



# Skeletal Muscle: A Significant Novel Neurohypophyseal Hormone-Secreting Organ

Sergio Adamo<sup>1</sup>, Eva Pigna<sup>1</sup>, Rosamaria Lugarà<sup>1</sup>, Viviana Moresi<sup>1</sup>, Dario Coletti<sup>1,2\*</sup> and Marina Bouché<sup>1</sup>

<sup>1</sup> Section of Histology & Medical Embryology, Department of Anatomical, Histological, Forensic and Orthopedic Sciences, Interuniversity Institute of Myology, Sapienza University of Rome, Rome, Italy, <sup>2</sup> Sorbonne Université, CNRS UMR 8256-INSERM ERL U1164, Biological Adaptation and Aging B2A, Paris, France

**Keywords:** vasopressin, oxytocin, myogenesis, muscle regeneration, satellite cell, muscle atrophy and hypertrophy, anabolizing agents

Vasopressin (arg<sup>8</sup>-vasopressin) and oxytocin are closely related nonapeptide hormones, synthesized as pre-hormones in the magnocellular neurons of the paraventricular and supraoptic nuclei of the hypothalamus. Vasopressin and oxytocin are secreted in response to a variety of physiological stimuli, serving such different functions as controlling water balance, milk ejection, uterine contraction, mood, and parental behavior (Lechan and Toni, 2000; Costa et al., 2014a).

## OPEN ACCESS

### Edited by:

Wataru Aoi,

Kyoto Prefectural University, Japan

### Reviewed by:

Antonio Torsello,

Università degli Studi di Milano

Bicocca, Italy

### \*Correspondence:

Dario Coletti

dario.coletti@uniroma1.it;

dario.coletti@upmc.fr

### Specialty section:

This article was submitted to

Striated Muscle Physiology,

a section of the journal

Frontiers in Physiology

**Received:** 09 September 2018

**Accepted:** 12 December 2018

**Published:** 08 January 2019

### Citation:

Adamo S, Pigna E, Lugarà R,

Moresi V, Coletti D and Bouché M

(2019) Skeletal Muscle: A Significant

Novel Neurohypophyseal

Hormone-Secreting Organ.

Front. Physiol. 9:1885.

doi: 10.3389/fphys.2018.01885

## MUSCLE AS A TARGET OF NEUROHYPOPHYSEAL HORMONES

Skeletal muscle or myogenic precursors have never been considered, until recently, as targets of neurohypophyseal hormones. However, very early evidence was provided by Wakelam and collaborators, who showed some mild effects of vasopressin on carbohydrate metabolism in myoblasts (Wakelam and Pette, 1982; Wakelam et al., 1987). These initial observations were corroborated by later studies showing that stimulation of primary chick embryo myoblasts or murine L6 and L5 myogenic cell lines with vasopressin, oxytocin, their analogs and antagonists resulted in the structure- and concentration-dependent activation of phospholipase C (PLC) signaling, the stimulation of myogenic differentiation, and the hypertrophy of newly formed muscle fibers (Teti et al., 1993; Nervi et al., 1995). Worth noting, PLC activates Protein Kinase C family members, among which the theta isoform plays an important role in both muscle differentiation and disease (Marrocco et al., 2014, 2017; Lozanoska-Ochser et al., 2018). The potent pro-myogenic effect of vasopressin was further characterized in myogenic cells cultured in a serum-free medium, a “clean” experimental model that allowed us to establish that L6 myogenic cells express the vasopressin V1a receptor (V1a-R) and that vasopressin elicits a complex signal transduction response in these cells (Minotti et al., 1998; Scicchitano, 2002, 2005; Naro et al., 2003; Toschi et al., 2011; Costa et al., 2014b). Moreover, it was found that V1a-R expression is modulated during the differentiation of L6 cells, probably in a post-translational manner (Alvisi et al., 2008).

Studies from other laboratories highlighted the presence of functional oxytocin receptors (OT-R) in human myoblasts derived from postnatal satellite cells (Breton et al., 2002), and in C2C12 myogenic cells which respond to oxytocin by activating the calcium–CaMKK–AMPK pathway (Lee et al., 2008). A recent *in vitro* study showed that C2C12 myoblasts express not only OT-R but also oxytocin and that the expression of both products increases upon myogenic differentiation of the cells (Berio et al., 2017). Furthermore, myotubes treated with 17 $\beta$ -estradiol overexpress oxytocin and OT-R genes by approximately 3- and 29-fold, respectively (Berio et al., 2017).

