Physiology International, Volume 106 (1), pp. 1–20 (2019)
DOI: 10.1556/2060.106.2019.07

Regulation of Wnt signaling by physical exercise in the cell biological processes of the locomotor system

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Received: October 17, 2017
Accepted: December 10, 2018

In the past decade, researches on Wnt signaling in cell biology have made remarkable progress regarding our understanding of embryonic development, bone formation, muscle injury and repair, neurogenesis, and tumorigenesis. The study also showed that physical activity can reverse age-dependent decline in skeletal muscle, preventing osteoporosis, regenerative neurogenesis, hippocampal function, cognitive ability, and neuromuscular junction formation, and the age-dependent recession is highly correlated with Wnt signaling pathways. However, how the biological processes in cell and physical activity during/following exercise affect the Wnt signaling path of the locomotor system is largely unknown. In this study, we first briefly introduce the important features of the cellular biological processes of exercise in the locomotor system. Then, we discuss Wnt signaling and review the very few studies that have examined Wnt signaling pathways in cellular biological processes of the locomotor system during physical exercise.

Keywords: physical exercise, Wnt signaling, cell biological processes, locomotor system, neuromuscular junction

Introduction

In parallel with the increasingly aging world population, numerous studies have focused on finding ways to improve quality of life (43, 71, 98), among which exercise, as a non-drug-based therapy, has attracted extensive attention in the past years (34, 110). The benefits of aerobic/resistance exercise in reducing the incidence of many conditions such as diabetes, osteoporosis, cardiovascular disease, and neurodegenerative diseases have been demonstrated (28, 67, 118). Adaptation to long-term high-intensity interval training/endurance exercise training can induce the accumulation of mRNA transcription proteins and affect numerous cell biological processes (74, 82).

It is widely accepted that the Wnt signal transduction cascade is a critical regulator in the development of many diseases, such as chronic obstructive pulmonary disease and chronic kidney disease (29, 66, 75). During the disease process, Wnt signaling has been reported as a general metabolic regulator (29). In addition, Wnt plays a critical role in modulations of cell growth, apoptosis, and in the process of gene expression of cell self-renewal (72).

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There are many signal pathways involved in an exercise that are parts of the cell biological processes of the locomotor system, but only few investigations of Wnt signaling regulation by physical exercise in cell biological processes of the locomotor system have been reported, and the relative mechanism is not clear. This study attempts to review the role of different modes of exercise training affecting Wnt signaling pathways in the cell biological processes of the locomotor system.

Wnt Signaling Pathways

Wnt proteins

Wnt signaling interacts with many biological pathways and has been implicated in numerous normal developmental and disease processes (30). At present, Wnt proteins are recognized as one of the major families of developmentally important signaling molecules, and mutations of Wnt genes display remarkable phenotypes in mice, *Caenorhabditis elegans*, and *Drosophila* (20). The Wnt gene is named according to the sequence homology of mouse Wnt-1 members (first known as int-1) (76, 102) and to wingless (wg) in *Drosophila* (19, 85). They encode secreted glycoproteins, usually 350–400 amino acids in length. Increasing numbers of homologous genes have been found in organisms ranging from mammals to the nematode *C. elegans*. As such, in *Drosophila*, Wg has been used as a model for elucidating Wnt gene function (9, 92).

Wnt signaling pathways

Wnt signaling pathways can be divided into two types, i.e., non-canonical and canonical pathways (2, 122). Non-canonical Wnt signaling is a signal transduction of Wnt- or Frizzled-initiated, which is independent of β-catenin transcription. Non-canonical Wnt pathways are diverse and in many cases are less characteristic/defined than canonical Wnt signaling pathways. They are divided into the following categories in clarity and simplicity: (1) Wnt/PCP signaling, (2) Wnt-cGMP/Ca^{2+} signaling, (3) Wnt-RAP1 signaling, (4) Wnt-ROR2 signaling, (5) Wnt-PKA signaling, (6) Wnt-GSK3-microtubule signaling, (7) Wnt-aPKC signaling, (8) Wnt-RYK signaling, and (9) Wnt-mTOR signaling (89).

Canonical Wnt pathways include Wnt protein, Fz protein, E-calcium mucin-cadherin (E), β-catenin, Disheveled (Dsh), APC complexes, the transcriptional factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors, and Ubiquitin (Ub) (97). Wnt pathways can be activated by binding a Wnt-protein ligand to a Frizzled family, which can pass the signal to proteins inside the cell. Wnt family members are secreted glycoproteins who bind to cell surface receptors such as the Frizzled. The canonical Wnt signal pathway has been described as follows: Wnt → Frz → Dsh → β-catenin degradation → β-catenin accumulation → enters into cell nucleus → TCF/LEF → gene transcription (e.g., c-myc and cyclinD1; Fig. 1) (40). The proteasomal degradation of β-catenin degradation requires phosphorylation, which can lead to the accumulation of β-catenin (61, 83). However, to facilitate Wnt signaling, low-density-related receptors 5 and 6 (LRP5/6), as co-receptors, play a key role in canonical Wnt signaling (53). Wnts are able to bind to one of the Frizzled family of seven transmembrane proteins and LRP5/6, which lead to the inhibition of GSK-3 activity and the activation of the canonical Wnt/β-catenin signaling cascade. Sost, Dkk, and Wise are Wnt inhibitors, which can bind to Lrp5/6 directly and prevent activation by Wnt lirands (42, 49).

