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Compensatory mechanisms in myoglobin deficient mice preserve NO homeostasis

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ABSTRACT

The mechanism for nitric oxide (NO) generation from reduction of nitrate (NO_3^-) and nitrite (NO_2^-) has gained increasing attention due to the potential beneficial effects of NO in cardiovascular diseases and exercise performance. We have previously shown in rodents that skeletal muscle is the major nitrate reservoir in the body and that exercise enhances the nitrate reduction pathway in the muscle tissue and have proposed that nitrate in muscle originates from diet, the futile cycle of nitric oxide synthase 1 (NOS1) and/or oxidation of NO by oxymyoglobin. In the present study, we tested the hypothesis that lack of myoglobin expression would decrease nitrate levels in skeletal muscle. We observed a modest but significant decrease of nitrate level in skeletal muscle of myoglobin deficient mice compared to littermate control mice (17.3 vs 12.8 nmol/g). In contrast, a NOS inhibitor, L-NAME or a low nitrite/nitrate diet treatment led to more pronounced decreases of nitrate levels in the skeletal muscle of both control and myoglobin deficient mice. Nitrite levels in the skeletal muscle of both types of mice were similar (0.48 vs 0.42 nmol/g). We also analyzed the expression of several proteins that are closely related to NO metabolism to examine the mechanism by which nitrate and nitrite levels are preserved in the absence of myoglobin. Western blot analyses suggest that the protein levels of xanthine oxidoreductase and sialin, a nitrate transporter, both increased in the skeletal muscle of myoglobin deficient mice. These results are compatible with our previously reported model of nitrate production in muscle and suggest that myoglobin deficiency activates compensatory mechanisms to sustain NO homeostasis.

1. Introduction

Nitric oxide (NO) is a gaseous signaling molecule that exerts various physiological functions such as vasodilation and regulation of platelet reactivity [1]. Once NO is produced from L-arginine and molecular oxygen by a family of endogenous nitric oxide synthase (NOS), it can be oxidized rapidly to nitrate (NO_3^-) and nitrite (NO_2^-) in blood and tissues [2]. These anions, once considered inert end products of NO generation, are now known to be able to be serially reduced back to NO in mammalian systems [3]. It has been shown that nitrate absorbed from diet can be converted to nitrite, mainly by commensal bacterial nitrate reductases in the oral cavity [4], and nitrite can be further reduced to NO by several pathways including deoxyhemoglobin [5], deoxymyoglobin [6,7], molybdenum containing enzymes such as xanthine oxidoreductase [8], and non-enzymatic reduction in the presence of protons [9,10]. This nitrate-nitrite-NO pathway is more relevant under hypoxic conditions where NOS activity is reduced [11]. Numerous studies examining the beneficial effects of dietary nitrate on cardiovascular diseases and exercise performance [12] have suggested that nitrate can be efficiently metabolized in vivo to NO.

In a previous report, we showed that skeletal muscle is the major nitrate storage organ in the body [13]. Compared to liver or blood, skeletal muscle contained significantly higher levels of nitrate and NOS1 deficient mice exhibited remarkably lower amount of nitrate in the skeletal muscle than wild type mice suggesting that some of NO produced by active NOS1 in skeletal muscle is oxidized to nitrate. When we examined the nitrate reduction activity of skeletal muscle in a rat exercise model, we found that the treadmill exercise enhanced nitrate conversion to nitrite and to NO in skeletal muscle [14]. These results suggest that skeletal muscle contributes to the dynamics of the NO cycle by producing and utilizing nitrate and nitrite.

Myoglobin, a monomeric cytosolic heme protein, belongs to the globin family and is mainly expressed in cardiac myocytes and striated muscles [15]. Since the three-dimensional structure of myoglobin was

Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal NOS (NOS1); XOR, xanthine oxidoreductase; L-NAME, Nω-Nitro-L-arginine methyl ester

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Table 1
Summary of studies of myoglobin functions using myoglobin deficient mice.

