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Wnt/β-catenin signaling pathway as an important mediator in muscle and bone crosstalk: A systematic review

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ABSTRACT

Background: The interaction between muscle and bone is shown to be clinically important but the underlying mechanisms are largely unknown. The canonical Wnt/β-catenin signaling pathway is reported to be involved in muscle-bone crosstalk, but its detailed function remains unclear. This systematic review aims to investigate and elucidate the role of the Wnt/β-catenin signaling pathways in muscle-bone crosstalk.

Methods: We conducted a literature search on the Web of Science, PubMed, EBSCO and Embase with keywords "Wnt*", "bone*" and "muscle*". A systematic review was completed according to the guideline of preferred reporting items of systematic reviews and meta-analyses (PRISMA). Data synthesis included species (human, animal or cell type used), treatments involved, outcome measures and key findings with respect to Wnts.

Results: Seventeen papers were published from 2007 to 2021 and were extracted from a total of 1529 search results in the databases of Web of Science (468 papers), PubMed (457 papers), EBSCO (371) and Embase (233). 12 Wnt family members were investigated in the papers, including Wnt1, Wnt2, Wnt2b, Wnt3a, Wnt4, Wnt5a, Wnt8a, Wnt8b, Wnt9a, Wnt10a, Wnt10b and Wnt16. Many studies showed that muscles were able to increase or decrease osteogenesis of bone, while bone increased myogenesis of muscle through Wnt/β-catenin signaling pathways. Wnt3a, Wnt4 and Wnt10b were shown to play important roles in the crosstalk between muscle and bone.

Conclusions: Wnt3a, Wnt4 and Wnt10b are found to play important mediatory roles in muscle-bone crosstalk. The role of Wnt4 was mostly found to regulate muscle from the bone side. Whilst the role of Wnt10b during muscle ageing was proposed, current evidence is insufficient to clarify the specific role of Wnt/β-catenin signaling in the interplay between sarcopenia and osteoporosis. More future studies are required to investigate the exact regulatory roles of Wnts in muscle-bone crosstalk in musculoskeletal disease models such as sarcopenia and osteoporosis.

Translational potential of this article: The systematic review provides an extensive overview to reveal the roles of Wnt/β-catenin signaling pathways in muscle-bone crosstalk. These results provide novel research directions to further understand the underlying mechanism of sarcopenia, osteoporosis, and their crosstalk, finally helping the future development of new therapeutic interventions.

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1. Introduction

The interrelationship between muscle and bone is intricate and dynamic. Extensive research has been devoted to understanding the biomechanical interactions and biochemical communications between these two tissues [1]. However, it is important to note that muscle-bone crosstalk is continuously influenced by their functional statuses during various stages of life, including development, aging and whether diseased. Sarcopenia and osteoporosis, both progressive disorders associated with aging, exemplify the link between muscle and bone in clinical studies [2,3]. These conditions involve the accelerated decline of tissue mass and function in both muscle [4] and bone [5–7],typically developing concurrently. Although the underlying mechanisms of their influence on each other remain unclear, the canonical Wnt/β-catenin signaling pathways are potential connectors between muscle and bone.

The canonical Wnt/β-catenin signaling pathway provides a potential underlying mechanism for WNTs to participate in bone formation and resorption, as demonstrated by previous studies [8,9]. WNTs is an integral part to the receptor activator of nuclear factor kappaB and nuclear factor kappaB ligand (RANK/RANKL) signaling pathways in bone metabolism [6]. In terms of bone formation, Wnt signaling activation was found to enhance osteogenic differentiation of bone marrow mesenchymal stem cells (BMSC), thereby promoting bone regeneration [10]. In terms of bone remodeling, the Wnt pathways were found to interact with WNT inhibitors such as Dickkopf-2 (DKK2) [11] and sclerostin [12], or mediators like Porcupine [13] and forkhead box O [14]. The inhibition by WNT inhibitors will impair the RANK/RANKL signaling pathways in bone metabolism. Similar to DKK and sclerostin, secreted frizzled-related protein (SFRP) inhibits WNTs by direct binding, impeding them from attachment to Frizzled receptors on the surface of osteoblasts and osteocytes [15,16]. In aging-associated bone loss, both glycogen synthase 3β (GSK3β) and axis inhibition protein (AXIN) were found to bind with adenomatous polyposis coli (APC) that subsequently led to degradation of β-catenin by proteasomes [17–19]. Abnormal inhibition of Wnt/β-catenin signaling pathway was shown to associate with osteoporosis and osteonecrosis in previous studies [20–23].

Meanwhile, the canonical Wnt/β-catenin signaling pathways were shown to participate in skeletal muscle activities [24–27]. Disruption of Wnt signaling during aging was reported to associate with the trans-differentiation of myoblasts to adipocytes that may have led to impaired muscle regenerative capacity [28,29]. Wnt can directly activate protein kinase B and the mammalian target of rapamycin (AKT/mTOR) anabolic growth signaling pathway responsible for maintenance of skeletal muscle size [27]. Overexpression of WNTs protein could increase the number of satellite cells and enhance muscle regeneration [30,31]. Although Wnt-induced signaling pathways in bone and muscle have been discussed in some narrative reviews [32–34], muscle-bone crosstalk mediated by canonical Wnt/β-catenin signaling pathway remains elusive. The objective of this systematic review is to summarize and analyze the roles of the canonical Wnt/β-catenin signaling pathway in the crosstalk between muscle and bone.

2. Materials and methods

2.1. Search strategy

A systematic literature search was conducted in Web of Science, PubMed, EBSCO and Embase with the keywords "Wnt*", "bone*" and "muscle*" (Last access to both on 30 May 2023). We combined the keywords as "Wnt* AND muscle* AND bone*" and followed Preferred reporting items of systematic reviews and meta-analyses (PRISMA) guidelines.