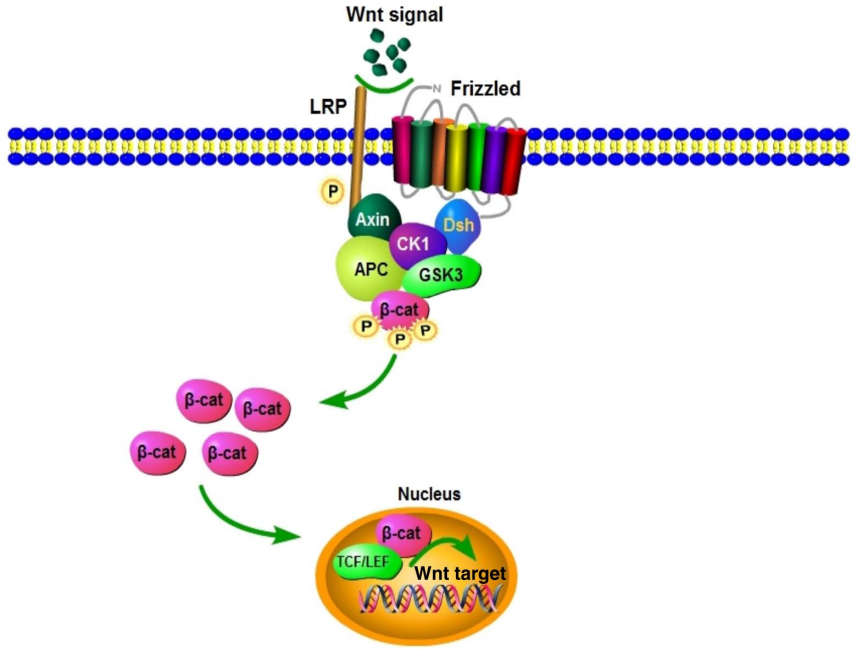

Fig. 1. The canonical Wnt pathways. When the ligand is bound in the extracellular space by one or more inhibitors and then intracellular β-catenin is degraded, Wnt signaling is turned off. The clear role of canonical Wnt signaling is to regulate the stability of β-catenin whose cytoplasmic concentrations are tightly regulated by the ubiquitin-proteosome degradation complex, which contains the scaffold protein Axon as well as β-catenin, casein kinase 1, glycogen synthase kinase-3β (GSK3β), and tumor suppressor protein adenomatous polyposis (APC)

Studies suggested that Wnt signaling pathways can control biological cell growth, apoptosis, and gene expression of self-renewal and survival. They also play an important role in muscle tissue repair, bone health, and neurogenesis (5, 27, 125).

Wnt Signaling Pathways in Exercise

Wnt signaling pathways in bone health

Both skeletal muscle and bone originate from the mesoderm, and are derived from somatic cells during embryonic development. Somatic cells differentiate into the dorsal dermal cells and ventral sclera, giving rise to skeletal muscle and bone. The balance between bone formation and resorption is important for the health of adult bones and the skeletal system. Breaking this balance in bone homeostasis leads to diseases, such as bone-related diseases, especially osteoporosis, in which bone mineral density (BMD) is decreased, bone micro-architecture deteriorated, and the risk of fracture increased.

Appropriate exercise is one of the most important positive stimuli for bone formation and appears to have positive effects on the prevention of osteoporosis (37). Appropriate exercise can enhance the bone mass, bone geometry, and bone strength in adults. In contrast, exhaustive or strenuous exercise has negative effects on the balance between bone formation and resorption, which may increase bone resorption and lead to stress-related fractures in military recruits and elite athletes (12).

Osteoporosis is a health problem that is more common in the elderly population. In mouse models, aerobic exercise increases serum levels of sex hormones and decreases levels of cytokines, such as IL-1, IL-6, and Cox-2, which can lead to osteoclastogenesis and bone resorption (60, 112). Xi et al. (28) showed that 5 weeks of medium intensity, but not low intensity, exercise could increase β-catenin mRNA expression in the tibia, and 9 weeks of exercise, at both medium and low intensity, increased β-catenin mRNA expression. Interestingly, they found that high-intensity exercise was able to maintain or even increase bone strength, while it had negative effects on bone mass. A previous study has demonstrated the effect of aerobic exercise on estrogen metabolism (93). Estradiol can enhance Wnt/β-catenin signaling during the menstrual cycle, whereas progesterone inhibits Wnt/β-catenin signaling (105). Kiel et al. (50) found that genetic variation in exons 10 and 18 of the LRP5 gene modulates Wnt signaling and the relationship between physical activity and BMD in men.

In addition, a study found that the expression of β-catenin, but not of Wnt1 or Lrp5, was increased after 5 weeks of medium-intensity exercise, suggesting that Akt/GSK3β might combine with Wnt/β-catenin signaling pathways at an early stage of exercise-mediated bone formation (22, 28, 90). These results indicate that activation of the Wnt signaling pathway plays a part in osteoporosis prevention in a senile rodent exercise model (28). A previous study indicated that the enhanced osteoblast differentiation induced by GSK3β was mediated by β-catenin downregulation (45).