Publications	Mb (+/+) *vs* Mb (−/−) mouse	Measurements	Results
Garry et al., Nature (1998) [17]	Intact animal and isolated cardiac and skeletal muscle	Exercise performance Ventilatory response to hypoxia Stroke work Time to fatigue	No significant difference between Mb (+/+) and (−/−)
Godecke et al., PNAS(1999) [18]	Blood and isolated hearts	Hb concentration and hematocrit Contractile parameters Oxygen consumption Basal coronary flow Cardiac energetics Capillary density Mitochondrial density	Increase in Hb concentration, basal coronary flow and capillary density in Mb (−/−)
Rassaf et al., Circ Res (2007) [7]	Isolated hearts and tissues	NO generation Cardiac function and energy status	NO generation decrease in Mb (−/−), nitrite effects on cardiac energy status seen only in Mb (+/+) heart
Hendgen-Cotta et al., PNAS (2008) [20]	Isolated hearts and tissues	NO generation Inhibition of respiration Oxidative damage Myocardial infarcts	Protective effects of nitrite against ischemia seen only in Mb (+/+) heart
Totzeck et al., Circulation (2012) [21]	Intact animal and aortic tissues	NO generation Hypoxic vasodilation Cardiac function	Nitrite-induced hypoxic vasodilation is dependent on Mb

solved [16], its role in oxygen delivery and storage has been extensively studied. About two decades ago, myoglobin deficient mice were generated by two groups [17,18]. Surprisingly these studies showed that the disruption of myoglobin presents no obvious defects in usual exercise capacity and cardiac function in these knockout mouse lines. More recently, new roles of myoglobin in the NO cycle has been suggested. In normoxic conditions, oxygenated myoglobin reacts with NO, forming metmyoglobin, and producing nitrate, thereby acting as a NO scavenger [19]. However, in severe hypoxic conditions, deoxygenated myoglobin can act as a nitrite reductase, reducing nitrite to NO and exhibiting protective effects against ischemic damages [6,7,20,21]. Table 1 summarizes experimental results from the myoglobin deficient mouse studies mentioned above.

In our previous reports, we demonstrated that nitrate stored in skeletal muscle is important for the maintenance of the NO cycle and proposed that the reaction of oxymyoglobin with NO as one of the sources of skeletal muscle nitrate [13,14]. In the current study, we tested whether myoglobin deficiency influences NO homeostasis in skeletal muscle by analyzing nitrite and nitrate levels in myoglobin deficient mice. We also measured the expression levels of several NO metabolism-related proteins: NOS, XOR and sialin. Our results show that nitrate levels in skeletal muscle of myoglobin deficient mice were moderately reduced, but with accompanying upregulation of XOR and sialin proteins. These results imply that compensatory mechanisms exist to sustain the NO pathway in the myoglobin deficient animals.

2. Materials and methods

Myoglobin (*Mb*) deficient mice were generated using CRISPR-Cas9 mediated gene targeting. Guide RNAs (gRNAs) targeting the heme binding domain of the *Mb* gene were designed using online tools available at http://crispr.mit.edu/. The pX330-U6-Chimeric-BB-Cbh-hSpCas9 plasmid (Addgene, Plasmid #42230, Watertown, MA) has a U6 promoter to drive expression of humanized *S.pyogenes* Cas9. The two gRNAs were individually cloned into the vector using *BbsI* sites. The gRNA expressing plasmids were microinjected to generate mouse lines in the C57Bl/6 background (Taconic, Rensselaer, NY). Genomic DNA from the mouse lines were amplified using primers targeting exon 2 of *Mb* and analyzed by sequencing. *Mb* gene knockout mice can be identified by PCR using the following primers: ACA ACAGCCACAGATAGTCAGGAG and CCTGTGCCTCAGTTTCCACATCT.

For diet manipulation, L-NAME (1 g/L) in drinking water or low nitrite/nitrate diet (Harlan Teklad, TD99366, South Easton, MA) was given for 3 days. All animal procedures were carried out according to recommendations in the Guide for the Care and Use of Laboratory Animals of NIH under NIDDK approved NIH protocol number K049-MMB-17 and K048-MMB-19.

Chemiluminescence assays for nitrite and nitrate in blood and tissues were performed according to previously published protocols [13,22,23]. Samples were weighed and homogenized with stop solution containing potassium ferricyanide, NEM and NP40 using GentleMacs (Miltenyi Biotec Inc, Auburn, CA). Proteins were precipitated by adding methanol (dilution 1:1) and subsequent centrifugation at 11000g for 15 min at 4 °C. Supernatants were used to determine nitrite and nitrate content (Sievers 280i Nitric Oxide Analyzer, GE Analytical Instruments).

