Nitric Oxide 82 (2019) 35–47

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Neuronal nitric oxide synthase (nNOS) splice variant function: Insights into nitric oxide signaling from skeletal muscle

Jordan E. Balke, Ling Zhang, Justin M. Percival*

Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine Miami, Florida, 33101, USA

ARTICLE INFO

Keywords:
nNOS
Neuronal nitric oxide synthase splice variants
Nitric oxide
sGC
Soluble guanylyl cyclase
PDE5
Phosphodiesterase 5 inhibitors
Duchenne muscular dystrophy skeletal muscle

ABSTRACT

Defects in neuronal nitric oxide synthase (nNOS) splice variant localization and signaling in skeletal muscle are a firmly established pathogenic characteristic of many neuromuscular diseases, including Duchenne and Becker muscular dystrophy (DMD and BMD, respectively). Therefore, substantial efforts have been made to understand and therapeutically target skeletal muscle nNOS isoform signaling. The purpose of this review is to summarize recent salient advances in understanding of the regulation, targeting, and function of nNOSμ and nNOSβ splice variants in normal and dystrophic skeletal muscle, primarily using findings from mouse models. The first focus of this review is how the differential targeting of nNOS splice variants creates spatially and functionally distinct nitric oxide (NO) signaling compartments at the sarcolemma, Golgi complex, and cytoplasm. Particular attention is given to the functions of sarcolemmal nNOSμ and limitations of current nNOS knockout models. The second major focus is to review current understanding of cGMP-mediated nNOS signaling in skeletal muscle and its emergence as a therapeutic target in DMD and BMD. Accordingly, we address the preclinical and clinical successes and setbacks with the testing of phosphodiesterase 5 inhibitors to redress nNOS signaling defects in DMD and BMD. In summary, this review of nNOS function in normal and dystrophic muscle aims to advance understanding how the messenger NO is harnessed for cellular signaling from a skeletal muscle perspective.

1. Introduction

Nitric oxide (NO) is one of the most powerful and unconventional signaling molecules in the body. NO can be synthesized from arginine in a reaction catalyzed by four neuronal nitric oxide synthase (nNOS) splice variants (α, β, γ, and μ). There is a fifth variant called nNOS-2, but it is unclear if it can synthesize NO. nNOS splice variants are highly expressed in skeletal muscle. Skeletal muscle expresses two nNOS splice forms: nNOSβ and nNOSμ. nNOSμ is the primary enzymatic source of NO in skeletal muscle [65,114]. Because of its widespread expression in skeletal muscle, which comprises ~40% of body mass, nNOSμ is responsible for the largest proportion of NOS enzyme-generated NO in the body. Importantly, NO from nNOS can be stored as nitrate making skeletal muscle the largest potential NO repository in the body [101]. Thus, skeletal muscle is a powerful model system for understanding nNOS enzyme function and NO biology [115].

Most of the interest in nNOS function in skeletal muscle originated from the discovery that the expression and sarcolemmal localization of nNOSμ were disrupted in skeletal muscles of individuals with Duchenne and Becker muscular dystrophy [20,27]. DMD and BMD are X-linked muscle wasting diseases (collectively known as dystrophinopathies) caused by mutations in the *DMD* gene that eliminate or reduce dystrophin protein expression, respectively [57,66]. The loss of dystrophin compromises the dystrophin protein complex, a multi-protein assembly that plays important structural and signaling roles in skeletal muscle. The use of novel nNOS knockout and transgenic mouse models to study nNOS regulation and function, outside and within the context of muscular dystrophy, have been highly informative for understanding nNOS splice variant biology. The purpose of this review is to provide an overview of how these findings have contributed to our understanding of NO signaling.

NO synthesized by nNOS splice forms exerts many of its regulatory effects through direct activation of NO-sensitive guanylyl cyclases (GC) which act like a receptor for NO (Fig. 1) [45,51]. GC is activated by the binding of NO to its prosthetic heme group, and inhibited by S-nitrosation of its cysteine thiols [15,110]. GCs catalyze the synthesis of the second messenger cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). NO signaling through GC and cGMP represents canonical NO-cGMP signaling. cGMP acts on downstream targets including cGMP-activated protein kinase (PKG) and cyclic

* Corresponding author. Department of Molecular and Cellular Pharmacology (R-189), University of Miami Miller School of Medicine, PO Box 016189, Miami, FL, 33101, USA.
E-mail address: j.percival@med.miami.edu (J.M. Percival).

https://doi.org/10.1016/j.niox.2018.11.004
Received 14 November 2018; Received in revised form 19 November 2018; Accepted 20 November 2018
Available online 29 November 2018
1089-8603/

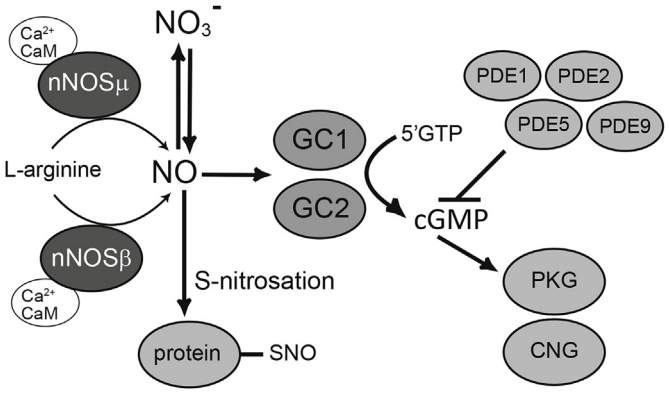

Fig. 1. Neuronal nitric oxide synthase (nNOS) splice variant signaling in skeletal muscle cells. Fully differentiated skeletal muscle cells express nNOSμ and nNOSβ. Ca^{2+}-dependent calmodulin binding activates nNOS enzymes to convert arginine substrate to nitric oxide (NO) and citrulline. NO can then bind and activate NO-sensitive guanylyl cyclases (GC1 and GC2) to synthesize the second messenger cyclic guanosine monophosphate (cGMP) from 5'-guanosine triphosphate. NO signaling through GC and cGMP represents canonical NO-cGMP signaling. NO can also signal by S-nitrosation through the formation of protein nitrosothiols (SNO). Alternatively, NO may be oxidized to nitrate and stored for later use. cGMP is hydrolyzed by some phosphodiesterases (PDEs) so that cGMP-hydrolyzing PDEs diminish or terminate cGMP-dependent NO signaling. cGMP also acts on other downstream effectors including cyclic nucleotide-gated ion channels (CNG) and cGMP activated protein kinase (PKG).

nucleotide-activated ion channels [30,111]. cGMP-dependent NO signaling is terminated by cGMP-hydrolyzing phosphodiesterases (PDE) such as PDE5 (Fig. 1) [44,77]. Thus, NO-cGMP signal transduction can be modulated at three distinct control points through the regulation of the catalytic activities of nNOS, GC, and PDEs. Deficits in nNOS activity in dystrophinopathies spurred successful preclinical testing, and less successful clinical testing of PDE5 inhibition as a therapeutic intervention for striated muscle dysfunction in DMD and BMD patients. Therefore, this review will also summarize what is known about NO-cGMP signaling in muscle and the use of PDE5 inhibitors to mitigate skeletal muscle dysfunction in patients with dystrophinopathy.

In summary, the overall goal of this review is to summarize recent advances in understanding of nNOS splice variant regulation and function from studies in normal and dystrophin-deficient skeletal muscle. In doing so, this review aims to provide new conceptual frameworks for understanding skeletal muscle NO signaling under physiological conditions and pathophysiological conditions that will be relevant and informative to the study of nNOS signaling in other cellular systems.

2. nNOS splice variant diversity in skeletal muscle

Rodent and especially human nNOS (*NOS1*) genes encode a diverse array of mRNA transcripts [19,21,72,123]. Murine splice variants are shown in Fig. 2. Transcriptional regulation and transcript diversity appear to have evolved as an important regulatory mechanisms to accommodate the wide range of nNOS biological functions. However, only a subset of *NOS1* transcripts translate into structural and functionally distinct nNOS protein splice variants or isoforms. Currently, there are at least five known nNOS splice variant proteins: nNOSα, nNOSβ, nNOSγ, nNOSμ and nNOS-2 (Fig. 3) [19,21,48,72,123]. nNOS transcript diversity comes primarily from exon 1 variant choice (and their associated promoters) and also from alterative splicing of exons within the open reading frame [124]. This is especially evident for the human *NOS1* gene, which encodes at least twelve exon 1 (exon 1a-1l) variants [124,130]. The murine and rat *NOS1* genes also encode several transcripts, but the diversity is not as extensive as in humans. Also, variable exon 1 sequences are poorly conserved between humans and mice [19,21,72,123]. In mice, five distinct 5′ untranslated regions have been identified that are spliced to a common acceptor in exon 2 [19,21]. Exon 1 expression is tissue-specific, for example, exons 1a, 1b, and 1c are robustly expressed in human skeletal muscle tissue [124]. Exon 1 choice results in unique 5' untranslated regions that have distinct translational efficiencies and can be influenced by muscle cell differentiation state. For example, exon 1a translation is higher in differentiated murine C2C12 myotubes than myoblasts [124].

2.1. nNOSμ splice variant

nNOS transcript diversity also comes from alternative splicing of exons within the open reading frame, which plays a prominent role in establishing a skeletal muscle-specific nNOS splice variant expression profile. This is exemplified by the induction of nNOSμ expression during muscle cell differentiation (Table 1). Immature primary myoblasts and myotubes express nNOSα. It remains unclear if nNOSβ is expressed in immature muscle cells. During differentiation, a 102 base pair (34 amino acid) mu (μ) cassette is inserted by alternative splicing between exons 16 and 17 resulting in the generation of nNOSμ (Figs. 2 and 3) [114]. Thus, nNOSμ is expressed as muscle cells differentiate into myotubes, which can express both nNOSα and nNOSμ. However, fully differentiated myofibers in skeletal muscle tissue express only nNOSμ, as well as nNOSβ (Table 1) [114]. Indeed, nNOSμ is the predominant splice form accounting for 95% of total nNOS expression, and 85% of NOS activity in mature skeletal muscle [61,115]. Thus, nNOSβ is a small, but as we will see, important source of NO, while nNOSα expression and function may be relevant to incompletely differentiated immature muscle cells. It is important to note that cultured immortalized or primary skeletal muscle cell lines do not develop or retain the fully differentiated state due to lack of innervation, endocrine factors, and other essential features of the in vivo environment. In addition, nNOSμ expression is not restricted to skeletal muscle cells, but can also be found in cardiac myocytes (although this was recently questioned), the urogenital tract, and in regions of the brain including the cerebellum [50,61,78,129].

Details of how mu insert inclusion impacts the structure and function of nNOSμ, to facilitate isoform-specific differences between nNOSμ and nNOSα, are slowly emerging. nNOSμ has a longer half-life than nNOSα (50 versus 12 min, respectively) suggesting a role for the mu insert in protein stability and/or turnover [69]. Initial reports suggested that nNOSμ and nNOSα had similar Vmax and Km values for the oxidation of arginine substrate to NO suggesting comparable catalytic behavior [69,114]. The regulation of nNOSμ and nNOSα by Ca^{2+}/calmodulin binding were also similar. However, recent biochemical studies suggest that the mu insert accelerates electron transfer in the absence of calmodulin and slows it in the presence of calmodulin [69,92]. Also, the magnitude of calmodulin binding-stimulated electron transfer between flavin mononucleotide and heme groups and the formation of the heme-nitrosyl complex was smaller in nNOSμ relative to nNOSα [92]. Uncoupled nNOSμ generated less superoxide than nNOSα, and calcium-induced superoxide production from nNOSμ was lower than from nNOSα [60]. nNOSα activity lead to greater increases in the nitrated second messenger 8-nitroguanosine 3′,5′-cyclic monophosphate (8-nitro-cGMP). 8-nitro-cGMP is a potent signaling molecule that participates in both canonical NO-cGMP signaling and in non-canonical protein S-guanylation [109]. S-guanylation is a post-translational modification involving covalent attachment of cGMP moieties to protein thiols that modulates the activity of proteins including those regulating mitochondrial permeability-transition pore opening [102]. Finally, the mu insert contains five serine residues in close proximity to basic amino acids that may represent potential phosphorylation sites, so that mu inclusion could influence post-translational regulatory control of nNOSμ function. Taken together, these studies suggest isoform-specific differences in isozyme stability and catalytic activity between nNOSμ and nNOSα that may differentially impact downstream second

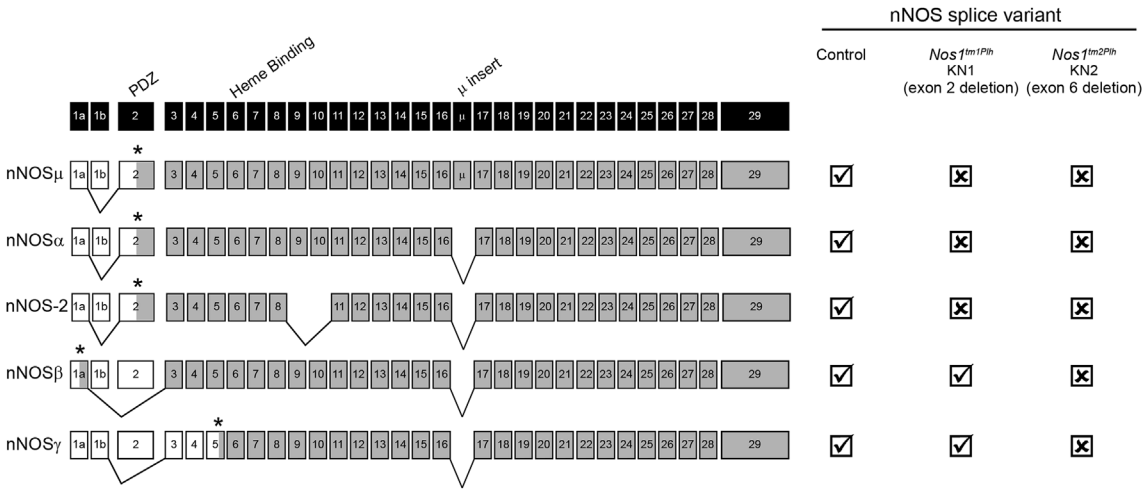

Fig. 2. Exon structure of murine NOS1 (nNOS) splice variant transcripts. The murine NOS1 gene encodes 31 exons (numbered black boxes) that are selectively used to generate at least five splice variants: nNOSμ, nNOSα, nNOS-2, nNOSβ and nNOSγ. Coding exons for each splice variant are shown as numbered gray boxes. Exons encoding the PDZ (PSD95/Dlg1/ZO1) protein-protein interaction domain, heme binding domain and μ-insert are shown. Non-coding exon sequences used to generate unique 5′ untranslated sequences are white. Asterisks mark translation initiation sites. nNOS splice variant expression in control and the two murine nNOS knockout models is shown on the right. A tick mark indicates expression. A cross mark indicates absence of expression and/or activity. Currently, two nNOS knockout models exist with distinct isoform expression profiles: first knockout of nNOS (KN1, exon 2 deletion) and second knockout of nNOS (KN2, exon 6 deletion). KN1 and KN2 mice are also known as *NOS1*^{tm1Plh} and *NOS1*^{tm2Plh} mice, respectively.

