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# Exercise-dependent regulation of glial cell line-derived neurotrophic factor (GDNF) expression in skeletal muscle and its importance for the neuromuscular system

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**Abstract** The focus of this review is to highlight the importance of glial cell line-derived neurotrophic factor (GDNF) for the motor nervous system. GDNF is the most potent survival factor for motor neurons, where it enhances maintenance and survival of both developing and mature motor neurons *in vivo* and *in vitro*. GDNF aids in neuromuscular junction formation, maintenance, and plasticity, where skeletal muscle-derived GDNF may be responsible for this phenomenon. Increased levels of physical activity can increase GDNF protein levels in skeletal muscle, where alterations in acetylcholine and acetylcholine receptor activation may be involved in regulation of these changes observed. With inactivity and disuse, GDNF expression shows different patterns of regulation in the central and peripheral nervous systems. Due to its potent effects for motor neurons, GDNF is being extensively studied in neuromuscular diseases.

**Keywords** glial cell line-derived neurotrophic factor, neuromuscular junction, motor neurons, skeletal muscle

## Neurotrophic factors

Neurotrophic factors are a family of small peptides that have been identified as important factors for the development, survival, plasticity, and function of neurons both in the central nervous system (CNS) and peripheral nervous systems (PNS) (Henderson et al., 1994; Allen and Dawbarn, 2006). The basic model of target-derived neurotrophic factors proposes that a target tissue produces a molecular signal that is recognized by innervating neurons and is capable of enhancing the growth and survivability of those neurons during development (Purves et al., 1988). Neurotrophic factors can be classified in several subgroups including the neurotrophin family, which consist of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4), the neurocytokine family, which includes ciliary neurotrophic factor, leukemia inhibitory factor (LIF) and interleukins, and the glial cell line-derived neurotrophic factor (GDNF) family, which includes GDNF,

neurturin (NTRN), artemin (ARTN), and persephin (PSPN).

## GDNF characterization and signaling

GDNF was first purified from B49 glial cells and was identified as a potent survival factor for dopaminergic neurons in the CNS (Lin et al., 1993; Schatz et al., 1999). Later, GDNF was reported to be a trophic factor for other populations of neurons in the PNS including spinal motor neurons (Henderson et al., 1994; Niles et al., 2004; Caumont et al., 2006; Sharma, 2006), where it has been identified as one of the most potent neurotrophic factors for motor neurons to date (Henderson et al., 1994; Oppenheim et al., 1995; Trupp et al., 1995; Yan et al., 1995; Nguyen et al., 1998). GDNF is synthesized as an inactive 211 amino acid precursor form, pre-proGDNF (Lin et al., 1993; Airaksinen and Saarma, 2002). The mature forms of GDNF are expressed in two splice variants, GDNF<sub>633</sub> and GDNF<sub>555</sub>, that are disulfide-bonded homodimers of two 134-residue long glycosylated polypeptide chains and are obtained after proteolytic cleavage during secretion (Suter-Crazzolara and Unsicker, 1994; Springer et al., 1995; Airaksinen and Saarma, 2002). The mature forms are thought to be released in a biologically

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active state and are able to initiate signaling with tyrosine kinase receptors (Lee et al., 2001). All the members of the GDNF family induce signaling through binding with high affinity to the GDNF family receptor  $\alpha$  (GFR- $\alpha$ 1-4), which are anchored to the plasma membrane by a glycosyl-phosphatidylinositol (GPI) anchor. Specifically, GDNF binds to GFR- $\alpha$ 1, NTRN binds to GFR- $\alpha$ 2, ARTN binds to GFR- $\alpha$ 3, and PSPN binds to GFR- $\alpha$ 4, however there is cross-talk among the ligands to other receptors in which they bind with lower affinity. The GDNF-GFR- $\alpha$ 1-GPI complex interacts with two RET molecules, which are single-pass transmembrane proteins, thus initiating homodimerization and tyrosine autophosphorylation (Airaksinen and Saarma, 2002). This in turn triggers downstream signaling cascades including phosphatidylinositol 3-kinase, extracellular regulated kinase, and mitogen-activated protein kinase (Soler et al., 1999). These same pathways have been linked to neuronal survival, synaptic plasticity, neurite outgrowth, and the enhancement of neurotransmission (Kaplan and Miller, 2000; Yang et al., 2001).