2.2. Search criteria

The inclusive criteria were: 1) pre-clinical or clinical studies on Wnt expression in muscle and/or bone; 2) all types of Wnt-related agents including biological, physical or chemical agents; 3) muscle or bone-related outcomes; 4) full-text literature in English. Meanwhile, the exclusive criteria were: 1) non-English papers; 2) without full-text access; 3) non-Wnt related; 4) non-skeletal muscle or bone related; 5) review articles; and 6) conference abstracts.

2.3. Selection of studies

Study selection was conducted by two independent reviewers. Initially, screening excluded obviously irrelevant papers based on titles and abstracts. Subsequently, the remaining potentially relevant articles were reviewed according to the inclusion and exclusion criteria. In case of disagreements between the two reviewers, a discussion was held to reach a consensus.

2.4. Data extraction

Reviewers extracted data from the included studies. The data were species and model systems (human, animal or cell type used), treatments involved, expression of Wnt-related genes or proteins, signaling pathways involved, outcome measures and key findings.

2.5. Data analysis

As high data heterogeneity exists in terms of study design, species or models, treatments and methodologies, a meta-analysis was inappropriate. Therefore, a qualitative systematic review was conducted.

3. Results

3.1. Search results

A total of 1529 publications were retrieved from Web of Science (468 papers), PubMed (457 papers), EBSCO (371 papers) and Embase (233). 813 publications remained after the removal of 716 duplicates. Titles and abstracts inspection excluded 743 studies due to irrelevance to the Wnt pathway in muscle or bone. 70 papers with full text remained for further eligibility check. The systematic review finally included 17 studies according to the inclusion and exclusion criteria. Fig. 1 shows the flow diagram summarizing the selection process. **Supplementary data 1** shows the details of the literature search.

3.2. Study characteristics

The 17 included studies were all pre-clinical studies published from 2007 to 2021. In 7 of these papers, both *in vitro* and *in vivo* experiments were conducted to investigate the crosstalk of bone and muscle, while 6 of them included *in vitro* experiments only and 4 of them included *in vivo* animal experiments only. Table 1 summarized the study characteristics.

3.3. Species and model systems

For the species involved in these studies, 6 studies used C2C12 myoblastic cell line as muscle cell models [35–40]. For bone cell models, 5 studies used BMSCs [39,41–44], 2 studies used primary osteocytes [37,38], 1 study used calvarial bone cells [39], 3 studies used MLO-Y4 [40,45] and OCY454 [39] as osteocytic cells, 5 studies used MC3T3 [36–38,45] and 2T3 [37,40] as osteoblastic cells. For *in vivo* animal models, 6 studies investigated both bone and muscle using C57BL/6 mice model [37,38,40,43,46], while several studies employed specialized disease models including BALB/c mice with the absence of a thymus that is incapable of generating T-cells [39], G93A transgenic mice with over-expressing mutant *SOD1* and causing neurodegeneration of spinal motor neurons and paralysis [47], as well as *Splotch-delayed* mice with allelic mutations on chromosome 1 [48]. Moreover, there were 2 studies

