Are genuine changes in protein expression being overlooked? Avoiding pitfalls in Western blotting quantification using AMPK and calsequestrin 1 as the proteins of interest

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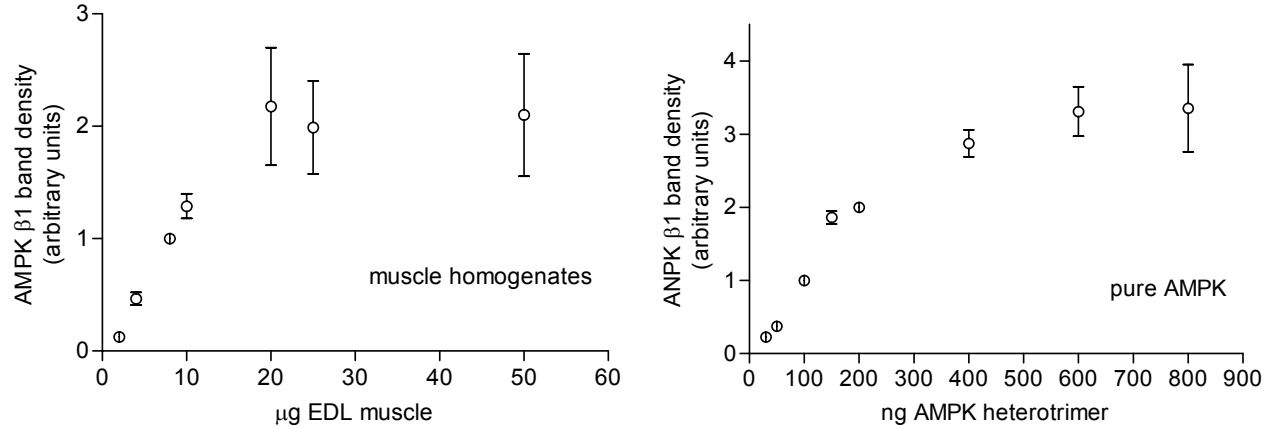
Western blotting has long been used in skeletal muscle physiology and biochemistry to examine relative amounts of specific proteins between treatment groups. An underlying assumption is that a linear, directly proportional, response exists between the amount of sample loaded and the density obtained for the protein band(s) of interest. Such a relationship does not usually exist and the present study highlights inadequacies in analysing proteins using arbitrary density units without the use of internal reference standard curves. It demonstrates that in order to correctly estimate relative changes in protein amounts, it is essential to establish the amount of sample that needs to be used and quantify the dynamic range over which the protein of interest can be detected.

Male Long-Evans hooded rats (6-8 months old) were sacrificed using a lethal overdose of fluothane in accordance with the La Trobe University Ethics Committee and the extensor digitorum longus (EDL) muscles excised. Whole muscle homogenates were used, that is, muscle samples were not subject to any centrifugation prior to Western blotting, in order to ensure that the entire pool of muscle proteins were represented. By ensuring that no proteins were inadvertently spun out, the absolute amounts of given proteins were able to be determined. A very sensitive Western blotting technique was used to detect calsequestrin 1 (CSQ1) and AMP kinase (AMPK) in as little as 2 μg total muscle homogenate (< 0.5 μg total protein). Using an anti-AMPK β1 isoform antibody, the concentration of AMPK in rat EDL muscle was determined to be ~60 μM. Standard curves prepared from both homogenates and pure protein samples of CSQ1 and AMPK demonstrated that loading too much sample (homogenates or pure proteins) would hinder seeing the true changes caused by some intervention, because the standard curves were no longer proportional with the amount of sample loaded (see Figure). This occurred with ~50 μg total muscle (~12 μg total protein) and 20 μg (~5 μg total protein) for CSQ1 and AMPK, respectively.



It was found, that extrapolation from a standard curve which although linear was not directly proportional, could result in an error of at least four-fold. This finding suggests the possibility that true changes due to a given treatment or intervention are not being detected. In conclusion, a simplistic approach of "less is more", (*i.e.* using small amounts of sample) enables a much clearer and more accurate outcome when using Western blotting for either absolute or relative amounts of particular proteins.