Western blotting was performed to analyze the levels of myoglobin, NOS1 (nNOS), NOS3 (eNOS), XOR, sialin and GAPDH protein in the mixture of Gluteus maximus, Vastus lateralis and Rectus femoris skeletal muscle of three control and myoglobin deficient mice respectively. Muscle tissues were homogenized in RIPA buffer (Sigma, Cat. #R0278) containing protease inhibitors (CalBiochem, #539134) and protein concentration was measured by bicinchoninic acid (BCA) assay (Thermo Scientific, #23227). Denatured samples (50 μg) were run on SDS-PAGE and then transferred to nitrocellulose membranes. The membranes were incubated with primary antibodies (Anti-myoglobin: Santa Cruz Biotechnology, sc-25607; Anti-sialin: Alpha Diagnostics, SIAL11-A; Anti-XOR: Abcam, ab133268; Anti-NOS3: Abcam, ab76198; Anti-NOS1: BD Transduction Laboratories, 610309; Anti-GAPDH: Cell Signaling, 97166) overnight at 4 °C. Goat-anti-mouse or goat-anti-rabbit antibodies conjugated with horseradish peroxidase (Jackson Immunoresearch) were used as secondary antibodies and followed by ECL detection (Thermo Scientific, #34095). Band density was quantified using NIH Image J software.

Values represent means ± standard deviation. Data were analyzed by Student's t-test (p value < 0.05, statistically significant).

3. Results

3.1. Nitrate and nitrite levels in myoglobin deficient and littermate control mice

We hypothesized that nitrate levels in skeletal muscle of myoglobin deficient mice would be much lower than that of control mice because oxygenated myoglobin contributes to nitrate formation by reacting with NO. Compared to control skeletal muscle, myoglobin deficient muscle showed a decrease in nitrate levels by 26% (17.3 vs 12.8 nmol/g). Nitrate levels in liver and whole blood were similar in control and myoglobin deficient mice (Fig. 1A). Treatment with L-NAME or low NOx diet for 3 days also led to decreased skeletal muscle nitrate levels in both control and myoglobin deficient mice (67% and 72% decrease for control and 65% and 67% for myoglobin deficient mice, respectively).

Nitrite levels in skeletal muscle and liver did not differ in myoglobin deficient mice compared to those of control mice, but blood nitrite levels of myoglobin deficient mice were higher than that of control mice (0.44 vs 0.31 nmol/g) (Fig. 1B). L-NAME or low NOx diet treatment

