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Nitric oxide bioavailability for red blood cell deformability in the microcirculation: A review of recent progress

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ABSTRACT

The rheological properties of red blood cells (RBCs) play an important role in their microcirculation. RBCs can elastically deform in response to mechanical forces to pass through narrow vessels for effective gas exchange in peripheral tissues. Decreased RBC deformability is observed in lifestyle-related diseases such as diabetes mellitus, hypercholesterolemia, and hypertension, which are pathological conditions linked to increased oxidative stress and decreased nitric oxide (NO) bioavailability. Redox-sensitive cysteine residues on RBC cytoskeletal proteins, such as α- and β-spectrins, responsible for membrane flexibility, are affected by prolonged oxidative stress, leading to reversible and irreversible oxidative modifications and decreased RBC deformability. However, endogenously, and exogenously generated NO protects RBC membrane flexibility from further oxidative modification by shielding redox-sensitive cysteine residues with a glutathione cap. Recent studies have shown that nitrate-rich diets and moderate exercise can enhance NO production to increase RBC deformability by increasing the interplay between RBCs and vascular endothelium-mediated NO bioavailability for microcirculation. This review focuses on the molecular mechanism of RBC- and non-RBC-mediated NO generation, and how diet- and exercise-derived NO exert prophylactic effects against decreased RBC deformability in lifestyle-related diseases with vascular endothelial dysfunction.

1. Introduction

While studies on the factors affecting blood flow in the circulation have been discussed from the viewpoint of vascular pathophysiology [1], another discussion on this issue has emerged from the perspective of rheological properties of red blood cells (RBCs) in the microcirculation [2]. The shape and stability of RBCs are maintained through cytoskeletal support in the plasma membrane. RBCs must change their shape to pass through capillaries for effective gas exchange of oxygen and carbon dioxide in peripheral tissues. This RBC shape flexibility is referred to as the deformability essential feature of RBCs to travel through small capillaries rather than large vessels [3]. RBC membranes composed of lipid bilayers are flexible with an underlying mesh-like configuration of cytoskeletal proteins, which provides an efficient distribution of forces and thus aids in morphological responses to mechanical stress [4]. RBCs are the most abundant cells in the body (around 70% of all the cells composing the body) [5] and may perceive changes in circulating plasma concentrations of glucose and cholesterol, which could influence RBC membrane flexibility. Decreased RBC deformability is observed in pathological conditions linked to increased oxidative stress or decreased nitric oxide (NO) bioavailability, such as diabetes mellitus (DM), hypercholesterolemia, and hypertension [6–8]. Treatment with oxidants and NO has been shown to affect RBC deformability in *in vitro* and *in vivo* studies [2,9,10], suggesting that redox balance affecting the RBC membrane and cytoskeletal proteins is closely related to RBC deformability in health and disease [11].

The present review focused on recent knowledge regarding the molecular mechanism of RBC- and non-RBC-mediated NO generation, which plays a key role in RBC deformability in the microcirculation. It also examined how diet- and exercise-derived NO exert prophylactic effects against the decrease in RBC deformability in vascular endothelial dysfunction associated with lifestyle-related diseases such as DM,

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hypercholesterolemia, and hypertension [12].

2. RBC- and non-RBC-mediated NO generation prevent RBC deformability from oxidative stress

RBC cytoskeletal proteins, including α- and β-spectrin chains, structurally form an elastic mesh-like network with RBC membranes to enable RBC deformation and flow through narrow capillaries in the microcirculation [13]. However, cysteine residues of α- and β-spectrins in RBC membranes are targets for oxidative modification, causing altered RBC deformability in various diseases [14,15]. Schwartz et al. reported that in patients with DM, protein glycosylation may induce oxidative damage to these spectrins. This causes irreversible oxidation of cysteine residues (cysteic acid, the final oxidation product of cysteine) and subsequently decreases RBC deformability, leading to impaired microcirculation and tissue perfusion [14].

In general, cysteine residues flanked by cationic amino acids in proteins serve as dynamic regulators of protein function *in vitro* and *in vivo* [16]. They also serve as redox sensors, followed by the oxidative modification of sulfenic (-SOH), sulfinic ($-SO_2H$), and sulfonoic ($-SO_3H$) acid formation under continued oxidative stress, causing reversible and/or irreversible protein dysfunctions [17] (Fig. 1). However, NO exposure derived either from exogenous (NO donors) or endogenous (NO synthase: NOS) sources induces *S*-nitrosation of these active thiols (SH of cysteine residues) by adding NO^+. Moreover, it elicits cell protection by the post-translational addition of glutathione to the protein thiols (S-glutathionylation, so-called glutathione cap) to protect and shield the redox-sensitive cysteine residues from further irreversible protein oxidation [12,18] (Fig. 1). In addition to redox-regulated protein functions by NO, gasotransmitter, hydrogen sulfide (H_2S) also plays an important role in the interplay with NO and the potential impact on vasorelaxation and oxidative system in RBCs [19]. H_2S is produced in RBC by 3-mercaptopyruvate sulfurtransferase [20], generating thiosulfate and polysulfides [20,21] These H_2S-induced protein *S*-sulfhydration have been shown to not only protect the cysteine residues from oxidation by elevating levels of cellular antioxidants and lowering levels of reactive oxygen species (ROS) [22], but also increase NO bioavailability by leading to *S*-nitrosation on cysteine residues in RBCs [21] (Fig. 1).

