Journal of Physiology (2002), **541.2**, *p.* 333

DOI: 10.1113/jphysiol.2002.023705
www.jphysiol.org

PERSPECTIVES

Lactate shuttle – between but not within cells?

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There appears to be general agreement among many authors on key aspects of the lactate shuttle concept, i.e. that the production, exchange and utilization of lactate are major means of providing energy and material for gluconeogenesis (see Brooks, 2002, for review). Other functions such as signalling cellular events through influences on adenine nucleotide energy charge and redox may be associated. However, the concept of the intracellular lactate shuttle goes further, since it includes the idea that mitochondria are sites of lactate oxidation accomplished through lactate dehydrogenase (LDH) and controlled to some extent by the redox state (Brooks *et al*. 1999). In this issue of *The Journal of Physiology* workers from two different laboratories (Rasmussen *et al*. 2002; Sahlin *et al*. 2002) dissent from the refined concept, but do agree 'it is well documented that lactate can be released by one muscle and oxidized by another muscle' (Sahlin *et al.* 2002).

The basis of their disagreement stems from their inability to replicate findings of muscle mitochondrial LDH or lactate oxidation by mitochondria of rat, mouse or human muscle. These results present a major challenge to the idea of the existence of an intracellular lactate shuttle *in vivo*. It is therefore desirable that the data and the conceptual issues concerning the lactate shuttle hypothesis be aired fully.

What might reconcile the different findings and views? We need to consider whether the two groups used procedures that were more or less likely to obtain positive results, as well as possibly overlooking key findings reported in the literature, including very persuasive results of studies carried out using non-invasive techniques *in vivo*.

Rasmussen *et al.* (2002) asserted that 'mitochondria were obtained in high purity and integrity' and Sahlin *et al.* (2002) made a similar claim. Each paper describes muscle being extensively homogenized in the presence of proteolytic enzymes with protein pellets obtained by centrifugation being subsequently treated with detergents. Any of these procedures could be responsible for the inability to confirm earlier results. We have shown previously that, rather than being discrete organelles (Kirkwood *et al.* 1986), mitochondria exist as extensions of an extensive reticulum; hence, mitochondrial isolation results in disruption. In my view both groups obtained membrane fragments with some functions preserved, but others lost. For instance, in Table 1 of Rasmussen *et al.* (2002) there is evidence that the matrix enzyme citrate synthase was lost to the supernatant during homogenization. Thus, there is concern that a mitochondrial constituent such as LDH (purported to exist in the inner membrane and inter-membrane space; Brooks *et al.* 1999) could be as labile as a matrix constituent during isolation.

The authors used a protease to free mitochondria from muscle debris, although we have long known that there can be loss of particular mitochondrial constituents due to proteolytic digestion (Pande & Blanchaer, 1970). It is a pity that they did not provide any data on, for example, the ability of their preparations to translocate adenine nucleotides or oxidize fatty acids. Such data would have mitigated criticism that their results were due to LDH loss in preparation.

Mitochondrial LDH has been visualized by electron microscopy several times (Brooks, 2002). As well, by confocal microscopy and immunofluorescence, succinic dehydrogenase and LDH were reported to colocalize in mouse muscle (Nakae *et al.* 1999). Additionally, rat (McClelland & Brooks, 2002) and human (Dubouchaud *et al.* 2000) mitochondrial preparations all appear to contain LDH, visualized by Western blotting. It is regrettable that Rasmussen *et al.* (2002) and Sahlin *et al.* (2002) did not use immunolocalization to assess the presence of LDH epitopes remaining after isolation.

Given the tenuous nature of assays of enzymatic function after tissue fragmentation, observations of lactate metabolism *in vivo* have power for testing the lactate shuttle concepts. Much of the extant data on lactate oxidation in cardiac and skeletal muscles of humans and other mammals using isotope tracers, classical arterial-venous mass balance techniques, and their combination, reviewed recently appears to be in favour of lactate shuttling *in vivo* (Brooks, 2002). Moreover, results from magnetic resonance spectroscopy (MRS), mainly on heart, are supportive. Initially, Laughlin *et al.* (1993) reported that infusion of ^{13}C-pyruvate into working canine hearts labelled cytosolic alanine and lactate pools, but ^{13}C-lactate did not label either pyruvate or alanine, which would be expected if cytosolic LDH were a major player. Instead glutamate, transaminated from the tricarboxcylic acid cycle (i.e. mitochondrial) intermediate α-ketoglutarate, was labelled. More recently, Chatham *et al.* (2001), again using MRS, compared cardiac oxidation rates of ^{13}C-glucose and ^{13}C-lactate and showed preferential oxidation of lactate as well as mitochondrial, but not cytosolic, lactate oxidation. Similar data have been provided by Bertocci & Lujan (1999) on working rat skeletal muscle.

Finally, we need to consider the following: net lactate oxidation in the cytosol of working muscle is unlikely (Brooks *et al.* 1999), which makes intracellular lactate shuttling more viable. Resting skeletal muscle continuously takes up glucose, and releases lactate. Lactate production occurs in muscle cytosol because the K_{eq} of LDH is 10^4, and muscle isoforms with low K_M values for pyruvate predominate. Muscle glucose uptake and glycogenolysis rise during contractions as do the pyruvate concentration and the [lactate]/[pyruvate] ratio, with resulting increases in lactate production. In contrast, the mitochondrial electron transport chain (ETC) is relatively *more* oxidized during exercise than at rest, and a high lactate/pyruvate ratio in cytosol and a low pH in the matrix favour mitochondrial lactate influx and its oxidation as the environment facilitates removal of both pyruvate (via TCA cycle) and NADH (via ETC). Unfortunately, measurements of mitochondrial redox potential in working muscle are not robust, but ^{13}C-MRS data show mitochondrial, not cytosolic, lactate-to-pyruvate conversion (see above).

In summary, the efforts of Rasmussen *et al.* (2002) and Sahlin *et al.* (2002) show why it is very hard to understand events *in vivo* from studies on cell fragments alone. Their methods were different from those employed by us and leave open the possibility of LDH loss during isolation. Hence, there remains a major need to know definitively when, where and how lactate is oxidized in muscle. The efforts of the authors in raising these critical issues regarding the viability of the lactate shuttle concept are appreciated as part of what must be an ongoing debate.

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