Almeida et al. (3) reported an age-related increase in lipid oxidation-produced 4-hydroxynonenal as well as increased expression of lipoxygenase and peroxisome proliferator-activated receptor-γ in the skeleton of 4-month-old mice, suggesting that a high-expressing allele of the lipoxygenase Alox15 induced renewed Wnt signaling that had been decreased (3). Other studies have found that in the early stages of fracture repair, target gene expression (c-myc and connexin 43) levels, and the Wnt signaling pathway were upregulated, except for LEF-1 expression, suggesting that the activation of Wnt signaling pathways in the process of fracture repair affects the fracture-healing process (114). Studies also found that the Wnt signaling pathway plays an important role in the stem cells of osteoblasts. Yamada et al. (115) found that the Wnt signaling pathway participates in the osteoblastic differentiation of hMSCs and secreted frizzled-related proteins, thus oppositely controlling osteoblastogenesis through canonical and non-canonical pathways. Therefore, Wnt signaling pathways play a crucial role in fracture repair, in the process of transformation of mesenchymal stem cells to osteoblasts, and in osteoblast differentiation and bone metabolism. Thus, Wnt intervention is an important target of bone loss in a variety of drug treatment interventions in astronauts (23).

In bone canaliculi, shear stress has been induced by fluid flow and then the synthesis of the activation of integrins was induced, which triggers the Wnt signaling pathway (31). This pathway is thus important in osteocytes and osteoblasts. In osteocytes, the Wnt pathway can prevent apoptosis and transmit signals to other bone cells as well as to osteoblasts for differentiation, proliferation, and the synthesis of the bone matrix.

Osteocytes were able to sense mechanical load. Physical activity can increase the resistance to bone fractures and may be used as a prophylactic tool against osteoporosis (31). The mammalian skeleton was reconfigured to accommodate the load environment, which also helps to increase and maintain maximum BMD. The prevention effectiveness of physical exercise in osteoporosis depends on strain magnitude, strain rate, cycle number, strain frequency, and rest periods, and an increase in BMD is found only in loaded parts of the skeleton. To address the problem of bone loss in the elderly, a lot of researches have focused

on mechanical signals, which can help maintain bone health (86, 100). Through mechanical exercise, signals can be regulated by Wnt signaling pathways that affect bone formation. Arnsdorf et al. (6), investigating β-catenin translocation and Wnt protein expression, found that oscillatory fluid flow stress can regulate the bone marrow mesenchymal stem cells of mice from C3H10T1/2 in the non-canonical and canonical Wnt signaling pathway. Non-canonical Wnt5a signaling involving Ror2 and RhoA, as well as N-cadherin mediated β-catenin signaling, is necessary for mechanically induced osteogenic differentiation (Fig. 2). It has been found that sclerostin (SOST) was an antagonist for Wnt signaling. The loss of SOST was able to induce Wnt signaling hyperactivation, which has also been found in sclerosteosis (88). Consistent with this, previous studies demonstrated that downregulation of *Sost* was an obligatory step for mechanical loading to activate Wnt signal and induce osteogenesis (Table I) (16, 99).

Wnt signaling pathways in muscles

The long-term net balance of skeletal muscle protein synthesis and the decomposition of the synthesis rate significantly affect the balance of proteins in the whole body and determine the state of muscle mass (69). Long-term suppression of synthesis or upregulated breakdown could lead to muscle atrophy; this mechanism plays an important role in a variety of disease states, including HIV/AIDS, cancer, renal failure, sepsis, diabetes, aging, and bed rest (25, 58, 59, 91).

Physical exercise plays an important role in the intervention for this negative balance, such as in sarcopenia, which also has an effect on satellite cells in the skeletal muscle during aging. Satellite cells play a major role in the regeneration and recovery of muscles, because they have the ability to produce a large amount of new muscle fibers within a few days.

