SHORT COMMUNICATION

Osteoprotegerin and β_2 -Agonists Mitigate Muscular Dystrophy in Slow- and Fast-Twitch Skeletal Muscles

Sébastien S. Dufresne,^{*} Antoine Boulanger-Piette,^{*} and Jérôme Frenette^{*†}

From the Centre Hospitalier Universitaire de Québec—Centre de Recherche du Centre Hospitalier de l'Université Laval^{*} and the Department of Rehabilitation,[†] Faculty of Medicine, Université Laval, Quebec City, Quebec, Canada

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Address correspondence to
Jérôme Frenette, P.T., Ph.D.,
Centre Hospitalier Universitaire
de Québec—Centre de Recherche
du Centre Hospitalier de
l'Université Laval, 2705 Blvd
Laurier, Room RC-9500,
Quebec City, QC, Canada G1V
4G2. E-mail: jerome.frenette@crchul.ulaval.ca.

Our recent work showed that daily injections of osteoprotegerin (OPG)—immunoglobulin fragment complex (OPG-Fc) completely restore the function of fast-twitch extensor digitorum longus muscles in dystrophic mdx mice, a murine model of Duchenne muscular dystrophy. However, despite marked improvements, OPG-Fc was not as effective in preventing the loss of function of slow-twitch soleus and diaphragm muscles. Because β_2 -agonists enhance the function of slow- and fast-twitch dystrophic muscles and because their use is limited by their adverse effects on bone and cardiac tissues, we hypothesized that OPG-Fc, a bone and skeletal muscle protector, acts synergistically with β_2 -agonists and potentiates their positive effects on skeletal muscles. We observed that the content of β_2 -adrenergic receptors, which are mainly expressed in skeletal muscle, is significantly reduced in dystrophic muscles but is rescued by the injection of OPG-Fc. Most important, OPG-Fc combined with a low dose of formoterol, a member of a new generation of β_2 -agonists, histologically and functionally rescued slow-twitch dystrophic muscles. This combination of therapeutic agents, which have already been tested and approved for human use, may open up new therapeutic avenues for Duchenne muscular dystrophy and possibly other neuromuscular diseases. (*Am J Pathol* 2017, 187: 498–504; <http://dx.doi.org/10.1016/j.ajpath.2016.11.006>)

Duchenne muscular dystrophy (DMD) is a lethal severe genetic muscle disease caused by a mutation in the gene encoding dystrophin, resulting in a complete absence of this protein.¹ Dystrophin is a cytoplasmic protein that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix through the cell membrane. Many muscle proteins, such as dystroglycan, sarcoglycan, vinculin, and talin, colocalize with dystrophin at the costamere and myotendinous junction.^{2,3} The absence of dystrophin in DMD weakens the dystroglycan complex, increasing the susceptibility of muscle fibers to contraction-induced injuries.⁴

The sympathetic nervous system is a major regulator of muscle mass mediated by β -adrenoceptors (ARs).⁵ β_2 -ARs are predominantly expressed in airways and skeletal muscle. Chronic injections of β_2 -selective agonist induce skeletal muscle hypertrophy through cAMP and the phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin

pathway.⁶ New generation of β_2 -agonists, such as formoterol, has a significant anabolic effect at micromolar concentrations⁷ and protects against contraction-induced muscle damage in mdx dystrophic mice.⁸ However, chronic treatment for months with β_2 -agonists is associated with some undesirable adverse effects, including increased heart rate and heart hypertrophy.⁹ Moreover, β_2 -agonists can also activate the receptor-activator of NF- κ B (RANK), the receptor-activator of NF- κ B ligand (RANKL), a key promoter of osteoclastogenesis and bone remodeling.¹⁰ The third protagonist, osteoprotegerin (OPG), binds to RANKL, exerting an inhibitory effect on the preosteoclastic differentiation process.¹¹

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Thus, the potential adverse effects on heart and bone limit the use of β_2 -agonists for the treatment of DMD.¹²