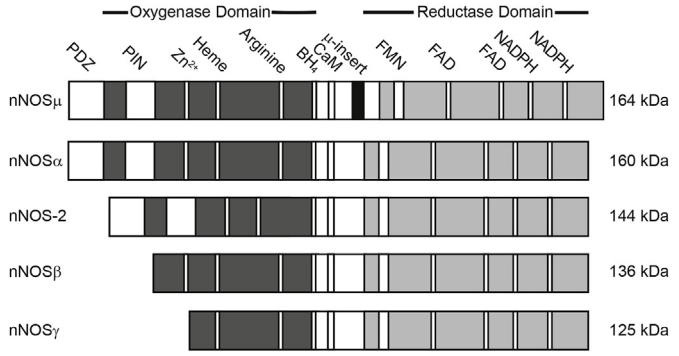

Fig. 3. nNOS splice variant protein domain structure. nNOS is composed of two domains-the amino terminus oxygenase and carboxyl terminus reductase domains. Calmodulin (CaM) modulates the flow of electrons from NADPH (nicotinamide-adenine dinucleotide phosphate) bound at the reductase domain to the heme bound at the oxygenase domain. The amino terminus of nNOSμ, nNOSα and nNOS-2 (but not nNOSβ and nNOSγ) contains a PDZ (PSD95/Dlg/ZO1) protein-protein interaction domain encoded by exon 2. nNOS-2 and nNOSγ lack sequence, including a Zn^{2+} binding site, necessary for catalytic activity in mice. The oxygenase domain contains binding sites for zinc (Zn^{2+}), arginine substrate, heme and the cofactor BH4 [(6R-)-5, 6, 7, 8-tetrahydrobiopterin]. The oxygenase domain also contains a protein inhibitor of nNOS (PIN) binding domain. The reductase domain contains binding sites for NADPH and the cofactors FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide). nNOS splice variant molecular weights are shown on the right of each variant. Note also that KN1 mice lack exon 2 encoding the PDZ domain and KN2 mice lack exon 6 encoding the heme domain.

messenger redox signaling, particularly through protein S-guanylation.

The findings that nNOSμ and nNOSα have distinct isoform-specific stability and catalytic behavior that differentially affect downstream redox signaling provide strong evidence that nNOSμ and nNOSα are not functionally redundant. Also, because nNOSα, but not nNOSμ, is expressed in immature skeletal muscle cells, these two closely related nNOS splice variants may have isoform-specific roles in muscle cell differentiation. The importance of considering nNOS isoform-specific function in skeletal muscle is further highlighted by the discovery of Golgi complex-localized nNOSβ, suggesting a novel role for NO in trafficking in muscle, and by compelling evidence of unique roles of nNOSμ and nNOSβ in regulating muscle structure, strength and fatigue resistance and blood delivery [96,120]. In addition, nNOS isoform-specific function is an important and under-appreciated consideration for non-splice variant selective pharmacological approaches used to explore nNOS functional diversity. Nevertheless, enhanced understanding and consideration of nNOS isoform-specific function will undoubtedly assist efforts in understanding how NO is able to perform so many diverse roles in many cell types, not just in skeletal muscle.

2.2. *nNOSβ and nNOSγ splice variants*

Studies in brain and skeletal muscle have been instrumental in defining nNOS splice variant diversity. nNOSα was the first nNOS enzyme to be discovered from studies in brain [17,18]. The existence of nNOSβ and nNOSγ splice variants was also demonstrated during characterization of the brains of the first nNOS knockout (KN1 or first knockout of nNOS) mouse (Fig. 2) [19,38,59]. Subsequent studies in skeletal muscle uncovered the existence of nNOSμ. nNOSα contains a translational start codon and amino terminal PDZ domain in exon 2; therefore, exon 2 was targeted for deletion [59]. However, significant amounts of residual NOS activity were detected in different brain regions of homozygous nNOS exon 2 null KN1 mice, with levels up to 7% of wild type [38,59]. Subsequent analyses revealed expression of two alternative transcripts: the high abundance 5'a transcript and low

Table 1
The relationship between catalytically active nNOS splice variant expression and skeletal muscle cell differentiation state.

Myoblasts (in vitro) (immature and proliferating)	Myotubes (in vitro) (differentiated)	Myofibers (in vivo) (fully differentiated)
nNOSα	nNOSα	nNOSβ
nNOSβ?	nNOSβ?	nNOSμ
	nNOSμ	

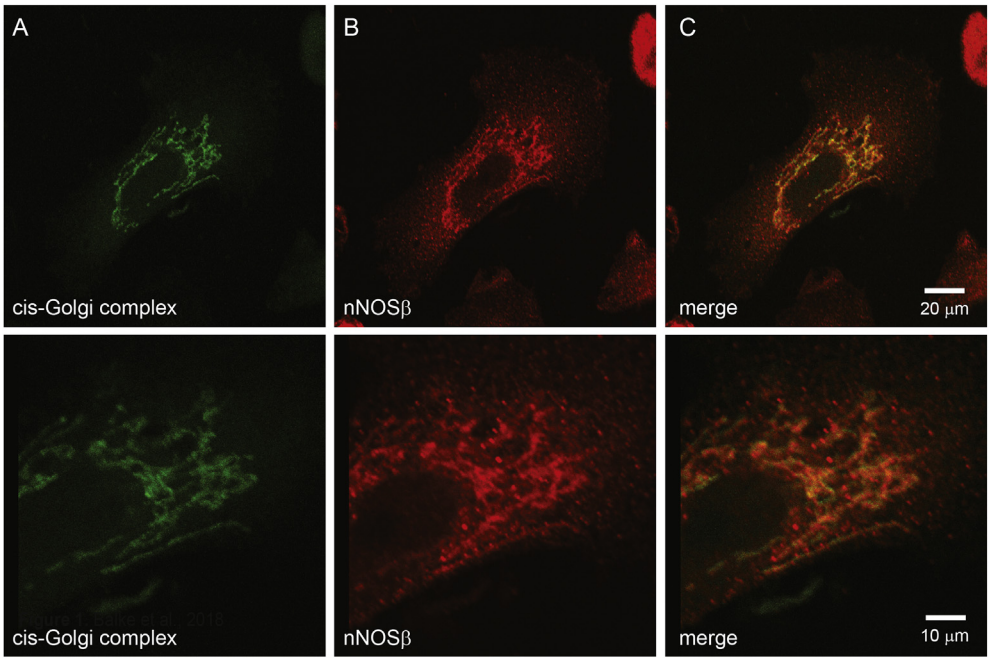

Fig. 4. Epitope-tagged nNOSβ localizes to the Golgi complex. **(A–C)** Confocal micrographs showing that transiently expressed epitope-tagged human nNOSβ localizes to the Golgi complex in cultured HeLa cells. Human nNOSβ cDNA containing a carboxyl terminus hemagglutinin (HA) epitope was electroporated into HeLa cells, and its localization evaluated 24 h later by confocal microscopy. **(A)** The cis-Golgi membrane network was labeled with Alexa 488-conjugated anti-GM130 antibody. **(B)** nNOSβ was visualized using an anti-HA polyclonal antibody and Alex 568-donkey anti-rabbit secondary antibody. **(C)** In agreement with findings in murine skeletal muscle and neurons, nNOSβ also targeted to the tubular cis-Golgi membrane network in human cells indicated by the extensive yellow coloring. Single optical sections captured by confocal laser scanning microscopy are shown. High magnification images for A-C are shown below each image to highlight the extensive colocalization (indicated by yellow color) of nNOSβ with the membrane tubules of the cis-Golgi compartment.

abundance 5'b transcript [19]. The 5'a transcript contains exon 1a with an in-frame non-canonical CUG translation initiation site. In 5'a transcripts, exon 2 is removed resulting in the splicing of exon 1a to exon 3 with translational initiation from the CUG codon giving rise to the 136 kDa nNOSβ splice variant (Figs. 2 and 3). Deletion of exon 2 also occurs in rats and humans [72,123,124].

nNOSβ mRNA and protein accounts for only about 5% of total nNOS mRNA and protein in wild type brain and skeletal muscle [61]. The murine nNOSβ isoform contains six amino acid residues (MRGLGS) at its NH2-terminus, and lacks the PDZ domain and mu insert. Heterologous transfection assays showed that nNOSβ has comparable catalytic activity to nNOSα [19]. The alternative use of exon 1 variants and exon 2 in humans and mice differentially targets nNOS splice variants to distinct cellular membranes. Exon 2 targets nNOSμ to the sarcolemma, while exon 1a targets nNOSβ to the Golgi complex (Fig. 4) [96]. Alternatively, translation initiation may occur at an AUG codon within exon 5 to give rise to the 125 kDa protein nNOSγ which lacks enzymatic activity in mice and may not be expressed in skeletal muscle cells [19,21]. Murine nNOSγ is homologous to a testis-specific variant of nNOS in humans called TnNOS, which is expressed in Leydig cells and exhibits Ca^{2+}-dependent catalytic activity [122]. This suggests species-specific differences in nNOSγ function between mice and men.

3. nNOS knockout mouse models and their considerations

Small amounts of NO (in the low picomolar range) exert profound physiological effects. Because it is challenging to achieve full and selective nNOS blockade pharmacologically, murine nNOS knockout models can be used to circumvent the challenge of NO potency. However, nNOS isoform diversity also poses distinct challenges for generating nNOS knockout models. Indeed, the ubiquitously studied whole body “nNOS knockout” KN1 mouse is not a true knockout, but in fact a partial and isoform-selective nNOS knockout (Fig. 2) [59]. KN1 mice cannot express nNOSα, nNOSμ and nNOS-2, but can still express catalytically active nNOSβ, and inactive nNOSγ (Fig. 2). Indeed, significant brain-region specific residual nNOSβ activities remain in KN1 mice. These original exon 2-targeted nNOS knockout mice have been referred as the KN1 (first knockout of nNOS), to distinguish them from a second whole body exon 6-targeted nNOS knockout mouse model known as the KN2 (second knockout of nNOS) as discussed below [53].

KN1 mice (also known as *NOS1*^{tm1plh}) are commercially available from The Jackson Laboratory.

Another important characteristic of KN1 mice is that they exhibit upregulation of nNOSβ expression and activity, particularly in the cortex and striatum [38]. We also observe these findings and detect increased expression of Golgi complex-associated nNOSβ in cortical neurons and other neuronal cell types (Fig. 5, unpublished). Therefore, increased nNOSβ could compensate in part for the loss of exon 2-containing nNOS enzymes (nNOSα, nNOSμ, and nNOS-2) in KN1 mice. Another consideration when interpreting the phenotypes of KN1 mice is that the genetic background of the mouse may influence the phenotype. For a review see Ref. [93]. For example, tibialis anterior muscles from KN1 mice in a mixed B6/129, congenic and partially congenic C57BL/6J backgrounds exhibited lowered, normal, and improved fatigue resistance, respectively [29,33,95,96]. These data suggest an important role for strain-specific genetic modifiers in modulating skeletal muscle fatigue resistance in KN1 mice. In addition, sex is also an important variable for NO-cGMP signaling and cannot be ruled out as an important modifier in the C57BL/6J background. Indeed, strain and sex are also important experimental variables in the functions of the key target of NO, guanylyl cyclase [22,23,37]. Thus, strain and sex-specific genetic modifiers appear to modulate the functions of NO-cGMP signaling.

To generate a nNOS knockout mouse model lacking all nNOS splice variant activity, two labs independently generated whole body nNOS null mice by targeting exon 6 for deletion [53,91]. Exon 6 is common to all splice variants and encodes the heme cofactor binding domain which is essential for catalytic activity. So, even if nNOS splice variants were expressed, they would be inactive. This targeting strategy eliminated all active nNOS with nNOS activity and protein expression undetectable in all tissues tested, including the brain and skeletal muscle [53]. To differentiate between the two nNOS knockout models, exon 6 targeted nNOS null mice were designated KN2 (second knockout of nNOS). We have also used this nomenclature and use it from this point on. KN2 mice (also known as *NOS1*^{tm2plh}) are also commercially available in the C57BL/6J background from The Jackson Laboratory.