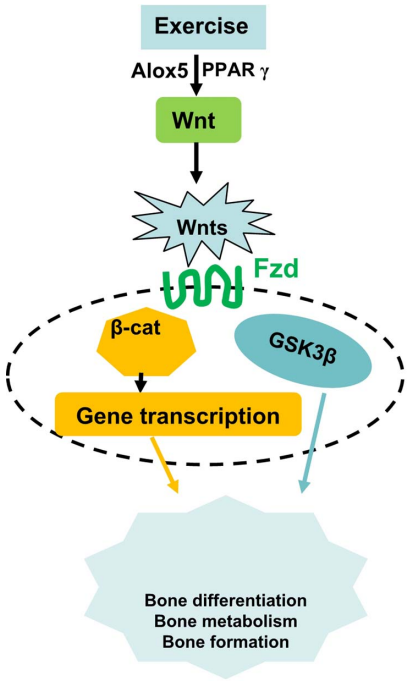

Fig. 2. The role of exercise on bone health by activating the Wnt signal pathway

Table I. Effect of Wnt signaling regulation by exercise on bone health

Molecular mediators	Classification	Modes of exercise training	Mechanism	Ref.
Wnt1, LRP5, and β-catenin	Inbred SAMP6 and SAMR1 mice	Medium-intensity aerobic exercise	Wnt signaling pathways were involved in osteoporosis prevention by exercise in a duration and intensity-dependent manner	(28)
Cox2, IL-1β2, and IL-6	Wistar female rats	Aerobic exercise	Exercise increased bone mass in post-menopausal osteoporosis rat models by inhibiting bone resorption and increasing bone formation	(60)
Akt and GSK3β	Marrow-derived mesenchymal stem cells	Mechanical strain	Reapplication of cyclic strain within a 24-h period leads to amplification of both Akt activation and its subsequent inhibition of GSK3β	(90)
β-Catenin, Wisp1,Cox2, PI3-kinase, and PGE2	CIMC-4 cells	Mechanical strain	Mechanical activation of Akt and inactivation of GSK3β contribute to β-catenin translocation independently of Wnt signaling through LRP5, and exercise can augment multiple strategies that increase bone	(22)
SFRP3 and SFRP4	Human mesenchymal stromal cells (hMSCs)		SFRPs contrarily control osteoblastogenesis through canonical and non-canonical pathways	(115)
The LRP5 gene	868 men and 929 women	Aerobic exercise	Wnt-LRP5 may play a role in the adaptation of bone to mechanical load in humans and may explain some gender-related differences in bone mass	(50)

Exercise can change the composition of muscle fibers, improve the performance and metabolism of muscles, and play a positive role in regulating satellite cells (38). During the aging process, the number of satellite cells decreases, which has been shown to be associated with a decline in muscle mass as well as with various functional characteristics (38). During the initial stage, the active presence of canonical Wnt signaling in satellite cells is a response to injury. The inhibition of Wnt signaling can lead to an obvious decrease in satellite-cell proliferation (80).

Fujimaki et al. (38) studied male C57BL/6J mice using a running wheel for 4 weeks. They found that, in the skeletal muscles of adult and aged mice, voluntary wheel running activated satellite cells and satellite-cell proliferation, and the levels of Wnt3, Wnt5a, and Wnt5b (canonical Wnt signaling) significantly increased after 4 weeks of voluntary running in both adult and aged mice. The change in Wnt signaling cascades however did not induce fibrosis in either group of mice. Canonical Wnt/β-catenin signaling was upregulated after mild voluntary wheel-running exercise (87). Because of the remodeling of MyoD and Myf5 promoter chromatin after exercise, the state of satellite cells ranged from quiescent to activated, which proved to be effective in preventing the development of sarcopenia (38). This study shows that exercise-induced Wnt does not cause fibrosis in aged skeletal muscles and rescues the activity of satellite cells.

In skeletal muscles, both insulin and exercise decrease GSK3β activity, and treadmill-running exercise *in vivo* significantly decreases β-catenin phosphorylation in both muscle types, with complete dephosphorylation being elicited by maximal exercise (8). In addition, resistance-training can increase the Wnt/β-catenin signaling (57, 94). In downhill-running exercise, the GSK3β activity and LEF1 protein expression were decreased, but other components of Wnt signaling were not affected (4).

Wnt and Shhare are extracellular signaling molecules, which are secreted from the surrounding environment and determine the developmental fate of skeletal muscles. Physiological stimuli including exercise and hyper muscular contraction are known to upregulate Notch and Wnt signaling in young muscles (8). However, the role of Wnt signaling in the repair of impaired aged skeletal muscles is controversial. Some studies suggest that there is an exacerbation of Wnt signaling in aged skeletal muscles. Although Wnt signaling is required for completion of muscle repair, the incongruous elevation of Wnt signaling in aged skeletal muscles may result in dysfunctional myogenic capability and acceleration of aging (121). Liu et al. (65) used the klotho mouse model (klotho-deficient mice show accelerated aging) and observed increased Wnt signaling that was associated with cell senescence and a decreased life span. Brack et al. (17) performed parabiotic experiments between young and aged mice and demonstrated that there was decreased Wnt signaling (increased GSK3β expression) in aged myogenic cells when exposed to young mouse serum, and increased Wnt signaling (decreased GSK3 expression) in young myogenic cells when paired with aged mice (Fig. 3). In addition, using a fusion protein of the Wnt receptor, Frizzled, (Frizzled-Fc), Brack et al. (17) indirectly measured increased levels of Wnt signaling components in the circulation of aged mice. When Frizzled-Fc was incubated with serum from aged mice, there was decreased Wnt activity; however, young serum incubated with Frizzled-Fc did not induce a change in the degree of Wnt signaling.

