



CORRESPONDENCE

Lactate production and exercise-induced metabolic acidosis: guilty or not guilty?

To the Editors:

In the excellent and very interesting chapter concerning clinical exercise testing published in the *European Respiratory Monograph* on lung function testing, ROCA and RABINOVITCH [1] wrote the following. "Increased lactate production is responsible for the fall in muscle pH, which in turn may play a role in determining exercise intolerance in these patients (COPD patients). Premature lactic acidosis during exercise in COPD patients has been associated with reduced oxidative enzyme concentrations in the lower limb muscles."

We would like to emphasise that the widespread belief that lactic acid is produced in working muscles as a result of oxygen insufficiency, and that it directly causes exercise-induced metabolic acidosis, has been criticised. In addition, numerous findings on different species are discordant with the prevailing theory [2].

We all accept that when looking at the end result of metabolism during intense exercise, the observed metabolic acidosis coincides with an accumulation of lactate, but does it mean that lactate production is guilty?

Recently, combining a large series of papers, ROBERGS *et al.* [3] have presented convincing empirical observations that lactate is a consequence rather than a cause of cellular events that cause acidosis.

From the transformation of a glucose molecule to two pyruvate molecules, three reactions release four protons, and one reaction driven by pyruvate kinase consumes two protons. The conversion of two pyruvate molecules to two lactate molecules by lactate dehydrogenase (LDH) consumes two protons. Another source of protons is adenosine triphosphate (ATP) hydrolysis.

During high-intensity exercise, as underlined by ROBERGS *et al.* [3], there is an increasing dependence on ATP supplied by glycolysis. Under these conditions, there is a greater rate of cytosolic proton release from glycolysis and ATP hydrolysis. The cell buffering capacity can be exceeded and acidosis develops. As a consequence of the favourable bioenergetics for the LDH reaction, lactate production increases. Lactate production does not seem to cause the acidosis and, furthermore, appears to play the role of a "sink" for protons produced during catabolism and ATP hydrolysis, thereby reducing acidosis.

In conclusion, due to "iso-time" apparition of lactate and metabolic acidosis, it remains that for clinical application lactate accumulation is obviously a good, indirect marker of cellular metabolic acidosis.

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Activation of human lung mast cells by monomeric immunoglobulin E

To the Editors:

Immunoglobulin (Ig) preparations contain variable proportions of Ig aggregates [1], which behave like true immune

complexes and are able to activate inflammatory cells by crosslinking Fc receptors [2]. The validity of the study by CRUSE *et al.* [3], which appeared in a previous issue of the *European Respiratory Journal* and showed that monomeric IgE activates

TABLE 1 Binding of immune complexes

Immune complexes	Binding to apoptotic neutrophils mean fluorescence	Reduction in binding %
None	3.4	
Uncentrifuged	92.3	
14000 × g for 20 min	86.8	2.4
300000 × g for 60 min	16.0	82.0

human lung mast cells, requires demonstration that there were no IgE aggregates in the IgE preparations. The authors proposed that centrifugation at 14,000 × g for 20 min would remove any aggregated IgE, but it is unlikely that this was effective. We have examined the effect of centrifugation on binding of IgG-containing immune complexes to the low-affinity IgG receptor FcγRIIA on apoptotic neutrophils. Fluorescent immune complexes were generated by combining monoclonal mouse IgG1 anti-fetuin with fluorescein-conjugated fetuin in conditions of antigen excess, and then subjected to centrifugation at 14,000 × g or 300,000 × g for 20 or 60 min, respectively. Binding of immune complexes in the post-centrifugation supernatants to apoptotic human neutrophils was measured by flow cytometry as previously described [4]. The results are presented in table 1.

These results demonstrate that centrifugation at 14,000 × g for 20 min does not effectively deplete immune complexes. Centrifugation at 300,000 × g for 60 min removes the majority of immune complex binding, but this would be inadequate for studies of cellular activation in which even immunoglobulin dimers may induce functional responses [5]. In contrast to centrifugation, size-exclusion chromatography would be a reliable method for purifying monomeric immunoglobulin E for use in mast cell activation studies.

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From the authors:

We are writing in response to the letter to the *European Respiratory Journal* by S. Hart and I. Dransfield regarding our recent paper [1]. We thank them for bringing to our attention that the centrifugation of our immunoglobulin (Ig)-E preparation at 14,000 × g for 20 min might be insufficient to remove IgE aggregates or multimers. We are, however, confident that the human myeloma IgE that we used in our experiments is free from such complexes (a belief that is shared by the manufacturer Calbiochem Novabiochem, Nottingham, UK).

Purified Ig preparations often contain immune aggregates. This has been demonstrated in several rodent studies [2–4]. These studies have also demonstrated that the efficacy of such IgE aggregates at initiating a response in mast cells is very poor compared with that of monomeric IgE.

These studies used high-performance liquid chromatography [2, 3], or size-exclusion chromatography [4] to ensure that their IgE preparations were truly monomeric, and recorded an array of responses with the monomeric forms. There are many recent studies which agree that monomeric IgE induces an array of responses in mast cells [5–8]. In our study we used IgE that was purified from the plasma of a myeloma patient. These preparations, therefore, are paraproteins that, as evidenced in IgE multiple myeloma, do not readily form dimers such as IgA or pentamers such as IgM, in the absence of a soluble antigen. The preparations have been assayed by the manufacturer using immunoelectrophoresis and produced a single arc, which further suggests a lack of aggregates or multimers. Any affinity for binding epitopes on the IgE molecules themselves would be very low. However, if there are intermolecular interactions with the IgE molecules at the receptor sites on the mast cell surface, as has been suggested in a recent mathematical model [9], this would not disprove the theory that monomeric IgE is an important activator of mast cell secretion, as the cross-linking of IgE by such interactions would occur *in vivo* at the same concentrations (the concentrations we used were experienced *in vivo*).

Therefore, we are not mimicking allergen exposure, but instead representing the physiological response to increased serum immunoglobulin E.

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