To date, all studies of nNOS function in skeletal muscle and other tissues have used whole body KN1 (in both B6; 129 and C57BL/6J backgrounds) or KN2 mice. While nNOS activity in KN1 mice is very low (less than 10% of wild type NOS activity levels in the brain,

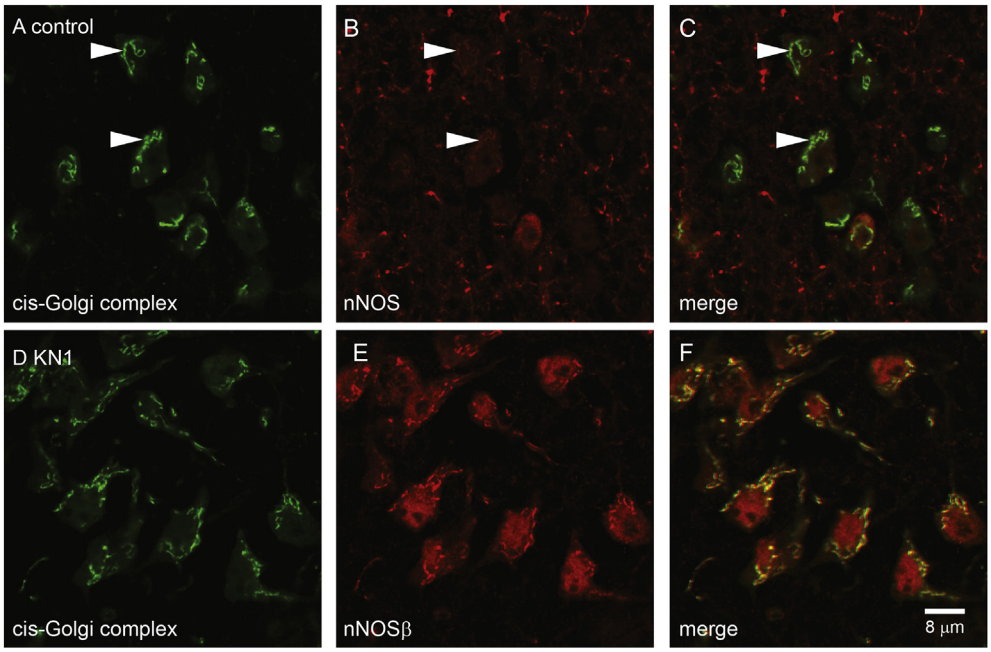

Fig. 5. Golgi complex-associated nNOSβ is upregulated in neurons of KN1 mice. nNOSβ is upregulated in cortical and other neurons in KN1 mouse brains [34]. **(A–C)** Confocal micrographs of nNOS splice variant localization in cortical neurons within cryosections from wild type mouse brains. **(A)** The cis-Golgi compartment was labeled with Alexa 488-conjugated anti-GM130 antibody and showed a typical perinuclear localization (arrowhead). **(B)** nNOS was detected using a pan-specific anti-nNOS polyclonal antibody and an Alexa 568-donkey anti-rabbit secondary antibody. nNOS localized to puncta (nNOSα) and was weakly detected at the cis-Golgi complex (nNOSβ) (arrowhead). **(C)** Merge image showing low levels of nNOSβ at the Golgi in wild type neurons. **(D–F)** Expected increase in expression of nNOSβ at the cis-Golgi compartment in cortical neurons from KN1 mice lacking expression of nNOSα, nNOSμ, and nNOS-2. **(D)** cis-Golgi compartment marked by GM130 in cortical neurons from KN1 mice. **(E)** In KN1 cortical neurons, there is a substantial increase in nNOS associated with the cis-Golgi consistent with nNOSβ upregulation [34]. **(F)** Merge image showing high levels of Golgi nNOSβ in KN1 cortical neurons indicated by extensive yellow coloring.

attributable in part to nNOSβ upregulation), the additional loss of this small activity leads to substantial phenotypic differences. KN2 mice exhibit severe systemic dysfunction compared to KN1 mice on congenic C57BL/6J backgrounds. Therefore, although nNOSβ activity is low, it is potent and is sufficient to preserve a large degree of organismal homeostasis in mice. This is consistent with a central principle of NO signaling that small amounts of NO can have profound effects under physiological conditions. KN2 mice manifest severe multi-system pathologies relative to KN1 mice. Unlike KN1 mice, KN2 mice are not born at expected mendelian ratios supporting an important role for nNOS in development. KN2 mice also exhibit severely stunted growth, driven in part by pyloric stenosis and gastrointestinal tract dysfunction [53]. These data are consistent with severe impairment of gastrointestinal tract function in NO-sensitive guanylyl cyclase null mice [46]. KN2 mice also exhibit high levels of postnatal mortality with half of KN2 mice dying by 4 weeks of age, despite liquid diet feeding to improve nutrition. Male and female KN2 mice are also infertile due to hormone secretion defects suggesting an important neuroendocrine role for Golgi-localized nNOSβ [53].

Furthermore, KN2 mice exhibit a severe myopathy characterized by reduced skeletal muscle growth and strength and resistance to contraction-induced fatigue [96]. Accordingly, the involuntary running performance of KN2 mice is extremely poor (unpublished observations). These findings are in direct contrast to KN1 mice in the same C57BL/6J background, which show normal muscle strength and fatigue resistance [96]. The muscle strength and fatigue phenotypes of KN1 mice have been reviewed previously and are not discussed further here [93]. Skeletal muscles from KN2 mice exhibit poor hypertrophic growth, but no overt histological evidence of muscle damage. Mitochondrial localization and ultrastructure is also compromised suggesting mitochondrial dysfunction as a possible causal factor in reduced muscle growth and weakness in KN2 mice [96]. Similarly, nNOS inhibition disrupted the localization of the Golgi complex that like mitochondria resides in the subsarcolemmal space. Defects in Golgi complex and mitochondrial organelle localization in KN2 mice were likely caused by defects in subsarcolemmal microtubule lattice organization [83,96]. The localization of nNOSβ to the Golgi complex and deficit s in neuroendocrine hormone secretion in KN2, but not KN1 mice, both support a role for Golgi nNOSβ in regulating intracellular trafficking and/or secretion in muscle and other tissues [58].

In summary, the systemic inhibition of all nNOS activity has catastrophic consequences for skeletal muscle. However, it remains to be determined whether the loss of skeletal muscle-expressed nNOS activity is solely responsible for skeletal muscle dysfunction because loss of nNOS activity in tissues outside muscle could also indirectly contribute to skeletal muscle dysfunction. Regardless, the substantial differences in skeletal muscle phenotypes between KN1 and KN2 mice suggest that nNOSβ may be an important regulator of skeletal muscle function.

4. Spatially and functionally distinct nNOS splice variant compartments in skeletal muscle

NO signaling is highly compartmentalized in many cell types. Over the past decade, compelling evidence has emerged from studies of normal and dystrophic skeletal muscle showing that NO signaling is also highly compartmentalized in fully differentiated skeletal muscle cells. Compartmentalization is achieved in part by the differential targeting of nNOSμ and nNOSβ splice variants to specific subcellular destinations (Fig. 6) [93]. Recently, the mechanisms controlling the differential targeting of nNOS splice forms, particularly nNOSμ, as well as the functional consequences of targeting have become clearer.

4.1. Targeting of nNOSμ to the sarcolemma

Fully differentiated skeletal muscle cells express at least two active alternative spliced nNOS enzymes: nNOSμ and nNOSβ [13,19,96,114]. nNOSμ has been most studied because impairment of its function contributes to skeletal muscle dysfunction in Duchenne and Becker muscular dystrophy [20,27]. nNOSμ localizes to the sarcolemma, neuromuscular junction and cytoplasm in rodent and human skeletal muscle cells [3,26,27,65]. However, in human skeletal muscle ~80% of nNOSμ localizes to the sarcolemma compared with ~50% in mice, suggesting species-specific differences in muscle nNOS distribution [26]. nNOSμ is scaffolded to the sarcolemma by the dystrophin glycoprotein complex (DGC) (Fig. 6). Sarcolemmal nNOSμ association requires expression of α-syntrophin, dystrophin, and α-dystrobrevin at the sarcolemma [3,5,19,20,27,52]. In other words, dystrophin, α-syntrophin, and α-dystrobrevin are each necessary, but not sufficient, for

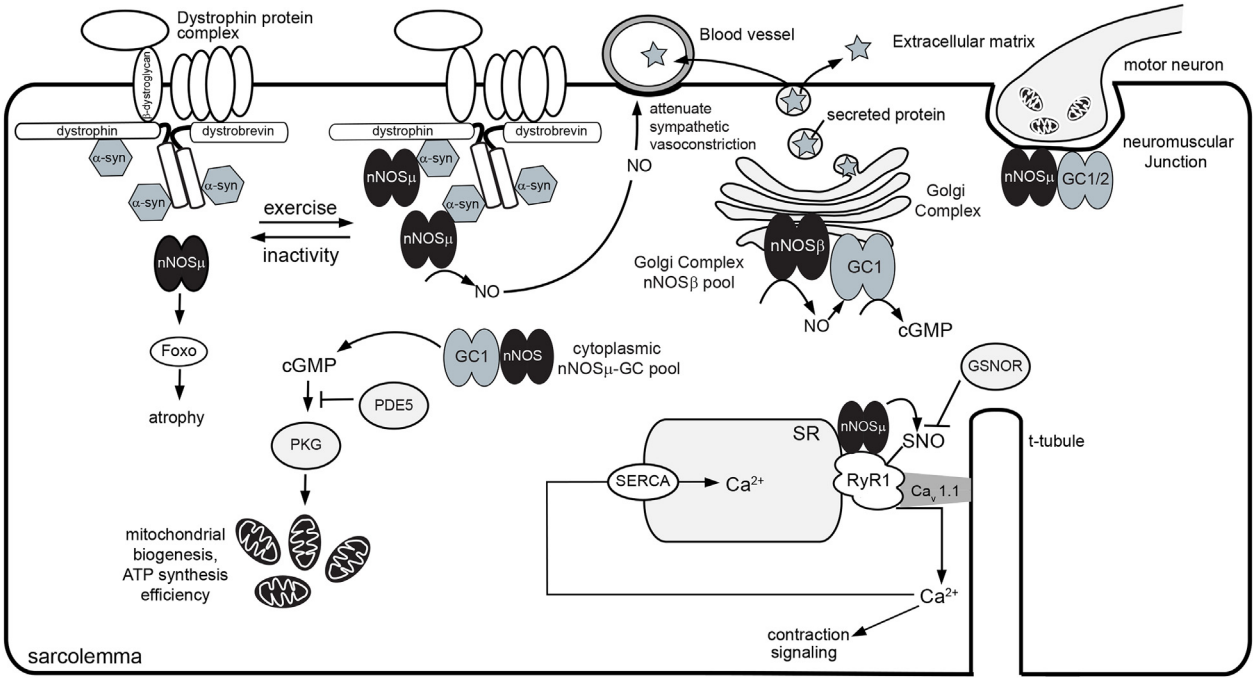

Fig. 6. Model showing spatially and functionally distinct nNOS splice variant signaling compartments in skeletal muscle cells. nNOSμ is localized to the sarcolemma, cytoplasm, sarcoplasmic reticulum (SR) and at postsynaptic membranes of the neuromuscular junction. At the sarcolemma, nNOSμ is bound to α-syntrophin which in turn binds sites within spectrin-like repeat 17 and the carboxyl terminus of dystrophin. nNOSμ expression is dynamically regulated because it increases with organismal activity and decreases with inactivity which may initiate FoxO-mediated muscle atrophy. Only sarcolemmal nNOSμ can oppose sympathetic vasoconstriction to promote local blood delivery to contracting muscles indicating an isoform-specific role for nNOSμ. nNOSβ localizes to the Golgi complex, the hub of the classical secretory pathway suggesting a distinct function for nNOSβ in trafficking. NO-sensitive guanylyl cyclase (GC)-the "NO-receptor"-localizes to a subset of nNOS subcellular domains supporting the existence of spatially and functionally distinct NO-cGMP signaling compartments in skeletal muscle. CaM: calmodulin, Cav1.1: voltage-dependent calcium channel, Foxo: forkhead box 03; GC: guanylyl cyclase, GSNOR: S-nitrosoglutathione reductase, nNOS: neuronal nitric oxide synthase; PDE5: phosphodiesterase 5, PKG: protein kinase G, RyR1: ryanodine receptor 1, SERCA: sarcoplasmic reticulum Ca^{2+}-ATPase, α -syn: α -syntrophin.

the anchoring of nNOSμ to the sarcolemma (Fig. 6). nNOSμ binds directly to α-1 syntrophin through the docking of an amino terminus beta finger containing a pseudo SXV motif into the peptide binding site of the PDZ domain of α1 syntrophin [56]. This binding mode leaves the amino terminal PDZ domain of nNOSμ free to bind other ligands. For example, the nNOSμ PDZ domain can bind the muscle form of phosphofructokinase, which catalyzes the first rate limiting step of glycolysis [43]. α-1 syntrophin binds at two syntrophin binding sites in the carboxyl terminus of dystrophin and the dystrophin-related protein α-dystrobrevin [6,88,117].

In addition to carboxyl terminus syntrophin binding sites, sequences within the rod domain of dystrophin are also key determinants of nNOSμ association with the sarcolemma [4,27,126]. Studies in BMD patients and mdx mice whose skeletal muscles naturally or artificially express internally truncated dystrophin proteins (e.g., dystrophin lacking exons 17–48, or exons 45–47 or exons 10–42), showed that nNOSμ did not localize to the sarcolemma, even in the presence of α-syntrophin [27,126]. Studies in Becker patients identified sequence within exons 45–48 encoding part of the internal rod domain of dystrophin as a key additional site required for nNOSμ sarcolemmal localization [126]. This site was further refined to a short 10 amino acid microdomain within spectrin-like repeat 17 of dystrophin, representing an important advance in the understanding of nNOSμ targeting [67,68]. Thus, nNOSμ localization required the co-operation of both α-syntrophin, and spectrin-like repeat 17 in the rod domain of dystrophin.

