Calpain-1 and calpain-3 are not autolysed with exhaustive exercise in humans

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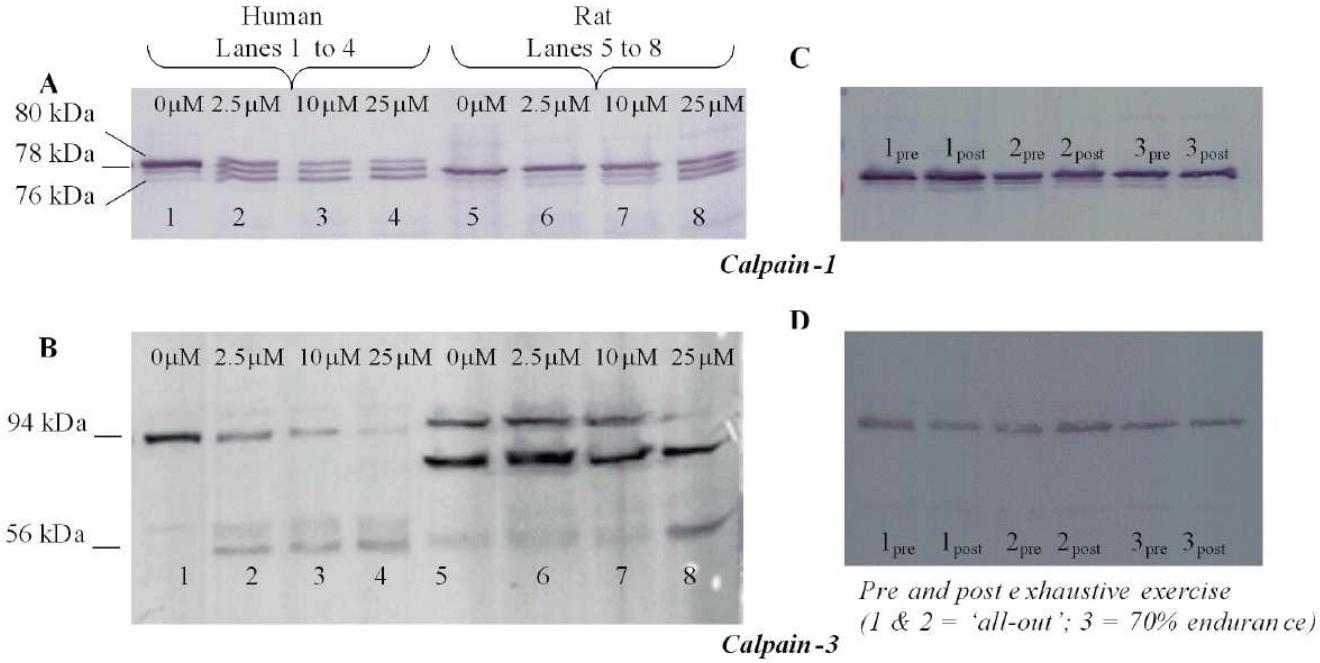
Calpain-1 and calpain-3 are Ca^{2+}-dependent proteases found in skeletal muscle. Autolysis of the calpains is observed by Western blotting as the cleaving of the full-length proteins to shorter products (see the Figure, A and B), which results in their activation. Biochemical assays suggest that calpain-1 becomes proteolytically active in the presence of 3-200 µM Ca^{2+}. Although calpain-3 is poorly understood, its activation is proposed to be much more Ca^{2+}-sensitive (~1 µM) than calpain-1. Adult Long Evans hooded rats were killed by an overdose of halothane, as approved by the Animal Ethics Committee at La Trobe University and the extensor digitorum longus (EDL) muscles were removed. Human muscle samples were obtained from the vastus lateralis using the needle biopsy technique. These samples were left over from a completed study which was approved by the Deakin University Human Ethics Committee. As shown in the Figure (A and B), we characterised the Ca^{2+}-dependence of autolysis of the calpains in human muscle samples and rat EDL muscle samples homogenised in solutions mimicking the intracellular environment at various [Ca^{2+}] (0, 2.5, 10 and 25 µM).



Autolysis of calpain-3 was found to occur over a similar [Ca^{2+}] range as that for calpain-1, and both calpains displayed a seemingly higher Ca^{2+}-sensitivity in human compared to rat muscle homogenates, with ~15 % autolysis observed following 1 min exposure to 2.5 µM Ca^{2+} in human muscle and almost none following 1-2 min exposure to the same [Ca^{2+}] in rat muscle. Since intracellular [Ca^{2+}] may transiently peak in the range found to activate calpain-1 and calpain-3, we examined the effect of two types of exhaustive cycling exercise (30 s "all-out", n=8 and 70 % VO2 peak until fatigue, n=3) on the amount of autolyzed calpain-1 or calpain-3 in human muscle. Following the sprint exercise, the percent decline in peak power was 45 ± 11 % (mean ± sd). In the endurance exercise trials, subjects cycled for 107 ± 27 min. Despite the exhaustive nature of the exercise, autolysis of calpain-1 or calpain-3 did not occur due to the exercise (Figure, C and D). These findings show that the time- and concentration-dependent changes in cytoplasmic [Ca^{2+}] occurring during concentric exercise fall near, but below that necessary to activate calpains *in vivo*.