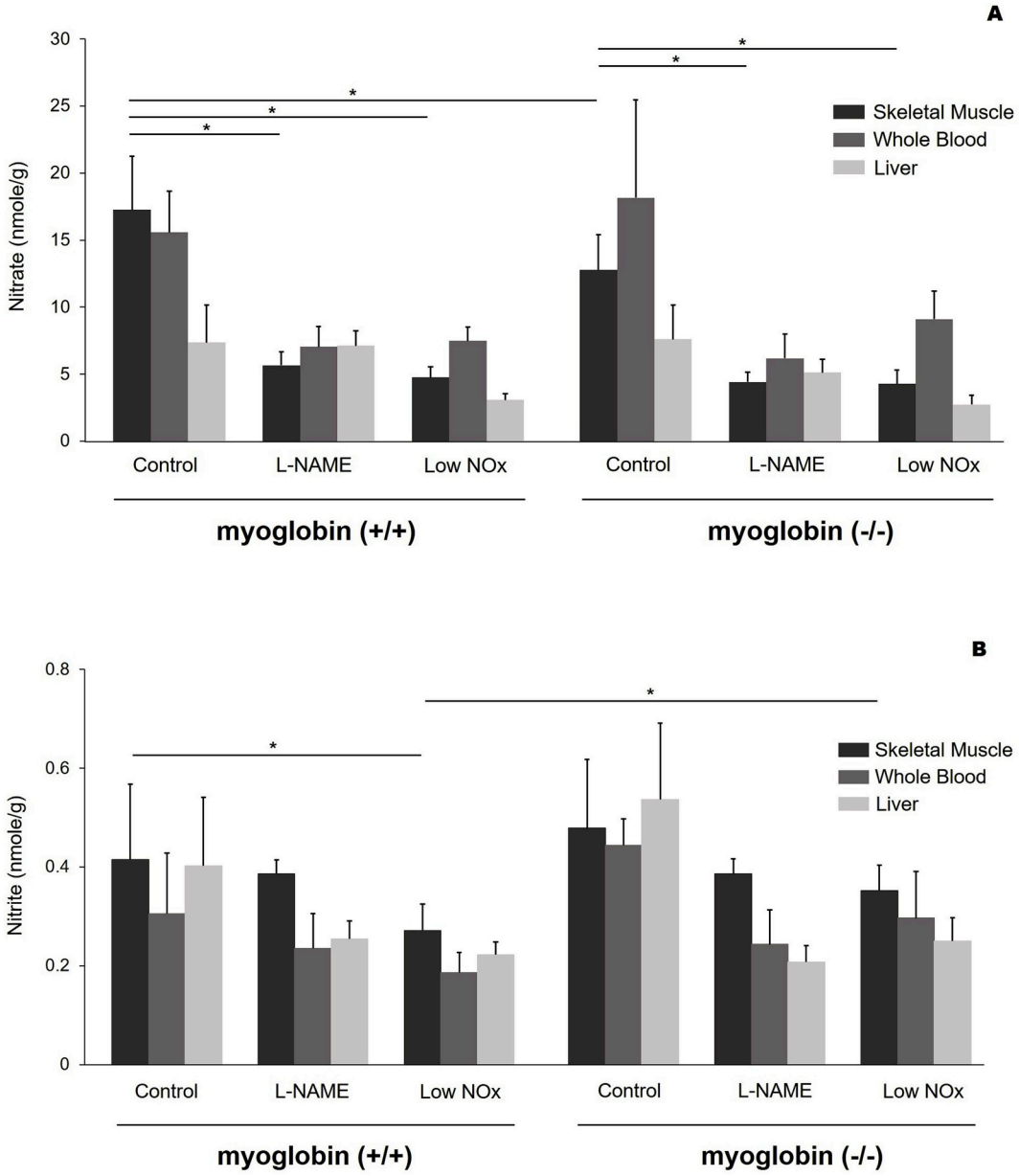

Fig. 1. Nitrate (A) and nitrite (B) levels in skeletal muscle, whole blood and liver of littermate control and myoglobin deficient mice. Control mice were on tap water and standard chow, L-NAME group mice received L-NAME (1 g/L) in drinking water and standard chow and low NOx group mice received tap water and low NOx diet for 3 days. Nitrate and nitrite contents were determined using a standard chemiluminescence method with vanadium chloride or tri-iodide solution respectively, n = 4–9 in each tissue, comparison between skeletal muscle samples, *p < 0.05.

decreased nitrite levels in both control and myoglobin deficient mice, but the effect of these treatments was more pronounced in liver than skeletal muscle or blood.

3.2. Elevation of NO metabolism-related protein levels in myoglobin deficient mice

Since myoglobin deficient mice still maintained about 74% of nitrate in skeletal muscle compared to control muscle, we tested whether there are other mechanisms that can provide skeletal muscle with nitrate in myoglobin deficient mice by assessing protein expression levels of NO pathway-related proteins. Western blotting analysis showed that several proteins were upregulated in myoglobin deficient skeletal muscle (Fig. 2A). Mean values of densitometric analyses of these Western blot bands are shown in Fig. 2B. Levels of XOR and sialin were increased in skeletal muscle samples of myoglobin deficient mice compared to that of control mice (85% and 89% for XOR and sialin, respectively). The NOS1 level was not statistically different although it showed a tendency to increase in myoglobin deficient muscle. NOS3 protein level did not change at all after myoglobin disruption.

4. Discussion

The first report on myoglobin deficient mice revealed no apparent myoglobin deficiency-related phenotypes, with normal behavior and exercise capacity [17]. The results from a second myoglobin deficient mouse line showed that these mice were normal in cardiac function because they developed compensatory mechanisms such as a higher capillary density and an increased coronary flow [18]. For more details, see Table 1. While myoglobin is not crucial for normal skeletal muscle or cardiac function in mice, myoglobin is known to be closely involved in the NO pathway by acting either as a nitrite reductase or as a NO di-