One model for sarcolemmal nNOSμ localization proposed a direct interaction between nNOSμ and dystrophin; however, in vitro studies have provided evidence both for and against such a model [27,68]. In addition, even if a direct interaction is possible, it is insufficient to localize nNOSμ to the sarcolemma in the absence of α-syntrophin. An important recent study suggests that spectrin-like repeat 17 contains a previously unrecognized α-syntrophin binding site that overlaps with the 10 amino acid microdomain [4]. This appears to solve the decades-old conundrum of the dual requirement for α-syntrophin and dystrophin for the localization of nNOSμ to the sarcolemma. Current evidence suggests a model whereby α-syntrophin recruits nNOSμ to the dystrophin complex by binding at syntrophin binding sites in the carboxyl terminus and spectrin-like repeat 17 of dystrophin (Fig. 6).

Not all nNOSμ localizes to the sarcolemma, at least in mice. In mice, about half of nNOSμ is localized to the sarcolemma, with the remainder localizing to the cytoplasm where it is often in a complex with α-syntrophin [26,62,118,120]. This contrasts with a study in human skeletal muscle showing that ~80% of nNOSμ is associated with the sarcolemma [26]. A fraction of cytoplasmic nNOSμ may associate with the ryanodine receptor 1 Ca^{2+} release channel (RyR1) at the sarcoplasmic reticulum where it may S-nitrosate and regulate RyR1 activity (Fig. 6) [39,40,75,107]. In cultured C2C12 muscle cells, α-syntrophin may shuttle nNOSα from the cytoplasm to the nucleus enabling nNOSα to S-nitrosate target proteins such as cAMP response element binding protein (CREB) and ultimately mitochondrial biogenesis [8,63]. Therefore, in immature skeletal muscle cells, nNOSα and α-syntrophin may localize to nuclei and the cytoplasm and this localization may be under dynamic regulation.

4.2. *nNOSβ is targeted to the Golgi complex*

nNOSβ is localized to the cis-Golgi network of the Golgi complex, the organellar hub of the classical secretory pathway in different cell types including skeletal muscle cells and neurons (Figs. 4 and 5) [47,96]. The Golgi complex is responsible for trafficking, modifying, and packaging proteins and lipids into distinct vesicles for delivery to intra- or extracellular destinations [127]. The cis-Golgi

subcompartment receives cargo from the endoplasmic reticulum. In rodent skeletal muscle cells, the Golgi complex is heavily concentrated in the subsarcolemmal space, with the highest densities in oxidative muscles [97,98,103]. Accordingly, higher nNOSβ densities are found in soleus than glycolytic tibialis anterior muscles [96]. As discussed above, the amino terminus of nNOSβ is encoded by exon 1 in mice and humans creating a unique amino terminus, which likely plays a direct role in the targeting of nNOSβ to the cis-Golgi compartment. The role of nNOSβ in the secretory pathway remains to be deciphered and is an ongoing area of interest of our laboratory.

5. The functional importance of sarcolemmal localization of nNOSμ in normal and dystrophin-deficient muscle

One fundamental and incompletely understood question is the functional importance of the localization of nNOSμ to the sarcolemma. This is important to understand because sarcolemmal nNOSμ expression is a positive biomarker of dystrophin-glycoprotein complex integrity, skeletal muscle health and exercise capacity under both physiological and neuromuscular disease states [42,93,126]. Understanding the functions of nNOSμ at the sarcolemma is particularly relevant to dystrophinopathies because defects in nNOSμ expression, localization, and function are a firmly established pathogenic feature of DMD and BMD [20,27]. In individuals with DMD and BMD, as well as mdx mice (the murine model of DMD), loss of normal dystrophin (BMD patients can express mutant truncated dystrophin) leads to a secondary impairment of nNOSμ expression and signaling, and mislocalization of both nNOSμ and nNOSβ [20,27,47]. Also, while there are sarcolemmal and cytoplasmic pools of nNOSμ in skeletal muscle, the relative importance of each to dystrophic pathology remains to be fully understood.

The sarcolemmal nNOSμ pool has received the most attention with strong evidence for a role in vasomodulation, and perhaps muscle atrophy. During hindlimb unloading, dislocation of nNOSμ from the sarcolemma to the cytoplasm was proposed to promote muscle atrophy by activating Foxo-dependent expression of atrogin1/MAFbx and MURF1 atrogenes [118]. However, the importance of dislocation was questioned because of concomitant large decreases in nNOSμ expression that may reduce or eliminate nNOSμ expression at both the sarcolemma and cytoplasm [113]. Accordingly, preservation of nNOSμ expression protects against atrophy [71]. In addition, chronic dislocation of nNOSμ in α-syntrophin null mice does not cause atrophy or enhance muscle atrophy during unloading [96,118]. It is possible that loss of sarcolemma nNOSμ would be apparent only in muscles that have low nNOSμ levels (e.g. soleus) compared to other muscles. Thus, a reduction in nNOSμ expression upon unloading may eliminate sarcolemmal nNOSμ labeling in the soleus, but this effect may not be apparent in other muscles with higher nNOSμ expression such as the tibialis anterior. This suggests that reduced nNOSμ expression, not nNOSμ dislocation from the sarcolemma, may be the key to protecting against unloading-induced atrophy in KN1 mice. Therefore, the role of sarcolemmal nNOSμ in atrophy requires further clarification.

Compelling evidence of a distinct role for sarcolemmal nNOSμ comes from studies of the mechanisms governing blood flow in contracting rodent and human skeletal muscles [119,120]. A specific role for sarcolemmal nNOSμ in opposing α-adrenergic receptor activated sympathetic vasoconstriction was established using α-syntrophin-null mice that express near wild type control nNOSμ protein levels, but cannot localize nNOSμ to the sarcolemma [120]. Mice lacking α-syntrophin exhibit unopposed α-adrenergic sympathetic vasoconstriction during contraction in response to epinephrine [120]. The ability to attenuate sympathetic vasoconstriction was rescued by re-expression of skeletal muscle α-syntrophin, which in turn restored sarcolemmal nNOSμ localization [120]. Furthermore, sympathetic vasoconstriction was also unopposed in mice whose muscles expressed a mutant α-syntrophin lacking the PDZ domain critical for binding nNOSμ [120]. Importantly, NO from cytoplasmic nNOSμ and nNOSβ were unable to oppose vasoconstriction in the absence of sarcolemmal nNOSμ demonstrating that the ability of NO to locally attenuate sympathetic vasoconstriction in contracting muscles required nNOSμ localization to the sarcolemma. Collectively, these data provide compelling evidence for an isoform-specific role for nNOSμ at the sarcolemma in opposing sympathetic vasoconstriction during exercise. They also provide strong evidence for a spatially and functionally distinct NO compartment within skeletal muscle.

The question then arises: what is the purpose of the vasomodulatory role of nNOSμ? Ostensibly, blood flow is matched closely to the metabolic demands of working muscle, but it is currently unclear what those metabolic demands might be. α-syntrophin null and KN1 mice (on congenic C57BL/6J backgrounds) do not show overt signs of skeletal muscle damage, weakness, lowered fatigue resistance, or exercise intolerance, despite unopposed sympathetic vasoconstriction [3,62,96]. Therefore, at least in wild type murine skeletal muscle, the inability to oppose local sympathetic vasoconstriction is not necessarily deleterious for muscle integrity or exercise performance. In other words, the vascular dysregulation in mice lacking sarcolemma-localized nNOSμ may not cause muscle contractile dysfunction, at least in normal muscle.

However, this vascular dysregulation could be deleterious in dystrophin-deficient skeletal muscles, which also exhibit unopposed sympathetic vasoconstriction due to the loss of sarcolemmal nNOSμ [108,119]. This may be because dystrophin-deficient skeletal muscle is more susceptible to damage and oxidatively stressed, so that even modest ischemic stress may be deleterious for muscle [10]. This possibility is also supported by preclinical gene therapy experiments in mdx mice, which show that the most therapeutically efficacious microdystrophin constructs are those that restore sarcolemmal nNOSμ [67]. Unlike microdystrophins (e.g., ΔH2-R19 microdystrophin) that increase total nNOSμ expression, but do not restore sarcolemmal nNOSμ, microdystrophins (e.g., ΔH2-R15 microdystrophin) that restored sarcolemmal nNOSμ localization exhibited normal attenuation of sympathetic vasoconstriction, improved perfusion of contracting muscle, and increased running exercise performance [67]. Interestingly, only the ΔH2-R15 microdystrophin construct reduced exercise-induced muscle inflammation. These data support a role for sarcolemmal nNOSμ in opposing sympathetic vasoconstriction, inflammation and exercise intolerance in dystrophin-deficient skeletal muscle.

6. Redresssing defective sarcolemmal nNOSμ vasomodulation with phosphodiesterase 5 inhibitors

Phosphodiesterase 5 (PDE5) inhibitors, such as short-acting sildenafil and long-acting tadalafil, successfully mitigate defects in vascular control resulting from the loss of sarcolemmal nNOSμ in the mdx mouse model of DMD [10,64,100]. PDE5 is highly expressed in smooth muscle and terminates some NO-cGMP signaling pathways by hydrolyzing cGMP, thereby promoting vasoconstriction [44]. Accordingly, PDE5 inhibitors are potent vasodilators. Long-term treatments of mdx mice with tadalafil or sildenafil reduced contraction-induced muscle damage and fibrosis and decreased diaphragm muscle weakness [10,100]. Although increases in cGMP can promote mitochondrial biogenesis, PDE5 inhibition did not impact mitochondrial content or deficits in ATP synthesis in mdx mice [99]. Acute PDE5 inhibition increased blood flow and cage activity following mild exercise, suggesting increased cGMP can reduce post-exercise fatigue in mdx mice [64]. Taken together, these data are in agreement with findings that improved muscle perfusion may reduce muscle damage and increase exercise tolerance in mdx mice [64,67]. In addition, these findings suggest that PDE5 inhibitors reduce skeletal muscle dysfunction in mdx mice.

It is important to note that PDE5 is only one of several cGMP-hydrolyzing PDEs in skeletal muscle and that PDE5 does not negatively regulate all nNOS-stimulated cGMP [16]. In other words, PDE5 mediates only a subset of nNOS functions; therefore, PDE5 inhibition alone will be insufficient to restore all nNOS-dependent dysfunction in

dystrophin-deficient muscle. In addition, the efficacy of PDE5 inhibitors is critically dependent on nNOS expression because NO is required to stimulate GC to synthesize cGMP, some of which is then hydrolyzed by PDE5 [64,94].

Like mdx mice, individuals with BMD and DMD also exhibit sympathetic vasoconstriction during exercise [80,86,108]. Taking advantage of the finding that PDE5 inhibition recapitulates some blood delivery functions of nNOSμ in mice, BMD and DMD patients were subject to mild simulated orthostatic stress to trigger sympathetic constriction of microvessels in contracting forearm skeletal muscles, then treated with a single dose of either sildenafil or tadalafil [80,86]. Both PDE5 inhibitors improved exercising forearm muscle perfusion in all DMD patients, and all but one BMD patient [80]. Therefore, sarcolemmal nNOSμ may regulate sympathetic vasoconstriction through a cGMP-dependent and PDE5-modulated pathway in humans. Acute oral treatments with the sodium nitrate (a source of vasodilating NO) similarly reduced sympathetic vasoconstriction and post-exercise hyperemia in individuals with BMD [87]. In contrast, one month of sildenafil treatment did not significantly impact brachial artery blood flow during maximal handgrip exercise or 6-min walk test times [128]. Reasons for this discrepancy are unclear, but may be caused by substantial reductions in PDE5 expression observed in some BMD patients in this study, and/or significant methodological differences in the application of sympathetic stress or duration of treatment.

The surprising finding that like nNOSμ, PDE5 is also downregulated in patients with dystrophinopathy is highly significant because it would produce a compensatory and protective increase NO-cGMP signaling. Therefore, nature may have already "led the way" by inhibiting PDE5 in many dystrophinopathy patients, thereby negating further clinical attempts to inhibit PDE5. Indeed, it was recently shown in a large phase 3 clinical trial in 330 DMD patients that a 48 week long tadalafil treatment did not slow the decline in 6 min walk distance, or other secondary measures of motor function compared with placebo (ClinicalTrials.gov Identifier: NCT01359670). It is worth noting that chronic PDE5 inhibition was safely tolerated in DMD patients. Indeed, in the three long-term trials to date, there is no evidence of unexpected adverse events resulting from long term PDE5 inhibition in DMD and BMD patients (NCT01359670) [73,128].

Collectively, these data suggest a role for nNOSμ-cGMP-PDE5 signaling in opposing sympathetic vasoconstriction in mice and humans. However, the therapeutic utility of sustained relief of sympathetic vasoconstriction by PDE5 inhibition for mitigating dystrophic pathogenesis is in question. Indeed, recent data suggest that PDE5 inhibition is a less than ideal approach for mitigating defects in cGMP-dependent nNOS enzyme signaling in striated muscle because of reduced PDE5 expression, and because PDE5 does not mediate all cGMP-dependent functions of nNOS enzymes (see below). However, this does not preclude further testing of PDE5 inhibitors to treat other NO-cGMP-modulated features of dystrophin-deficiency pathology, such as cognitive dysfunction, that negatively impact quality of life [7].