In cognitively impaired aged rats, the activation of Wnt was decreased with general aging, whereas exercise seemed to activate the status of Wnt pathway signaling (27). Wu et al. (113) found in animal studies that electrical stimulation (ES) increased the expression of Hey 1 (Notch target gene) and Pitx2 (a Wnt responsive gene), suggesting that the activation

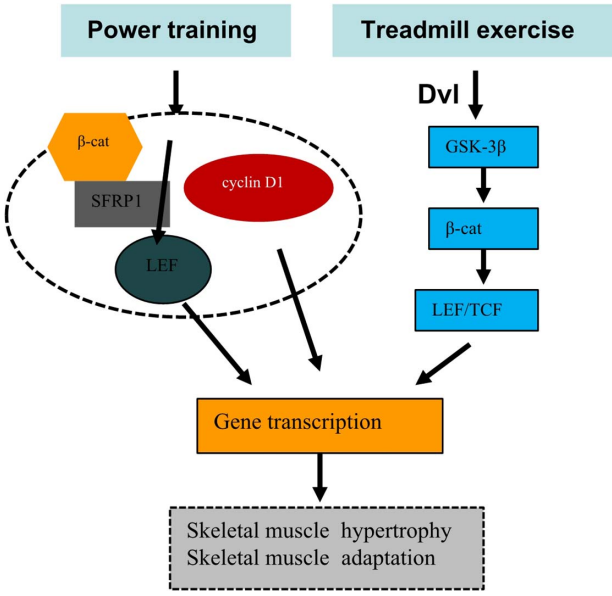

Fig. 3. The role of exercise on muscle health by activating the Wnt signal pathway

of the Notch and Wnt pathways coincides with the movement-related muscle cell potential change. According to a recent study by Naito et al. (73), the increasing secretion of C1q activates the Wnt signaling pathway in aging muscles and leads to the development of muscle fibrosis. Cellular senescence was promoted by a continuous Wnt exposure in a mouse model of accelerated aging (65), and age-related elevation of Wnt signaling causes fibrosis in skeletal muscles, resulting in sarcopenia (77). The level of C1q may be a novel biomarker of sarcopenia, and the aging-induced increase in C1q secretion activates the Wnt signaling pathway in muscles, leading to the development of muscle fibrosis (107). Furthermore, Okada et al. (77) found that C1q-induced activation of Wnt signaling can lead to fiber-type shift and demonstrated that the status of Wnt signaling may be a target to prevent skeletal myopathy in CHF. Consistent with this, it has been demonstrated that resistance training was able to prevent muscle fibrosis and atrophy via downregulation of C1q-induced Wnt signaling (Table II) (7, 44).

Wnt signaling pathways and cranial nerve function

Hippocampal neurogenesis. Adult hippocampal neurogenesis plays a key role in memory and mood or anxiety-related behaviors, and environmental factors such as exercise, environmental enrichment, hippocampal-dependent learning, ES, stress, age, and pharmacological manipulations have all been shown to affect adult hippocampal neurogenesis, memory, depression, and neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (124). Physical activity can reduce these harmful effects of the aging process. At the brain level, long-term moderate exercise regulates synaptic plasticity and neurogenesis in which the canonical Wnt/β-catenin signaling pathway participates in neural protection and synaptic plasticity (10). Physical activity increases the vascularization in the frontal cortex and the hippocampus, thereby increasing oxygen delivery and neuronal survival and neurogenesis (104). Running has also been shown to increase neurogenesis and dendritic complexity as well as the length of granule cell processes in the dentate gyrus in

Table II. Effect of Wnt signaling regulation by exercise on muscle

Molecular mediators	Classification	Modes of exercise training	Conclusion	Ref.
Wnt3/5a/5b, GSK-3β, and β-catenin	C57BL/6J mice	Aerobic exercise	Aerobic exercise upregulated canonical Wnt/β-catenin signaling in skeletal muscle	(38)
Dvl, Axin, β-catenin, and GSK3β	Sprague–Dawley rats	Aerobic exercise	Exercise *in vivo*, but not insulin, increases the association between Dvl and GSK-3β in skeletal muscle, an event paralleled by β-catenin dephosphorylation	(8)
Wnt3a, β-catenin, GSK3β, LEF1	C57BL/J6 male mice	Eccentric exercise	DHR decreased GSK3β and increased LEF1 protein expression but did not affect other components of Wnt signaling	(4)
Akt, p-Akt Thr(308) and Ser(473), GSK3α, P-GSK3α; cateninSer(33/37) Thr(41)	Eight healthy human subjects	Aerobic exercise	Exercise can regulate Akt, GSK3, and β-catenin signaling in human skeletal muscle, and as such identify them as possible molecular mediators of exercise's effect on metabolic and transcriptional processes in skeletal muscle	(87)
Wnt4 and β-catenin	Healthy and resistance-trained men	Resistance exercise	ULB resistance exercise increased Wnt4/β-catenin signaling	(94)
WNT1, Frizzled 1, SFRP1, DVL1, and β-catenin	Male subjects	Resistance raining	Resistance-training regimens activated expression of components of the WNT signaling pathway at the gene and protein levels	(57)
C1q	69 men and 62 women	Resistance training	Serum C1q level was significantly higher in middle-aged and older adults than in young adults, and serum C1q level significantly correlated with muscle mass and muscle strength	(107)
C1q, glycogen synthase kinaseβ/β-catenin	Young and aged senescence-accelerated mouse	Resistance training	Resistance training ameliorated muscle fibrosis and atrophy in aged mice/decreased circulating and muscle C1q levels/attenuated muscle Wnt signaling activation	(44)

both wild and captive-bred rats (21, 35, 84). Several research groups have shown that the importance of running is a key factor of lifestyle by stimulating neurogenesis significantly and is critical for Wnt signaling (95). The effects of exercise on hippocampal function are related to Wnt signaling pathways (124). After 2 months of aerobic exercise, neurogenesis is significantly increased in the mouse's dentate gyrus (1).