While cell cultures provide useful models to define, under controlled conditions, the effects of vasopressin or oxytocin on myogenic differentiation at the molecular level, *in vivo* data support the idea that both neurohypophyseal hormones play physiological roles in skeletal muscle. Indeed, a role for neurohypophyseal hormones in prenatal muscle development was first suggested by the presence of immunoreactive vasopressin in human fetal and neonatal skeletal muscle (Smith et al., 1992). Moreover, a >120-fold increase in oxytocin expression was observed in bovine muscle during early to mid-fetal calf development, coincident with active myofiber formation (De Jager et al., 2011), findings in keeping with the above reported *in vitro* data.

In an *in vivo* murine experimental model, we reported that V1a-R expression, measurable under basal conditions in muscle, was strongly up-regulated in the early phase of regeneration (9 h after injury) and gradually decreased in the following days, along with the regeneration process. In this model, vasopressin administration promoted muscle regeneration. Furthermore, overexpression of V1a-R in muscle sufficed to dramatically enhance post-injury muscle regeneration, without administering exogenous vasopressin (Toschi et al., 2011). In an *in vivo* mouse model of tumor necrosis factor (TNF)-inhibited muscle regeneration, the administration of vasopressin rescued the inhibitory effect of TNF, likely through a mechanism involving the modulation of HSP70 levels (Moresi et al., 2009). Again, TNF-mediated muscle atrophy was rescued by the overexpression of the V1a-R *in vivo* (Costa et al., 2014b).

Altogether, the *in vivo* evidence supports the notion that both vasopressin and oxytocin have potent effects, in the development, regeneration and homeostasis of skeletal muscle.

Further insights came from studies conducted in aged mice (Elabd et al., 2014). The authors focused on the reduced muscle regeneration and muscle atrophy (sarcopenia) occurring in aging. While it is known that aging is accompanied by reduced physiological levels of sex steroids, the authors found that circulating oxytocin level is also reduced. Interestingly, satellite cells from aged animals exhibited a significantly lower OT-R expression than those from young animals. Moreover, comparing young and aged mice treated with an oxytocin selective antagonist or with exogenous oxytocin, respectively, the authors demonstrated that oxytocin is required for efficient muscle regeneration (Elabd et al., 2014). The impaired muscle regeneration of aged mice was shown to depend primarily upon reduced proliferation of satellite cells, a phenomenon rescued by exogenous oxytocin administration. The oxytocin effect on satellite cell proliferation was reported to be mediated by the MAPK/ERK pathway (Elabd et al., 2014). In line with the above findings, muscle regeneration in oxytocin KO mice was severely compromised. Young oxytocin KO mice displayed a premature decline in muscle regeneration, as well as muscle fibrosis and fat infiltration, showing a muscle phenotype characteristic of sarcopenia (Elabd et al., 2014).

Therefore, both neurohypophyseal hormones appear to regulate positively muscle homeostasis in different models: oxytocin, in the aging and KO mouse models (Elabd et al., 2014);

and vasopressin, in injured muscle or TNF-induced muscle wasting (Moresi et al., 2009; Toschi et al., 2011; Costa et al., 2014b).

The apparent overlapping between the observed effects of the two neurohypophyseal hormones in the regulation of muscle differentiation and trophism may depend on the fact that both V1a-R and OT-R cross-bind their ligands, albeit with different affinities (Barberis et al., 1998; Gupta et al., 2008). Furthermore, the results obtained in the myogenic L6 cell line used as an experimental tool to show the effect of neurohypophyseal hormones probably depend on a peculiar expression of the receptors for these hormones, in comparison to other myogenic cell types.