7. Beyond blood flow: sarcolemmal nNOSμ and dystrophin-deficient muscle contraction

A recent study that addressed the consequences of restoring sarcolemmal nNOSμ alone in dystrophin-deficient muscle revealed an important new function for sarcolemmal nNOSμ in muscle contraction [104]. Targeting of nNOSμ to the sarcolemma was achieved in the absence of dystrophin by attaching a short plasma membrane-directing C-terminal palmitoylation signal sequence from the K-ras oncogene to the carboxyl terminus of nNOSμ. Forced skeletal muscle-specific sarcolemmal targeting of nNOSμ in mdx mice substantially reduced susceptibility to lengthening contraction-induced injury in limb muscles and contraction-induced fatigue in both limb and respiratory muscles [104]. These findings suggest a role for sarcolemmal nNOSμ in increased susceptibility to lengthening contraction-induced injury and fatigue which are hallmark causes of weakness in mdx mice and individuals with DMD [35].

Forced sarcolemmal nNOSμ localization enhanced the expression and sarcolemmal localization of the dystrophin homolog utrophin, which can partly compensate for the loss of dystrophin [34]. This finding suggested an NO-induced utrophin-based structural remodeling decreased the susceptibility to lengthening contraction-induced injury, and perhaps fatigue in mdx muscle [34,104]. These data agree with a previous study showing that loss of nNOS increased lengthening contraction-induced injury in mdx mice [47]. Collectively, these data suggest an important role for nNOS in resisting contraction-induced injury in dystrophin-null muscle. In contrast, nNOSμ overexpressed in the cytoplasm had no impact on hindlimb or respiratory skeletal muscle force output, highlighting the fact that the localization of nNOSμ to the sarcolemma is necessary for its ability to regulate muscle contractility and to ameliorate dystrophic muscle dysfunction [104]. Importantly, this study provides additional evidence of how PDE5 inhibition cannot recapitulate fully the loss of nNOSμ function in mdx mice. PDE5 inhibition has no effect on the contractile performance of dystrophin-deficient tibialis anterior skeletal muscle in situ, and modestly increased diaphragm muscle specific force [100].

While targeting of nNOSμ to the sarcolemma contributes to the diversity of NO signaling function in skeletal muscle, the question then arises as to the function of cytoplasmic nNOSμ and nNOSβ in normal and dystrophic muscle. Little is known about the role of Golgi-associated nNOSβ because this an emerging area of research. However, nNOSβ is mislocalized in murine dystrophin-deficient muscles and this may be due to impaired subsarcolemmal microtubule lattice formation that disrupts the distribution of the Golgi complex and/or reduced association of nNOSβ with the Golgi [47,98]. Given that the localization of nNOS in muscle is pivotal for its function, it is possible that defects in nNOSβ localization in mdx muscle also contribute to muscle dysfunction. This possibility remains to be tested.

These findings suggest several important points about nNOSμ function, the regulation of NO signaling, and potential therapies designed to restore nNOS signaling in skeletal muscle. First, they expand the function of sarcolemmal nNOSμ beyond attenuation of sympathetic vasoconstriction to the regulation of resistance to contraction-induced fatigue and injury. Indeed, current data suggest that opposing sympathetic vasoconstriction and contractile regulation are separate functions of sarcolemmal nNOSμ. Second, the unique functions of sarcolemmal and cytoplasmic nNOSμ support the conceptual framework that the differential targeting of nNOSμ to the sarcolemma and cytoplasm facilitates the creation of spatially and functionally distinct NO compartments which contributes to NO signaling diversity in vivo. Third, it strengthens the argument that NO signaling is local and not diffusion limited, which was an unlikely proposition in skeletal muscle given the high abundance of potent NO scavengers such as myoglobin. Indeed, studies in the cardiovascular and central nervous system support this contention [12,51,116]. Fourth, these findings, in combination with previous studies of PDE5 inhibitors in dystrophic muscle suggest that while PDE5 may mediate some of the vascular control exerted by sarcolemmal nNOSμ, it may not participate in the control of muscle force output. Thus, PDE5 inhibition cannot fully recapitulate impaired nNOSμ function in mdx muscle, which is an important limitation of PDE5-based therapy for dystrophinopathies.

8. The roles of cytoplasmic nNOSμ in dystrophic muscle

Several studies hypothesized that in dystrophin-deficient muscles, residual cytoplasmic nNOSμ expression may drive nitrosative stress, exacerbating muscle damage and weakness [28,31]. However, skeletal muscles from mdx mice crossed with KN1 mice (KN1mdx) that lack nNOSα and residual muscle nNOSμ activity exhibited no change in biomarkers of muscle damage or degeneration, but did show a modest increase in specific force (strength) associated with decreased RyR1 S-

nitrosation [28,31,75]. In contrast, increasing cytoplasmic nNOSμ expression selectively in the skeletal muscles of mdx mice did not affect established muscle weakness [104]. In addition, muscles from mice lacking all nNOS activity (KN2mdx mice), and thus lacking all nNOS-derived nitrosative stress as well as normal nNOS function, are weaker and more inflamed than mdx controls. Taken together these findings argue against cytoplasmic nNOSμ-mediated nitrosative stress as a primary driver of dystrophic muscle dysfunction [47].

Increased RyR1 S-nitrosation by iNOS was also postulated to drive muscle damage and weakness in mdx mice [14]. However, genetic inhibition of iNOS function in mdx mice had no impact on muscle strength and little to no impact on muscle damage, despite reducing pro-inflammatory M1 type iNOS-expressing macrophages in young mdx mice [14,74,121]. Therefore, iNOS- RyR1 S-nitrosation may not play a major role in dystrophic pathogenesis. Also, it is important to note that increased RyR1 S-nitrosation is not necessarily deleterious or result in changes in Ca^{2+} handling. Genetic inhibition of the denitrosylase S-nitrosoglutathione reductase (GSNOR) increased RyR1 S-nitrosation, skeletal muscle strength, and fatigue resistance in situ, without affecting Ca^{2+} handling [84].

Furthermore, several studies suggest that activation of cytoplasmic NO signaling pathways can be protective in mdx mice. Macrophages are a major component of the inflammatory infiltrate in dystrophin-deficient skeletal muscles [2,105,121,125]. Transgenic overexpression of nNOSα substantially decreased the infiltration and cytolytic activity of macrophages in mdx mice [125]. In young mdx mouse muscle, pro-cytolytic M1 class macrophages predominate, while in early adulthood there is a switch to an M2 macrophage type that may favor muscle regeneration [121]. Because nNOSα is not expressed in mature muscle cells, nNOSα likely exerted its anti-inflammatory effects through activation of downstream targets of nNOSμ or nNOSβ; however, current evidence supports a primary role for nNOSβ. Skeletal muscles from KN1mdx mice show no histological evidence of increased inflammation relative to mdx controls [28,31,75]. In contrast, KN2mdx mice lacking all nNOS activity manifested greater skeletal muscle macrophage infiltration [47]. KN2mdx mice were generated by breeding mdx and KN2 mice thereby systemically eliminating all nNOS splice form activity including nNOSβ and residual active nNOSμ in skeletal muscle. These findings are consistent with an important immunomodulatory role for nNOS in mdx mice and suggest that nNOSβ may negatively regulate inflammatory cell infiltration in dystrophin-deficient muscle.

Greater skeletal muscle inflammation in KN2mdx mice was associated with more severe muscle damage and weakness than KN1mdx mice [47]. Unlike KN1mdx mice, KN2mdx mice show substantial declines in hallmark features of dystrophic muscle pathology. First, KN2mdx mice exhibited impaired compensatory hypertrophic growth and greater force deficits during lengthening contractions. Second, remarkably the loss of dystrophin “rescued” the poor fatigue resistance observed in nNOS null (KN2) mice [47]. This finding suggested a previously unrecognized compensatory mechanism(s) in mdx mice that sustains normal muscle fatigue resistance in the absence of nNOS. Third, genetic nNOS inhibition reduced the number of centrally nucleated fibers, suggesting impaired regeneration consistent with an important role for NO in muscle repair [106]. These findings suggest that nNOS plays a greater role in mitigating dystrophic pathology than previously recognized. In addition, they suggest the possibility that nNOSβ may play an important role in dystrophic pathogenesis, and may represent a new therapeutic target in dystrophinopathies.

9. NO-sensitive guanylyl cyclase compartments in skeletal muscle cells

nNOS splice forms can exert many of their regulatory effects through direct activation of NO-sensitive guanylyl cyclase (GC) (Fig. 1). GCs are direct physiological targets of NO in skeletal muscle and other cell types and act like an “NO receptor” to mediate cGMP-dependent NO signaling functions in the cardiovascular, gastrointestinal, and central nervous systems [9,36,45]. GCs catalyze the conversion of GTP to cGMP and pyrophosphate [54]. GCs exists as two heterodimeric isoforms, GC1 and GC2, which are comprised of α1β1 and α2β1 GC subunits, respectively. α1, α2, β1 GC subunit mRNA and protein are expressed in human and rodent skeletal muscle tissues, specifically in the muscle cells themselves and in cells of the microvasculature [49,81,83,112]. In mice, GC1, but not GC2, expression parallels the oxidative capacity of skeletal muscle with highest levels of α1 and β1 protein expression in diaphragm muscles, and the lowest in gastrocnemius muscles [83]. This is opposite to nNOSμ expression which is highest in glycolytic muscles [65]. These data suggest skeletal-muscle specific differences in cGMP synthesis capacity and NO-cGMP signal propagation.

Under resting conditions, NO stably binds the heme group associated with the β1 subunit of GC, resulting in low rates of cGMP synthesis; however, under activating conditions additional NO binds to non-heme sites in GC further increasing cGMP synthesis [24,36]. Resting human and rodent skeletal muscle tissues exhibit low levels of GC activity (relative to other organs such as the lung) that are similar between oxidative and glycolytic murine muscle types [9,54,83]. Microvasculature-expressed GC activity makes a greater contribution to total resting GC activity in oxidative muscles because they are more highly vascularized. Thus, NO stimulates greater increases in GC activity in oxidative muscles compared with glycolytic muscles in mice [9,83]. In resting skeletal muscle, nNOSμ and nNOSβ expression is necessary for normal GC activity and also for GC activation by agonists including NO donors, heme-dependent GC stimulators, and heme-independent GC activators [83]. In other words, GC agonists (NO donors, GC stimulators, or GC activators) cannot fully increase GC activity in the absence of active nNOS, even in muscle biochemical homogenates in vitro. These findings suggest that NO donors (or GC agonists) do not fully recapitulate all aspects of regulation of GC by nNOS in skeletal muscle. Nonetheless, these data indicate that GC1 is an important target and effector for nNOS in skeletal muscle cells.

The mechanisms underpinning the dependence of GC activity on nNOS remain to be determined; however, normal regulation of GC1 activity may require an interaction with nNOSμ because the α1 GC subunit and nNOSμ are found in a complex in skeletal muscle [83]. Most studies suggest that GC is not localized to the sarcolemma suggesting that GC1 and nNOSμ are part of a cytosolic complex and that GC enzymes localize to a subset of nNOS compartments within muscle cells [41,49,83]. GC1 is localized in close proximity to nNOSβ at the cis-Golgi compartment supporting a role for NO-cGMP signaling in the classical secretory pathway [83]. In addition, like nNOSμ, GC1 and GC2 localize to the postsynaptic zone of the neuromuscular junction in human and rodent skeletal muscles [41,83,112]. In summary, GC localizes to a subset of nNOSμ and nNOSβ splice variant compartments including the Golgi complex, cytoplasm, and neuromuscular junction in skeletal muscle. These findings support the contention that GC is an important effector for nNOS and that differential targeting of nNOS and GC isoforms facilitates the creation of spatially and functionally distinct NO-cGMP signaling compartments in skeletal muscle cells.

10. NO-sensitive guanylyl cyclase function in skeletal muscle cells

The question then arises: what are the functions of GC isoforms in skeletal muscle? Unfortunately, GC isoform function in skeletal muscle has received little attention. However, there is increasing interest in GC function given substantial reductions in cGMP synthesis in Duchenne and Becker muscular dystrophy patients and the emergence of NO-cGMP signaling pathways as a clinically actionable therapeutic target in dystrophinopathies [25,94]. While GC function is poorly understood, some data exist regarding the roles of cGMP in skeletal muscle contraction. Skeletal muscle cGMP levels are increased in a NO-dependent manner during contraction [70,79]. Increasing cGMP with non-

selective cGMP analogues and phosphodiesterase inhibitors modestly inhibited rat diaphragm force output [1,65]. However, opposing findings were reported showing that inhibition of GC using non-specific LY835843 reduced maximal shortening velocity and maximal tetanic force output in murine extensor digitorum longus muscles [79,85]. Additional evidence suggesting that cGMP does not depress muscle contractility comes from studies with the highly selective PDE5 inhibitor sildenafil. Chronic increases in cGMP resulting from sildenafil treatment had no impact on wild type or mdx diaphragm or tibialis anterior muscle specific force (strength) or contraction-induced fatigue resistance ex vivo and in situ, respectively [100]. Therefore, the majority of pharmacological evidence suggests that cGMP does not play a major role in regulating skeletal muscle strength.

A recent genetic loss-of-function study in α1 GC subunit null mice (GC1 null) also suggests that cGMP does not regulate skeletal muscle strength, but instead indicates a role for cGMP in muscle fatigue resistance. GC1 null mice exhibit a mild myopathy characterized by lowered contraction-induced fatigue resistance and poor force recovery after a fatiguing protocol in situ [83]. GC1 inhibition had no impact on specific force output at maximal or submaximal stimulation frequencies, as seen previously in KN1 mice. Lowered fatigue resistance in GC1 null mice is consistent with reduced shortening velocity and force output in murine extensor digitorum longus muscles treated with GC inhibitor LY835843 [79]. Together, the majority of evidence suggests that cGMP does not decrease skeletal muscle strength, but that cGMP synthesis by GC1 is necessary for the normal fatigue resistance of skeletal muscle.