Evidence indicates that Wnt signaling can modulate adult hippocampal neurogenesis (103) that controls synaptic plasticity; regulates long-term potentiation; regulates neuronal development, differentiation, and synaptogenesis; and inhibits GSK3β activity (26, 30). The Wnt signaling pathway is dysregulated in neurodegenerative diseases (13).

A study found that the Dkk-1 induction was correlated with lower levels of sirtuin1 and increased levels of the acetylated form of p53 in sedentary (SED) animals, whereas they found increased levels of sirtuin1 and reduction in the acetylated form of p53 in the hippocampus of EXE (exercising) and NoEXE (environmentally enriched) animals (10). These results show that exercise and enrichment can increase the expression of this antiapoptotic protein. Axin1 is a scaffold protein that promotes the GSK3β-mediated phosphorylation and subsequent degradation of β-catenin (61). Axin2 is generally regarded as a transcriptional target of β-catenin and therefore a general indicator of Wnt pathway activity. Axin2 constitutes a negative feedback loop that controls Wnt signaling activity (68). Bayod et al. (10) showed higher levels of Axin1 and Axin2 protein levels in SED animals compared to the NoEXE or the EXE groups and an increase in protein levels of the active form of GSK3 in SED rats compared to the NoEXE and the EXE groups. This shows that long-term moderate exercise can activate Wnt pathways in the hippocampus of adult rats, and that it is related to DKK1, Axin1, and Axin2 (Fig. 4).

Alzheimer's disease (AD). There is evidence that the dysfunction of the Wnt pathway is involved in neurodegeneration in various central nervous system diseases, including AD and ischemia (24, 47). The key step of regulation is to regulate the Wnt/β-catenin signaling pathway and the central scaffold protein Axin1 APC, GSK3(3α/β), and CKI (30, 61). β-TrCP is part of the E3 Ub ligase complex, the protein containing the F protein, and the phosphorylated form of the β-catenin is not recognized. Thus, β-catenin is ubiquitinated and ultimately degraded by proteasomes. When the Wnt ligand binds to the Frizzled receptor and the low-density lipoprotein-related protein 5/6 (LRP5/6) co-receptor, the Wnt pathway is activated, resulting in the stabilization and nuclear translocation of the β-catenin. In the nucleus, β-catenin binds to TCF/LEF transcription factors to activate the Wnt-reactive target

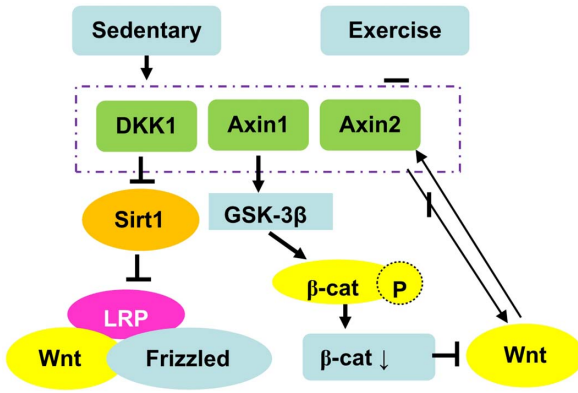

Fig. 4. Long-term moderate exercise can activate Wnt pathways in the hippocampus of adult rats, and that it is related to DKK1, Axin1, and Axin2

gene, which plays a key role in neuroprotection and synaptic plasticity in the mature nervous system (46).

Physical activity can reduce the deleterious effects of AD at the cerebral level in animal models (101). Okamoto et al. found that exercise significantly increase *de novo* expression of Wnt3 and thereby rescue impaired neurogenesis in aged animals. Furthermore, the decreased expression of Dcx, NeuroD1, and retrotransposon L1 was related to Wnt3 (79). Physical activity preserves cognition in the aging brain, demonstrated by Um et al., who trained mice at 24 months of age for 12 weeks (at a speed of 12 m/min, 60 min/day, 5 days/week, on a 0% gradient), and found that treadmill-exercised mice showed improved cognitive function in water maze tests (46). A study demonstrated that treadmill exercise can restore memory through increasing neurogenesis by activating Wnt signaling in AD (51).

Wnt signal pathways can also affect cognitive ability, as Gerenu et al. (39) showed in 4-month-old Tg2576 mice that were cognitively trained. AD-like pathologies were measured, and the results indicated that levels of hippocampal post-synaptic markers (PSD95 and NR1) were increased, in which Arc and β-catenin were involved in synaptic formation, and that β-catenin was a key effector molecule in the Wnt signaling pathway. These findings show that physical activity is one of the most important protective factors that can delay or prevent AD. Early and long-time exercise can therefore serve as a kind of cognitive stimulation through Wnt signaling pathways, which affects the nervous system and thereby reduces the occurrence of AD.

