Reply by the authors to the comments of Mortimer Lorber, MD

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We sincerely appreciate Dr. Lorber's interest in our work, but we cannot agree with his conclusion that we "...analyzed not the normal circulating red cell mass..." Dr. Lorber makes the incorrect assumption that we repeatedly removed erythrocytes during the washing procedure used to generate the purified erythrocyte preparations. Our methods state clearly that whole blood was centrifuged at $500 \times g$ at 4°C for 10 min, and the plasma, buffy coat and upper most layer of erythrocytes were removed by aspiration and discarded. The remaining erythrocytes were then washed three times in buffer. The only erythrocytes discarded are those removed along with the buffy coat, less than 1% of the total erythrocyte mass. The additional washes are used to ensure that the erythrocytes are devoid of any contaminating substances that could be present in the plasma including ATP and medications. Importantly, these washes do not remove additional erythrocytes. Moreover, since the centrifugation is performed at $500 \times g$ with no gradient added for the separation of erythrocytes based on their density, we clearly have studied an erythrocyte population comprised of erythrocytes of all ages.

It is important to note that this approach results in an erythrocyte population that can be subsequently subdivided by density gradient centrifugation into distinct groups of cells based on differences in their densities. We have performed such separations using Percoll gradients with healthy human erythrocytes (unpublished observation). In addition, Subasinghe and Spence used an identical washing procedure to obtain rabbit erythrocytes that were subsequently fractionated by age using a similar Percoll gradient centrifugation.[1] Thus, in deference to the theoretical concern raised by Dr. Lorber, erythrocytes prepared by our method clearly contain both old and young cells based on their density.

Taken together, we do not consider Dr. Lorber's concerns to be pertinent to our work as we clearly have studied a diverse erythrocyte population that is entirely representative of the circulating erythrocyte mass *in vivo*. However, we will include additional information in the methods sections of future manuscripts to prevent any similar misinterpretation on the part of the reader.

REFERENCE

1. Subasinghe W, Spence DM. Simultaneous determination of cell aging and ATP release from erythrocytes and its implications in type 2 diabetes. *Anal Chem Acta* 2008;**618**:227–33