The findings from studies of muscle fatigue resistance in GC1 null mice, in combination with those from S-nitrosoglutathione reductase null ($GSNOR^{-/-}$) mice, illuminate potentially distinct roles for NO-cGMP signaling and NO−S-nitrosylation-based signaling in skeletal muscle fatigue resistance [83,84]. GC1 inhibition decreases muscle fatigue resistance, while GSNOR inhibition does the opposite and promotes fatigue resistance. These findings suggest that nNOS positively regulates muscle fatigue resistance through both cGMP and S-nitrosothiol-dependent mechanisms, with perhaps a more major role for S-nitrosothiols. In addition, GC1 null, but not $GSNOR^{-/-}$ mice, show deficits in force recovery following fatiguing exercise [83,84]. Therefore, nNOS-NO may regulate force recovery following exercise primarily through a cGMP-dependent mechanism.

In addition to a possible role in muscle fatigue resistance, several studies suggest that NO-cGMP signaling may regulate mitochondrial content and ATP synthesis in skeletal muscle cells. NO donors, GC agonists, and cGMP analogues drive the formation of functional mitochondria through increased expression of transcriptional regulators including PGC1α in cultured rat L6 skeletal muscle cells [76,89,90]. NO-cGMP may stimulate mitochondrial biogenesis through activation of cGMP-activation of protein kinase G because systemic overexpression of a PKG Iα transgene caused the formation of abnormally large mitochondria in skeletal muscle in vivo [82]. In addition, several studies also suggested that nNOS-cGMP signaling regulates skeletal muscle cell mitochondrial integrity, in part through the control of dynamin-related protein 1-mediated fission [32,33,96]. However, total mitochondrial content in skeletal muscles of GC1 null mice is similar to controls [83]. Non-invasive metabolic spectroscopy showed that skeletal muscle mitochondrial ATP synthesis capacity and efficiency were also unaffected by GC1 inhibition [83]. These data suggest that GC1, the major source of NO-stimulated cGMP in striated muscle, is dispensable for establishing mitochondrial mass and ATP synthesis in resting muscle.

Similarly, skeletal muscles from wild type and mdx mice treated for three months with sildenafil exhibited a two-fold increase in cGMP levels but normal mitochondrial mass and intermyofibrillar and subsarcolemmal mitochondrial densities [83,99]. These data indicate that increased cGMP does not necessarily increase mitochondrial mass and suggest a more complex relationship between cGMP and mitochondrial biogenesis, at least in vivo. However, sildenafil treatment increased the oxygen cost of ATP synthesis indicating a reduction in ATP synthesis efficiency [83]. Inefficient mitochondria oxidize more substrates such as glucose and fatty acids to make the same amount of ATP [55]. Because skeletal muscle is 40% of body mass, increased muscle mitochondrial inefficiency can significantly increase whole body energy expenditure promoting a negative caloric energy balance and weight loss [55]. Indeed, chronically increasing NO-cGMP signaling by PDE5 inhibition or PKG overexpression in skeletal muscle and other tissues protects against diet-induced obesity in mice [11,82]. Importantly, these data illustrate a crucial point that although skeletal muscle has low levels of cGMP and cGMP synthesis relative to other tissues, small changes in skeletal muscle cGMP levels could have profound physiological effects on systemic energy balance through modulation of mitochondria bioenergetics [9]. In summary, these data support an important role for NO-cGMP signaling in skeletal muscle bioenergetics and suggest new directions for understanding how skeletal muscle modulates systemic energy balance.

11. Future directions

Studies of nNOS splice variants in both normal and dystrophic skeletal muscle have provided substantial new insights and understanding of the regulation and function of NO signaling. However, it is clear that we have much to learn regarding nNOS isoform functional diversity because the in vivo roles of nNOSβ, nNOSγ, and nNOS-2 are unknown. Going forward closer attention needs to be given to isoform-specific behavior of nNOS isoforms, particularly the short splice variants nNOSβ, nNOSγ, and to the interpretation of current nNOS knockout models. In addition, while powerful mouse models currently exist, it is clear that we will need more precise mouse models to define the skeletal muscle-specific roles of nNOSμ and nNOSβ. To this end, we have generated two different lines of knockout mice whose skeletal muscles lack both nNOSβ and nNOSμ or nNOSβ alone. Also, given that nNOS signaling pathways are important modulators of dystrophic pathogenesis and attractive drug targets in DMD and BMD, new approaches are needed to explore and harness the therapeutic potential of nNOS signaling in dystrophinopathies.

Funding

This work was supported by grants to JMP from the National Institutes of Health [1R03AR066805-01] and the Department of Defense Duchenne Muscular Dystrophy Research Program [MD140021].

References

[1] R.Z. Abraham, L. Kobzik, M.R. Moody, M.B. Reid, J.S. Stamler, Cyclic GMP is a second messenger by which nitric oxide inhibits diaphragm contraction, Comp. Biochem. Physiol. Mol. Integr. Physiol. 119 (1998) 177–183.
[2] S. Acharyya, S.A. Villalta, N. Bakkar, T. Bupha-Intr, P.M. Janssen, M. Carathers, Z.W. Li, A.A. Beg, S. Ghosh, Z. Sahenk, M. Weinstein, K.L. Gardner, J.A. Rafael-Fortney, M. Karin, J.G. Tidball, A.S. Baldwin, D.C. Guttridge, Interplay of IKK/NF-kappaB signaling in macrophages and myofibers promotes muscle degeneration in Duchenne muscular dystrophy, J. Clin. Invest. 117 (2007) 889–901.
[3] M.E. Adams, N. Kramarcy, S.P. Krall, S.G. Rossi, R.L. Rotundo, R. Sealock, S.C. Froehner, Absence of alpha-syntrophin leads to structurally aberrant neuromuscular synapses deficient in utrophin, J. Cell Biol. 150 (2000) 1385–1398.
[4] M.E. Adams, G.L. Odom, M.J. Kim, J.S. Chamberlain, S.C. Froehner, Syntrophin binds directly to multiple spectrin-like repeats in dystrophin and mediates binding of nNOS to repeats 16-17, Hum. Mol. Genet. 27 (17) (2018 Sep 1) 2978–2985.
[5] M.E. Adams, Y. Tesch, J.M. Percival, D.E. Albrecht, J.I. Conhaim, K. Anderson, S.C. Froehner, Differential targeting of nNOS and AQP4 to dystrophin-deficient sarcolemma by membrane-directed alpha-dystrobrevin, J. Cell Sci. 121 (2008) 48–54.
[6] A.H. Ahn, C.A. Freener, E. Gussoni, M. Yoshida, E. Ozawa, L.M. Kunkel, The three human syntrophin genes are expressed in diverse tissues, have distinct chromosomal locations, and each bind to dystrophin and its relatives, J. Biol. Chem. 271 (1996) 2724–2730.
[7] M.S. Alexander, M.J. Gasperini, P.T. Tsai, D.E. Gibbs, J.M. Spinazzola, J.L. Marshall, M.J. Feyder, M.T. Pletcher, E.L. Chekler, C.A. Morris, M. Sahin,