Our laboratory has previously demonstrated that OPG-immunoglobulin fragment complex (OPG-Fc) treatment mitigates and restores function of fast-twitch muscles in young mdx mice.¹³ Because β_2 -agonists, as opposed to OPG-Fc, are effective for enhancing or preventing the loss of function of slow-twitch muscles, we tested whether a combined treatment of OPG-Fc with a low dose of formoterol (10 $\mu\text{g/kg}$ per day i.p.) would be superior to OPG-Fc and β_2 -agonist alone. We show that OPG-Fc and a low dose of formoterol act synergistically on force production of slow-twitch soleus (Sol), but not on fast-twitch extensor digitorum longus (EDL) of mdx mice. We also showed that the β_2 -AR content of dystrophic Sol and EDL muscles was significantly lower than that of control muscles and that OPG-Fc rescued the β_2 -AR content of dystrophic Sol and EDL muscles. The combination of these two pharmacological agents provides a new and potentially effective treatment for rescuing the functions of slow- and fast-twitch dystrophic muscles and may counteract the negative effects of β_2 -agonists on bone.

Materials and Methods

Animals

Male wild-type (*C57BL/10ScSnJ*) and mdx dystrophic (*C57BL/10ScSn-Dmd^{mdx/J}*) mice, bred in our animal facility and weighing 14 to 15 g, were used for this protocol. Food and water were provided ad libitum. Mice were injected i.p. with OPG-Fc (1 mg/kg per d; R&D Systems, Minneapolis, MN) or formoterol (10 or 100 $\mu\text{g/kg}$ per d, β_2 -agonist; Sigma-Aldrich, Oakville, ON, Canada) alone or in combination for 10 days starting at 25 days of age. Changes in body weight and fluid consumption were adjusted to maintain the effective dose. Mice injected with equal volumes of phosphate-buffered saline were used as control. At the end of the different experimental procedures, mice were euthanized by cervical dislocation under anesthesia. All of the procedures were approved by the Université Laval Research Center Animal Care and Use Committee on the basis of the Canadian Council on Animal Care guidelines.

Isometric Contractile Properties

Mice received buprenorphine as an analgesic (0.1 mg/kg) at least 15 minutes before being anesthetized with pentobarbital sodium (50 mg/kg). The right Sol and EDL were dissected and incubated in a buffered physiological salt solution (Krebs-Ringer), as previously described by Dufresne et al.¹⁴ The maximum tetanic tension (P_0 , g) values were obtained using a 305B-LR dual-mode lever arm system controlled by dynamic muscle control and data acquisition software (Dynamic Muscle Data Analysis software version 6.1; Aurora Scientific, Aurora, ON, Canada), and the maximum specific tetanic tension (sP_0 , N/cm²) was obtained by dividing the wet weight by the

optimal muscle length, multiplied by the muscle density (1.06 g/cm³), multiplied by the fiber/muscle length ratio. At the end of the contractile property measurements, the tendons were removed, and the muscles were weighed, embedded in tissue-freezing medium (Triangle Biomedical Sciences, Durham, NC), frozen in liquid nitrogen, and stored at -80°C until used for the immunohistochemical and immunofluorescence assays.

Immunohistochemical and Immunofluorescence Analyses

Transversal sections (10 μm thick) of Sol and EDL were immunolabeled for 2 hours at room temperature with anti-pan-macrophage F4/80 (1:100; Serotec, Oxford, UK) or anti-neutrophil Ly-6G and Ly-6C (Gr-1) antibodies (1:300; BD Pharmingen, Mississauga, ON, Canada) to identify macrophages and neutrophils, respectively. Positive cells were counted at $\times 400$ magnification, and the total areas of the sections were determined and multiplied by the thicknesses to express the number of each cell type per cubic millimeter. The concentrations of inflammatory cells were measured in duplicate in two different sections of muscles. Fiber typing and areas occupied by type I, IIA, IIX, and IIB fibers were determined, as described by Schiaffino et al.¹⁵ Briefly, muscle sections were incubated with the following antibodies: anti-MyHC I (Novus Biological, Oakville, ON, Canada), anti-MyHC IIA (SC-71; Developmental Studies Hybridoma Bank, Iowa City, IA), anti-MyHC IIX (6H1; Developmental Studies Hybridoma Bank), anti-MyHC IIB (BF-F3; Developmental Studies Hybridoma Bank), and Alexa Fluor secondary antibodies (Invitrogen, Waltham, MA). The myofiber cross-sectional area of each fiber type was analyzed with ImageJ software version 1.41 (NIH, Bethesda, MD; <http://imagej.nih.gov/ij>).