## GDNF's role for the neuromuscular system

### Developing motor neurons

GDNF was found to be 75-, 650-, and 2500-fold more potent in preventing programmed cell death in cultured motor neurons than BDNF, rat ciliary neurotrophic factor (CNTF) and human cholinergic differentiation factor leukemia inhibitory factor, respectively. In this same study, administration of GDNF prevented death of nearly all facial motor neurons in neonatal rats and was the only trophic factor capable of averting axotomy-induced atrophy *in vivo* (Henderson et al., 1994). GDNF treatment was shown to prevent death and atrophy of nearly 100% of axotomy-induced motor neurons in chick embryos (Oppenheim et al., 1995) and facial motor neurons in neonatal rats as compared to axotomized controls in which less than 6% of motor neurons remained (Yan et al., 1995). In addition, over-expression of GDNF in developing muscle showed an increase in survival of nearly all motor neuron populations (spinal and cranial) during naturally occurring cell death compared to GDNF-deficient embryos (Oppenheim et al., 2000), where the survival of motor neurons during programmed cell death depends on GDNF secreted specifically by skeletal muscle (Angka et al., 2008). Excessive cell death was observed in double mutant embryonic mice lacking both skeletal muscle and their trophic factors. In contrast, these double mutants treated with GDNF had the same level of motor neuron survival as that in wild type mice (Angka et al., 2008). Although GDNF has been characterized as a neurotrophic factor for alpha-motor neurons (Henderson et al., 1994), differential effects on motor neuron subtypes have been observed during development. Recently, it has been

reported that gamma-motor neurons express higher levels of GDNF receptor (GFR- $\alpha$ 1) compared to alpha-motor neurons in the early postnatal period, suggesting that early gamma-motor neurons depend on muscle spindle-derived GDNF (Shneider et al., 2009; Kanning et al., 2010). GDNF also plays a chemoattractant role in axon pathfinding during development. Studies have shown that GDNF acts with ephrinA/ephrinA receptor, the complex that induces motor neuron repulsion, to ensure the correct connection of motor axons in limbs of animal models (Kramer et al., 2006; Dudanova et al., 2010). These studies indicate that developing and axotomized motor neurons depend on access to GDNF for survival, and possibly from muscle-derived GDNF rather than centrally-derived GDNF.

### Mature motor neurons

GDNF availability has been found to be crucial for the maintenance of mature adult motor neurons. Disease and nerve injury studies have revealed the trophic importance of GDNF to motor neurons in adults. Administration of GDNF prevented neuronal loss and maintained motor function in a mouse model of Huntington's disease (Ebert et al., 2010). Also, GDNF synthesized from healthy mouse femur bone marrow was sufficient to slow motor neuron degeneration in transgenic mice with muscle *deficient/osteochondrodystrophy* mutation (*mdf/ocd*: Pastor et al., 2011).

GDNF mRNA was shown to be upregulated at the distal part of injured nerves, skeletal muscle, and Schwann cells after transection (Naveilhan et al., 1997; Frostick et al., 1998; Lie and Weis, 1998). The primary GDNF receptor, GFR- $\alpha$ 1, was also increased at the distal part of the nerve after nerve lesion (Frostick et al., 1998). This elevation of GDNF mRNA may suggest an immediate trophic need by the damaged nerve. More importantly, GDNF mRNA remained elevated for five or six weeks; in contrast with members of the neurotrophin family whose upregulation existed for only two weeks (Frostick et al., 1998; Michalski et al., 2008). From this, it can be suggested that adult motor neurons depend primarily on GDNF as their trophic factor for survival and that GDNF is critical for the maintenance of the neuromuscular system.