J.F. Harms, C.J. Schmidt, R.J. Kleiman, L.M. Kunkel, Reversal of neurobehavioral social deficits in dystrophic mice using inhibitors of phosphodiesterases PDE5A and PDE9A, Transl. Psychiatry 6 (2016) e901.
[8] K. Aquilano, S. Baldelli, M.R. Ciriolo, Nuclear recruitment of neuronal nitric-oxide synthase by alpha-syntrophin is crucial for the induction of mitochondrial biogenesis, J. Biol. Chem. 289 (2014) 365–378.
[9] W.P. Arnold, C.K. Mittal, S. Katsuki, F. Murad, Nitric oxide activates guanylate cyclase and increases guanosine 3':5'-cyclic monophosphate levels in various tissue preparations, Proc. Natl. Acad. Sci. U. S. A. 74 (1977) 3203–3207.
[10] A. Asai, N. Sahani, M. Kaneki, Y. Ouchi, J.A. Martyn, S.E. Yasuhara, Primary role of functional ischemia, quantitative evidence for the two-hit mechanism, and phosphodiesterase-5 inhibitor therapy in mouse muscular dystrophy, PLoS One 2 (2007) e806.
[11] J.E. Ayala, D.P. Bracy, B.M. Julien, J.N. Rottman, P.T. Fueger, D.H. Wasserman, Chronic treatment with sildenafil improves energy balance and insulin action in high fat-fed conscious mice, Diabetes 56 (2007) 1025–1033.
[12] L.A. Barouch, R.W. Harrison, M.W. Skaf, G.O. Rosas, T.P. Cappola, Z.A. Kobeissi, I.A. Hobai, C.A. Lemmon, A.L. Burnett, B. O'Rourke, E.R. Rodriguez, P.L. Huang, J.A. Lima, D.E. Berkowitz, J.M. Hare, Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms, Nature 416 (2002) 337–339.
[13] O. Baum, S. Schlappi, F.A. Huber-Abel, A. Weichert, H. Hoppeler, A. Zakrzewicz, The beta-isoform of neuronal nitric oxide synthase (nNOS) lacking the PDZ domain is localized at the sarcolemma, FEBS Lett. 585 (2011) 3219–3223.
[14] A.M. Bellinger, S. Reiken, C. Carlson, M. Mongillo, X. Liu, L. Rothman, S. Matecki, A. Lacampagne, A.R. Marks, Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle, Nat. Med. 15 (2009) 325–330.
[15] A. Beuve, Thiol-based redox modulation of soluble guanylyl cyclase, the nitric oxide receptor, Antioxidants Redox Signal. 26 (2017) 137–149.
[16] T.J. Bloom, Age-related alterations in cyclic nucleotide phosphodiesterase activity in dystrophic mouse leg muscle, Can. J. Physiol. Pharmacol. 83 (2005) 1055–1060.
[17] D.S. Bredt, P.M. Hwang, C.E. Glatt, C. Lowenstein, R.R. Reed, S.H. Snyder, Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase, Nature 351 (1991) 714–718.
[18] D.S. Bredt, S.H. Snyder, Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 682–685.
[19] J.E. Brenman, D.S. Chao, S.H. Gee, A.W. McGee, S.E. Craven, D.R. Santillano, Z. Wu, F. Huang, H. Xia, M.F. Peters, S.C. Froehner, D.S. Bredt, Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains, Cell 84 (1996) 757–767.
[20] J.E. Brenman, D.S. Chao, H. Xia, K. Aldape, D.S. Bredt, Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy, Cell 82 (1995) 743–752.
[21] J.E. Brenman, H. Xia, D.S. Chao, S.M. Black, D.S. Bredt, Regulation of neuronal nitric oxide synthase through alternative transcripts, Dev. Neurosci. 19 (1997) 224–231.
[22] E.S. Buys, M.J. Raher, A. Kirby, M. Shahid, D.M. Baron, S.R. Hayton, L.T. Tainsh, P.Y. Sips, K.M. Rauwerdink, Q. Yan, R.E. Tainsh, H.R. Shakartzi, C. Stevens, K. Decaluwe, G. Rodrigues-Machado Mda, R. Malhotra, J. Van de Voorde, T. Wang, P. Brouckaert, M.J. Daly, K.D. Bloch, Genetic modifiers of hypertension in soluble guanylate cyclase alpha1-deficient mice, J. Clin. Invest. 122 (2012) 2316–2325.
[23] E.S. Buys, P. Sips, P. Vermeersch, M.J. Raher, E. Rogge, F. Ichinose, M. Dewerchin, K.D. Bloch, S. Janssens, P. Brouckaert, Gender-specific hypertension and responsiveness to nitric oxide in sGCalpha1 knockout mice, Cardiovasc. Res. 79 (2008) 179–186.
[24] S.P. Cary, J.A. Winger, E.R. Derbyshire, M.A. Marletta, Nitric oxide signaling: no longer simply on or off, Trends Biochem. Sci. 31 (2006) 231–239.
[25] C. Cerri, N. Canal, L. Frattola, Guanylate cyclase activity in normal and diseased human muscle, J. Neurol. Neurosurg. Psychiatry 41 (1978) 805–808.
[26] W.J. Chang, S.T. Iannaccone, K.S. Lau, B.S. Masters, T.J. McCabe, K. McMillan, R.C. Padre, M.J. Spencer, J.G. Tidball, J.T. Stull, Neuronal nitric oxide synthase and dystrophin-deficient muscular dystrophy, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 9142–9147.
[27] D.S. Chao, J.R. Gorospe, J.E. Brenman, J.A. Rafael, M.F. Peters, S.C. Froehner, E.P. Hoffman, J.S. Chamberlain, D.S. Bredt, Selective loss of sarcolemmal nitric oxide synthase in Becker muscular dystrophy, J. Exp. Med. 184 (1996) 609–618.
[28] D.S. Chao, F. Silvagno, D.S. Bredt, Muscular dystrophy in mdx mice despite lack of neuronal nitric oxide synthase, J. Neurochem. 71 (1998) 784–789.
[29] J.E. Church, S.M. Gehrig, A. Chee, T. Naim, J. Trieu, G.K. McConell, G.S. Lynch, Early functional muscle regeneration after myotoxic injury in mice is unaffected by nNOS absence, Am. J. Physiol. Regul. Integr. Comp. Physiol. 301 (2011) R1358–R1366.
[30] K.B. Craven, W.N. Zagotta, CNG and HCN channels: two peas, one pod, Annu. Rev. Physiol. 68 (2006) 375–401.
[31] R.H. Crosbie, V. Straub, H.Y. Yun, J.C. Lee, J.A. Rafael, J.S. Chamberlain, V.L. Dawson, T.M. Dawson, K.P. Campbell, Mdx muscle pathology is independent of nNOS perturbation, Hum. Mol. Genet. 7 (1998) 823–829.
[32] C. De Palma, S. Falcone, S. Pisoni, S. Cipolat, C. Panzeri, S. Pambianco, A. Pisconti, R. Allevi, M.T. Bassi, G. Cossu, T. Pozzan, S. Moncada, L. Scorrano, S. Brunelli, E. Clementi, Nitric oxide inhibition of Drp1-mediated mitochondrial fission is critical for myogenic differentiation, Cell Death Differ. 17 (2010) 1684–1696.
[33] C. De Palma, F. Morisi, S. Pambianco, E. Assi, T. Touvier, S. Russo, C. Perrotta, V. Romanello, S. Carnio, V. Cappello, P. Pellegrino, C. Moscheni, M.T. Bassi, M. Sandri, D. Cervia, E. Clementi, Deficient nitric oxide signalling impairs skeletal muscle growth and performance: involvement of mitochondrial dysregulation, Skeletal Muscle 4 (22) (2014).
[34] A.E. Deconinck, J.A. Rafael, J.A. Skinner, S.C. Brown, A.C. Potter, L. Metzinger, D.J. Watt, J.G. Dickson, J.M. Tinsley, K.E. Davies, Utrophin-dystrophin-deficient mice as a model for Duchenne muscular dystrophy, Cell 90 (1997) 717–727.
[35] C. Dellorusso, R.W. Crawford, J.S. Chamberlain, S.V. Brooks, Tibialis anterior muscles in mdx mice are highly susceptible to contraction-induced injury, J. Muscle Res. Cell Motil. 22 (2001) 467–475.
[36] E.R. Derbyshire, M.A. Marletta, Structure and regulation of soluble guanylate cyclase, Annu. Rev. Biochem. 81 (2012) 533–559.
[37] A.C. Dordea, S. Vandenwijngaert, V. Garcia, R.E. Tainsh, D.I. Nathan, K. Allen, M.J. Raher, L.T. Tainsh, F. Zhang, W.S. Lieb, S. Mikelman, A. Kirby, C. Stevens, R. Thoonen, A.G. Hindle, P.Y. Sips, J.R. Falck, M.J. Daly, P. Brouckaert, K.D. Bloch, D.B. Bloch, R. Malhotra, M.L. Schwartzman, E.S. Buys, Androgen-sensitive hypertension associated with soluble guanylate cyclase-alpha1 deficiency is mediated by 20-HETE, Am. J. Physiol. Heart Circ. Physiol. 310 (2016) H1790–H1800.
[38] M.J. Eliasson, S. Blackshaw, M.J. Schell, S.H. Snyder, Neuronal nitric oxide synthase alternatively spliced forms: prominent functional localizations in the brain, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 3396–3401.
[39] J.P. Eu, J.M. Hare, D.T. Hess, M. Skaf, J. Sun, I. Cardenas-Navina, Q.A. Sun, M. Dewhirst, G. Meissner, J.S. Stamler, Concerted regulation of skeletal muscle contractility by oxygen tension and endogenous nitric oxide, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15229–15234.
[40] J.P. Eu, J. Sun, L. Xu, J.S. Stamler, G. Meissner, The skeletal muscle calcium release channel: coupled O2 sensor and NO signaling functions, Cell 102 (2000) 499–509.
[41] M. Feussner, H. Richter, O. Baum, R. Gossrau, Association of soluble guanylate cyclase with the sarcolemma of mammalian skeletal muscle fibers, Acta Histochem. 103 (2001) 265–277.
[42] E.L. Finanger Hedderick, J.L. Simmers, A. Soleimani, E. Andres-Mateos, R. Marx, D.C. Files, L. King, T.O. Crawford, A.M. Corse, R.D. Cohn, Loss of sarcolemmal nNOS is common in acquired and inherited neuromuscular disorders, Neurology 76 (2011) 960–967.
[43] B.L. Firestein, D.S. Bredt, Interaction of neuronal nitric-oxide synthase and phosphofructokinase-M, J. Biol. Chem. 274 (1999) 10545–10550.
[44] S.H. Francis, M.A. Blount, J.D. Corbin, Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions, Physiol. Rev. 91 (2011) 651–690.
[45] A. Friebe, D. Koesling, The function of NO-sensitive guanylyl cyclase: what we can learn from genetic mouse models, Nitric Oxide 21 (2009) 149–156.
[46] A. Friebe, E. Mergia, O. Dangel, A. Lange, D. Koesling, Fatal gastrointestinal obstruction and hypertension in mice lacking nitric oxide-sensitive guanylyl cyclase, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 7699–7704.
[47] S.C. Froehner, S.M. Reed, K.N. Anderson, P.L. Huang, J.M. Percival, Loss of nNOS inhibits compensatory muscle hypertrophy and exacerbates inflammation and eccentric contraction-induced damage in mdx mice, Hum. Mol. Genet. 24 (2) (2015 Jan 15) 492–505.
[48] H. Fujisawa, T. Ogura, Y. Kurashima, T. Yokoyama, J. Yamashita, H. Esumi, Expression of two types of nitric oxide synthase mRNA in human neuroblastoma cell lines, J. Neurochem. 63 (1994) 140–145.
[49] T. Fukutani, S. Iino, Y. Nojyo, The expression of soluble guanylate cyclase in the vasculature of rat skeletal muscle, Arch. Histol. Cytol. 72 (2009) 117–126.
[50] J.F. Garbincius, D.E. Michele, Dystrophin-glycoprotein complex regulates muscle nitric oxide production through mechanoregulation of AMPK signaling, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 13663–13668.
[51] J. Garthwaite, New insight into the functioning of nitric oxide-receptive guanylyl cyclase: physiological and pharmacological implications, Mol. Cell. Biochem. 334 (2010) 221–232.
[52] R.M. Grady, H. Zhou, J.M. Cunningham, M.D. Henry, K.P. Campbell, J.R. Sanes, Maturation and maintenance of the neuromuscular synapse: genetic evidence for roles of the dystrophin–glycoprotein complex, Neuron 25 (2000) 279–293.
[53] R. Gyurko, S. Leupen, P.L. Huang, Deletion of exon 6 of the neuronal nitric oxide synthase gene in mice results in hypogonadism and infertility, Endocrinology 143 (2002) 2767–2774.
[54] J.G. Hardman, E.W. Sutherland, Guanyl cyclase, an enzyme catalyzing the formation of guanosine 3',5'-monophosphate from guanosine trihosphate, J. Biol. Chem. 244 (1969) 6363–6370.
[55] M.E. Harper, K. Green, M.D. Brand, The efficiency of cellular energy transduction and its implications for obesity, Annu. Rev. Nutr. 28 (2008) 13–33.
[56] B.J. Hillier, K.S. Christopherson, K.E. Prehoda, D.S. Bredt, W.A. Lim, Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS-syntrophin complex, Science 284 (1999) 812–815.
[57] E.P. Hoffman, R.H. Brown Jr., L.M. Kunkel, Dystrophin: the protein product of the Duchenne muscular dystrophy locus, Cell 51 (1987) 919–928.
[58] C. Hoffmann, C. Weigert, Skeletal muscle as an endocrine organ: the role of myokines in exercise adaptations, Cold Spring Harb Perspect Med 7 (11) (2017 Nov 1) pii: a029793.
[59] P.L. Huang, T.M. Dawson, D.S. Bredt, S.H. Snyder, M.C. Fishman, Targeted disruption of the neuronal nitric oxide synthase gene, Cell 75 (1993) 1273–1286.
[60] H. Ihara, A. Kitamura, S. Kasamatsu, T. Ida, Y. Kakihana, H. Tsutsuki, T. Sawa, Y. Watanabe, T. Akaike, Superoxide generation from nNOS splice variants and its potential involvement in redox signal regulation, Biochem. J. 474 (2017) 1149–1162.
[61] H. Ihara, M. Kuwamura, M. Atsuta, I. Nihonmatsu, T. Okada, M. Mukamoto, S. Kozaki, Expression of neuronal nitric oxide synthase variant, nNOS-mu, in rat brain, Nitric Oxide 15 (2006) 13–19.

[62] S. Kameya, Y. Miyagoe, I. Nonaka, T. Ikemoto, M. Endo, K. Hanaoka, Y. Nabeshima, S. Takeda, alpha1-syntrophin gene disruption results in the absence of neuronal-type nitric-oxide synthase at the sarcolemma but does not induce muscle degeneration, J. Biol. Chem. 274 (1999) 2193–2200.

[63] M.J. Kim, S.H. Hwang, J.A. Lim, S.C. Froehner, M.E. Adams, H.S. Kim, Alpha-syntrophin modulates myogenin expression in differentiating myoblasts, PLoS One 5 (2010) e15355.

[64] Y.M. Kobayashi, E.P. Rader, R.W. Crawford, N.K. Iyengar, D.R. Thedens, J.A. Faulkner, S.V. Parikh, R.M. Weiss, J.S. Chamberlain, S.A. Moore, K.P. Campbell, Sarcolemma-localized nNOS is required to maintain activity after mild exercise, Nature 456 (2008) 511–515.

[65] L. Kobzik, M.B. Reid, D.S. Bredt, J.S. Stamler, Nitric oxide in skeletal muscle, Nature 372 (1994) 546–548.

[66] M. Koenig, E.P. Hoffman, C.J. Bertelson, A.P. Monaco, C. Feener, L.M. Kunkel, Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals, Cell 50 (1987) 509–517.

[67] Y. Lai, G.D. Thomas, Y. Yue, H.T. Yang, D. Li, C. Long, L. Judge, B. Bostick, J.S. Chamberlain, R.L. Terjung, D. Duan, Dystrophins carrying spectrin-like repeats 16 and 17 anchor nNOS to the sarcolemma and enhance exercise performance in a mouse model of muscular dystrophy, J. Clin. Invest. 119 (2009) 624–635.

[68] Y. Lai, J. Zhao, Y. Yue, D. Duan, alpha2 and alpha3 helices of dystrophin R16 and R17 frame a microdomain in the alpha1 helix of dystrophin R17 for neuronal NOS binding, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 525–530.

[69] R. Laine, P.R. de Montellano, Neuronal nitric oxide synthase isoforms alpha and mu are closely related calpain-sensitive proteins, Mol. Pharmacol. 54 (1998) 305–312.

[70] K.S. Lau, R.W. Grange, W.J. Chang, K.E. Kamm, I. Sarelius, J.T. Stull, Skeletal muscle contractions stimulate cGMP formation and attenuate vascular smooth muscle myosin phosphorylation via nitric oxide, FEBS Lett. 431 (1998) 71–74.

[71] J.M. Lawler, M. Kunst, J.M. Hord, Y. Lee, K. Joshi, R.E. Botchlett, A. Ramirez, D.A. Martinez, EUK-134 ameliorates nNOSmu translocation and skeletal muscle fiber atrophy during short-term mechanical unloading, Am. J. Physiol. Regul. Integr. Comp. Physiol. 306 (2014) R470–R482.

[72] M.A. Lee, L. Cai, N. Hubner, Y.A. Lee, K. Lindpaintner, Tissue- and development-specific expression of multiple alternatively spliced transcripts of rat neuronal nitric oxide synthase, J. Clin. Invest. 100 (1997) 1507–1512.

[73] D.G. Leung, D.A. Herzka, W.R. Thompson, B. He, G. Bibat, G. Tennekoon, S.D. Russell, K.H. Schuleri, A.C. Lardo, D.A. Kass, R.E. Thompson, D.P. Judge, K.R. Wagner, Sildenafil does not improve cardiomyopathy in Duchenne/Becker muscular dystrophy, Ann. Neurol. 76 (2014) 541–549.

[74] D. Li, J.H. Shin, D. Duan, iNOS ablation does not improve specific force of the extensor digitorum longus muscle in dystrophin-deficient mdx4cv mice, PLoS One 6 (2011) e21618.

[75] D. Li, Y. Yue, Y. Lai, C.H. Hakim, D. Duan, Nitrosative stress elicited by nNOSmicro delocalization inhibits muscle force in dystrophin-null mice, J. Pathol. 223 (2011) 88–98.

[76] V.A. Lira, D.L. Brown, A.K. Lira, A.N. Kavazis, Q.A. Soltow, E.H. Zeanah, D.S. Criswell, Nitric oxide and AMPK cooperatively regulate PGC-1 in skeletal muscle cells, J. Physiol. 588 (2010) 3551–3566.

[77] K. Loughney, T.R. Hill, V.A. Florio, L. Uher, G.J. Rosman, S.L. Wolda, B.A. Jones, M.L. Howard, L.M. McAllister-Lucas, W.K. Sonnenburg, S.H. Francis, J.D. Corbin, J.A. Beavo, K. Ferguson, Isolation and characterization of cDNAs encoding PDE5A, a human cGMP-binding, cGMP-specific 3',5'-cyclic nucleotide phosphodiesterase, Gene 216 (1998) 139–147.

[78] T. Magee, A.M. Fuentes, H. Garban, T. Rajavashisth, D. Marquez, J.A. Rodriguez, J. Rajfer, N.F. Gonzalez-Cadavid, Cloning of a novel neuronal nitric oxide synthase expressed in penis and lower urinary tract, Biochem. Biophys. Res. Commun. 226 (1996) 145–151.

[79] G. Marechal, G. Beckers-Bleukx, Effect of nitric oxide on the maximal velocity of shortening of a mouse skeletal muscle, Pflügers Archiv 436 (1998) 906–913.