Hematoxylin and Eosin Staining and Serum Creatine Kinase Assays

Hematoxylin and eosin staining (Sigma-Aldrich) was used to visualize the number of centrally nucleated fibers and muscle integrity and quantify the extent of muscle damage on approximately 100 myofibers per muscle. The damaged area was defined as an area not occupied by normal or regenerating muscle fibers. Sections were examined with an inverted microscope (Nikon, Mississauga, ON, Canada) and analyzed with ImageJ software version 1.41.

Blood collection was performed by cardiac puncture, and samples were centrifuged at $10,000 \times g$ at 4°C for 10 minutes. The serum creatine kinase activity was determined according to the manufacturer's instructions (BioVision, Milpitas, CA). The activity was expressed as U/L and used to detect indirectly muscle damage.

Western Blots

For Western blotting, Sol and EDL muscles from mice treated for 10 days with OPG-Fc (1 mg/kg per day) or

formoterol (10 or 100 $\mu\text{g/kg}$ per day) alone or in combination were homogenized in lysis buffer, separated on 9% SDS-polyacrylamide gels, and transferred to polyvinylidene difluoride membranes. The protein bands were immunoblotted with an anti- β_2 -AR primary antibody overnight at 4°C (1:500; Santa Cruz Biotechnology, Dallas, TX), followed by a horseradish peroxidase-conjugated secondary antibody (1:1000; Santa Cruz Biotechnology). Bands were revealed using the ECL-Plus chemiluminescent detection system (PerkinElmer, Waltham, MA). Images of the membranes were acquired, scanned, and analyzed using Quantity One software version 4.6.6 (Bio-Rad, Mississauga, ON, Canada). Glyceraldehyde-3-phosphate dehydrogenase (1:5000; Santa Cruz Biotechnology) was used as a loading control. Images of the membranes were acquired, scanned, and analyzed using Quantity One software version 4.6.6.

Statistical Analysis

The data were analyzed by two-way analysis of variance to determine whether the variations among the experimental groups were significant (InStat software version 3; GraphPad Software, Inc., La Jolla, CA). When a significant *F* ratio was obtained, a posteriori test was performed

Figure 1 Osteoprotegerin (OPG)—immunoglobulin fragment complex (OPG-Fc) restores the expression of β_2 -adrenoceptor (B_2R) content in dystrophic soleus (Sol) and extensor digitorum longus (EDL) muscles. Wild-type control or mdx mice were injected with phosphate-buffered saline (PBS) or OPG-Fc and/or formoterol for 10 days and included the following six groups: i) mdx PBS-injected mice (mdx PBS), ii–iii) mdx formoterol-injected mice at two different doses, 10 or 100 $\mu\text{g/kg}$ (mdx FORMO-10 and mdx FORMO-100, respectively), iv) mdx OPG-injected mice at a dose of 1 mg/kg, or v–vi) mdx injected with a combination of OPG and two different doses of formoterol (mdx FORMO-10 + OPG and mdx FORMO-100 + OPG). Data are expressed as percentage increase or decrease relative to wild-type PBS-injected mice. Dystrophic muscles have a 17% to 28% reduction of the β_2 -adrenoceptor content, whereas OPG-Fc, but not formoterol, rescues completely its expression. The dashed line is the visual representation of wild-type data. All data are expressed as means $\pm$ SEM. *n* = 10 independent experiments per group. **P* < 0.05. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Figure 2 Formoterol and osteoprotegerin (OPG)—immunoglobulin fragment complex (OPG-Fc) act synergistically in slow-twitch soleus (Sol) muscles, but not in fast-twitch extensor digitorum longus (EDL) muscles. mdx mice were injected with phosphate-buffered saline (PBS) or OPG-Fc and/or formoterol for 10 days and included the same six groups defined in Figure 1: i) mdx PBS-injected mice (mdx PBS), ii–iii) mdx formoterol-injected mice at two different doses, 10 or 100 $\mu\text{g/kg}$ (mdx FORMO-10 and mdx FORMO-100, respectively), iv) mdx OPG-injected mice at a dose of 1 mg/kg, or v–vi) mdx injected with a combination of OPG and two different doses of formoterol (mdx FORMO-10 + OPG and mdx FORMO-100 + OPG). Maximum specific force decreased in dystrophic Sol muscles, and formoterol (100 $\mu\text{g/kg}$) or OPG-Fc (1 mg/kg) significantly increased the force production of dystrophic Sol muscles. The combination of formoterol and OPG-Fc acts synergistically, and the force production of dystrophic Sol muscle was superior to formoterol or OPG-Fc alone. The lower or higher doses of formoterol combined with OPG-Fc were not different. Maximum specific force also decreased in dystrophic EDL muscles, but formoterol (100 $\mu\text{g/kg}$) or OPG-Fc (1 mg/kg) treatments significantly increased the force production of dystrophic EDL muscles. The combination of any doses of formoterol and OPG-Fc treatment was not superior to OPG-Fc alone for fast-twitch EDL muscles. The dashed line is the visual representation of wild-type data. All data are expressed as means $\pm$ SEM. *n* = 8 independent experiments per group. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