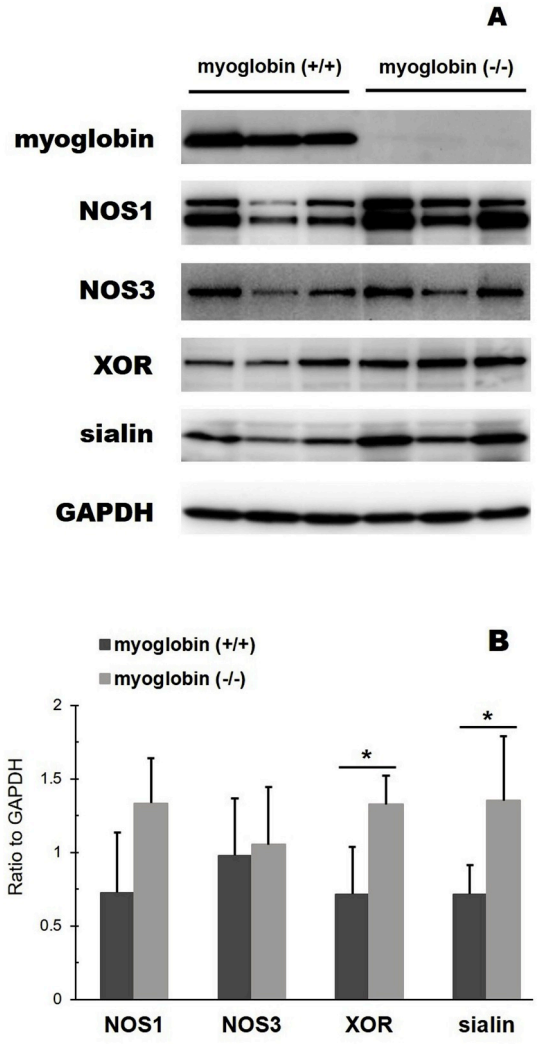

Fig. 2. Western blotting images (A) and densitometric analyses (B) of myoglobin, NOS1, NOS3, XOR, sialin and GAPDH in muscle samples from three different control and myoglobin deficient mice. Skeletal muscle homogenate samples (50 μg) were run on SDS-PAGE and transferred onto nitrocellulose membranes for Western blotting. Primary antibodies were incubated overnight at 4 °C, then horseradish peroxidase conjugated secondary antibodies were used for ECL detection. Band density was quantified using NIH Image J software, *$p < 0.05$.

oxygenase [24]. Using rats, we previously identified skeletal muscle as a mammalian nitrate reservoir and a critical NO source [13,14]. We also identified XOR as a skeletal muscle nitrate reductase at basal conditions and that its nitrate reduction activity increased after exercise [13,14]. We then hypothesized that the nitrate reservoir in skeletal muscle originates from at least three independent sources: dietary nitrate, the futile cycle of NOS1 and oxidation of NO by oxymyoglobin. We have shown the importance of diet and an active NOS for levels of nitrate in skeletal muscle [13,14,25].

To better understand the contribution of myoglobin to nitrate levels and NO signaling in skeletal muscle, we created myoglobin deficient mice and analyzed nitrite and nitrate levels in skeletal muscle as well as in blood and liver for comparison. We hypothesized that the loss of myoglobin would reduce nitrate levels in skeletal muscle, and the magnitude of the effect would be comparable to what we previously measured in NOS1 deficient mouse. In skeletal muscle, NOS1 is a predominant isoform of NOS responsible for NO production and we showed that NOS1 knockout mice have dramatically reduced nitrate levels in skeletal muscle compared to wild type mice (88% decrease) [13] suggesting that NOS1 is critical to maintain the skeletal muscle nitrate reservoir. In the present study, we analyzed nitrate levels and observed a modest but significant decrease (about 26%) of nitrate in myoglobin deficient muscle compared to control muscle. The fact that NOS is also known to directly generate nitrate through a futile cycle [26] seems to partly account for this relatively smaller change in nitrate levels, because it can produce nitrate without a reaction with oxymyoglobin. We observed a difference in the baseline of muscle nitrate levels between wild type mice in our previous publications [13,14] and the current genetically altered mice (both myoglobin $+/+$ and $-/-$), perhaps due to CRISPR/Cas9 process itself or to other environmental differences, especially with regard to nitrate intake and microbiome variation. To confirm the importance of NOS in the nitrate levels in skeletal muscle, we treated mice with L-NAME and measured nitrate levels. As expected, L-NAME treatment for 3 days in drinking water decreased muscle nitrate levels by 67% in control mice and by 66% in myoglobin deficient mice demonstrating NOS as a critical nitrate source in skeletal muscle. In addition to endogenous sources, diet rich in green leafy vegetables or beetroot is also a great exogenous source of nitrate in mammals [27]. Standard rodent chow was reported to contain considerably higher nitrate levels compared to the low NOx diet we used in our study [25,28] and we observed 72% decrease of nitrate in control muscle and 66% decrease of nitrate in myoglobin deficient muscle compared to control muscle respectively when we fed mice the low NOx diet for 3 days. These data indicate that muscle nitrate content in these animals is affected largely by NOS-derived pathway and direct exogenous source rather than myoglobin-derived NO oxidation. However, there could be other sources of nitrate that are not currently identified, as demonstrated by Feelisch's group [29].