Riccio et al. recently reported that incubation of banked RBCs with NO donors resulted in tenfold greater levels of *S*-nitroso-hemoglobin (SNO-Hb) and increased *S*-nitrosation of RBC cytoskeletal and membrane proteins, including α- and β-spectrin chains, compared to untreated control or sham RBCs. This resulted in significantly increased RBC deformability and reduced adhesion to cultured endothelial cells [13]. However, it should be noted that the effects of extracellular NO on RBC deformability are not without controversy because they have not been consistently confirmed in other studies [15,23]. Oxidative stress induced by tert-buthylhydroperoxide (t-BuOOH) dose-dependently decreased RBC deformability, whereas it was not affected by treatment with increasing concentrations of the NO donor, nitrosated cysteine (CysNO). In contrast, pretreatment with CysNO protected RBC from t-BuOOH-induced decrease in RBC deformability, suggesting a possible prophylactic effect of exogenous NO by preventing cysteine residues from following oxidative modifications, rather than their restoration from already existing oxidation [11,15].

In addition to exogenous NO generation, including from the vascular endothelium, RBCs themselves contribute to the NO-dependent regulation of vascular homeostasis by RBC-mediated endogenous NO production and its bioavailability in the microcirculation [24]. Grau et al. demonstrated that RBC–NOS–produced NO improved membrane flexibility and RBC deformability under normoxic conditions through direct *S*-nitrosation of cytoskeletal proteins along with α- and β-spectrins [25]. It is possible that RBC-mediated NO production within the microvasculature, where RBCs are in close contact with the endothelium, plays a key role in microcirculation, particularly in diseases such as DM and hypertension, which are characterized by decreased vascular eNOS expression and activity.

In addition, Grau et al. indicated a different mechanism of RBC-derived NO generation under normoxic and hypoxic conditions, suggesting that RBC–NOS–mediated RBC deformability is decreased under hypoxia, while the addition of nitrite prevented a decrease in RBC deformability by sustaining *S*-nitrosation of RBC α- and β-spectrins through the compensatory mechanism of deoxy-Hb-catalyzed nitrite reduction to NO [25]. Although there has been debate on how NO produced in RBC escapes from scavenging by RBC Hb [26], it has been suggested that the reaction of nitrite and deoxyHb forms a less reactive intermediate such as dinitrogen trioxide (N_2O_3) to escape and release NO activity out of Hb [26]. This increases RBC deformability and induces vasodilation to increase blood flow to low-oxygen tissues where it is needed [27].

3. NO-mediated regulation of mechanoproperties of RBCs in microcirculation

Most of the endogenous NO generated in RBCs is oxidized to nitrate (a stable oxidation end product of NO and nitrite) by oxyHb, and then excreted in the urine. However, some NO provides NO activity by forming nitrosyl-Hb [Hb (FeII) NO], followed by the formation of *S*-nitroso-Hb [SNO-Hb] to transnitrosate cysteine residues of RBC proteins, including cytoskeletal proteins, for basal RBC deformability (left panel in Fig. 2). In contrast, RBC–NOS–derived NO is also oxidized and stored in RBCs as nitrite, together with dietary nitrite derived from the enterosalivary circulation. This nitrite is reduced to NO by deoxyHb and xanthine oxidoreductase during capillary transit and hypoxic