Depression. Exercise shows positive effects on depression prevention and on mental disorders such as autism, via the Wnt signaling pathway. Research indicated that β-catenin plays an important role in nervous systemic diseases, including depression (11, 41, 62). Running increases the amount of time spent in the open arms of the elevated plus maze and reduces anxiety (14). This effect is consistent with the effects of chronic treatment with the antidepressant fluoxetine (56). Okamoto et al. (78) reported that in the hippocampus, several antidepressants can increase WNT signaling, including downstream regulation of DVL and GSK3β. Wilkinson et al. (109) also showed that polymorphisms in several WNT-DVL-GSK3β pathway genes, including DVL2, are correlated with depression and certain neuropathological findings. Dias et al. injected Herpes simplex virus–green fluorescence protein into experimental mice of 7–9 weeks of age and found that the mice presented similarities to the social avoidance phenotype of depression. The overexpression of β-catenin, however, inhibited this phenotype and in the forced swim group and the water maze group, they found that β-catenin mediated the antidepressant phenotype (33).

The experimental group in the previous experiments confirmed that the nucleus accumbens shows an increase in the Wnt signaling pathway in depression model rats (78). Studies (32, 106) by Wassink and De Ferrari additionally found that autism may be associated with the breakdown of Wnt signaling pathways. Lijam et al. (63) reported that when Wnt pathway-related Dvl-1 is knocked out, mice showed the social behavior typical of autism, indicating that Wnt signaling pathways are associated with autism. These studies suggest that the changes in Wnt signaling pathways after exercise could be used for a therapeutic approach for depression and autism.

The β-catenin protein plays an important role in the mature central nervous system and in the development of behavioral resilience. It can activate a network that includes Dicer1 and downstream mRNAs, and its dysfunction has been connected to several neuropsychiatric disorders including depression. Thus, these mechanisms present a foundation for the development of novel therapeutic targets to promote stress resilience (33).

Wnt signaling pathways and NMJ

The NMJ is a cholinergic synapse that rapidly conveys signals from motoneurons to muscle cells to control muscle contraction. Neuromuscular junction formation requires proper interaction between motoneurons and muscle cells. NMJ formation requires reciprocal interactions between nerve terminals and muscle cells.

The formation of abnormal NMJ leads to neurological dysfunction, including congenital myasthenic syndrome and myasthenia gravis. Muscle-specific tyrosine kinase receptor (MuSK) and its co-receptor LRP4 are required for NMJ formation (18, 111). MuSK contains a Frizzled-like domain (cysteine-rich domain) in its extracellular region, and several Wnt molecules, including Wnt4, Wnt11, and Wnt9a, can be mediated by the Frizzled-like domains *in vitro* (48, 96, 119). Before muscle innervation, acetylcholine receptors (AChRs) begin to aggregate in a broad central and prospective synaptic region of the muscle, and activation of the MuSK–LRP4 complex regulates the prepatterning step (36, 64, 108, 117). Then, the MuSK–LRP4 complex is further stimulated by the neural Agrin, which induces multiple signaling pathways leading to clustering and remodeling of aneural AChR clusters (52, 120). When tyrosine is phosphorylated, MuSK activates signaling cascades that promote synapse-specific transcription and anchoring of AChRs (18, 64). Wnt signaling pathways take place simultaneously during the formation of vertebrate and invertebrate neuromuscular junctions, and some Wnts are essential for the formation of NMJs (Table III).

Table III. Effect of Wnts in NMJ formation

Classification		Target	Mechanism	Members	Ref.
Vertebrate	Zebrafish	Prepatterning of AChRs	MuSK Fz-like domain	Wnt11r and Wnt4a	(49, 103)
	Rats	Post-synaptic currents		Wnt-5a	(110)
	Mice or rat	AChR clustering, post-synaptic apparatus, and motor axon outgrowth	Agrin, LRP4, and MuSK, MuSK phosphorylation	Wnt3 or Wnt3a, Wnt11 and Wnt4, Wnt 2, Wnt4, Wnt7a, Wnt9a, and Wnt11	(31, 74, 112, 127)
	C2C12	AChR clusters	MuSK and LRP4	Wnt9a, 9b, 10b, 11, and 16	(127)
Invertebrate	*Drosophila*	Development of neuromuscular synapses	Wnt/Fz signaling and Neto	Wnt1, Wnt2, and Wnt7b	(54, 55, 66, 86)
	Caenorhabditis elegans	Synaptic function	RIG-3, CAM-1, and Wnt	Wnt/LIN-44	(55, 87)
			CWN-2, LIN-17, and post-synaptic receptor levels	β-Catenins	

AChR: acetylcholine receptor; NMJ: neuromuscular junction formation

Agrin, which is a ligand supplied by motor neurons, is the key member in vertebrate neuromuscular synapse formation and maintenance. Agrin binds LRP4, then stimulates further associations between LRP4 and MuSK, and increases MuSK phosphorylation (18, 123), essential for the induction and maintenance of AChR clustering at nascent synaptic sites (18, 55). In contrast, Ach is a type of antagonist, which depolarizes muscles and extinguishes AChR clusters, and is not directly opposed to nerve terminals that supply Agrin focally (18, 55).

Physical training leads to significant adjustments in NMJs. Most exercise interventions that have been investigated were types of endurance training (54, 70). Calcitonin gene-related peptide (CGRP) acts upon a wide variety of systems. Parnow et al. studied the results of endurance training (60 min/day at 30 m/min) and resistance (2-m wire mesh tower) on slow-twitch (soleus) and fast-twitch (Tibialis anterior) muscle fibers and the sciatic nerve and found that there was no difference between muscles of different features and that both types of training significantly increased CGRP and AChR levels. However, the action of CGRP on hypertrophy of the NMJ is not known (81).