To determine if NO metabolism-related proteins were influenced by myoglobin disruption, we performed Western blotting for NOS1, NOS3, XOR and sialin using control and myoglobin deficient muscle homogenates. We found by densitometric analyses significant increases in XOR and sialin protein levels in myoglobin deficient muscle compared to control muscle, of 85% and 89% for XOR and sialin, respectively. NOS1 increase (by 84%) did not reach statistical significance. Sialin was identified as a nitrate transporter and known to concentrate nitrate from blood to salivary glands [30]. To our knowledge, it has not been established yet whether sialin is involved in nitrate transport in skeletal muscle. We confirmed that sialin is expressed in mouse skeletal muscle and its expression was upregulated upon myoglobin deletion. In our previous studies, we showed that XOR is a major mammalian nitrate reductase in skeletal muscle [13,14] and myoglobin deficient muscle used in the current study had elevated levels of XOR. These results suggest that multiple compensatory pathways were activated in skeletal muscle to sustain NO metabolism in myoglobin deficient mice.

Fig. 3 is a hypothetical scheme that shows our observations on how the NO-nitrite-nitrate cycle is affected by different factors such as diet and the changes in these protein levels.

In conclusion, our results demonstrate that nitrate levels in skeletal muscle represent the dynamics of NO metabolic processes. Engineered myoglobin deficiency, the endogenous NOS enzymes and nitrate in the diet all contributed to variation of nitrate amount in skeletal muscle, albeit to different levels. Particularly, in the case of myoglobin deletion by genetic modification, compensatory mechanisms, such as XOR and sialin upregulation and small increases in NOS1 in skeletal muscle appear to develop to maintain the NO signaling pathway. NOS3 levels do not appear to change. These results imply that adequate nitrate levels in skeletal muscle need to be preserved for normal physiology.

Contributions

JWP, BP and ANS designed the experiments and wrote the manuscript. JWP and BP performed the research and analyzed the data. SD and CTN generated the myoglobin knockout mice and provided help with animal experiment design. All authors contributed to data interpretation and commented on the manuscript.