## MUSCLE TISSUE AS A SOURCE OF VASOPRESSIN AND OXYTOCIN

Interestingly, the possibility that myogenic cells express one of the neurohypophyseal hormones, as suggested by the pioneering results of Smith in prenatal human muscle for vasopressin (Smith et al., 1992), was recently proposed again (Berio et al., 2017), with regard to oxytocin and OT-R. Based on these observations, muscle can thus be added to the list of previously unrecognized sites of oxytocin expression, such as testes, ovaries, heart and lungs (Assinder et al., 2000; Jankowski et al., 2004; Kiss and Mikkelsen, 2005; Gutkowska and Jankowski, 2012). Further results supporting this hypothesis, were obtained in studies aimed to investigate the mechanisms triggered by the administration of steroids used to increase muscle mass in livestock farming. Cattle regularly treated with anabolizing agents displayed dramatically enhanced oxytocin mRNA expression in skeletal muscle, accompanied by a ~50-fold higher level of circulating oxytocin (De Jager et al., 2011). Intriguingly, the authors provided evidence that the hypertrophying effect of anabolic steroids is prevalently mediated by OT-R signaling. Furthermore, in a more recent study, Divari reported that serum levels of oxytocin increased dramatically in cattle regularly treated with 17 $\beta$ -estradiol, but not with either dexamethasone or placebo. This administration of 17 $\beta$ -estradiol also resulted in increased (33-fold) skeletal muscle expression of the oxytocin-precursor mRNA (Divari et al., 2013). Increased expression of the oxytocin-precursor mRNA was also found in the muscle of sheep subjected to chronic treatment with a combination of 17 $\beta$ -estradiol and the synthetic androgen trenbolone acetate (TBA). Also the circulating oxytocin level increased in steroid-treated sheep compared to placebo-treated controls (Kongsuwan et al., 2012). Together, the above studies indicate a correlation between the steroid-induced muscle hypertrophy and the increased expression of both oxytocin and OT-R in skeletal muscle.

The mechanisms underlying regulation by steroids of oxytocin expression remain to be fully elucidated. The oxytocin promoter does not possess a classical Estrogen Response Element, whereas it has a high affinity binding-site for nuclear orphan receptors/estrogen related receptor alpha (ERR $\alpha$ ). Koochi reported that the estrogen dependent control of the oxytocin

promoter is independent of classical Estrogen Receptor (ER) binding, but requires a functional  $ERR\alpha$  (Koochi et al., 2005). These authors further demonstrated that the estrogenic stimulation of the OT-R occurs through the ERK/MAPK-mediated stimulation of the transcriptional activity of  $ERR\alpha$ . The up-regulation of oxytocin expression by this non-classical mechanism may, in turn, sustain an autocrine feed-forward oxytocin/OT-R loop which amplifies the response to oxytocin, as shown in bone (Colaïanni et al., 2012; Berio et al., 2017).

Worth noting, exercise (i.e., muscle contraction) represents a physiological stimulus increasing the levels of circulating neurohypophyseal hormones, as well as their expression (or the expression of their receptors) in several tissues beside skeletal muscle (Martins et al., 2005). Based on several studies in man and other mammals, it is clear that exercise induces a five-fold increase in the circulating levels of vasopressin (Melin et al., 1980; Convertino et al., 1981; Alexander et al., 1991). This increase, associated to the beneficial effects of exercise on muscle homeostasis, suggests a model whereby physical activity stimulates muscle secretion of the neurohypophyseal hormones and induces a generalized sensitization to these factors through the up-regulation of their receptors in various districts. An exercise-mediated increase in vasopressin and/or oxytocin could ultimately contribute to maintaining muscle homeostasis and add to the additional benefits of exercise, including an increased life span and general well-being.

## FINAL REMARKS

The *in vitro* and *in vivo* studies discussed above suggest that skeletal muscle is a target of neurohypophyseal hormones, which regulate muscle homeostasis and function in both physiological and pathological conditions. On the other hand, skeletal muscle has been recognized as the source of a wide range of circulating factors, namely myokines, which regulate a number of different functions with paracrine or endocrine mechanisms (Pedersen

and Febbraio, 2008; Hoffmann and Weigert, 2017). In the light of its abundance in the organism, skeletal muscle may thus be regarded as the largest endocrine gland in the body. In this article, we discuss *in vitro* and *in vivo* studies showing that oxytocin is synthesized by muscle and that its secretion significantly contributes to the level of circulating hormones. This activity appears to be central to the mechanisms which regulate muscle homeostasis, to contribute to muscle hypertrophic responses and to be altered in atrophic conditions. As a whole, this evidence suggests that OT and AVP be considered as potential myokines. In addition, these considerations suggest a potential therapeutic use of these molecules, along with more selective and potent analogs, in atrophic and muscle wasting conditions, such as sarcopenia and cachexia, and as a tool in adjuvant therapies against muscular dystrophies and neuromuscular diseases.