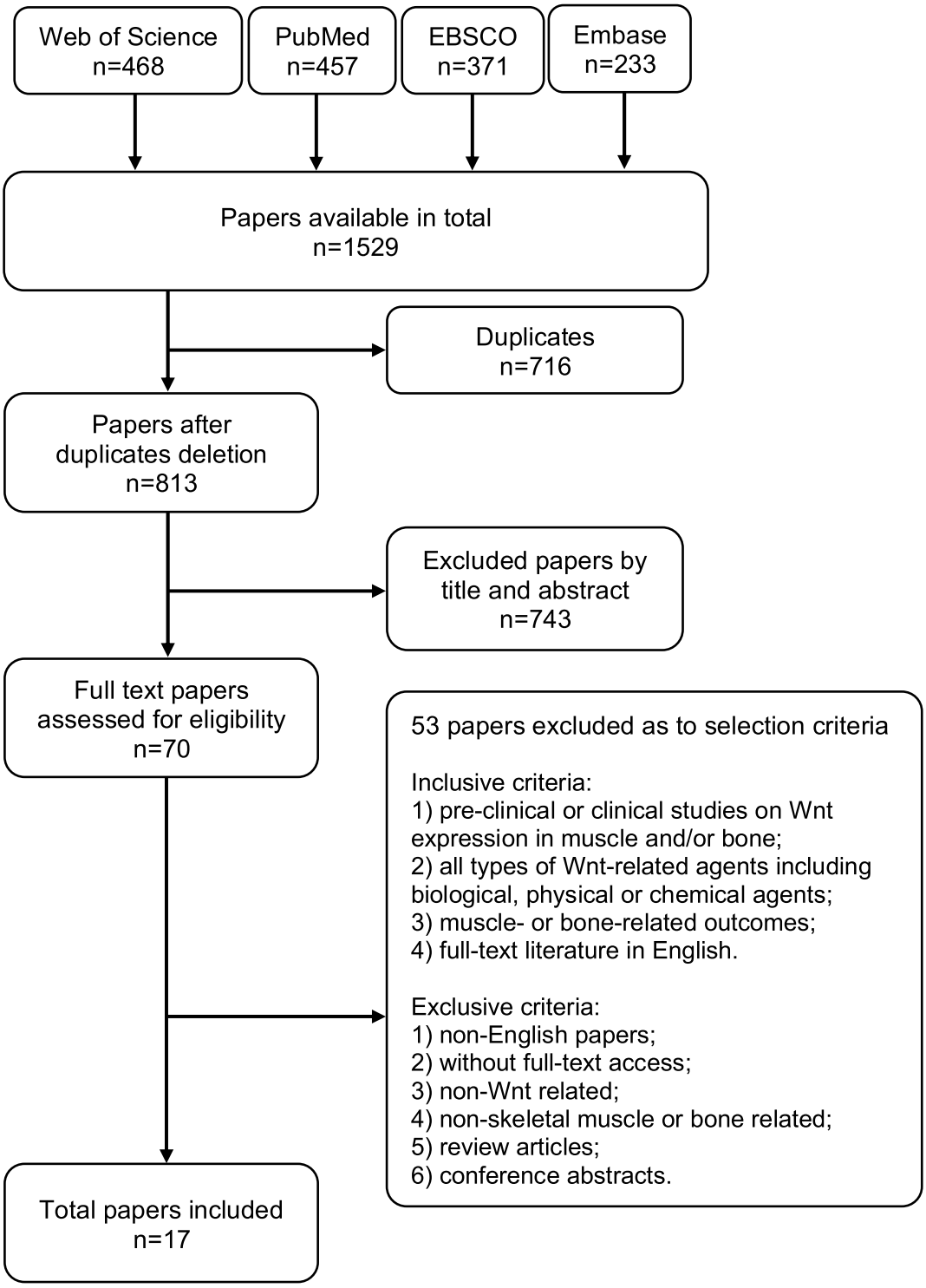

Figure 1. The flow diagram of the selection process.

using female Wistar rats with spinal cord injury (SCI) [49] and Sprague–Dawley rat model with excellent reproductive performance [50], and 1 using rabbit model [51]. In summary, the more frequently used models were murine C2C12 myoblastic cells for muscle cells, MC3T3 osteoblasts and BMSCs for bone cells, C57BL/6 mice models to study changes in both bone and muscle. Table 1 presents the details of species and model system.

3.4. Treatments

Different exposures were used in the 17 studies. Two studies directly applied Wnt as the treatment or intervention. In one study, WNT3A protein was provided to the bone marrow cells for the induction of myogenic differentiation [35]. *Wnt10a* gene was knocked out to assess the change of bone and muscle in mice [44]. Some studies used Wnt-related proteins as the treatments, such as brain and muscle ARNT-like 1 (BMAL1) [36], transcription factor Forkhead box protein C2 (FOXC2) [37], sclerostin [41], irisin [43] and fibroblast growth

Table 1
Characteristics of included studies.

Study type		Species and model system	Treatment	Outcome measures	Key findings with Wnt
Shang et al., 2007 [35] rowhead	Cell	Bone marrow mesenchymal stem cells (BMSC) from adult male Sprague–Dawley rat	Active Wnt3a proteins	Wnt3a, β-catenin	1 Wnt3a signaling induced β-catenin nuclear translocation and activated the Wnt pathway in BMSC; 2 WNT3A increased BMSC proliferation more rapidly; 3 Wnt3a inhibited the adipogenic differentiation in BMSC; 4 Active proteins WNT3A treatment not only could increase BMSC proliferation more rapidly but also activated primary mouse BMSCs from adult male Sprague–Dawley rat to myogenic differentiation by increasing the proteins expression of muscle formation markers
He et al., 2013 [36] rowhead	Cell	BMSC from old male C57BL/6 mice	Brain and muscle ARNT-like 1 (Bmal1)	Wnt3a, β-catenin, T cell factor 1 (TCF1)	1WNT3A, β-catenin and TCF1 decreased during osteo-induction by adding Dickkopf-related protein 1(DKK1); 2The Wnts inhibition by DKK1 down-regulated the BMSC osteogenesis; 3BMSC osteogenesis in old male C57BL/6 mice was decreased or suppressed by the inhibition of WNT3A, β-catenin and TCF1 using DKK1, that could be rescued by muscle-derived *Bmal1* over-expression
Gozo et al., 2013 [37] rowhead	Cell	C2C12 myoblasts cells	Forkhead box protein C2 (Foxc2)	Wnt4, β-catenin, T cell factor protein/lymphoid enhancer factor (TCF/LEF)	1WNT4 and TCF/LEF was up-regulated in C2C12 by FOXC2; 2WNT4, β-catenin and TCF/LEF up-regulation by FOXC2 inhibit myogenesis of C2C12; 3Genetically modified C2C12 myoblasts with sustained *Foxc2* expression would induce the expression of WNT4 and transdifferentiate to osteoblastic lineage
Xu et al., 2018 [38]	Cell	C2C12 myoblasts and MC3T3-E1 osteoblastic precursor cells	miR-27a-3p from exosomes derived from C2C12 myoblasts	β-catenin, Adenomatous polyposis coli (APC)	1Wnt/β-catenin pathway activation promoted MC3T3-E1 osteoblast differentiation by miR-27a-3p suppressing APC; 2miR-27a-3p of C2C12-derived exosome suppressed APC and activated Wnts pathway, promoting MC3T3-E1 osteoblastic precursor differentiation
Qin et al., 2017 [39] rowhead	Cell	BMSC, OCY454 osteocytic cells and MC3T3-E1 osteoblastic precursor cells	Myostatin-modified osteocytic exosomes (MMOE) from OCY454 osteocytic cells	β-catenin, TCF7, glycogen synthase 3β (GSK3β), sclerostin	1β-catenin and TCF7 levels was decreased while GSK3β was increased by MMOE in MC3T3; 2Wnt/β-catenin pathway down-regulation inhibited osteoblastic differentiation and activity by MMOE; 3The reduction of β-catenin and the increase of GSK3β were completely reversed by osteocyte-derived exosomal miR-218; 4Muscle-derived myostatin decreased osteoblastic differentiation and activities by down-regulating Wnt signaling pathway
Huang et al., 2017 [40] rowhead	Cell	MLO-Y4 osteocyte-like cells, MC3T3-E1 osteoblastic precursor cells, 2T3 osteoblast-like cells, primary osteocytes from five-month-old male C57BL/6 mice, and C2C12 myoblasts	Treatment of C2C12 cells with primary osteocytes conditioned media (CM), MLO-Y4 CM, 2T3 CM, MC3T3-E1 CM, and Wnt3a	Wnt3a, β-catenin, Axin2	1*Wnt3a* enhanced myogenic differentiation of C2C12 cells by inducing translocation of β-catenin to the nucleus of C2C12 cells; 2Wnt3a effect on myogenic differentiation of C2C12 was inhibited by sclerostin; 3WNT3A enhance Ca^{2+} release from the sarcoplasmic reticulum and modulate expression of key Ca^{2+} signaling in C2C12 cells; 4Wnt3a mRNA upregulation induced by fluid flow stress in MLO-Y4 osteocytes promoted the myogenesis of C2C12 myoblasts by inducing nuclear translocation of β-catenin