(Tukey's protected least-significant differences test). The level of significance was set at *P* < 0.05.

Results

OPG-Fc Rescues the Expression of β_2 -Adrenergic Receptor

The β_2 -AR content was significantly reduced by 17% and 28%, respectively, in dystrophic Sol and EDL muscles compared with wild-type muscles. OPG-Fc treatment completely rescued β_2 -AR expression in both dystrophic muscles, whereas the low and high doses of formoterol treatment had no significant impact on β_2 -adrenergic receptor content in dystrophic muscles (Figure 1).

OPG-Fc Acts Synergistically with Formoterol to Enhance Dystrophic Slow-Twitch Muscle Function

We next investigated the effect of OPG-Fc and/or low or high doses of formoterol on the contractile properties of

Figure 3 Osteoprotegerin (OPG)–immunoglobulin fragment complex (OPG-Fc) and formoterol significantly improve muscle integrity and reduce muscle inflammation in slow-twitch Sol muscles of mdx mice. mdx mice were injected with phosphate-buffered saline (PBS) or OPG-Fc and/or formoterol for 10 days and included five groups: i) mdx PBS-injected mice (mdx PBS), ii–iii) mdx formoterol-injected mice at two different doses, 10 or 100 $\mu\text{g}/\text{kg}$ (mdx FORMO-10 and mdx FORMO-100, respectively), iv) mdx OPG-injected mice at a dose of 1 mg/kg, or v–vi) mdx injected with a combination of OPG and two different doses of formoterol (mdx FORMO-10 + OPG and mdx FORMO-100 + OPG). Representative hematoxylin and eosin staining (A), quantification of muscle damage (B), serum creatine kinase (CK) activity (C), and percentage of centrally nucleated fibers of Sol (D) and EDL (E) showed that OPG-Fc (1 mg/kg per day) or the high dose of formoterol (100 $\mu\text{g}/\text{kg}$ per day) or OPG-Fc plus a low dose of formoterol (10 $\mu\text{g}/\text{kg}$ per day) reduce cell infiltration and damage in dystrophic Sol muscles. Immunohistochemical analyses demonstrated that the numbers of neutrophils (anti-Ly6C/Ly6G; F) and pan-macrophages (anti-F4/80; G) are reduced significantly after OPG-Fc and/or formoterol treatments. Healthy muscles from wild-type mice are shown as controls. The dotted line is the visual representation of wild-type (WT) data. All data are expressed as means $\pm$ SEM (B–G). $n = 6$ to 8 independent experiments per group (B–G). * $P < 0.05$, ** $P < 0.01$. Scale bar = 100 μm (A).