[80] E.A. Martin, R. Barresi, B.J. Byrne, E.I. Tsimerinov, B.L. Scott, A.E. Walker, S.V. Gurudevan, F. Anene, R.M. Elashoff, G.D. Thomas, R.G. Victor, Tadalafil alleviates muscle ischemia in patients with Becker muscular dystrophy, Sci. Transl. Med. 4 (2012) 162ra155.

[81] E. Mergia, M. Russwurm, G. Zoidl, D. Koesling, Major occurrence of the new alpha2beta1 isoform of NO-sensitive guanylyl cyclase in brain, Cell. Signal. 15 (2003) 189–195.

[82] K. Miyashita, H. Itoh, H. Tsujimoto, N. Tamura, Y. Fukunaga, M. Sone, K. Yamahara, D. Taura, M. Inuzuka, T. Sonoyama, K. Nakao, Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity, Diabetes 58 (2009) 2880–2892.

[83] Y. Moon, J.E. Balke, D. Madorma, M.P. Siegel, G. Knowels, P. Brouckaert, E.S. Buys, D.J. Marcinek, J.M. Percival, Nitric oxide regulates skeletal muscle fatigue, fiber type, microtubule organization, and mitochondrial ATP synthesis efficiency through cGMP-dependent mechanisms, Antioxidants Redox Signal. 26 (17) (2017 Jun 10) 966–985.

[84] Y. Moon, Y. Cao, J. Zhu, Y. Xu, W. Balkan, E.S. Buys, F. Diaz, W.G. Kerrick, J.M. Hare, J.M. Percival, GSNOR deficiency enhances in situ skeletal muscle strength, fatigue resistance, and RyR1 S-nitrosylation without impacting mitochondrial content and activity, Antioxidants Redox Signal. 26 (4) (2017 Feb 1) 165–181.

[85] A. Mulsch, R. Busse, S. Liebau, U. Forstermann, LY 83583 interferes with the release of endothelium-derived relaxing factor and inhibits soluble guanylate cyclase, J. Pharmacol. Exp. Therapeut. 247 (1988) 283–288.

[86] M.D. Nelson, F. Rader, X. Tang, J. Tavyev, S.F. Nelson, M.C. Miceli, R.M. Elashoff, H.L. Sweeney, R.G. Victor, PDE5 inhibition alleviates functional muscle ischemia in boys with Duchenne muscular dystrophy, Neurology 82 (2014) 2085–2091.

[87] M.D. Nelson, R. Rosenberry, R. Barresi, E.I. Tsimerinov, F. Rader, X. Tang, O. Mason, A. Schwartz, T. Stabler, S. Shidban, N. Mobaligh, S. Hogan, R. Elashoff, J.D. Allen, R.G. Victor, Sodium nitrate alleviates functional muscle ischaemia in patients with Becker muscular dystrophy, J. Physiol. (2015).

[88] S.E. Newey, M.A. Benson, C.P. Ponting, K.E. Davies, D.J. Blake, Alternative splicing of dystrobrevin regulates the stoichiometry of syntrophin binding to the dystrophin protein complex, Curr. Biol. 10 (2000) 1295–1298.

[89] E. Nisoli, E. Clementi, C. Paolucci, V. Cozzi, C. Tonello, C. Sciorati, R. Bracale, A. Valerio, M. Francolini, S. Moncada, M.O. Carruba, Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide, Science 299 (2003) 896–899.

[90] E. Nisoli, S. Falcone, C. Tonello, V. Cozzi, L. Palomba, M. Fiorani, A. Pisconti, S. Brunelli, A. Cardile, M. Francolini, O. Cantoni, M.O. Carruba, S. Moncada, E. Clementi, Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 16507–16512.

[91] M.A. Packer, Y. Stasiv, A. Benraiss, E. Chmielnicki, A. Grinberg, H. Westphal, S.A. Goldman, G. Enikolopov, Nitric oxide negatively regulates mammalian adult neurogenesis, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9566–9571.

[92] S.P. Panda, W. Li, P. Venkatakrishnan, L. Chen, A.V. Astashkin, B.S. Masters, C. Feng, L.J. Roman, Differential calmodulin-modulatory and electron transfer properties of neuronal nitric oxide synthase mu compared to the alpha variant, FEBS Lett. 587 (2013) 3973–3978.

[93] J.M. Percival, nNOS regulation of skeletal muscle fatigue and exercise performance, Biophys. Rev. 209–217 (2011).

[94] J.M. Percival, C.M. Adamo, J.A. Beavo, S.C. Froehner, Evaluation of the therapeutic utility of phosphodiesterase 5A inhibition in the mdx mouse model of duchenne muscular dystrophy, Handb. Exp. Pharmacol. 323–44 (2011).

[95] J.M. Percival, K.N. Anderson, P. Gregorevic, J.S. Chamberlain, S.C. Froehner, Functional deficits in nNOSmu-deficient skeletal muscle: myopathy in nNOS knockout mice, PLoS One 3 (2008) e3387.

[96] J.M. Percival, K.N. Anderson, P. Huang, M.E. Adams, S.C. Froehner, Golgi and sarcolemmal neuronal NOS differentially regulate contraction-induced fatigue and vasoconstriction in exercising mouse skeletal muscle, J. Clin. Invest. 120 (2010) 816–826.

[97] J.M. Percival, S.C. Froehner, Golgi complex organization in skeletal muscle: a role for Golgi-mediated glycosylation in muscular dystrophies? Traffic 8 (2007) 184–194.

[98] J.M. Percival, P. Gregorevic, G.L. Odom, G.B. Banks, J.S. Chamberlain, S.C. Froehner, rAAV6-microdystrophin rescues aberrant Golgi complex organization in mdx skeletal muscles, Traffic 8 (2007) 1424–1439.

[99] J.M. Percival, M.P. Siegel, G. Knowels, D.J. Marcinek, Defects in mitochondrial localization and ATP synthesis in the mdx mouse model of Duchenne muscular dystrophy are not alleviated by PDE5 inhibition, Hum. Mol. Genet. 22 (2013) 153–167.

[100] J.M. Percival, N.P. Whitehead, M.E. Adams, C.M. Adamo, J.A. Beavo, S.C. Froehner, Sildenafil reduces respiratory muscle weakness and fibrosis in the mdx mouse model of Duchenne muscular dystrophy, J. Pathol. 228 (2012) 77–87.

[101] 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.

[102] M.M. Rahaman, T. Sawa, A.K. Ahtesham, S. Khan, H. Inoue, A. Irie, S. Fujii, T. Akaike, S-guanylation proteomics for redox-based mitochondrial signaling, Antioxidants Redox Signal. 20 (2014) 295–307.

[103] E. Ralston, Z. Lu, T. Ploug, The organization of the Golgi complex and microtubules in skeletal muscle is fiber type-dependent, J. Neurosci. 19 (1999) 10694–10705.

[104] D.L. Rebolledo, M.J. Kim, N.P. Whitehead, M.E. Adams, S.C. Froehner, Sarcolemmal targeting of nNOSmu improves contractile function of mdx muscle, Hum. Mol. Genet. 25 (2016) 158–166.

[105] A.S. Rosenberg, M. Puig, K. Nagaraju, E.P. Hoffman, S.A. Villalta, V.A. Rao, L.M. Wakefield, J. Woodcock, Immune-mediated pathology in Duchenne muscular dystrophy, Sci. Transl. Med. 7 (2015) 299rv4.

[106] P. Rovere-Querini, E. Clementi, S. Brunelli, Nitric oxide and muscle repair: multiple actions converging on therapeutic efficacy, Eur. J. Pharmacol. (2013).

[107] M. Salanova, G. Schiffl, J. Rittweger, D. Felsenberg, D. Blottner, Ryanodine receptor type-1 (RyR1) expression and protein S-nitrosylation pattern in human soleus myofibres following bed rest and exercise countermeasure, Histochem. Cell Biol. 130 (2008) 105–118.

[108] M. Sander, B. Chavoshan, S.A. Harris, S.T. Iannaccone, J.T. Stull, G.D. Thomas, R.G. Victor, Functional muscle ischemia in neuronal nitric oxide synthase-deficient skeletal muscle of children with Duchenne muscular dystrophy, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 13818–13823.

[109] T. Sawa, M.H. Zaki, T. Okamoto, T. Akuta, Y. Tokutomi, S. Kim-Mitsuyama, H. Ihara, A. Kobayashi, M. Yamamoto, S. Fujii, H. Arimoto, T. Akaike, Protein S-guanylation by the biological signal 8-nitroguanosine 3',5'-cyclic monophosphate, Nat. Chem. Biol. 3 (2007) 727–735.

[110] N. Sayed, P. Baskaran, X. Ma, F. van den Akker, A. Beuve, Desensitization of soluble guanylyl cyclase, the NO receptor, by S-nitrosylation, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12312–12317.

[111] J. Schlossmann, R. Feil, F. Hofmann, Insights into cGMP signalling derived from cGMP kinase knockout mice, Front. Biosci. 10 (2005) 1279–1289.

[112] B.G. Schoser, S. Behrends, Soluble guanylyl cyclase is localized at the neuromuscular junction in human skeletal muscle, Neuroreport 12 (2001) 979–981.

[113] B.S. Shenkman, T.L. Nemirovskaya, Y.N. Lomonosova, No-dependent signaling pathways in unloaded skeletal muscle, Front. Physiol. 6 (2015) 298.

[114] F. Silvagno, H. Xia, D.S. Bredt, Neuronal nitric-oxide synthase-mu, an alternatively spliced isoform expressed in differentiated skeletal muscle, J. Biol. Chem. 271 (1996) 11204–11208.
[115] J.S. Stamler, G. Meissner, Physiology of nitric oxide in skeletal muscle, Physiol. Rev. 81 (2001) 209–237.
[116] A.C. Straub, A.W. Lohman, M. Billaud, S.R. Johnstone, S.T. Dwyer, M.Y. Lee, P.S. Bortz, A.K. Best, L. Columbus, B. Gaston, B.E. Isakson, Endothelial cell expression of haemoglobin alpha regulates nitric oxide signalling, Nature 491 (2012) 473–477.
[117] A. Suzuki, M. Yoshida, E. Ozawa, Mammalian alpha 1- and beta 1-syntrophin bind to the alternative splice-prone region of the dystrophin COOH terminus, J. Cell Biol. 128 (1995) 373–381.
[118] N. Suzuki, N. Motohashi, A. Uezumi, S. Fukada, T. Yoshimura, Y. Itoyama, M. Aoki, Y. Miyagoe-Suzuki, S. Takeda, NO production results in suspension-induced muscle atrophy through dislocation of neuronal NOS, J. Clin. Invest. 117 (2007) 2468–2476.
[119] G.D. Thomas, M. Sander, K.S. Lau, P.L. Huang, J.T. Stull, R.G. Victor, Impaired metabolic modulation of alpha-adrenergic vasoconstriction in dystrophin-deficient skeletal muscle, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 15090–15095.
[120] G.D. Thomas, P.W. Shaul, I.S. Yuhanna, S.C. Froehner, M.E. Adams, Vasomodulation by skeletal muscle-derived nitric oxide requires alpha-syntrophin-mediated sarcolemmal localization of neuronal Nitric oxide synthase, Circ. Res. 92 (2003) 554–560.
[121] S.A. Villalta, H.X. Nguyen, B. Deng, T. Gotoh, J.G. Tidball, Shifts in macrophage phenotypes and macrophage competition for arginine metabolism affect the severity of muscle pathology in muscular dystrophy, Hum. Mol. Genet. 18 (2009) 482–496.
[122] Y. Wang, M.S. Goligorsky, M. Lin, J.N. Wilcox, P.A. Marsden, A novel, testis-specific mRNA transcript encoding an NH2-terminal truncated nitric-oxide synthase, J. Biol. Chem. 272 (1997) 11392–11401.
[123] Y. Wang, D.C. Newton, P.A. Marsden, Neuronal NOS: gene structure, mRNA diversity, and functional relevance, Crit. Rev. Neurobiol. 13 (1999) 21–43.
[124] Y. Wang, D.C. Newton, G.B. Robb, C.L. Kau, T.L. Miller, A.H. Cheung, A.V. Hall, S. VanDamme, J.N. Wilcox, P.A. Marsden, RNA diversity has profound effects on the translation of neuronal nitric oxide synthase, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 12150–12155.
[125] M. Wehling, M.J. Spencer, J.G. Tidball, A nitric oxide synthase transgene ameliorates muscular dystrophy in mdx mice, J. Cell Biol. 155 (2001) 123–131.
[126] K.E. Wells, S. Torelli, Q. Lu, S.C. Brown, T. Partridge, F. Muntoni, D.J. Wells, Relocalization of neuronal nitric oxide synthase (nNOS) as a marker for complete restoration of the dystrophin associated protein complex in skeletal muscle, Neuromuscul. Disord. 13 (2003) 21–31.
[127] C. Wilson, R. Venditti, L.R. Rega, A. Colanzi, G. D'Angelo, M.A. De Matteis, The Golgi apparatus: an organelle with multiple complex functions, Biochem. J. 433 (2011) 1–9.
[128] N. Witting, C. Kruuse, B. Nyhuus, K.P. Prahm, G. Citirak, S.J. Lundgaard, S. von Huth, N. Vejlstrup, U. Lindberg, T.O. Krag, J. Vissing, Effect of sildenafil on skeletal and cardiac muscle in Becker muscular dystrophy, Ann. Neurol. 76 (2014) 550–557.
[129] K.Y. Xu, D.L. Huso, T.M. Dawson, D.S. Bredt, L.C. Becker, Nitric oxide synthase in cardiac sarcoplasmic reticulum, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 657–662.
[130] T. Zhang, P. Haws, Q. Wu, Multiple variable first exons: a mechanism for cell- and tissue-specific gene regulation, Genome Res. 14 (2004) 79–89.