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Table 1 (*continued*)

Study type		Species and model system	Treatment	Outcome measures	Key findings with Wnt
Magarò et al., 2021 [41]	Cell; Animal	2T3 osteoblast-like cells, C2C12 myoblasts, male C57BL/6 mice	Electro-transfer of a plasmid containing the sclerostin gene	WNT1 inducible signaling pathway protein 2 (WISP2), sclerostin	1WISP2 was consistently reduced in 2T3 cells by adding the C2C12 culture supernatants at Day 1, 6 and 14; 2Sclerostin derived from skeletal muscle was also shown to reduce osteogenesis and induce trabecular bone loss by inhibiting the Wnt/β-catenin pathway
Kawao et al., 2020 [42]	Cell; Animal	Male C57BL/6J mice	Hindlimb unloading	Wnt10b, Wnt10a, Wnt8a, Wnt8b, Wnt5a, Wnt9a, β-catenin, DKK2, sclerostin	1*Wnt5a Wnt8a, Wnt8b, Wnt9a, Wnt10a and Wnt10b*, and decreased in mice soleus by hindlimb unloading, yet *Wnt8b* and *Wnt10b* significantly increased by hyper-gravity with 3 g versus 1 g; 2Wnt/β-catenin pathway activation was significantly induced by shear stress suppressing *Dkk2* in C2C12; 3Mechanical unloading down-regulated Wnt pathway of muscle and serum by increased DKK2
Chen et al., 2020 [43]	Cell; Animal	BMSC; femur and tibia of male C57BL/6 mice (*Ex vivo*)	Irisin	β-catenin, LEF1, TCF4, sclerostin	1Wnt/β-catenin signaling pathways were enhanced by irisin stimulating autophagy and osteogenic differentiation of BMSC; 2Muscle-derived irisin was shown to enhance the expression of Wnt pathway markers such as β-catenin, LEF-1 and TCF-4
Tsukamoto et al., 2019[a] [44]	Cell; Animal	BMSC; male C57BL/6 mice	*Wnt10a* knockout	Wnt10a, β-catenin	1*Wnt10a* deletion decreased osteogenic activity and adipogenesis in bone marrow, but *Wnt10a* deletion did not decrease muscle weight; 2*Wnt10a* deletion decreased β-catenin in BMSC; 3*Wnt10a* deletion decreased myostatin expression in muscle and adipose tissues
Jähn et al., 2012 [45]	Cell; Animal	MLO-Y4 osteocyte-like cells, primary osteocytes, and MC3T3 osteoblastic cells, C2C12 myoblasts; Intact extensor digitorum longus (EDL) or soleus muscle derived from 4-month-old male C57BL/6 mice	C2C12 myotube conditioned media, conditioned media from *ex vivo* electrically stimulated, intact extensor digitorum longus or soleus muscle	β-catenin	1β-catenin siRNA abrogated apoptosis of osteocytes by C2C12 myotube conditioned media; 2Wnt/β-catenin pathway activation protected against osteocytes death by C2C12 myotube conditioned media; 3The activation of the Wnt/β-catenin pathway protected osteocytes from cell death by adding conditioned media from C2C12 myotubes and extensor digitorum longus derived from 4-month-old male C57BL/6 mice
Adhikary et al., 2019[a] [46]	Cell; Animal	Calvarial bone cells from mice pups, C2C12 myoblasts, primary BMSC from adult male mice; male BALB/c mice	Fibroblast growth factor 2 (FGF-2)	Wnt10b, GSK3β, β-catenin, sclerostin, DKK1	1WNT10B was significantly increased in osteoblast cells by myokine FGF-2; 2WNT10B was down-regulated in osteoblast cells by dexamethasone yet WNT10B could not rescued by FGF2; 3β-catenin nuclear translocation was activated in osteoblast cells by FGF-2 but was inhibited by dexamethasone; 4GSK3β was inhibited in osteoblast cells by FGF-2; 5 WNT10B was down-regulated by DKK1 and sclerostin in osteoblast derived from calvarial bone of mice pups that can be restored by the application of myokine FGF-2 that opposes both DKK1 and sclerostin
Zhu et al., 2015 [47]	Cell; Animal	Female *SOD1* (G93A) transgenic mice	Amyotrophic lateral sclerosis	β-catenin, LRP5/6, sclerostin	1β-catenin was drastically reduced in BMSCs from G93A transgenic mice; 2β-catenin decreased in osteoblast on bone surface from muscle atrophic mice; 3Sclerostin expression was drastically increased but β-catenin expression was decreased by amyotrophic lateral sclerosis in osteoblasts on bone surfaces
Rolfe et al., 2014 [48]	Animal	Males and female heterozygous *Splotch-delayed (Pax3Spd/+)* mice	Limb skeletal muscle absence (*Splotch-delayed*)	Wnt2, Wnt2b, Wnt4, Wnt16, R-Spondin 2 (RSPO2) and Rspo3, secreted frizzled-related protein 2 (SFRP2), LRP5/6, TCF15, DKK2	1*Tcf15* was down-regulated in developing muscle-less humeri; 2Wnt/β-catenin inhibitors *Dkk2* and *Sfrp2* were up-regulated; 3The absence of limb skeletal muscle from *Splotch-delayed* mice increased the