## AUTHOR CONTRIBUTIONS

SA, MB drafted the manuscript and approved the final version. EP, RL contributed to manuscript writing. DC, VM provided important interpretations, critically revised the manuscript. All authors provided final approval of the opinion content.

## ACKNOWLEDGMENTS

The authors are grateful to Pr. Giulio Cossu, University of Manchester, UK, and to Anna Luisa Mazzotti for critical reading and editing of the manuscript, respectively. SA is funded by Sapienza University research projects 2016 (SapMedi2016) and 2017 (RM11715C78539BD8). DC is funded by AFM (2017–20603), ANR (2013-J13R191), EFEM 2016, IBPS (2014), NIH (2013-1R01CA108857-01subcontractor). MB is funded by Sapienza University of Rome research projects 2017 (RP11715C7D238352), and by Duchenne Parent Project Onlus.

## REFERENCES

- Alexander, S. L., Irvine, C. H. G., Ellis, M. J., and Donald, R. A. (1991). The effect of acute exercise on the secretion of corticotropin-releasing factor, arginine vasopressin, and adrenocorticotropin as measured in pituitary venous blood from the horse. *Endocrinology* 128, 65–72. doi: 10.1210/endo-128-1-65
- Alvisi, M., De Arcangelis, V., Ciccone, L., Palombi, V., Alessandrini, M., Nemoz, G., et al. (2008). V1a vasopressin receptor expression is modulated during myogenic differentiation. *Differentiation* 76, 371–380. doi: 10.1111/j.1432-0436.2007.00231.x
- Assinder, S. J., Carey, M., Parkinson, T., and Nicholson, H. D. (2000). Oxytocin and vasopressin expression in the ovine testis and epididymis: changes with the onset of spermatogenesis. *Biol. Reprod.* 63, 448–456. doi: 10.1095/biolreprod63.2.448
- Barberis, C., Mouillac, B., and Durroux, T. (1998). Structural bases of vasopressin/oxytocin receptor function. *J. Endocrinol.* 156, 223–229. doi: 10.1677/joe.0.1560223
- Berio, E., Divari, S., Starvaggi Cucuzza, L., Biolatti, B., and Cannizzo, F. T. (2017). 17  $\beta$ -estradiol upregulates oxytocin and the oxytocin receptor in C2C12 myotubes. *PeerJ* 5:e3124. doi: 10.7717/peerj.3124
- Breton, C., Haeggeli, C., Barberis, C., Heitz, F., Bader, C. R., Bernheim, L., et al. (2002). Presence of functional oxytocin receptors in cultured human myoblasts. *J. Clin. Endocrinol. Metab.* 87, 1415–1418. doi: 10.1210/jcem.87.3.8537
- Colaïanni, G., Sun, L., Di Benedetto, A., Tamma, R., Zhu, L. L., Cao, J., et al. (2012). Bone marrow oxytocin mediates the anabolic action of estrogen on the skeleton. *J. Biol. Chem.* 287, 29159–29167. doi: 10.1074/jbc.M112.365049
- Convertino, V. A., Keil, L. C., Bernauer, E. M., and Greenleaf, J. E. (1981). Plasma volume, osmolality, vasopressin, and renin activity during graded exercise in man. *J. Appl. Physiol.* 50, 123–128. doi: 10.1152/jappl.1981.50.1.123
- Costa, A., Rossi, E., Scicchitano, B. M., Coletti, D., Moresi, V., and Adamo, S. (2014a). Neurohypophyseal hormones: novel actors of striated muscle development and homeostasis. *Eur. J. Transl. Myol.* 24:3790. doi: 10.4081/bam.2014.3.217
- Costa, A., Toschi, A., Murfun, I., Pelosi, L., Sica, G., Adamo, S., et al. (2014b). Local overexpression of V1a-vasopressin receptor enhances regeneration in tumor necrosis factor-induced muscle atrophy. *Biomed Res. Int.* 2014:235426. doi: 10.1155/2014/235426
- De Jager, N., Hudson, N. J., Reverter, A., Wang, Y.-H., Nagaraj, S. H., Cafe, L. M., et al. (2011). Chronic exposure to anabolic steroids induces the muscle expression of oxytocin and a more than fiftyfold increase in circulating oxytocin in cattle. *Physiol. Genomics.* 43, 467–478. doi: 10.1152/physiolgenomics.00226.2010