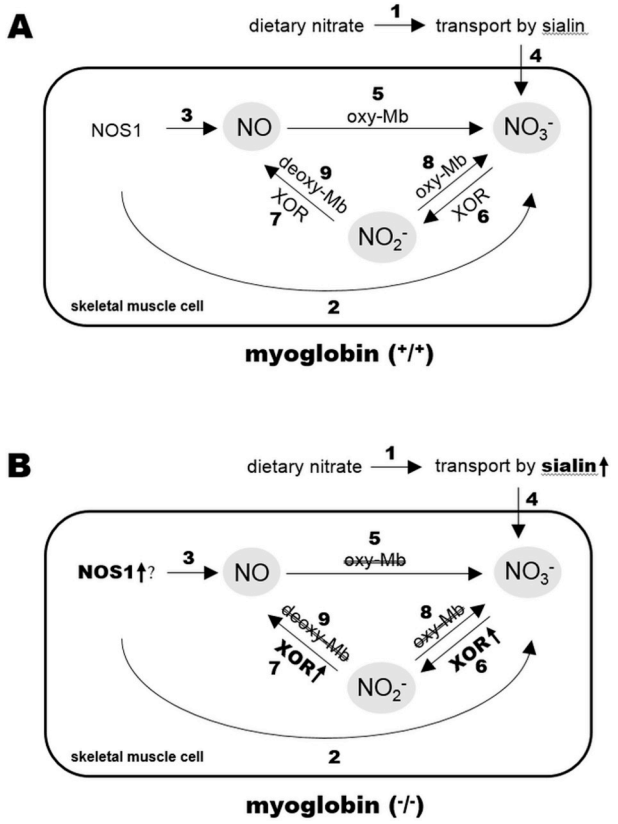

Fig. 3. Effects of myoglobin disruption on NO/nitrite/nitrate pathways in mice. This schematic representation of NO/nitrite/nitrate pathways compares myoglobin knockout mice (myoglobin $^{-/-}$) with their littermate controls (myoglobin $^{+/+}$). (A) In control mice (myoglobin $^{+/+}$), the main nitrate sources are diet (1) and the futile cycle of NOS1 residing in skeletal muscle cells (2), as well as from NO produced by NOS1 (3). Dietary nitrate is transported into skeletal muscle tissue by sialin (4). Within the muscle cell, NO is oxidized to nitrate by oxyMb (5); nitrate is reduced by XOR to nitrite (6) and NO (7). Nitrite can be also oxidized to nitrate by oxyMb (8) or reduced to NO by deoxyMb (9). (B) In myoglobin knockout mice (myoglobin $^{-/-}$), the (5), (8) and (9) pathways are reduced because of the lack of the myoglobin protein. Although there could be some activity due to other heme proteins, such detailed information is not available. To compensate for myoglobin deletion, several pathways are upregulated – namely, sialin transport into the cell (1) and the XOR-related pathways (6) and (7). The NOS1-related pathways (2) and (3) may also be elevated, but the change in level did not reach statistical significance.

Conflicts of interest

Alan N. Schechter is listed as a co-inventor on several patents issued to the National Institutes of Health for the use of nitrite salts for the treatment of cardiovascular diseases. He receives royalties based on NIH licensing of these patents for clinical development but no other compensation. These arrangements do not affect his adherence to NO journal policies. The authors declare that they have no conflicts of interest.

