Metabolic rates in red blood cells under shear studied by Rheo-NMR

J. Milius[1], P. W. Kuchel[2], D. Shishmarev[3], S. J. Stevenson[1], T. I. Brox[1] P. Galvosas[1]
[1]MacDiamid Institute for Advanced Materials and Nanotechnology, School of Chemical and Physical Sciences, Victoria University Wellington, Wellington, New Zealand
[2]School of Life and Environmental Sciences, University of Sydney, NSW, Australia
[3]John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia

Introduction: Red blood cells (RBCs) are responsible for oxygen transport in many living organisms including humans. RBCs experience very different environmental conditions in different parts of the cardiovascular system. In major vessels, blood flow rates are large and mechanical shear stress for RBCs is small to moderate. The opposite is true for blood flowing through arterioles and venules: flow rates drop significantly while shear rates increase and reach values of up to 2000 s^{-1} in healthy humans [1]. Even more mechanical stress and deformation is applied to RBCs in capillaries with diameters that are only half that of an RBC.
It has been shown that mechanical deformation modifies the conversion rate from glucose to lactate in the main metabolic pathway of RBCs [2]. Static compression of RBCs in gelatin gel resulted in an enhancement of the metabolic rate by ~80% [2]. Here we report for the first time that metabolic rate is also enhanced when mechanical stress is applied dynamically through continuous shear at physiological shear rates.
Methods: Rheo-NMR has been used over the last 3 decades as a complementary rheological tool for the study of non-Newtonian and complex fluids [3,4]. We use a cylindrical Taylor-Couette cell adapted for use in a liquid-state high resolution NMR probe in a 400 MHz Bruker Avance spectrometer. The RBC samples were prepared from fresh human blood, under Human Ethics Clearances, as in [2]. Time series of ^{13}C NMR spectra were continuously recorded over 12 h while applying a shear rate of 1005.3 s^{-1}. After 12 h, the shear was stopped while recording spectra continued for another 4 h. Spectra were processed using MestreNova software in which peak integral values were extracted for glucose and lactate plotted against time. Metabolic rates were estimated from the slopes of the progress curves.
Results and Discussion: The rate of conversion of glucose to lactate in the RBCs under shear was 2.1 ± 0.2 mmol [liter RBC]$^{-1}$ h^{-1} while this rate dropped to 0.8 ± 0.1 mmol [liter RBC]$^{-1}$ h^{-1} after shear was stopped. This corresponded to a 2.5-fold enhancement of the metabolic activity in the RBCs under shear, thus confirming that the cells not only respond to static shape distortion but also to mechanical stress imposed by shear flow, as would be present *in vivo*.
Conclusions: We have demonstrated that RBCs respond with changes in their metabolic activity to mechanical deformation caused by shear flow. Shear rates were chosen to be similar to the physiological shear rates that are present in the cardiovascular system of humans. The detected difference in the metabolic rate is consistent with the operation of the mechanosensitive non-selective cation channel PIEZO1 as first seen with static RBC compression in compressed gelatin gels. However, in the latter samples, the RBCs have random orientations with respect to the direction of the strain/stress field, whereas in the rheometer, the alignment is uniform. This accounts for the even greater enhancement (2.5-fold compared with ~0.8-fold) of the metabolic rate under our conditions of uniform shear. The experimental set-up paves the way for studies of cation transport ($^{23}Na^{+}$ and $^{133}Cs^{+}$) in normal RBCs under physiological shear stress conditions and abnormal RBCs such as in sickle cell anaemia and malaria. In turn, this will improve our understanding of the fundamental biophysics of shape and volume regulation by cells in normal and disease states.
References: [1] Sakariassen, *Future Sci*. OA **1** (2015). [2] Kuchel, *Sci. Adv*. **3**, eaao1016 (2017). [3] Callaghan, *Rep. Prog. Phys*. **62** , 599-670 (1999). [4] Galvosas, *Mag. Reson. Chem*. DOI: 10.1002/mrc.4861 (2019)
JM acknowledges financial assistance from Bruker. PWK, DS and PG are supported by ARC DP190100510