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## The authors reply: Comment on: "Aldehyde dehydrogenases contribute to skeletal muscle homeostasis in healthy, aging, and Duchenne muscular dystrophy patients" by Etienne et al.

We wish to thank Drs Campos, Chen, and Ferreira for their careful and subtle appraisal of this study, for their rewarding proposals and discussion, and for underlining the perspectives. Our recent article<sup>1</sup> was the first step in investigating the complex contribution of aldehyde dehydrogenases (ALDHs) in skeletal muscle's homeostasis, regeneration, and myopathies.

As presented by these authors, Aldefluor<sup>®</sup> assay (ALDEF) alone cannot be considered a univocal marker for the analysis of heterogeneous populations of cells, and this is not specific to the skeletal muscle tissue. Indeed, the measurement of ALDEF relies on enzymatic activities, and there are still uncertainties regarding the metabolism of ALDEF by several or most ALDH isoenzymes, due to their structural evolution and diversity. All isoenzymes are not equivalent. Based on gene transfer experiments, nine isoenzymes have been recently proposed to metabolize ALDEF efficiently (ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, ALDH2, ALDH3A1, ALDH3A2, ALDH3B1, and ALDH5A1),<sup>2</sup> and the article<sup>1</sup> suggested that most of them were involved in muscle biology. New probes with dedicated isoenzyme selectivity (thiophene-bridged aldehydes<sup>3</sup>), or complementary to ALDEF (Alderred<sup>4</sup>), have been described or are under development. ALDEF labelling may be necessary but not sufficient to segregate subpopulations of cells. We then combined ALDEF labelling with known extracellular markers to improve the definition of cell types. Such combinations have been used in different fields such as haematology.<sup>5</sup> Previously, we distinguished two human muscle cell populations with ALDH activity (ALDEF<sup>+</sup>/CD34<sup>+</sup> and ALDEF<sup>+</sup>/CD34<sup>-</sup>) but a different intrinsic ability to engage in a myogenic programme.<sup>6</sup> In the study,<sup>1</sup> we investigated how these two main subpopulations are influenced by ageing and Duchenne muscular dystrophy (DMD), and we pinpointed important differences between healthy donors and DMD patients.

Relating to DEAB, it is a reference inhibitor provided with the Aldefluor<sup>®</sup> kit assay. It presents different selectivities, turnover, and mechanisms regarding the inhibition of the several ALDH isoenzymes, and it cannot cover their whole spectrum. This may be taken in consideration for fine tuning of mechanistic experiments *in vitro* and *in vivo*.<sup>2,7,8</sup> In the article,<sup>1</sup> DEAB was used classically as a negative control for ALDEF labelling. Other classes of inhibitors exist to perform functional assays with different selectivity and enzymatic features (reversible, irreversible, competitive, and non-competitive).<sup>7,8</sup> Extensive researches of modulators are done in the field of cancerology, since ALDHs are involved in the resistance to some chemotherapy agents<sup>9-12</sup> and in the field of cardiology where ALDH2 activation by dedicated molecules improves post-infarction remodelling as underlined by Drs Campos, Chen, and Ferreira.<sup>13,14</sup> The identification of specific modulators is and will be extremely valuable to cross-fertilize the research in the field of muscle physiology, regeneration, and fibrosis.

ALDEF may be very useful to prepare cells on important scales for the purpose of regenerative medicine but may present limitations regarding fundamental biology. This prompted us to look directly at the expression of isoenzymes, using histochemistry and molecular biology. The analysis of tissue distribution of ALDH isoenzymes emphasized the presence of several cell types linked to different functions afferent to muscle physiology: myogenesis, angiogenesis, and neural and stromal support. It revealed also differential presence of ALDH3A2, ALDH1L1, and ALDH9A1 between tissues from healthy and DMD patients, and some ALDH isoenzymes may become useful markers. Finally, the selective expression of some isoenzymes in crude tissue, dissociated mononucleated cells, and myogenic cultures suggests their involvement at several levels of muscle homeostasis. Interestingly, most of them, but not all, are able to metabolize ALDEF.<sup>2</sup>

The article<sup>1</sup> is primarily descriptive and does not provide direct mechanistic roles for ALDH isoenzymes in skeletal muscle regeneration and physiopathology. Indeed, ALDH isoenzymes are expressed in different cellular compartments and involved in several biochemical pathways whose link with muscle physiology is not always established. While the production of retinoic acid by some isoenzymes is involved in skeletal and cardiac muscle development, and the detoxification of several aldehydes' adducts plays roles in muscle homeostasis in the context of muscular dystrophies, ageing, or sarcopenia, the potential roles played by several ALDH metabolites (folate, glutamate, and succinate) have still to be explored in muscle. Nevertheless, the article<sup>1</sup> documented the involvement of ALDH1A3, ALDH1B1, ALDH2, ALDH3A2, ALDH7A1, ALDH8A1, and ALDH9A1, at least, in addition to

ALDH1A1 whose expression in muscle tissue has been previously documented.

Altogether, the use of targeted activators or inhibitors (pharmacological agents discussed above, siRNA, CRISPR-Cas control of gene expression, etc.) in step-by-step experiments *in vitro* and *in vivo* will help in understanding the respective contribution and complementarity of the isoenzymes expressed in skeletal muscle, to promote a better knowledge of muscle physiology and a better control of muscle pathology. We thank Drs Campos, Chen, and Ferreira who have shed light on the importance of ALDH in muscle tissue in these perspectives.

The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.<sup>15</sup>

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