dystrophic Sol and EDL muscles (Figure 2). A high dose of formoterol (100 µg/kg) increased the maximum specific force of dystrophic Sol and EDL muscles by 100% and 65%, respectively, whereas a low dose (10 µg/kg) had no significant effect. OPG-Fc (1 mg/kg) was significantly superior to any dose of formoterol, increasing the maximum specific force of dystrophic EDL muscles by 120%. However, the effect of OPG-Fc alone on the function of dystrophic Sol muscles was similar to that of the high dose of formoterol (100 µg/kg; 81% gain in force). More important, the combination of a low dose of formoterol (10 µg/kg) plus OPG-Fc (1 mg/kg) had a synergistic effect that was significantly superior to OPG-Fc alone in restoring the function of dystrophic Sol muscles (Figure 2). Interestingly, the effect of a high dose of formoterol plus OPG-Fc was similar to that of a low dose of formoterol plus OPG-Fc on force production by dystrophic Sol muscles. On the other hand, the effect of OPG-Fc alone on dystrophic EDL muscles was significantly superior to any dose of formoterol, whereas the combination of the two pharmacological agents did not provide any additional benefit for dystrophic EDL muscles. Like specific force, the maximum absolute isometric tetanic force (P0) showed similar differences between groups for Sol and EDL muscles (Supplemental Table S1). However, OPG-Fc and/or low or high doses of formoterol had no significant impact on time-to-peak twitch tension, half-relaxation time, muscle mass, and body weight (Supplemental Table S1).

OPG-Fc Is as Effective as Formoterol in Reducing Muscle Damage and Inflammation

The dystrophic features (centrally nucleated myofibers, necrotic myofibers, and serum creatine kinase) were

considerably reduced in dystrophic mice treated with OPG-Fc (1 mg/kg) or a high dose of formoterol or OPG-Fc and a low dose of formoterol (Figure 3, A–E). However, treatments had no impact on myofiber size and phenotype in Sol and EDL muscles (Supplemental Tables S2 and S3). Because chronic inflammation is a major problem in muscular dystrophy and contributes significantly to disease progression in DMD, we next compared the efficacy of OPG-Fc, formoterol, and both combined in reducing neutrophil and macrophage infiltrations. Consistent with the extent of muscle damage and creatine kinase activity, the number of neutrophils and macrophages was significantly reduced in dystrophic muscles from mice treated with OPG-Fc or a high dose of formoterol or OPG-Fc combined with a low dose of formoterol compared with phosphate-buffered saline–treated mdx mice (Figure 3, F and G). The low dose of formoterol had no significant impact on the extent of muscle damage area and the number of invaded inflammatory cells (Figure 3B). In terms of muscle damage and inflammation, the combined treatment of OPG-Fc with a low dose of formoterol was not superior to OPG-Fc or a high dose of formoterol alone.

Discussion

The RANK/RANKL/OPG pathway and the β -adrenergic system communicate and regulate bone homeostasis.¹⁰ RANKL/RANK interactions and adrenergic stimulation also control Ca^{2+} content and mobilization as well as SERCA [sarco(endo)plasmic reticulum Ca^{2+} ATPase, an ATP-dependent Ca^{2+} pump] activity in skeletal muscles.^{16,17} The loss of Ca^{2+} homeostasis leads to muscle degeneration, inflammation, and fibrosis, a signature of muscular dystrophy.¹⁸ The present study provides compelling evidence that

Figure 4 Schematic and hypothetical representation depicting how osteoprotegerin (OPG)—immunoglobulin fragment complex (OPG-Fc) and formoterol may interact in dystrophic muscles. β_2 -Agonists bind to adrenergic receptors, a class of G protein–coupled receptors that stimulate intracellular adenylyl cyclase, which catalyzes the conversion of ATP to cAMP, triggering skeletal muscle anabolism and SERCA activity. OPG binds to RANKL, preventing RANKL/RANK interactions and activation of the NF- κ B pathway. mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; TRAF, TNF receptor-associated factor.

the combination of a low dose of formoterol plus OPG-Fc is sufficient to rescue the function of slow- and fast-twitch dystrophic skeletal muscles during the first peak of muscle degeneration at 3 to 5 weeks of age in mdx mice.