### Neuromuscular systems

The presence of GDNF in skeletal muscle at the neuromuscular junction (NMJ) suggests a target-derived action on motor neurons, in which GDNF is retrogradely transported by axons to the target neuron's cell body, through a receptor-mediated process (Nguyen et al., 1998). GDNF has also been shown to be secreted by astrocytes of the CNS, exerting its effects on the PNS by anterograde transport (Zhao et al., 2004). GDNF appears to increase acetylcholine receptor (AChR) density in the absence of innervation, indicating an autocrine action, since both the ligand and its receptor are

expressed in skeletal muscle (Yang and Nelson, 2004). Studies examining transgenic mice overexpressing GDNF in skeletal muscle (*myo*-GDNF) and transgenic mice overexpressing GDNF in astrocytes (*GFAP*-GDNF) showed that the classical retrograde transport may have more physiologic effects for treatment of amyotrophic lateral sclerosis (ALS) compared to the anterograde direction (Li et al., 2007).

GDNF has been shown to be critical for NMJ formation, maintenance, and plasticity both *in vivo* and *in vitro*. Studies have shown that during synaptic formation, the number of axons innervating skeletal muscle depends on the concentration of GDNF protein available (Nguyen et al., 1998). Elevated GDNF expression causes hyperinnervation at the NMJ of transgenic *myo*-GDNF mice, whereas *myo*-neurotrophin-3 (NT-3) mice and *myo*-neurotrophin-4 (NT-4) mice have no effect (Nguyen et al., 1998). Adult mice overexpressing GDNF under myosin light chain 1 (MLC1) promoter were shown to maintain hyperinnervation of skeletal muscles, showing a 70% increase in the number of endplates when compared to wild-type animals (Zwick et al., 2001). Multiple innervations of endplates is also observed when continuous administration of exogenous GDNF is initiated after birth into adulthood (Keller-Peck et al., 2001). In addition to GDNF-induced hyperinnervation, continuous treatment with GDNF in *Xenopus* nerve-muscle coculture enhanced axonal growth by increasing the length of neurites in motor neurons (Wang et al., 2002). These results suggest that GDNF plays a significant role in synaptic maintenance and remodeling of the NMJ into adulthood.

GDNF acts on both presynaptic and postsynaptic apparatus during synaptic transmission. GDNF increases spontaneous neurotransmitter release in neonatal mice. Of the neurotrophic factors examined: GDNF, BDNF, NT-4, LIF, insulin-like growth factor (IGF), and IGF-2, GDNF was the only one to increase frequency of spontaneous neurotransmitter release by twofold as compared to the other neurotrophic factors (Ribchester et al., 1998). Other studies show that GDNF not only potentiates spontaneous synaptic transmission, but also enhances evoked transmitter release. Treatment with GDNF increased the spontaneous frequency of neurotransmitter release 5-fold and the evoked neurotransmitter release twofold in *Xenopus* nerve-muscle cocultures (Wang et al., 2001). GDNF elicits a small increase in quantal size without affecting the average rise and decay times of synaptic currents (Wang et al., 2002). Exogenous application of other neurotrophic factors, such as BDNF, can increase the frequency of spontaneous synaptic currents (Stoop and Poo, 1996). It was found that GDNF induces activity in the presynaptic terminal by enhancing expression of frequenin, an N-type calcium binding protein, hence facilitating calcium influx into the nerve terminal (Wang et al., 2001). Again, the action of GDNF on frequenin and calcium influx was specific to GDNF because other molecules such as NT-3 and NT-4 had no effect (Wang et al., 2001). In the postsynaptic cell, GDNF was shown to increase the size of AChR aggregates

(Wang et al., 2002) as well as the insertion rate of these receptors (Yang and Nelson, 2004). Taken together, it can be suggested that GDNF promotes axon branching, enhances synaptic formation, maintains synaptic connection, and modulates mammalian NMJ through both presynaptic and postsynaptic actions.