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Table 1 (*continued*)

Study type		Species and model system	Treatment	Outcome measures	Key findings with Wnt
					Wnt inhibitors *Dkk2* and *Sfrp2* in humeral bone, although *Wnt2*, *Wnt2b*, *Wnt4 and Wnt16* mRNA were upregulated
Qin et al., 2013 [49]	Animal	Female spinal cord injury (SCI) rats	Muscles electrical stimulation of the hindlimb stimulated after SCI by using implantable microstimulators	Wnt1, Wnt3a, Wnt5a, Wnt16, DKK1, sclerostin, SFRP2	1WNT3A expression was greatly increased in osteoblasts from SCI rats by muscle electrical stimulation, but WNT5A decreased instead; 2Wnt/β-catenin inhibitors DKK1, SFRP2 and sclerostin increased in osteoblasts; 3Wnt/β-catenin inhibitors DKK1, SFRP2, and sclerostin were decreased in osteoblasts by muscle electrical stimulation; 4WNT3A protein expression was significantly increased in osteoblasts of SCI rats by muscle electrical stimulation
Macias et al., 2012[a] [50]	Animal	Male Sprague–Dawley rats	Simulated resistance training (SRT) combined with alendronate during hindlimb unloading (HU)	Sclerostin	1Wnt/β-catenin inactivation was induced by hindlimb unloading; 2Muscle stimulation by resistance training was shown to have activated the Wnts pathway and suppressed sclerostin, which restored cortical bone formation in osteocytes in hindlimb unloaded male Sprague–Dawley rats
Rodriguez et al., 2021 [51]	Animal	Adult female Rabbits	Bilateral posterolateral spine arthrodesis with autologous iliac crest bone graft	Wnt1, Wnt2, Wnt3a, Wnt4, Wnt5a, LRP6, GSK3β, β-catenin, sclerostin, DKK1	1*Wnt3a* steadily increased in lumber spines over time 6-weeks after arthrodesis; 2*Wnt5* increased in lumber spines over the first 4-weeks then decreased at week-6 after arthrodesis; 3Wnt/β-catenin inhibitor sclerostin increased sharply in muscle at week-3 then returned to baseline at week-6; 4Lumbar spine fusions in the rabbit model significantly increased the gene expression of *Wnt1*, *Wnt3a*, *Wnt4* in muscle at week-1 but the gene expressions returned to baseline levels by week-3

[a] Indirect muscle and bone crosstalk.

factor 2 (FGF-2) [39,46]. Some studies used exosomes from C2C12 myoblasts [38], or OCY454 osteocytic cells [39], and conditioned medium from bone cells [40] or muscle cells [45]. Some treatments were directly related to muscles, in which one study used hindlimb unloading for mice [42], one study used limb skeletal muscle absence [48], one study used amyotrophic lateral sclerosis [47], one study used single-channel and programmable implantable microstimulators to induce contraction of hindlimb muscles in SCI model [49], one study used simulated resistance training combined with alendronate treatment during hindlimb unloading of rats [50]. Also, one study used bilateral posterolateral spine arthrodesis with autologous iliac crest bone graft for rabbits [51]. All treatments are summarized in Table 1.

3.5. Outcome measures

As shown in Tables 1 and in order to investigate the Wnt/β-catenin signaling pathways, 10 studies took *Wnt* mRNA or WNT proteins as the key outcome measures, including Wnt1 [49,51], Wnt2 [48], Wnt2b [48], Wnt3a [35,36,40,49,51], Wnt4 [37,48,51], Wnt5a [42,49,51], Wnt8a [42], Wnt8b [42], Wnt9a [42], Wnt10a [42,44], Wnt10b [42,46], Wnt16 [48,49], and other Wnts [40,42]. 13 studies measured β-catenin mRNA or proteins [35–40,42–47,51]. As the receptor of WNT proteins, low-density lipoprotein receptor-related protein 5 and 6 (LRP5 and LRP6) were also measured [47,48,51]. GSK3β [39,46,51], Axin2 [40], T-cell factor 1 (TCF1) [36,37], TCF4 [43], TCF7 [39], TCF15 [48] and lymphoid enhancer factor 1 (LEF1) [43] were measured at the mRNA or protein levels as important downstream factors in the Wnt/β-catenin signaling pathways. Sclerostin [39,41–43,46,47,49–51], DKK1 [36,39,42,46,49,51], DKK2 [39,42,48], DKK3 [42] and SFRP2 [39,48] were measured as inhibitors for Wnt signaling.

