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Freezer Study: An Investigation of the Stability of Skeletal Muscle Proteins after prolong freezer storage

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The long-term effect of protein stability following sample cryo-storage has not been well-documented, particularly with respect to skeletal muscle. Those longitudinal studies that have focused on the effects of freezing samples have predominantly examined whole blood, sera, and plasma and have reported varying results, with the detection of some proteins sensitive to degradation and others not. The current study aimed to identify if protein integrity and overall abundance are affected after long-term freezer storage. The longitudinal study qualitatively and quantitatively compared the relative abundance of heat shock proteins (HSPs) in human skeletal muscle frozen at different temperatures and for different lengths of time. Human *vastus lateralis* muscle biopsies (Pre, 3h, 24h, and 7d) taken from a subset of the same individuals from a previously published study were used (Frankenberg *et al.*, 2014a, Frankenberg *et al.*, 2014b), under the same human ethics approvals. Participants engaged in an initial eccentric exercise bout (ECC1) and a repeated bout of the same exercise, 7 days after ECC1 (ECC2). Samples were either previously prepared by crude cell fractionation and stored in sample loading buffer (SDS) at -20˚C for 9 years or freshly prepared from raw tissue which had been stored at -80˚C for 17 years. Storage of prepared muscle samples at -20˚C resulted in the noticeable degradation and/or absence of the abundant muscle protein, whilst the abundance of actin along with total and phosphorylated small heat shock protein (HSP27 and αB-crystallin) were seemingly stable following storage. Following ~17 years storage at -80˚C, the abundance of smHSP proteins in whole muscle and the translocation events established as a result of ECC1 (Frankenberg *et al.*, 2014a, Frankenberg *et al.*, 2014b) in crude cell fractions were still observed. These findings reinforce that long-term freezer storage at -80˚C may be suitable to maintain human skeletal tissue without incurring any loss in protein integrity, including post-translational modification, cellular localisation, and abundance of proteins.

Frankenberg NT, Lamb GD, Overgaard K, Murphy RM, Vissing K (2014a) Small heat shock proteins translocate to the cytoskeleton in human skeletal muscle following eccentric exercise independently of phosphorylation. *Journal of Applied Physiology* 116: 1463-1472

Frankenberg NT, Lamb GD, Vissing K, Murphy RM (2014b) Subcellular fractionation reveals HSP72 does not associate with SERCA in human skeletal muscle following damaging eccentric and concentric exercise. *J Appl Physiol (1985)* 116: 1503-11