## GDNF expression following exercise

A role for neurotrophic factors has been proposed as contributing to exercise-induced changes in the nervous system (Wehrwein et al., 2002; Adlard and Cotman, 2004; McCullough et al., 2011). Neurotrophic factors, such as BDNF, IGF-1, and vascular endothelial growth factor (Wu et al., 2008; Trejo et al., 2001; Fabel et al., 2003), have also been suggested to play a role in exercise-mediated neuroprotective effects, where levels of expression of BDNF and NT-3 are increased in the spinal cord and skeletal muscle following both involuntary and voluntary exercise (Gómez-Pinilla et al., 2001, 2002). A relationship between neurotrophic factors and physical activity has been demonstrated with concentrations of NT-4 in skeletal muscle changing in proportion to the intensity of exercise (Funakoshi et al., 1995). We have previously shown that 4 weeks of treadmill training and 2 weeks of forced wheel running can increase GDNF protein content in rat skeletal muscle, while decreases in GDNF protein content were observed with hindlimb suspension (Wehrwein et al., 2002; McCullough et al., 2011) (Table 1). Following nerve injury, exercise increases GDNF mRNA in rat skeletal muscle (Dupont-Versteegden et al., 2004). Injection of botulinum toxin type A into skeletal muscle has been shown to block the beneficial effects of voluntary exercise on BDNF expression, suggesting that ongoing neuromuscular activity is important in regulating neurotrophic factor expression in skeletal muscle (Gómez-Pinilla et al., 2002).

## Exercise-induced GDNF expression following spinal cord injury

Spinal cord injuries interfere with an individual's daily activity. However, no standard treatment for spinal cord injury (SCI) has been implemented as it requires various treatments according to the site of injury. Neurotrophic factors, such as BDNF and GDNF, have been regarded as potential candidates for SCI treatment (Cote et al., 2011). Particularly, levels of GDNF protein and its mRNA are upregulated after SCI, indicating a trophic role to the damaged spinal cord (Hashimoto et al., 2005; Zhou et al., 2008). *In vivo* and *in vitro* administration of exogenous GDNF have shown promising results. GDNF enhances axonal regeneration and myelination *in vivo* following SCI whereas in a coculture of Schwann cells and neurons of the dorsal root ganglia, GDNF increases myelin sheath cells

**Table 1** Regulation of GDNF protein production

Factor	Effect	Site	References
Walk training	-	EDL	McCullough et al., 2011; Wehrwein et al. 2002
	+	SOL	
Stretch	none	EDL	McCullough et al., 2011
	+/-	SOL	
Electrical stimulation	-	EDL	McCullough et al., 2011
	+	SOL	
Hindlimb suspension	-	SOL	Wehrwein et al. 2002
	+	Pectoralis major	
Cholinergic neurons	-	Skeletal muscle cells in culture	Vianney and Spitsbergen, 2011
nAChR activation	-	Skeletal muscle cells in culture	Vianney and Spitsbergen, 2011
Spinal cord injury	+	Skeletal muscle	Lie and Weis, 1998; Suzuki et al., 1988a; Trupp et al., 1995; Naveilhan et al., 1997
	+	Spinal cord	
Spinal cord injury and exercise	+	SOL	Dupont-Versteegden et al., 2004
	+	Spinal cord	
Aging	-	Skeletal muscle	Nagano and Suzuki, 2003

(Zhang et al., 2009). In SCI animal models, GDNF protein was shown to increase 14-fold in animals subjected to exercise as compared to sedentary control groups (Dupont-Versteegden et al., 2004), suggesting that the release of GDNF may be exercise-dependent. Interestingly, the increased levels of GDNF in exercised animals correlate with SCI recovery (Cote et al., 2011) supporting that exercise training can be beneficial for restoration of injured spinal cord via increasing release of GDNF.