- Divari, S., Pregel, P., Cannizzo, F. T., Starvaggi Cucuzza, L., Brina, N., and Biolatti, B. (2013). Oxytocin precursor gene expression in bovine skeletal muscle is regulated by 17 $\beta$ -oestradiol and dexamethasone. *Food Chem.* 141, 4358–4366. doi: 10.1016/j.foodchem.2013.07.029
- Elabd, C., Cousin, W., Upadhyayula, P., Chen, R. Y., Chooljian, M. S., Li, J., et al. (2014). Oxytocin is an age-specific circulating hormone that is necessary for muscle maintenance and regeneration. *Nat. Commun.* 5:4082. doi: 10.1038/ncomms5082
- Gupta, J., Russell, R. J., Wayman, C. P., Hurley, D., and Jackson, V. M. (2008). Oxytocin-induced contractions within rat and rabbit ejaculatory tissues are mediated by vasopressin V1A receptors and not oxytocin receptors. *Br. J. Pharmacol.* 155, 118–126. doi: 10.1038/bjp.2008.226
- Gutkowska, J., and Jankowski, M. (2012). Oxytocin revisited: its role in cardiovascular regulation. *J. Neuroendocrinol.* 24, 599–608. doi: 10.1111/j.1365-2826.2011.02235.x
- Hoffmann, C., and Weigert, C. (2017). Skeletal muscle as an endocrine organ: The role of myokines in exercise adaptations. *Cold Spring Harb. Perspect. Med.* 7: a029793. doi: 10.1101/cshperspect.a029793
- Jankowski, M., Danalache, B., Wang, D., Bhat, P., Hajjar, F., Marcinkiewicz, M., et al. (2004). Oxytocin in cardiac ontogeny. *Proc. Natl. Acad. Sci. U.S.A.* 101, 13074–13079. doi: 10.1073/pnas.0405324101
- Kiss, A., and Mikkelsen, J. D. (2005). Oxytocin - Anatomy and functional assignments: a minireview. *Endocr. Regul.* 39, 97–105.
- Kongsuwan, K., Knox, M. R., Allingham, P. G., Pearson, R., and Dalrymple, B. P. (2012). The effect of combination treatment with trenbolone acetate and estradiol-17 $\beta$  on skeletal muscle expression and plasma concentrations of oxytocin in sheep. *Domest. Anim. Endocrinol.* 43, 67–73. doi: 10.1016/j.domaniend.2012.02.004
- Koohi, M. K., Ivell, R., and Walther, N. (2005). Transcriptional activation of the oxytocin promoter by oestrogens uses a novel non-classical mechanism of oestrogen receptor action. *J. Neuroendocrinol.* 17, 197–207. doi: 10.1111/j.1365-2826.2005.01298.x
- Lechan, R. M., and Toni, R. (2000). *Functional Anatomy of the Hypothalamus and Pituitary*. South Dartmouth, MA: Endotext.
- Lee, E. S., Uhm, K. O., Lee, Y. M., Kwon, J., Park, S. H., and Soo, K. H. (2008). Oxytocin stimulates glucose uptake in skeletal muscle cells through the calcium-CaMKK-AMPK pathway. *Regul. Pept.* 151, 71–74. doi: 10.1016/j.regpep.2008.05.001
- Lozanoska-Ochser, B., Benedetti, A., Rizzo, G., Marrocco, V., Di Maggio, R., Fiore, P., et al. (2018). Targeting early PKC $\theta$ -dependent T-cell infiltration of dystrophic muscle reduces disease severity in a mouse model of muscular dystrophy. *J. Pathol.* 244, 323–333. doi: 10.1002/path.5016
- Marrocco, V., Fiore, P., Benedetti, A., Pisu, S., Rizzuto, E., Musarò, A., et al. (2017). Pharmacological inhibition of pkc $\theta$  counteracts muscle disease in a mouse model of duchenne muscular dystrophy. *EBioMedicine* 16, 150–161. doi: 10.1016/j.ebiom.2017.01.001
- Marrocco, V., Fiore, P., Madaro, L., Crupi, A., Lozanoska-Ochser, B., and Bouché, M. (2014). Targeting PKC $\theta$  in skeletal muscle and muscle diseases: good or bad? *Biochem. Soc. Trans.* 42, 1550–1555. doi: 10.1042/BST20140207