3.6. The role of Wnt/β-catenin pathway in muscle affecting osteogenesis

Muscle or muscle-derived factors was shown to increase osteogenesis of osteoblastic cells in the musculoskeletal system through the activation of Wnt/β-catenin pathway. In the included *in vitro* studies, miR-27a-3p of C2C12-derived exosome suppressed APC and activated Wnts pathway, promoting MC3T3-E1 osteoblastic precursor differentiation [38]. During the process, C2C12 exosome containing miR-27a-3p down-regulated its direct target APC, and up-regulated β-catenin protein in the MC3T3-E1 cells, yet the effect was not observed in miR-27a-3p-deprived C2C12 exosomes. These findings showed a novel viewpoint that exosomes from muscles are capable of entering pre-osteoblasts and promoting differentiation, which extend our understanding of the muscle-bone crosstalk. BMSC osteogenesis in old male C57BL/6 mice was decreased or suppressed by the inhibition of WNT3A, β-catenin and TCF1 using DKK1, that could be rescued by muscle-derived *Bmal1* over-expression [36]. Given aging progression together with reduced osteogenesis of BMSC, these results demonstrate that by targeting muscle-derived Bmal1 may prevent the aging of bone and promote osteogenic differentiation of MSC through the Wnt/β-catenin pathway. WNT10B was down-regulated by DKK1 and sclerostin in osteoblast derived from calvarial bone of mice pups that can be restored by the application of myokine FGF-2 that opposes both DKK1 and sclerostin [46]. During the process, FGF-2 inhibited the function of GSK3β and thus enhanced the nuclear translocation of β-catenin in the

osteoblastic cells. This study reveals the role of exogenous myokine FGF-2 in helping Wnt pathway to maintain osteoblastogenesis.

Genetically modified C2C12 myoblasts with sustained *Foxc2* expression would induce the expression of WNT4 and transdifferentiate to osteoblastic lineage with expression of Ras homologous Guanosine-5′-triphosphate (RhoGTP) and bone morphogenetic protein 4 (BMP4) [37]. FOXC2 is therefore, shown to up-regulate WNT4, β-catenin and TCF/LEF to regulate myogenesis or the trans-differentiation of C2C12 myoblasts. Muscle or muscle-derived factors promoted BMSCs to differentiate into osteocytes; muscle-derived irisin was shown to enhance the expression of Wnt pathway markers such as β-catenin, LEF-1 and TCF-4 [43]. It also upregulated the expression of bone formation markers such as Runx2, alkaline phosphatase and osteocalcin, and decreased the expression of sclerostin. These results suggested that muscle-derived irisin plays a role in the osteogenesis of bone via the Wnt pathway. Muscle or muscle-derived factors can also protect osteocytes through the Wnt pathway. A study showed that the activation of the Wnt/β-catenin pathway protected osteocytes from cell death by adding conditioned media from C2C12 myotubes and extensor digitorum longus derived from 4-month-old male C57BL/6 mice [45]. In which, the conditioned media stimulated more β-catenin nuclear translocation and β-catenin knock-down decreased the positive effects of the conditioned media on dexamethasone-induced apoptosis of osteocytes.

In animal studies, WNT3A protein expression was significantly increased in osteoblasts of SCI rats by muscle electrical stimulation [49], with the expression of Wnt pathway inhibitors DKK1, SFRP2 and sclerostin were decreased in osteoblasts by the stimulation. These key gene expression changes suggested that muscle electrical stimulation motivated a net increase in Wnt pathway in bone marrow cell-derived osteoblasts. Moreover, another form of muscle stimulation by resistance training was also shown to have activated the Wnts pathway and suppressed sclerostin, which restored cortical bone formation in osteocytes in hindlimb unloaded male Sprague–Dawley rats [50].

On the contrary, some skeletal muscle- or muscle-derived factors were reported to decrease osteogenesis of bone cells through inhibition of the Wnt pathway. Muscle-derived myostatin decreased osteoblastic differentiation and activities by down-regulating Wnt signaling pathway [39]. In this study, β-catenin and TCF7 levels were significantly reduced by myostatin-modified osteocytic exosomes in MC3T3-E1 osteoblastic precursor cells, yet the reduction of β-catenin and the increase of GSK3β were completely reversed by osteocyte-derived exosomal miR-218. The results indicated that muscle-derived myostatin could suppress osteogenesis by inhibiting miR-218 further to decrease β-catenin and increase GSK3β. In an animal study using C57BL/6 mice, sclerostin derived from skeletal muscle was also shown to reduce osteogenesis and induce trabecular bone loss by inhibiting the Wnt/β-catenin pathway [41], yet the results are limited to clarify the specific mechanism. Another animal study revealed that the mRNA expression of *Wnt5a, Wnt8a, Wnt8b, Wnt9a, Wnt10a, Wnt10b* were decreased with the increased expression of *Dkk2* in the skeletal muscles in the disuse- and microgravity-induced bone loss model [42]. The main reason was that mechanical unloading down-regulated Wnt pathway of muscle and serum by increased DKK2. There were two animal studies supporting this observation of muscle on bone. In a study using amyotrophic lateral sclerosis mice model, sclerostin expression was drastically increased but β-catenin expression was decreased by amyotrophic lateral sclerosis in osteoblasts on bone surfaces [47]. Rolfe et al. reported that the absence of limb skeletal muscle from *Splotch-delayed* mice increased the Wnt inhibitors *Dkk2* and *Sfrp2* in humeral bone, although *Wnt2, Wnt2b, Wnt4 and Wnt1*6 mRNA were upregulated [48]. Therefore, these results showed that Wnt/β-catenin pathway activation or inhibition by muscle secreted factors can contribute to the increase or decrease of osteogenesis.