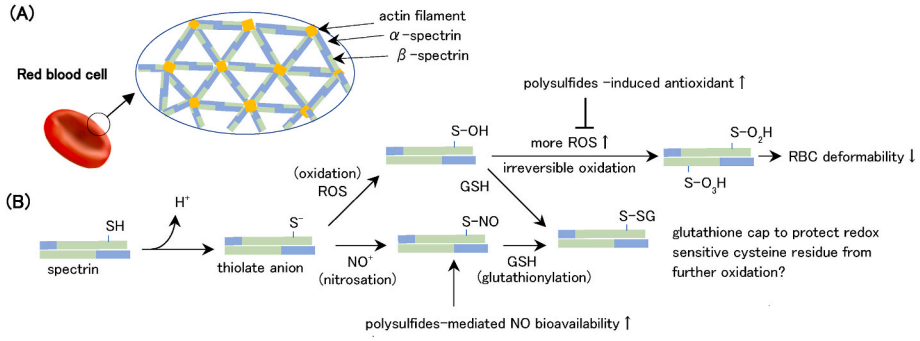

Fig. 1. Proposed redox modifications of spectrin cysteine in RBC by oxidation, nitrosation, and glutathionylation
(A) Spectrin fibrils, main protein component of the RBC cytoskeleton, are connecting to each other by nodes of actin filaments with the formation of an elastic network under RBC membrane. (B) Cysteine residues of spectrins are targets for reversible oxidative modification such as sulfenic (-SOH), followed by more oxidative sulfinic ($-SO_2H$), and sulfonoic ($-SO_3H$) acid formation under continued oxidative stress, subsequently causing decreased RBC deformability [52]. However, NO^+ nitrosylates the sensitive thiols to shield the cysteine residues from further oxidation (glutathione cap). RBC produces H_2S, followed by *S*-sulfhydration and formation of polysulfides (-S-SH) groups on cysteine residues. Although its potential role and mechanism need to be further investigated, it may protect the cysteine residues from oxidation by elevating levels of cellular antioxidants and lowering levels of reactive oxygen species (ROS) [22], and also may increase NO bioavailability by leading to *S*-nitrosation events on cysteine residues in RBCs [53].ROS, reactive oxygen species; RBC, red blood cell; GSH, glutathione; NO, nitric oxide.

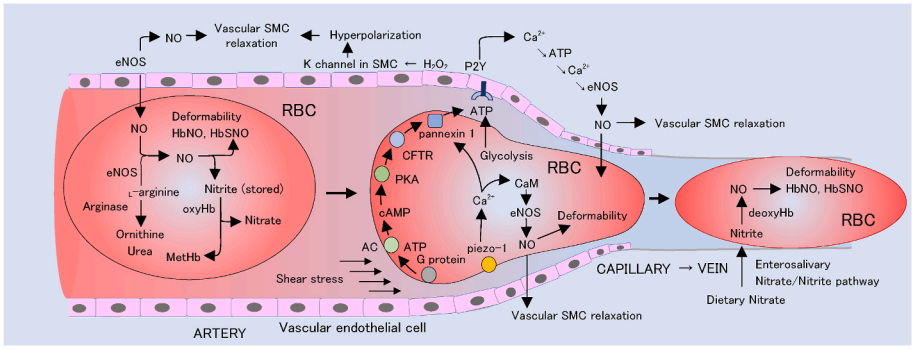

Fig. 2. Molecular regulation of microcirculation by NO-mediated RBC mechanoproperties

Vascular endothelium- and RBC-mediated NO generation cooperate to enhance NO-dependent RBC deformability for microcirculation. NO generated by vascular and RBC eNOS provides NO activity by forming HbNO and HbSNO to transnitrosate cysteine residues of RBC cytoskeletal proteins for basal RBC deformability (left panel in Figure). In peripheral capillaries, mechanical shear stress activates piezo-1 → increases intracellular calcium concentration → dissociates eNOS from caveolae into the cytoplasm → activates eNOS → relaxes vascular smooth muscle and contributes to RBC deformability (middle panel in Figure). In addition, various stimuli to RBC membrane activate membrane-bound AC through G protein-mediated signaling → increase cAMP and activate PKA → phosphorylate CFTR → activate pannexin-1 → release ATP from RBCs. Pannexin-1 is also activated by shear-induced calcium influx for ATP release from RBC, followed by ATP binding to the P2Y receptor on ECs → calcium release from endothelial intracellular stores to the cytoplasm → eNOS activation for vasodilation. Nitrite stored in RBCs (derived from NO oxidation and dietary sources) is reduced to NO by deoxyHb and xanthine oxidoreductase during capillary transit and hypoxic conditions for NO bioavailability in the microcirculation (right panel in Figure). Considering the mechanism mentioned above, there may be a complicated interplay between NO bioavailability and RBC deformability in the vascular endothelium and RBCs. Please refer to the details provided in the text. NO, nitric oxide; eNOS, endothelial nitric oxide synthase; HbNO, hemoglobin NO; HbSNO, S-nitroso-hemoglobin; OxyHb, oxyhemoglobin; MetHb, methemoglobin; RBC, red blood cell; AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CFTR, cyclic fibrosis transmembrane conductance regulator; deoxyHb, deoxyhemoglobin; SMC, smooth muscle cell; CaM, calmodulin.

conditions, respectively (right panel in Fig. 2) [12,26,28].

In peripheral capillaries, microcirculation is regulated by NO-mediated RBC mechanoproperties [29]. When RBCs pass through the microvasculature, shear stress stimulates RBC membrane deformation, which induces a transient increase in intracellular calcium concentration and subsequent vasodilation through the mechanically activated cation channel piezo-1 [30,31]. It is hypothesized that external mechanical stimuli (e.g., elongation of the RBC) bend the mechanosensitive channel piezo-1 incorporated in the RBC plasma membrane, allowing the opening of the piezo-1 channel for calcium ion influx, leading to the release of vasoactive molecules such as eNOS-derived NO and ATP [4,31] through the mechanotransductive pathway (middle panel in Fig. 2), as described below.

