J Physiol 594.11 (2016) pp 3161–3162

The Journal of Physiology

LETTER

Inactivation of the intrinsic muscle clock does not cause sarcopenia

The role of the muscle intrinsic molecular clock has been the object of recent investigations based on the selective inactivation of the *Bmal1* gene in skeletal muscle. Using an inducible knockout model, in which *Bmal1* is inactivated solely in adult mouse skeletal muscle (iMS*Bmal1*$^{-/-}$), Schroder and colleagues recently reported a number of changes in muscle structure and function, and concluded that 'Disruption of the molecular clock in adult skeletal muscle is sufficient to induce changes in skeletal muscle similar to those seen in the *Bmal1* knockout mouse (*Bmal1*$^{-/-}$), a model of advanced ageing' (Schroder *et al.* 2015). However, both circadian locomotor activity and total activity levels are markedly affected in the germline but not in the inducible *Bmal1*$^{-/-}$ model and it was recently shown that rhythmic muscle activity is sufficient to drive part of the circadian muscle transcriptome independently of the endogenous clock (Dyar *et al.* 2015). Using muscle-specific inactivation of *Bmal1* since early developmental stages (mKO), a model better comparable to the germline *Bmal1* gene KO, we previously reported that the muscle phenotype of mKO mice is strikingly different from whole body KO (Dyar *et al.* 2014). Mice with germline *Bmal1* KO stop growing around 16 weeks of age, display progressive and dramatic muscle atrophy, and die between 26 and 52 weeks of age (Bunger *et al.* 2000; Kondratov *et al.* 2006). In contrast, life span was normal in mKO mice and muscle weight was even significantly increased compared to controls (Dyar *et al.* 2014). Although normalized muscle force was decreased, muscle histology and ultrastructure were apparently normal even at advanced age, with no sign of muscle fibre atrophy, degeneration/regeneration or fibrosis. In conclusion, our results suggest that the dramatic sarcopenia observed in germline *Bmal1* KO mice is not due to the loss of cell-autonomous function of *Bmal1* in muscle fibres. This view is supported by the recent analyses of another *Bmal1* KO model, in which *Bmal1* was inducibly deleted in adult mice in all tissues. No significant change in life span, body and organ weight, or abnormal calcification was observed in these mice, showing that most phenotypes described in standard *Bmal1* KO mice are due to *Bmal1* effects during development (Yang *et al.* 2016). Although the decrease in body weight and longevity of germline *Bmal1*$^{-/-}$ mice was rescued by muscle-specific *Bmal1* overexpression, the transgene used in that study was driven by a constitutive α-skeletal actin promoter, leading to overexpression of non-cycling BMAL1 protein (McDearmon *et al.* 2006), thus likely affecting rhythmic transcription of BMAL1 target genes and BMAL1-dependent circadian oscillation of protein synthesis (Lipton *et al.* 2015).

Dyar *et al.* (2014) also used an inducible model of muscle-specific *Bmal1* KO and reported that muscle force is unchanged in these mice, whereas a decrease in muscle force was described in the Schroder study using the same inducible knockout model. Schroder *et al.* (2015) suggested that this discrepancy may be due to the fact that tamoxifen treatment for *Bmal1* recombination was started at 8 weeks of age in the Dyar study but at 12–16 weeks in the Schroder study, arguing that satellite cell fusion, still ongoing at 8 weeks, would dilute the Bmal1 negative myonuclei through addition of Bmal1 positive nuclei after the tamoxifen treatment. However, this interpretation is unlikely because we found that Bmal1 transcripts were drastically reduced in muscles from mice treated with tamoxifen at 8 weeks and examined 5 months later. The issue of satellite cell fusion during postnatal muscle development is the object of debate. It was reported that the total number of myonuclei in mouse extensor digitorum longus (EDL) muscle fibres increases up to 3 weeks after birth and then remains constant, suggesting that satellite cell fusion does not occur at subsequent postnatal stages (White *et al.* 2010). A more recent report revealed satellite cell fusion events even at later stages; however, the contribution of satellite cells to uninjured myofibres of mouse EDL was identical at 8, 12 and 27 postnatal weeks (Pawlikowski *et al.* 2015).

A more likely interpretation to account for the difference in muscle functional properties between the two studies, as also suggested by Schroder *et al.* (2015), is that most analyses were performed at 58 weeks post-tamoxifen treatment in the Schroder study but at 20 weeks in the Dyar study. An age-dependent increase in oxidative stress may be involved in this difference, as the loss of *Bmal1 per se* causes accumulation of reactive oxygen species (Kapre *et al.* 2011). This interpretation is supported by the different techniques used for muscle force measurements in the Dyar and Schroder studies. Whereas Dyar *et al.* (2014) performed force measurements *in vivo* in anaesthetized animals, Schroder *et al.* used an *ex vivo* approach whereby muscles were incubated for 30 min at 37°C. It was previously shown that incubation of mouse EDL muscle at 37°C, as opposed to the more generally used 22°C, leads to a marked reduction of muscle tetanic force of about 70% (Edwards *et al.* 2007). This force drop was apparently due to an increase in muscle superoxide production, as it was largely prevented by incubation of muscles at 37°C in the presence of Tempol, a superoxide dismutase mimetic. Oxidative damage would probably be higher in iMS*Bmal1*$^{-/-}$ than wild-type muscles, as the loss of *Bmal1 per se* was found to cause accumulation of reactive oxygen species in different tissues (Kapre *et al.* 2011; Lee *et al.* 2013; Jacobi *et al.* 2015). Another methodological point to be considered is that in the Schroder study the optimal length of the EDL muscle was determined by optimizing for twitch force (1 Hz). It is known that the optimal length for skeletal muscle fibres is not the same when measuring a single twitch or a full activation during a tetanic contraction (Close, 1972). Force production generated during a tetanus mostly depends on the overlap between thick and thin filaments, whereas the twitch is also affected by the amount of calcium released by the sarcoplasmic reticulum and the calcium sensitivity of the myofilaments, which in turn is sensitive to length changes. Indeed, the length at which optimal twitch tension is achieved is greater than that at which tetanic tension is maximal. Since Schroder *et al.* (2015) determined optimal muscle length using twitch tension, it is likely that tetanic contractions were performed on the downward slope of the force–length relationship and one cannot rule out

DOI: 10.1113/JP272127

the possibility that iMS*Bmal1*$^{-/-}$ muscles behave differently under these conditions compared to wild type muscles, possibly due to an effect of oxidative stress on the excitation–contraction coupling.

K. A. Dyar, S. Schiaffino and B. Blaauw

Venetian Institute of Molecular Medicine (VIMM), 35129 Padova, Italy

Email: bert.blaauw@unipd.it
Present address K. A. Dyar: Molecular Endocrinology, Institute for Diabetes and Obesity, Helmholtz Zentrum München, Germany

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Additional information

Competing interests

None declared.