3.7. The role Wnt/β-catenin pathway in bone increasing myogenesis

Bone or its derived factors can increase myogenesis through the Wnt pathway. *In vitro* studies showed that *Wnt3a* mRNA upregulation induced by fluid flow stress in MLO-Y4 osteocytes promoted the myogenesis of C2C12 myoblasts by inducing nuclear translocation of β-catenin [40], yet the Wnt3a effect on myogenic differentiation of C2C12 was inhibited by sclerostin. Importantly, the models of the *in vitro* and the *ex vivo* results supported the concept of bone to muscle by Wnts pathway. Also, active proteins WNT3A treatment not only could increase BMSC proliferation more rapidly but also activated primary mouse BMSCs from adult male Sprague–Dawley rat to myogenic differentiation by increasing the proteins expression of muscle formation markers paired box 7 (PAX7), myoblast determination protein 1 (MYOD1), myogenic factor 4/5 (MYF 4/5), myogenin and myosin heavy chain (MHC) [35]. However, no evidence is available for the ability of Wnt3a to induce myogenic differentiation of adult stem cells. Another study focusing on bone-to-muscle is the study conducted by Rodriguez-Feo et al. They reported that lumbar spine fusions in the rabbit model significantly increased the gene expression of *Wnt1, Wnt3a, Wnt4* in muscle at week-1 but the gene expressions returned to baseline levels by week-3 [51], indicating *Wnts* genes expressions in muscle were regulated dynamically with bone fusion. However, the study did not evaluate functional outcomes of the paraspinal muscles, although gene expressions of these muscles were dramatically changed. Interestingly, these results further confirmed that the paraspinal muscles may play an active role in osteogenesis through Wnts pathways. However, it requires more evidence to confirm how bone affects muscle via the Wnt/β-catenin signaling pathway. The important crosstalk from these studies are summarized in Fig. 2.

4. Discussion

This systematic review aims to explore the relationship between muscle and bone with a focus on the Wnt/β-catenin signaling pathway. Current evidence suggests that skeletal muscles possess the capacity to modulate osteogenesis of bone through Wnt/β-catenin signaling pathways. However, there are also reports indicating the reverse relationship, where bones influence muscle development through the Wnt/β-catenin signaling pathways.

Different Wnt family members have been analyzed in this systematic review, but only three Wnts including Wnt3a, Wnt4 and Wnt10b are extensively studied. Wnt3a is one of the most important and well-studied factors in muscle-bone crosstalk. During embryonic development, the WNT3A protein is highly expressed in the dorsal neural tube activating myogenic gene expression [52,53]. While postnatally, it participates in muscle proliferation and regeneration by inducing the expression of muscle regulator genes [54]. Additionally, Wnt3a has been identified as a critical regulator of osteogenesis in bone. Studies have shown that WNT3A protein accelerated bone repair in osteoporotic BALB/c mice [55] and re-established osteogenic capacity for osteoblasts [56] or bone in aging animals [57].

Among the studies investigating the muscle-bone crosstalk, 5 studies have focused on Wnt3a and its effects [35,36,40,49,51], which is the most intensively studied one among all Wnts. Shang et al. demonstrated the ability of WNT3A protein to induce myogenic differentiation in BMSC by increasing the expression of myogenic markers such as PAX7, myogenin and MHC in BMSC [35]. This was attributed to the involvement of the Wnt3a pathway in the self-renewal process of many tissue stem cells. Moreover, BMSCs, just like human embryonic stem cells, have the pluripotency characteristics to differentiate into many mesenchymal tissue lineages including myogenic lineages, therefore WNT3A was capable to induce myogenic differentiation of pluripotent BMSCs [58].

The Wnts were found to regulate the development, proliferation, differentiation, and migration of cells [59]. He et al. found that osteoinduction of BMSCs was suppressed by the inhibition of Wnt3a/β-catenin pathway using DKK1. DKK1 impaired the regulatory ability of Wnts and hence the self-renewal capacity of BMSC, yet the