Yang et al. (116) cultivated rat adipose-derived stem cells (ADSCs) with the CGRP gene (CGRP-ADSCs) in complete neural-induced medium. The results showed that Wnt 3a, Wnt 5a, and β-catenin regulate the neural differentiation of ADSCs, and that CGRP gene expression apparently was connected to canonical Wnt signals to promote the neurogenesis of ADSCs. Zhou et al. (126) also found that the Wnt/β-catenin signaling pathway may be involved in CGRP-promoted osteoblastic differentiation of bone marrow stromal stem cells.

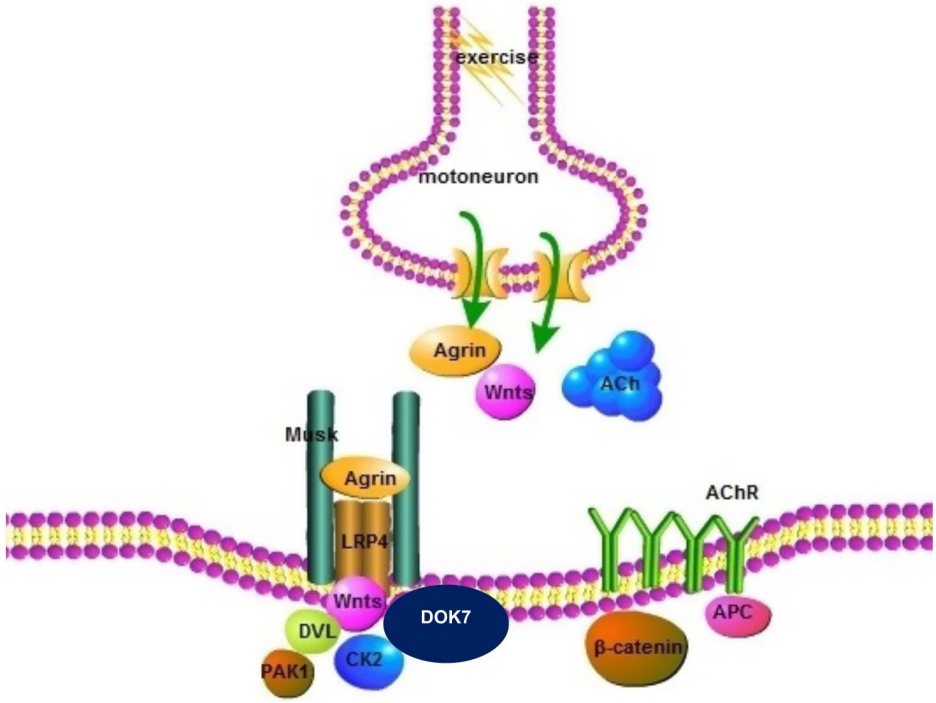

Fig. 5. Neuromuscular transmission regulated by physical exercise

Acetylcholinesterase plays a major role in neuromuscular transmission and is regulated by exercise. In fast-twitch muscles, the levels of total acetylcholinesterase can be affected by exercise, and synaptic acetylcholinesterase can increase markedly, but the levels of synaptic AChRs are not changed, suggesting that aerobic exercise elevates the synaptic acetylcholinesterase/receptor ratio (15). Strochlic et al. (96) found that Wnt4 dysfunction can decrease the number of prepatterned AChR clusters in mice embryos to 35% (E14 and E18.5 embryos), and when Wnt4 was overexpressed, the number of AChR clusters increased in cultured myotubes, indicating that Wnt4 directly affects post-synaptic differentiation (Fig. 5).

ES can induce muscle contraction to restore muscle mass, fiber-type distribution, and neuromuscular activity. In spinal cord injury, when the rat's soleus muscle was subjected to isometric resistance exercise by ES for 1 h per day for 1, 3, or 7 days, and 16 weeks, the results showed that ES increased the expression of Hey1 and Pitx2 and thereby Notch and Wnt signaling, suggesting that after 16 weeks of ES, neuromuscular junctions were repaired (113).

Discussion and Conclusions

The above review clearly shows how much is already known about the influence of physical activity on biological processes, including processes in muscles, bones, and the NMJ, as well as neurogenesis. Although several key studies and reviews have provided important insights into paracrine Wnt signaling between muscles and neurogenesis for the past 5 years, there are several important aspects that still need to be investigated and specifically the role of physical activity during the aging process and other important signaling pathways. Although it is evident that exercise plays an important role in the aging process, the intensity of exercise and the required time are still need to be studied. Exercise is particularly important in the prevention of diseases during the aging process. One fundamental question for future research will be, since animals (as well as humans) tend to be less active as they age, what happens during the biological process with decreasing exercise.

Acknowledgements

This study was funded by National Natural Science Foundation grants of China (61575065 and 11604104). SH and LY collected literature and drafted the manuscript. CW prepared the figures. TC-YL edited and revised the manuscript and approved the final version of the manuscript. This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare that there is no conflict of interest in this study.

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