- Martins, A. S., Crescenzi, A., Stern, J. E., Bordin, S., and Michelini, L. C. (2005). Hypertension and exercise training differentially affect oxytocin and oxytocin receptor expression in the brain. *Hypertension* 46, 1004–1009. doi: 10.1161/01.HYP.00001175812.03322.59
- Melin, B., Eclache, J. P., Geelen, G., Annat, G., Allevard, A. M., Jarsaillon, E., et al. (1980). Plasma AVP, neurophysin, renin activity, and aldosterone during submaximal exercise performed until exhaustion in trained and untrained men. *Eur. J. Appl. Physiol. Occup. Physiol.* 44, 141–151. doi: 10.1007/BF00421092
- Minotti, S., Scicchitano, B. M., Nervi, C., Scarpa, S., Lucarelli, M., Molinaro, M., et al. (1998). Vasopressin and insulin-like growth factors synergistically induce myogenesis in serum-free medium. *Cell Growth Differ. Mol. Biol. J. Am. Assoc. Cancer Res.* 9, 155–163.
- Moresi, V., Garcia-Alvarez, G., Pristerà, A., Rizzuto, E., Albertini, M. C., Rocchi, M., et al. (2009). Modulation of caspase activity regulates skeletal muscle regeneration and function in response to vasopressin and tumor necrosis factor. *PLoS ONE* 4:e5570. doi: 10.1371/journal.pone.0005570
- Naro, F., De Arcangelis, V., Sette, C., Ambrosio, C., Komati, H., Molinaro, M., et al. (2003). A bimodal modulation of the cAMP pathway is involved in the control of myogenic differentiation in l6 cells. *J. Biol. Chem.* 278, 49308–49315. doi: 10.1074/jbc.M306941200
- Nervi, C., Benedetti, L., Minasia, M., Molinaro, M., and Adamo, S. (1995). Arginine-vasopressin induces differentiation of skeletal myogenic cells and up-regulation of myogenin and Myf-5. *Cell Growth Differ.* 6, 81–89.
- Pedersen, B. K., and Febbraio, M. A. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol. Rev.* 88, 1379–1406. doi: 10.1152/physrev.90100.2007
- Scicchitano, B. M. (2002). AVP induces myogenesis through the transcriptional activation of the myocyte enhancer factor 2. *Mol. Endocrinol.* 16, 1407–1416. doi: 10.1210/me.16.6.1407
- Scicchitano, B. M. (2005). Vasopressin-dependent myogenic cell differentiation is mediated by both Ca<sup>2+</sup>/calmodulin-dependent kinase and calcineurin pathways. *Mol. Biol. Cell.* 16, 3632–3641. doi: 10.1091/mbc.E05-01-0055
- Smith, A., Stephen, R. I., Arkley, M. M., and McIntosh, N. (1992). Immunoreactive arginine vasopressin in human fetal and neonatal skeletal muscle. *Early Hum. Dev.* 28, 215–222.
- Teti, A., Naro, F., Molinaro, M., and Adamo, S. (1993). Transduction of arginine vasopressin signal in skeletal myogenic cells. *Am. J. Physiol.* 265(1 Pt 1), C113–121.
- Toschi, A., Severi, A., Coletti, D., Catizone, A., Musarò, A., Molinaro, M., et al. (2011). Skeletal muscle regeneration in mice is stimulated by local overexpression of V1a-vasopressin receptor. *Mol. Endocrinol.* 25, 1661–1673. doi: 10.1210/me.2011-1049
- Wakelam, M. J., Patterson, S., and Hanley, M. R. (1987). L6 skeletal muscle cells have functional V1-vasopressin receptors coupled to stimulated inositol phospholipid metabolism. *FEBS Lett.* 210, 181–184.
- Wakelam, M. J., and Pette, D. (1982). The control of glucose 1,6-bisphosphate by developmental state and hormonal stimulation in cultured muscle tissue. *Biochem. J.* 204, 765–769. doi: 10.1042/bj2040765

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Adamo, Pigna, Lugarà, Moresi, Coletti and Bouché. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.