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References

[1] L.J. Ignarro, Nitric oxide as a unique signaling molecule in the vascular system: a historical overview, J. Physiol. Pharmacol. 53 (2002) 503–514.
[2] S. Moncada, A. Higgs, The L-arginine-nitric oxide pathway, N. Engl. J. Med. 329 (1993) 2002–2012.
[3] J.O. Lundberg, E. Weitzberg, M.T. Gladwin, The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics, Nat. Rev. Drug Discov. 7 (2008) 156–167.
[4] M. Govoni, E.A. Jansson, E. Weitzberg, J.O. Lundberg, The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash, Nitric Oxide 19 (2008) 333–337.
[5] K. Cosby, K.S. Partovi, J.H. Crawford, R.P. Patel, C.D. Reiter, S. Martyr, B.K. Yang, M.A. Waclawiw, G. Zalos, X. Xu, K.T. Huang, H. Shields, D.B. Kim-Shapiro, A.N. Schechter, R.O. Cannon 3rd, M.T. Gladwin, Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation, Nat. Med. 9 (2003) 1498–1505.
[6] S. Shiva, Z. Huang, R. Grubina, J. Sun, L.A. Ringwood, P.H. MacArthur, X. Xu, E. Murphy, V.M. Darley-Usmar, M.T. Gladwin, Deoxymyoglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration, Circ. Res. 100 (2007) 654–661.
[7] T. Rassaf, U. Flogel, C. Drexhage, U. Hendgen-Cotta, M. Kelm, J. Schrader, Nitrite reductase function of deoxymyoglobin: oxygen sensor and regulator of cardiac energetics and function, Circ. Res. 100 (2007) 1749–1754.
[8] T.M. Millar, C.R. Stevens, N. Benjamin, R. Eisenthal, R. Harrison, D.R. Blake, Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions, FEBS Lett. 427 (1998) 225–228.
[9] N. Benjamin, F. O'Driscoll, H. Dougall, C. Duncan, L. Smith, M. Golden, H. McKenzie, Stomach NO synthesis, Nature 368 (1994) 502.
[10] J.O. Lundberg, E. Weitzberg, J.M. Lundberg, K. Alving, Intragastric nitric oxide production in humans: measurements in expelled air, Gut 35 (1994) 1543–1546.
[11] R.R. Giraldez, A. Panda, Y. Xia, S.P. Sanders, J.L. Zweier, Decreased nitric-oxide synthase activity causes impaired endothelium-dependent relaxation in the post-ischemic heart, J. Biol. Chem. 272 (1997) 21420–21426.
[12] M.N. Woessner, L.C. McIlvenna, J. Ortiz de Zevallos, C.J. Neil, J.D. Allen, Dietary nitrate supplementation in cardiovascular health: an ergogenic aid or exercise therapeutic? Am. J. Physiol. Heart Circ. Physiol. 314 (2018) H195–H212.
[13] B. Piknova, J.W. Park, K.M. Swanson, S. Dey, C.T. Noguchi, A.N. Schechter, Skeletal muscle as an endogenous nitrate reservoir, Nitric Oxide 47 (2015) 10–16.
[14] B. Piknova, J.W. Park, K. Kwan Jeff Lam, A.N. Schechter, Nitrate as a source of nitrite and nitric oxide during exercise hyperemia in rat skeletal muscle, Nitric Oxide 55–56 (2016) 54–61.
[15] S.B. Kanatous, P.P. Mammen, Regulation of myoglobin expression, J. Exp. Biol. 213 (2010) 2741–2747.
[16] J.C. Kendrew, G. Bodo, H.M. Dintzis, R.G. Parrish, H. Wyckoff, D.C. Phillips, A three-dimensional model of the myoglobin molecule obtained by x-ray analysis, Nature 181 (1958) 662–666.
[17] D.J. Garry, G.A. Ordway, J.N. Lorenz, N.B. Radford, E.R. Chin, R.W. Grange, R. Bassel-Duby, R.S. Williams, Mice without myoglobin, Nature 395 (1998) 905–908.
[18] A. Godecke, U. Flogel, K. Zanger, Z. Ding, J. Hirchenhain, U.K. Decking, J. Schrader, Disruption of myoglobin in mice induces multiple compensatory mechanisms, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 10495–10500.
[19] U. Flogel, M.W. Merx, A. Godecke, U.K. Decking, J. Schrader, Myoglobin: a scavenger of bioactive NO, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 735–740.
[20] U.B. Hendgen-Cotta, M.W. Merx, S. Shiva, J. Schmitz, S. Becher, J.P. Klare, H.J. Steinhoff, A. Goedecke, J. Schrader, M.T. Gladwin, M. Kelm, T. Rassaf, Nitrite reductase activity of myoglobin regulates respiration and cellular viability in myocardial ischemia-reperfusion injury, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 10256–10261.
[21] M. Totzeck, U.B. Hendgen-Cotta, P. Luedike, M. Berenbrink, J.P. Klare, H.J. Steinhoff, D. Semmler, S. Shiva, D. Williams, A. Kipar, M.T. Gladwin, J. Schrader, M. Kelm, A.R. Cossins, T. Rassaf, Nitrite regulates hypoxic vasodilation via myoglobin-dependent nitric oxide generation, Circulation 126 (2012) 325–334.
[22] B. Piknova, A.N. Schechter, Measurement of nitrite in blood samples using the ferricyanide-based hemoglobin oxidation assay, Methods Mol. Biol. 704 (2011) 39–56.
[23] A.G. Pinder, S.C. Rogers, A. Khalatbari, T.E. Ingram, P.E. James, The measurement of nitric oxide and its metabolites in biological samples by ozone-based chemiluminescence, Methods Mol. Biol. 476 (2008) 11–28.
[24] U.B. Hendgen-Cotta, M. Kelm, T. Rassaf, Myoglobin functions in the heart, Free Radic. Biol. Med. 73 (2014) 252–259.
[25] C.N. Gilliard, J.K. Lam, K.S. Cassel, J.W. Park, A.N. Schechter, B. Piknova, Effect of dietary nitrate levels on nitrate fluxes in rat skeletal muscle and liver, Nitric Oxide 75 (2018) 1–7.
[26] D.J. Stuehr, J. Santolini, Z.Q. Wang, C.C. Wei, S. Adak, Update on mechanism and catalytic regulation in the NO synthases, J. Biol. Chem. 279 (2004) 36167–36170.
[27] J.O. Lundberg, E. Weitzberg, J.A. Cole, N. Benjamin, Nitrate, bacteria and human health, Nat. Rev. Microbiol. 2 (2004) 593–602.
[28] N.J. Raat, A.C. Noguchi, V.B. Liu, N. Raghavachari, D. Liu, X. Xu, S. Shiva, P.J. Munson, M.T. Gladwin, Dietary nitrate and nitrite modulate blood and organ nitrite and the cellular ischemic stress response, Free Radic. Biol. Med. 47 (2009) 510–517.
[29] A.B. Milsom, B.O. Fernandez, M.F. Garcia-Saura, J. Rodriguez, M. Feelisch, Contributions of nitric oxide synthases, dietary nitrite/nitrate, and other sources to the formation of NO signaling products, Antioxidants Redox Signal. 17 (2012) 422–432.
[30] L. Qin, X. Liu, Q. Sun, Z. Fan, D. Xia, G. Ding, H.L. Ong, D. Adams, W.A. Gahl, C. Zheng, S. Qi, L. Jin, C. Zhang, L. Gu, J. He, D. Deng, I.S. Ambudkar, S. Wang, Sialin (SLC17A5) functions as a nitrate transporter in the plasma membrane, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 13434–13439.