Previous studies have shown that 1000 mg/kg per day of clenbuterol¹⁹ or 25 to 100 mg/kg per day of formoterol,²⁰ given for 4 weeks, is required to induce muscle hypertrophy and protect against muscular dystrophy. Mechanistically, β_2 -agonists activate, among others, protein kinase A, which phosphorylates phospholamban on serine 16 and activates SERCA (β_2 -agonists—protein kinase A—SERCA pathway). Interestingly, our findings showed that low doses of OPG-Fc potentiate the positive effects of formoterol on slow-twitch Sol muscles in young mdx mice. Indeed, the combination of formoterol and OPG-Fc increased the force production of dystrophic Sol muscles to a greater extent than the sum of the effects of OPG-Fc and formoterol alone. However, the possible cross talk between OPG-Fc and formoterol in skeletal muscles is poorly understood. Given that OPG-Fc increased the density of β_2 -AR and thus the number of potential binding sites for formoterol, it is possible that OPG may strengthen the downstream effect of β_2 -agonists on phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin signaling and/or the cAMP-dependent pathway by promoting protein synthesis and SERCA activity (Figure 4). However, the fact that OPG-Fc alone was largely superior to any dose of formoterol in rescuing fast-twitch dystrophic EDL muscles suggests that the inhibition of RANKL/RANK interactions by OPG-Fc is important in preventing subsequent NF- κ B activation and the progression of muscular dystrophy²¹ (Figure 4). OPG-Fc reduced the number of neutrophils and macrophages in dystrophic muscles but had no effect on muscle force production by healthy mice, suggesting that a pathological state is required for OPG-Fc to be effective. Although the mechanism by which OPG-Fc and formoterol work requires further investigation, our findings provide the first evidence that much less formoterol concentration is required to obtain the same beneficial effect in skeletal muscles, with potentially no or fewer adverse effects on heart and bone functions.

The present study convincingly shows that a low dose of formoterol combined with OPG-Fc acts synergistically on force production of Sol muscles and mitigates muscular dystrophy. The effects of prolonged OPG-Fc and β_2 -agonist treatments should be investigated in a more severe mouse model of muscular dystrophy. Because osteoporosis and muscle atrophy/degeneration worsen in tandem during neuromuscular diseases and because OPG-Fc has a beneficial effect on skeletal muscle and bone functions, we have high hopes that this combined treatment may open the way for new treatments for DMD patients.

Acknowledgments

S.S.D. and J.F. conceived the project and its design; S.S.D. performed experiments and data analysis; S.S.D., A.B.-P.,

and J.F. wrote the manuscript; and all authors checked for scientific content and approved the final manuscript.

Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2016.11.006>.