Mechanical shear stress promotes wall stretching to induce the dissociation of eNOS from caveolae into the cytoplasm, followed by eNOS activation and NO release by phosphorylation of eNOS at Ser1117 through binding of the Ca^{2+}/calmodulin complex [32]. Thereafter, RBC-generated NO contributes to smooth microcirculation by enhancing RBC deformability and diffusing endothelial cells into smooth muscle cells for vasodilatation (middle panel in Fig. 2).

ATP release from RBCs in response to various stimuli (including glycolysis pathway in RBCs) is an alternative signaling pathway for smooth microcirculation. Upon exposure of RBCs to stimuli (such as increased mechanical deformation, decreased extracellular pH, increased CO_2 concentration, and elevated temperature), membrane-bound adenylyl cyclase is activated via an unidentified G protein, leading to an increase in cAMP and protein kinase A (PKA) activation for subsequent phosphorylation of cyclic fibrosis transmembrane conductance regulator (CFTR) to induce ATP release from RBCs through ATP-releasing channel pannexin-1 [33,34]. It is also possible that shear-induced Ca^{2+} influx through the mechanism described above activates pannexin-1 directly to release ATP from RBCs [30]. Once released into the vascular lumen, ATP binds to the P2Y receptor on ECs, followed by the release of calcium from endothelial intracellular stores to the cytoplasm to activate eNOS for vasodilation (middle panel in Fig. 2) [4].

Considering the mechanism mentioned above, there may be a complicated interplay between NO bioavailability and RBC deformability in the vascular endothelium and RBCs. RBC membrane flexibility may be affected by oxidative and nitrosative modifications (e.g., enhanced formation of peroxynitrite, $ONOO^-$) of the RBC membranes/cytoskeletal proteins. Vascular endothelial dysfunction caused by DM, dyslipidemia, and hypertension reduces NO bioavailability in RBCs, leading to decreased RBC deformability, which further disturbs the RBC mechanoproperties linked to NO-mediated RBC deformability and ATP-mediated NO production in the vascular endothelium (Fig. 3).

4. Diet- and exercise-enhanced NO bioavailability for RBC deformability

Keymel et al. reported that RBC deformability was reduced in DM patients with coronary artery disease [35]. Babu also showed that RBC deformability was reduced in DM patients with normal cholesterol and was much more reduced in DM patients with hypercholesterolemia than in healthy subjects [36]. In animal studies, hypercholesterolemia induced in rabbits fed a cholesterol-rich diet altered the lipid composition of RBC membranes, resulting in decreased RBC deformability. However, this was significantly improved during *in vitro* incubation with an NO donor, cyclic GMP analog, or eNOS substrate arginine [7], suggesting that endogenous and exogenous NO pathway activation restores RBC deformability in the presence of hypercholesterolemia. Tsuda et al. also demonstrated that the fluidity of RBC membranes in hypertensive patients was more pronounced by NO donors than that of normotensive individuals. NO increased membrane fluidity, particularly in rigid RBC membranes caused by the hypertensive state [37]. These experimental and clinical studies suggest that NO-dependent RBC deformability participates in the regulation of the rheological behavior of RBCs to improve microcirculation in patients with DM, hypercholesterolemia, and hypertension. Based on these findings, diets and exercise that enhance NO bioavailability may be recommended to prevent a decrease in RBC deformability in persons with these diseases.

Nitrate, abundant in vegetables, is absorbed in the stomach and small intestine, enters the blood circulation, is concentrated in the salivary gland, and then is secreted into saliva via enterosalivary circulation. The salivary nitrate secreted during the chewing of food is converted to nitrite by commensal bacterial nitrate reductases in the oral cavity, followed by chemical reduction of nitrite to NO in the acidic stomach [12]. While this short-lived NO serves as a vasodilator in local gastric blood flow, it also serves as a signaling molecule, such as *S*-nitrosothiol (RSNO) and nitrite, after entering RBCs or diffusing into the plasma in the systemic circulation. Abu-Alghayth et al. recently reported that dietary supplementation of inorganic nitrate via beetroot juice significantly