## Potential mechanisms

Possible signaling mechanisms that may be impacting GDNF expression following exercise, include altered neurotransmitter release, activation of AChRs, electrical stimulation and stretch. A recent study has found that addition of cholinergic neurons inhibits GDNF secretion by skeletal muscle cells (Vianney and Spitsbergen, 2011) (Table 1). Total GDNF (in both cell culture medium and inside cells) was decreased 36% when cells were cocultured as compared to muscle cell cultures. Earlier reports show similarities to this finding where high amounts of GDNF are found after skeletal muscle denervation, where it may be acting as a first responder to provide trophic support for reinnervating neurons (Lie and Weis, 1998; Suzuki et al., 1998a, b). Together, these results suggest that upon synaptic contact, motor neurons may inhibit production of GDNF in skeletal muscle and only allow the amount of GDNF to be released that is needed to maintain normal structure and function of the neuron. Electrical stimulation at a low frequency (0.1 Hz) has been shown to alter GDNF protein content in rat skeletal muscle and pretreatment with  $\alpha$ -bungarotoxin blocks those effects (McCullough et al., 2011) (Table 1). Addition of  $\alpha$ -bungarotoxin to nerve-muscle cocultures reverses the inhibitory effects of

cholinergic neurons on skeletal muscles, suggesting that activation of nicotinic acetylcholine receptors (nAChRs) may be decreasing GDNF expression (Vianney and Spitsbergen, 2011) (Table 1). Others have found that neurotransmitters from sympathetic neurons can alter the level of secretion of NGF in vascular and bladder smooth muscle cells (Spitsbergen et al., 1995; Clemow et al., 1999). Together, these results suggest that ACh is acting on nAChRs to regulate neurotrophic factor expression in muscle cells, where increased nAChR activation leads to a decrease in neurotrophic factor expression. This inhibitory effect of neurotransmitters on GDNF expression may provide one explanation for elevated GDNF mRNA levels following denervated skeletal muscle from individuals suffering from ALS (Lie and Weis, 1998) and for enhanced survival of developing motor neurons following blockade of nAChRs (Oppenheim et al., 2000). Furthermore, a direct relationship between stretching of muscle and the amount of neurotransmitter released may be another reason for changes in neurotrophic factor expression following exercise (Chen and Grinnell, 1997). Stretching muscles *in vitro* was shown to increase GDNF protein content after only 4 h, where blockade of nAChRs followed by stretch leads to a large increase in GDNF protein content (McCullough et al., 2011). Thus, mechanical activity of skeletal muscles may be an important mediator to increase neurotrophic factor expression with exercise. Different modalities of exercise have been found to alter the nerve terminal area, such as with low-intensity exercise (Andonian and Fahim, 1987; McCullough et al., 2011) and with low-frequency electrostimulation (Mussini and Marchioro, 1991). It has been suggested that there is a difference in the amount of storage and release of ACh in skeletal muscles upon exercise demands (Dorlöchter et al., 1991; Deschenes et al., 1993; Wood and Slater, 1997; Reid et al., 1999). Further investigation needs to be done to

determine how exercise increases GDNF in the neuromuscular system.

## GDNF's role with aging

Deficiency of GDNF has been reported to accelerate age-related problems (Boger et al., 2006). Aged individuals show a decline of GDNF in substantia nigra that is associated with motor dysfunction (Salvatore et al., 2004). Because GDNF knockout mice do not survive due to undeveloped kidneys (Moore et al., 1996), researchers have been using heterozygous mice to study consequences of decreased GDNF expression. In a study conducted by Borger et al., a partial deletion of the *GDNF gene* in heterozygous mice caused an age-related motor dysfunction and a significant decrease in tyrosine hydroxylase (TH) expression compared to that in wild type mice (Boger et al., 2006). It had been reported that administration of GDNF improved motor function recovery in aged rat. This effect was shown to be mediated through the increase of TH in striatum and substantia nigra, which catalyzes the dopamine synthesis from their precursors, hence dopaminergic neurons activity (Salvatore et al., 2004). Thus GDNF may be important in maintaining motor function, possibly through TH enzyme expression. Furthermore, during aging, it has been observed that nerves start producing growth-associated protein 43 (GAP43) suggesting that aging nerves may be switching from transmitting function to growth mode in order to promote survival and reinnervation, which was reported in axotomized nerves (Fu and Gordon, 1997; Ulfhake et al., 2000).

