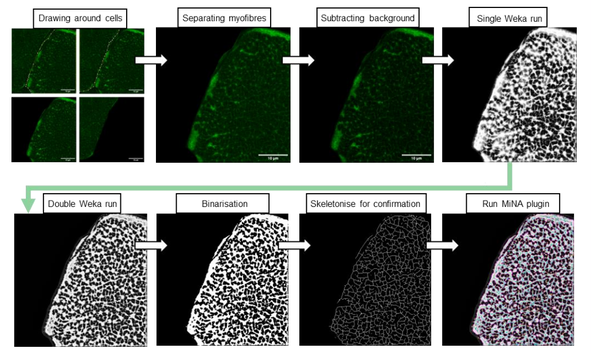
The same was observed on fluorescence microscopy

Nuclei stained with DAPI, actin stain with rhodamine phalloidin, mitochondria stained with MitoView

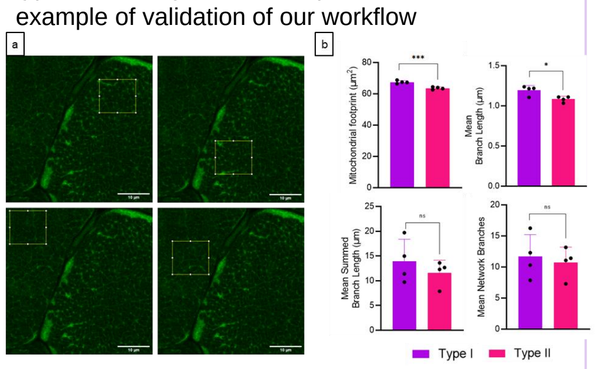


We next generated a workflow for mitochondrial analysis using existing functions and plugins in Fiji

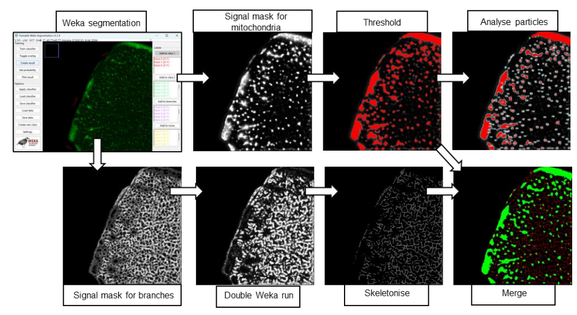
Trainable Weka Segmentation plugin in Fiji was used to accurately threshold and skeletonize mitochondrial network



Mitochondrial Network Analyser (MiNA) plugin was then used to obtain quantitative parameters of the mitochondrial network. Mitochondria in type I and II myofibres are quantified below as an example of validation of our workflow



The workflow can also be used to generate a map of mitochondria and their branches to aid in localisation of target of interest



Conclusion

The isopentane/histocassette combination ensures artefact-free preservation of entire skeletal muscle biopsy. Moreover, our Fiji workflow adopting the Trainable Weka Segmentation plugin provides a reliable method for mitochondrial analysis in skeletal muscle tissues, facilitating future studies in muscle health, nutrition and ageing.

Future Work

- A cross-sectional study on healthy volunteers across all ages to investigate role of inflammation in muscle ageing
- Super resolution microscopy techniques to obtain detailed images and explore interactions between inflammatory proteins and mitochondria
- African Turquoise Killifish experimental model to further explore and elucidate mechanisms via which dietary interventions impact muscle ageing