References

- Rosenberg AS, Puig M, Nagaraju K, Hoffman EP, Villalta SA, Rao VA, Wakefield LM, Woodcock J: Immune-mediated pathology in Duchenne muscular dystrophy. *Sci Transl Med* 2015, 7:299rv4
- Kukkurainen S, Määttä JA, Saeger J, Valjakka J, Vogel V, Hytönen VP: The talin-integrin interface under mechanical stress. *Mol Biosyst* 2014, 10:3217–3228
- Law DJ, Allen DL, Tidball JG: Talin, vinculin and DRP (utrophin) concentrations are increased at mdx myotendinous junctions following onset of necrosis. *J Cell Sci* 1994, 107(Pt 6): 1477–1483
- Call JA, Warren GL, Verma M, Lowe DA: Acute failure of action potential conduction in mdx muscle reveals new mechanism of contraction-induced force loss. *J Physiol* 2013, 591: 3765–3776
- Michel MC, Seifert R: Selectivity of pharmacological tools: implications for use in cell physiology: a review in the theme: cell signaling: proteins, pathways and mechanisms. *Am J Physiol Cell Physiol* 2015, 308:C505–C520
- Berdeaux R, Stewart R: cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. *Am J Physiol Endocrinol Metab* 2012, 303:E1–E17
- Harcourt LJ, Schertzer JD, Ryall JG, Lynch GS: Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue. *Neuromuscul Disord* 2007, 17: 47–55
- Gehrig SM, Koopman R, Naim T, Tjoarkarfa C, Lynch GS: Making fast-twitch dystrophic muscles bigger protects them from contraction injury and attenuates the dystrophic pathology. *Am J Pathol* 2010, 176: 29–33
- Cates CJ, Jaeschke R, Schmidt S, Ferrer M: Regular treatment with formoterol and inhaled steroids for chronic asthma: serious adverse events. *Cochrane Database Syst Rev* 2013, 6:CD006924
- Huang HH, Brennan TC, Muir MM, Mason RS: Functional α 1- and β 2-adrenergic receptors in human osteoblasts. *J Cell Physiol* 2009, 220:267–275
- Bonnet N, Pierroz DD, Ferrari SL: Adrenergic control of bone remodeling and its implications for the treatment of osteoporosis. *J Musculoskelet Neuronal Interact* 2008, 8:94–104
- Pierroz DD, Bonnet N, Bianchi EN, Boussein ML, Baldock PA, Rizzoli R, Ferrari SL: Deletion of β -adrenergic receptor 1, 2, or both leads to different bone phenotypes and response to mechanical stimulation. *J Bone Miner Res* 2012, 27:1252–1262
- Dufresne SS, Dumont NA, Bouchard P, Lavergne É, Penninger JM, Frenette J: Osteoprotegerin protects against muscular dystrophy. *Am J Pathol* 2015, 185:920–926
- Dufresne SS, Dumont NA, Boulanger-Piette A, Fajardo VA, Gamu D, Kake-Guena SA, David RO, Bouchard P, Lavergne É, Penninger JM, Pape PC, Tupling AR, Frenette J: Muscle RANK is a key regulator of calcium storage, SERCA activity, and function of fast-twitch skeletal muscles. *Am J Physiol Cell Physiol* 2016, 310:C663–C672
- Schiaffino S, Gorza L, Sartore S, Saggin L, Ausoni S, Vianello M, Gundersen K, Lomo T: Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. *J Muscle Res Cell Motil* 1989, 10:197–205

16. Yang Y-M, Kim MS, Son A, Hong JH, Kim K-H, Seo JT, Lee S-I, Shin DM: Alteration of RANKL-induced osteoclastogenesis in primary cultured osteoclasts from SERCA2^{+/-} mice. *J Bone Miner Res* 2009, 24:1763–1769
17. Briston SJ, Dibb KM, Solaro RJ, Eisner DA, Trafford AW: Balanced changes in Ca buffering by SERCA and troponin contribute to Ca handling during β -adrenergic stimulation in cardiac myocytes. *Cardiovasc Res* 2014, 104:347–354
18. Gehrig SM, van der Poel C, Sayer TA, Schertzer JD, Henstridge DC, Church JE, Lamon S, Russell AP, Davies KE, Febbraio MA, Lynch GS: Hsp72 preserves muscle function and slows progression of severe muscular dystrophy. *Nature* 2012, 484:394–398
19. Lynch GS, Hinkle RT, Faulkner JA: Power output of fast and slow skeletal muscles of mdx (dystrophic) and control mice after clenbuterol treatment. *Exp Physiol* 2000, 85:295–299
20. Ryall JG, Schertzer JD, Murphy KT, Allen AM, Lynch GS: Chronic β_2 -adrenoceptor stimulation impairs cardiac relaxation via reduced SR Ca²⁺-ATPase protein and activity. *Am J Physiol Heart Circ Physiol* 2008, 294:H2587–H2595
21. Acharyya S, Villalta SA, Bakkar N, Bupha-Intr T, Janssen PML, Carathers M, Li Z-W, Beg AA, Ghosh S, Sahenk Z, Weinstein M, Gardner KL, Rafael-Fortney JA, Karin M, Tidball JG, Baldwin AS, Guttridge DC: Interplay of IKK/NF- κ B signaling in macrophages and myofibers promotes muscle degeneration in Duchenne muscular dystrophy. *J Clin Invest* 2007, 117:889–901