During aging, GDNF protein and GDNF mRNA expression have shown differential regulation patterns in central and peripheral systems. While GDNF mRNA decreases with age in the mouse striatum (Blum and Weickert, 1995), it is upregulated in skeletal muscle (Ulfhake et al., 2000), an observation which was seen in denervated skeletal muscle with disease. From this, it can be suggested that aging NMJ may experience denervation and that increased GDNF expression might be promoting reinnervation (Ulfhake et al., 2000; Edström et al., 2007). However, GDNF protein content has been found to decrease with age (Nagano and Suzuki, 2003; McCullough et al., 2011). Likewise, *GFR $\alpha$ -1* and *RET* expressions are increased in aging motor neurons and in dorsal root ganglions (Bergman et al., 1999; Ulfhake et al., 2000). Neurotrophin tyrosine kinase receptors have also been found to be downregulated in aging motor neurons, implying a weakened signal transduction of neurotrophins with age-related neurodegeneration (Dragunow, 1998; Bergman et al., 1999; Connor and Johnson et al., 1999). With aging, some of the main changes that occur are loss of motor neurons (Jacob, 1998) and loss of input to cell bodies of motor neurons (Kullberg et al., 1998). Decreased levels of GDNF may be devastating for motor neuron function and structure with aging; however increased physical activity may be a mechanism to combat these changes.

## Conclusions

GDNF plays an important role for the development, maintenance, and survival of the neuromuscular system. GDNF causes hyperinnervation and continuous synaptic remodeling at the NMJ (Nguyen et al., 1998; Keller-Peck et al., 2001; Zwick et al., 2001; Zhao et al., 2004), and enhances motor and sensory nerve regeneration after injury (Houenou et al., 1996; Michalski et al., 2008). Furthermore, GDNF potentiates transmitter release and synaptic transmission (Wang et al., 2002) and enhances AChR insertion on postsynaptic cells (Yang et al., 2001; Yang and Nelson, 2004). GDNF expression has been shown to be upregulated after tissue damage, with the level of GDNF mRNA increasing after nerve injury (Naveilhan et al., 1997) in denervated muscle and in neuromuscular diseases (Lie and Weis, 1998; Suzuki et al., 1998a), suggesting a role in reinnervation and synaptic maintenance. Since GDNF expression has been found to decrease with age (Nagano and Suzuki, 2003; McCullough et al., 2011), this could negatively impact motor neuron structure and function because GDNF is known as one of the most potent survival factors for motor neurons (Henderson et al., 1994; Oppenheim et al., 1995). Increasing an individual's level of exercise and/or physical therapy may be a countermeasure to increase GDNF expression and protect motor neurons from degeneration.

## Abbreviations

Acetylcholine Receptor (AChR); Amyotrophic Lateral Sclerosis (ALS); Artermin (ARTN); Brain-Derived Neurotrophic Factor (BDNF); Central Nervous System (CNS); GDNF Family Receptor  $\alpha$  (*GFR $\alpha$* ); Glial Cell Line-Derived Neurotrophic Factor (GDNF); Glycosyl-Phosphatidylinositol (GPI); Insulin-Like Growth Factor (IGF); Leukaemia Inhibitory Factor (LIF); Nerve Growth Factor (NGF); Neuromuscular Junction (NMJ); Neurotrophin-3 (NT-3); Neurotrophin-4 (NT-4); Neurturin (NTRN); Nicotinic Acetylcholine receptor (nAChR); Peripheral Nervous Systems (PNS); Persephin (PSPN).

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