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Technical considerations when assessing gene expression in human skeletal muscle using quantitative real-time PCR

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Gene expression analysis by quantitative real-time PCR (qPCR) is common in skeletal muscle research and exercise science. The reproducibility and reliability of the data fundamentally depend on how the experiments are performed and interpreted. Despite the popularity of the assay, there is a considerable variation in experimental protocols and data analyses from different laboratories and a lack of consistency of proper quality control steps throughout the assay. In this study, we present the results from several experiments on various steps of the qPCR workflow and demonstrate how to accurately perform gene expression analysis using qPCR in human skeletal muscle samples. To assist researchers in obtaining more reliable data, we test some common issues in performing qPCR, including sample handling and preparation, quality of RNA extraction, the use of reference genes, and normalisation of the data. We found that mishandling muscle for a short period (≤ 10 minutes) before RNA extraction did not affect RNA quality, and isolated total RNA was preserved for up to one week at room temperature. However, we found that careful consideration of the normalisation method is crucial; the use of unstable reference genes led to significant differences in the final results. Likewise, the expression of individual genes can be normalised to total cDNA content; however, complete removal of RNA from cDNA samples is essential for obtaining accurate cDNA content.