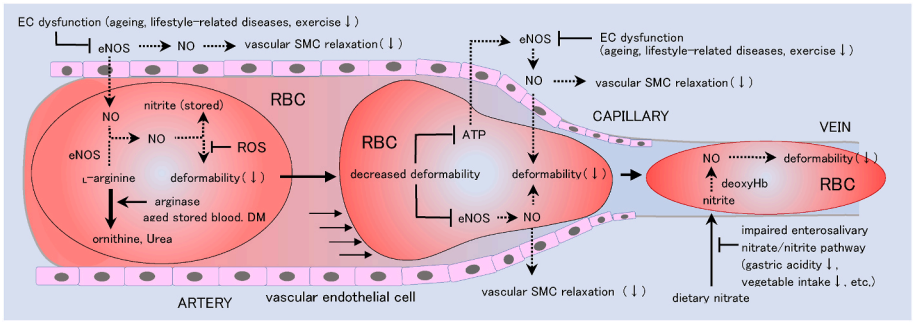

Fig. 3. Proposed molecular mechanism of decrease in RBC deformability following impaired NO generation and its bioavailability

Vascular EC dysfunction caused by ageing, DM, dyslipidemia, and hypertension reduces NO bioavailability and deformability of RBCs. Aged and stored blood increases RBC arginase activity leading to increased L-arginine consumption and decreased RBC deformability (left panel), which further disturb the RBC mechanoproperties linked to eNOS-mediated RBC NO production and ATP-mediated vascular NO production (middle panel). Impaired enterosalivary nitrate/nitrite pathway (gastric acidity↓ and vegetable intake↓ et al.) decreases plasma levels of nitrite and NO bioavailability in RBCs (right panel)

NO, nitric oxide; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; RBC, red blood cell; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; deoxyHb, deoxyhemoglobin; SMC, smooth muscle cell; DM, diabetes mellitus; ROS, reactive oxygen species.

increased the RBC and plasma levels of nitrate, nitrite, and RSNO in healthy human volunteers [38], suggesting that dietary nitrate could enhance RBC deformability and improve microcirculation by increasing NO bioavailability through the enterosalivary nitrate-nitrite-NO pathway [39].

In addition, compared with the ingestion of inorganic nitrate alone [40], beetroot juice containing nitrate and other components that influence the uptake and/or metabolism of nitrate, such as polyphenols, vitamin C, and sulfhydryl compounds, can provide more RSNO in and around RBCs through the reaction of sulfhydryl compounds (RSH groups) with N_2O_3, an NO^+ donors formed in the acidic stomach. RSNO transnitrosates the target proteins and peptides, including α- and β-spectrins in RBC cytoskeletal proteins, as well as albumin and glutathione, which contain cysteine residues to transfer and boost NO bioavailability in peripheral tissues [38,41,42].

Exercise also increases RBC deformability by shear stress-mediated NO generation from vessels and RBCs to improve microcirculation [43,44]. Tripette et al. showed that moderate exercise increased RBC-NOS activation and RBC deformability in healthy subjects and patients with microcirculatory disorders [45]. Suhr also showed that exercise-induced shear stress (running on a treadmill for 1 h) activated RBC-NOS and increased NO generation through the phosphatidyl-inositol 3 kinase/Akt kinase pathway and phosphorylation of RBC NOS-Ser1177, which subsequently increased RBC deformability (i.e., RBC elongation index measured by laser-assisted-optical-rotational red cell analyzer) during moderate exercise [46].

However, the effect of exercise on RBC deformability remains controversial. Suhr indicated possible downregulation of eNOS and NO generation in human RBCs following high-intensity exercise [47]. Yalcin also reported that high-intensity exercise (exhaustive swimming exercise in rats) reduces RBC deformability in untrained rats, whereas exercise training induces adaptive mechanisms that suppress such alterations in rats [48]. Acute and intensive exercise may generate more ROS, exceeding the antioxidant capacity (such as antioxidant vitamins, protein and non-protein thiols, and antioxidant enzymes) [49], and oxidize redox-sensitive cysteine residues on the proteins. This leads to decreased RBC deformability. Alternatively, continued moderate exercise could induce redox adaptations by enhancing transcriptional and protein levels of antioxidant activities [50], suggesting that RBC deformability may be altered by the cellular redox balance resulting from different exercise intensities and durations (i.e., moderate or intensive, and acute or chronic).

Exercise also increases RBC deformability by changing the distribution between young and old RBCs with different RBC deformability in the circulation. Regular endurance exercise upregulates catecholamines, cortisol, growth hormones, and insulin-like growth factor-1, which stimulate bone marrow activity and increase erythropoiesis, resulting in rapid circulating RBC turnover by removing old RBCs and mobilizing young RBCs [51]. In contrast to sedentary and untrained healthy individuals, trained individuals usually exhibit increased RBC deformability by increasing the proportion of young RBCs over old RBCs, with decreased deformability due to prolonged exposure to oxidative stress.

5. Conclusion and perspectives

In the microcirculation, blood flow is strongly dependent on the NO-mediated regulation of the mechanical properties of RBCs. Vascular endothelium and RBC-derived NO cooperate with each other to enhance NO-dependent RBC deformability in the microcirculation, where RBCs are in close contact with the vascular endothelium. Nitrate-rich diets and moderate exercise enhance NO bioavailability to increase RBC deformability through RBC- and non-RBC-mediated NO generation and may improve microcirculation, particularly in lifestyle-related diseases associated with vascular endothelial dysfunction. In addition, RBC cytoskeletal protein spectrin carries redox sensitive cysteine residues, and redox modifications such as oxidation, nitrosation, and sulfhydration may be related to each other to affect the deformability and the membrane stability of RBCs. Future studies would be directed at the studies focusing on the cross-talk of these post-translational modifications to regulate RBC deformability and rheology in health and disease.

Author contributions

Conception and design: J.K.; collection and assembly of data: K.O., I. M., and K.S.; manuscript writing: J.K. All authors have read and agreed to the published version of the manuscript.

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Informed consent statement

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Conflicts of interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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References

[1] C. Hahn, M.A. Schwartz, Mechanotransduction in vascular physiology and atherogenesis, Nat. Rev. Mol. Cell Biol. 10 (2009) 53–62.
[2] M. Bor-Kucukatay, R.B. Wenby, H.J. Meiselman, O.K. Baskurt, Effects of nitric oxide on red blood cell deformability, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H1577–H1584.
[3] B. Özüyaman, M. Grau, M. Kelm, M.W. Merx, P. Kleinbongard, N.O.S. RBC, Regulatory mechanisms and therapeutic aspects, Trends Mol. Med. 14 (2008) 314–322.
[4] K.J. Richardson, L. Kuck, M.J. Simmonds, Beyond oxygen transport: active role of erythrocytes in the regulation of blood flow, Am. J. Physiol. Heart Circ. Physiol. 319 (2020) H866–H872.
[5] E. Bianconi, A. Piovesan, F. Facchin, A. Beraudi, R. Casadei, F. Frabetti, L. Vitale, M.C. Pelleri, S. Tassani, F. Piva, S. Perez-Amodio, P. Strippoli, S. Canaider, An estimation of the number of cells in the human body, Ann. Hum. Biol. 40 (2013) 463–471.
[6] C.D. Brown, H.S. Ghali, Z. Zhao, L.L. Thomas, E.L.I.A. Friedman, Association of reduced red blood cell deformability and diabetic nephropathy, Kidney Int. 67 (2005) 295–300.
[7] T. Kuwai, J. Hayashi, Nitric oxide pathway activation and impaired red blood cell deformability with hypercholesterolemia, J. Atherosclerosis Thromb. 13 (2006) 286–294.
[8] G. Cicco, A. Pirrelli, Red blood cell (RBC) deformability, RBC aggregability and tissue oxygenation in hypertension, Clin. Hemorheol. Microcirc. 21 (1999) 169–177.
[9] J. Mohanty, E. Nagababu, J.M. Rifkind, Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging, Front. Physiol. 5 (2014) 84.
[10] J. Kim, H. Lee, S. Shin, Advances in the measurement of red blood cell deformability: a brief review, J. Cell. Biotechnol. 1 (2015) 63–79.
[11] F. Barbarino, L. Wäschenbach, V. Cavalho-Lemos, M. Dillenberger, K. Becker, H. Gohlke, M.M. Cortese-Krott, Targeting spectrin redox switches to regulate the mechanoproperties of red blood cells, Biol. Chem. 402 (2021) 317–331.
[12] J. Kobayashi, K. Ohtake, H. Uchida, NO-rich diet for lifestyle-related diseases, Nutrients 7 (2015) 4911–4937.
[13] D.A. Riccio, H. Zhu, M.W. Foster, B. Huang, C.L. Hofmann, G.M. Palmer, T. J. McMahon, Renitrosylation of banked human red blood cells improves deformability and reduces adhesivity, Transfusion 55 (2015) 2452–2463.
[14] R.S. Schwartz, J.W. Madsen, A.C. Rybicki, R.L. Nagel, Oxidation of spectrin and deformability defects in diabetic erythrocytes, Diabetes 40 (1991) 701–708.
[15] L. Diederich, T. Suvorava, R. Sansone, I.V.T.C. Keller, F. Barbarino, T.R. Sutton, C. M. Kramer, W. Lückstädt, B.E. Isakson, H. Gohlke, M. Feelisch, M. Kelm, M. M. Cortese-Krott, On the effects of reactive oxygen species and nitric oxide on red blood cell deformability, Front. Physiol. 9 (2018) 332.
[16] S. Biswas, A.S. Chida, I. Rahman, Redox modifications of protein–thiols: emerging roles in cell signaling, Biochem. Pharmacol. 71 (2006) 551–564.
[17] D. Barford, The role of cysteine residues as redox-sensitive regulatory switches, Curr. Opin. Struct. Biol. 14 (2004) 679–686.
[18] M.B. West, B.G. Hill, Y.T. Xuan, A. Bhatnagar, Protein glutathiolation by nitric oxide: an intracellular mechanism regulating redox protein modification, Faseb. J. 20 (2006) 1715–1717.
[19] D. Zhang, J. Du, C. Tang, Y. Huang, H. Jin, H_2S-induced sulfhydration: biological function and detection methodology, Front. Pharmacol. 8 (2017) 1–13.
[20] V. Vitvitsky, P.K. Yadav, A. Kurthen, R. Banerjee, Sulfide oxidation by a noncanonical pathway in red blood cells generates thiosulfate and polysulfides, J. Biol. Chem. 290 (2015) 8310–8320.
[21] M.M. Cortese-Krott, B.O. Fernandez, J.L.T. Santos, E. Mergia, M. Grman, P. Nagy, M. Kelm, A. Butler, M. Feelisch, Nitrosopersulfide (SSNO$^-$) accounts for sustained NO bioactivity of S-nitrosothiols following reaction with sulfide, Redox Biol. 2 (2014) 234–244.
[22] M. Peleli, S.I. Bibli, Z. Li, A. Chatzianastasiou, A. Varela, A. Katsouda, S. Zukunft, M. Bucci, V. Vellecco, C.H. Davos, N. Nagahara, G. Cirino, I. Fleming, D.J. Lefer, A. Papapetropoulos, Cardiovasculafr phenotype of mice lacking 3-mercaptopyruvate sulfurtransferase, Biochem. Pharmacol. 176 (2020), 113833.
[23] A.M. Belanger, C. Keggi, T. Kanias, M.T. Gladwin, D.B. Kim-Shapiro, Effects of nitric oxide and its congeners on sickle red blood cell deformability, Transfusion 55 (2015) 2464–2472.
[24] P. Horn, M.M. Cortese-Krott, S. Keymel, I. Kumara, S. Burghoff, J. Schrader, M. Kelm, P. Kleinbongard, Nitric oxide influences red blood cell velocity independently of changes in the vascular tone, Free Radic. Res. 45 (2011) 653–661.
[25] M. Grau, S. Pauly, J. Ali, K. Walpurgis, M. Thevis, W. Bloch, F. Suhr, RBC-NOS-dependent S-nitrosylation of cytoskeletal proteins improves RBC deformability, PLoS One 8 (2013), e56759.
[26] V. Kapil, R.S. Khambata, A. Jones, K. Rathod, G. Primus, G. Massimo, J.M. Fukuto, A. Ahluwalia, The noncanonical pathway for in vivo nitric oxide generation: the nitrate-nitrite-nitric oxide pathway, Pharamacol. Rev. 72 (2020) 692–766.
[27] M. Grau, A. Lauten, S. Hoeppener, B. Goebel, J. Brenig, C. Jung, W. Bloch, F. Suhr, Regulation of red blood cell deformability is independent of red blood cell-nitric oxide synthase under hypoxia, Clin. Hemorheol. Microcirc. 63 (2016) 199–215.
[28] A.W. DeMartino, D.B. Kim-Shapino, R.P. Patel, M.T. Gladwin, Nitrite and nitrate chemical biology and signaling, Br. J. Pharmacol. 176 (2019) 228–245.
[29] F. Leo, B. Hutzler, C.A. Ruddiman, B.E. Isakson, M.M. Cortese-Krott, Cellular microdomains for nitric oxide signaling in endothelium and red blood cells, Nitric Oxide 96 (2020) 44–53.
[30] E. Cinar, S. Zhou, J. DeCourcey, Y. Wang, R.E. Waugh, J. Wan, Piezo 1 regulates mechanotransductive release of ATP from human RBCs, Proc. Natl. Acad. Sci. USA 112 (2015) 11783–11788.
[31] L. Kuck, J.N. Peart, M.J. Simmonds, Piezo1 regulates shear-dependent nitric oxide production in human erythrocytes, Am. J. Physiol. Heart Circ. Physiol. 323 (2022) H24–H37.
[32] R. Erkens, T. Suvorava, C.M. Kramer, L.D. Diederich, M. Kelm, M.M. Cortese-Krott, Modulation of local and systemic heterocellular communication by mechanical forces: a role of endothelial nitric oxide synthase, Antioxidants Redox Signal. 26 (2017) 917–935.
[33] J. Wan, A.M. Forsyth, H.A. Stone, Red blood cell dynamics: from cell deformation to ATP release, Integr. Biol. 3 (2011) 972–981, 2011.
[34] E. Ugurel, E. Goksel, N. Cilek, E. Kaga, O. Yalcin, Proteomic analysis of the role of the adenylyl cyclase-cAMP pathway in red blood cell mechanical responses, Cells 11 (2022) 1250.
[35] S. Keymel, C. Heiss, P. Kleinbongard, M. Kelm, T. Lauer, Impaired red blood cell deformability in patients with coronary artery disease and diabetes mellitus, Horm. Metab. Res. 43 (2011) 760–765.
[36] N. Babu, Influence of hypercholesterolemia on deformability and shape parameters of erythrocytes in hyperglycemic subjects, Clin. Hemorheol. Microcirc. 41 (2009) 169–177.
[37] K. Tsuda, K. Kimura, I. Nishio, Y. Masuyama, Nitric oxide improves membrane fluidity of erythrocytes in essential hypertension: an electron paramagnetic resonance investigation, Biochem. Biophys. Res. Commun. 275 (2000) 946–954.
[38] M. Abu-Alghayth, A. Vanhatalo, L.J. Wylie, S.T.J. McDonagh, C. Thompson, S. Kadach, P. Kerr, M.J. Smallwood, A.M. Jones, P.G. Winyard, S-nitrosothiols, and other products of nitrate metabolism, are increased in multiple human blood compartments following ingestion of beetroot juice, Redox Biol. 43 (2021), 101974.
[39] N. Wajih, S. Basu, A. Jailwala, H.W. Kim, D. Ostrowski, A. Perlegas, C.A. Boldena, N.L. Buechlerc, M.T. Gladwin, D.L. Caudellf, E. Rahbarg, M.A. Alexander-Millerh, V. Vachharajani, D.B. Kim-Shapiro, Potential therapeutic action of nitrite in sickle cell disease, Redox Biol. 12 (2017) 1026–1039.
[40] J.O. Lundberg, M. Govoni, Inorganic nitrate is a possible source for systemic generation of nitric oxide, Free, Radic. Biol. Med. 37 (2004) 395–400.
[41] R. Marley, R.P. Patel, N. Orie, E. Ceaser, V. Darley-Usmar, K. Moore, Formation of nanomolar concentrations of S-nitroso-albumin in human plasma by nitric oxide, Free Radic. Biol. Med. 31 (2001) 688–696.
[42] D. Jourd'heuil, F.L. Jourd'heuil, M. Feelisch, Oxidation and nitrosation of thiols at low micromolar exposure to nitric oxide: evidence for a free radical mechanism, J. Biol. Chem. 278 (2003) 15720–15726.
[43] P. Ulker, N. Yaras, O. Yalcin, C. Celik-Ozenci, P.C. Johnson, H.J. Meiselman, O. K. Baskurt, Shear stress activation of nitric oxide synthase and increased nitric oxide levels in human red blood cells, Nitric Oxide 24 (2011) 184–191.
[44] P. Connes, M.J. Simmonds, J.F. Brun, O.K. Baskurt, Exercise hemorheology: classical data, recent findings and unresolved issues, Clin. Hemorheol. Microcirc. 53 (2013) 187–199.
[45] J. Tripette, P. Connes, E. Beltan, T. Chalabi, L. Marlin, R. Chout, O.K. Baskurt, O. Hue, M.D. Hardy-Dessources, Red blood cell deformability and aggregation, cell adhesion molecules, oxidative stress and nitric oxide markers after a short term, submaximal, exercise in sickle cell trait carriers, Clin. Hemorheol. Microcirc. 45 (2010) 39–52.
[46] F. Suhr, J. Brenig, R. Müller, H. Behrens, W. Bloch, M. Grau, Moderate exercise promotes human RBC-NOS activity, NO production and deformability through Akt kinase pathway, PLoS One 9 (2012), e45982.
[47] F. Suhr, S. Porten, T. Hertrich, K. Brixius, A. Schmidt, P. Platen, W. Bloch, Intensive exercise induces changes of endothelial nitric oxide synthase pattern in human erythrocytes, Nitric Oxide 20 (2009) 95–103.
[48] O. Yalcin, M. Bor-Kucukatay, U.K. Senturk, O.K. Baskurt, Effects of swimming exercise on red blood cell rheology in trained and untrained rats, J. Appl. Physiol. 88 (2000) 2074–2080.
[49] A.K. Banerjee, A. Mandal, D. Chanda, S. Chakraborti, Oxidant, antioxidant and physical exercise, Mol. Cell. Biochem. 253 (2003) 307–312.
[50] L. Ji, Modulation of skeletal muscle antioxidant defense by exercise: role of redox signaling, Free Radic. Biol. Med. 44 (2008) 142–152.
[51] H. Mairbäurl, Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells, Front. Physiol. 4 (2013) 332.
[52] P.S. Becker, C.M. Cohen, S.E. Lux, The effect of mild diamide oxidation on the structure and function of human erythrocyte spectrin, J. Biol. Chem. 261 (1986) 4620–4628.
[53] C. Szabo, Hydrogen sulfide, an enhancer of vascular nitric oxide signaling: mechanisms and implications, Am. J. Physiol. Cell Physiol. 312 (2017) C3–C15.