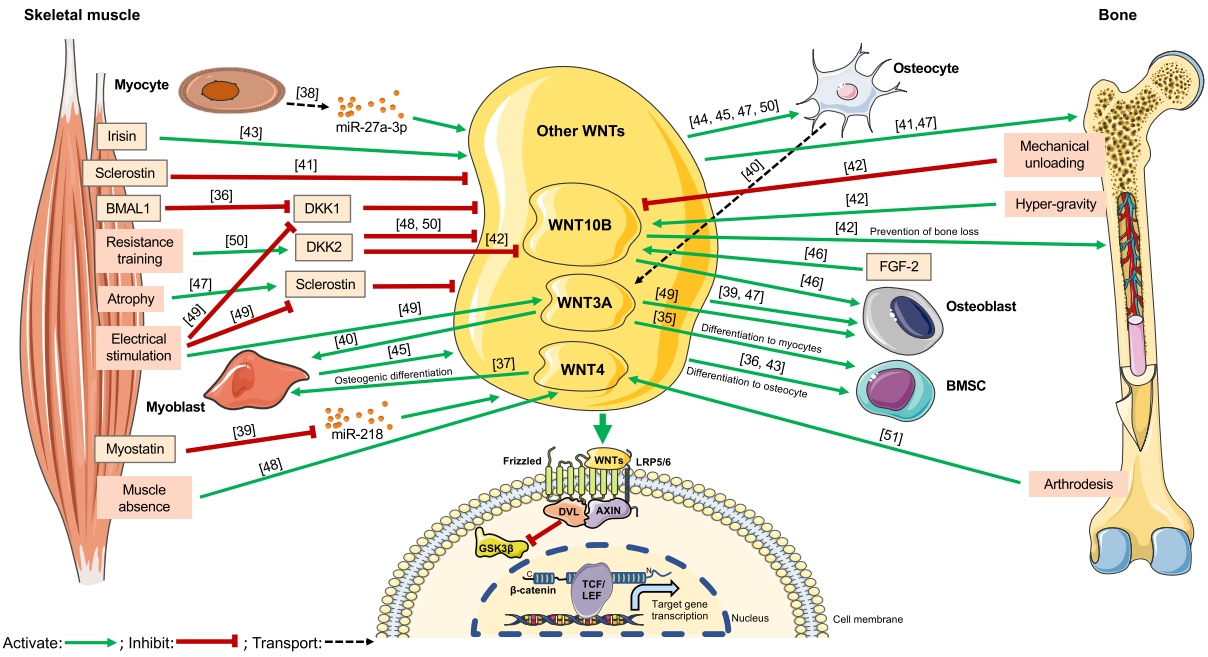

Figure 2. The muscle-bone cross-talk through various Wnt pathways

The figure presents a summary of the muscle-bone crosstalk studies. The left side of the figure illustrates that muscle or muscle-derived factors can either increase or decrease the osteogenesis of cells from the musculoskeletal system through Wnt3a, Wnt4, Wnt10b and other Wnts (Wnt1, Wnt2, Wnt2b, Wnt5a, Wnt8a, Wnt8b, Wnt9a, Wnt10a, and Wnt16). The right side of the figure shows that bone or its bone-derived factors can increase myogenesis through Wnt3a, Wnt4, Wnt10b and other Wnts. The center of the figure demonstrates how the Wnt pathway operates in the crosstalk: when the red/green lines start from muscle or end to bone, it indicates the muscle regulating bone via Wnts pathway; when the lines start from bone or end to muscle, it indicates the bone regulating muscle via Wnts pathway. Specifically, Wnts bind to both receptors of Frizzled and LRP5/6 in cell membranes, which result in the cytosolic tail of LRP5/6 binding to AXIN and DVL proteins. This inhibits the combination of GSK3β with β-catenin. Ultimately, as β-catenin accumulates in intracellular fluid, β-catenin enters the nucleus and binds to TCF/LEF facilitating target gene transcription.

AXIN: axis inhibition protein; Bmal1: muscle-derived brain and muscle ARNT-like 1; DKK: Dickkopf; DVL: disheveled; FGF-2: fibroblast growth factor 2; GSK3β: glycogen synthase 3β; LRP5/6: low-density-lipoprotein-related protein5/6; MSCs: mesenchymal stem cells; SOST: sclerostin; TCF/LEF: T cell factor protein/lymphoid enhancer factor.

inhibition could be rescued by muscle-derived *Bmal1* [36]. *Bmal1* probably had a synergistic effect on the Wnt3a pathway during BMSC osteogenesis. The expression of the *Wnt3a* increased by *Bmal1* mRNA alleviated the aging of BMSCs by increasing their proliferation and osteogenic differentiation in osteoporotic mice [60]. Huang et al. demonstrated that *Wnt3a* was more potent than *Wnt1* in increasing myogenesis [40], which further supported the ability of *Wnt3a* in differentiation induction. In the other two studies, interestingly, WNT3A expression was found to be greatly increased in osteoblasts by muscle stimulation (electrical stimulation) [49]. In contrast, the expression of *Wnt3a* and β-catenin inhibitor *Gsk3β* significantly increased in muscle at week-1 and returned to baseline levels at week-3 by bone stimulation (spinal fusion). However, Wnt3a inhibitor sclerostin increased sharply in muscle at week-3 and returned to baseline at week-6; meanwhile, *Wnt3a* steadily increased in lumber spines over 6 weeks after arthrodesis [51]. These changes of the Wnt/β-catenin pathway over a period of time indicate that the muscle-bone crosstalk is an intricate and dynamic process. These confirm Wnt3a to be an important factor in the muscle and bone. Wnt3a can be the potential target to regulate the muscle-bone crosstalk through the Wnt/β-catenin signaling pathway.

Wnt4 is another important factor in muscle-bone crosstalk. The Wnt4 pathway involves in the myogenic proliferation and differentiation of the skeletal muscle [61,62]. The overexpression of the *Wnt4* gene stimulates the proliferation of muscle satellite cells and differentiation of the myoblasts [61,62]. Moreover, *Wnt4* was reported to promote osteogenic differentiation of BMSCs [63,64], and inhibit bone loss in skeletal aging by enhancing osteogenesis as well as decreasing inflammation and osteoclast differentiation [65]. A decreased WNT4 expression was associated with decreased bone mineral density with aging [66]. There were three studies involving Wnt4 in muscle-bone crosstalk studies in the review [37,48,51]. Up-regulation of *Wnt4* gene expression shifted C2C12 myoblastic cell lineage commitment toward the osteogenesis [37], where WNT4 and FOXC2 derived from myoblasts can alter the commitment of C2C12 toward osteogenic differentiation. FOXC2 is an important upstream regulator of the *Wnt4* gene, which binds to its promoter to stimulate the transcription of the *Wnt4* gene. Hsu et al. also found that prolonged co-activation of *Wnt10b* and *Foxc2* improved osteogenic differentiation of BMSCs [67]. Taken together, the *Wnt4* gene participates in the crosstalk from muscle to bone mainly through its expression in bone induced by muscle-derived FOXC2, subsequently promoting BMSCs differentiation. Although *Wnt4* in muscle could be modulated by bone stimulation [51] and *Wnt4* in bone could be increased by muscle-related intervention [48], these studies did not go further to investigate the effect of the Wnt4 pathway between muscle and bone. In summary, Wnt4 is the potential candidate to regulate muscle influence on bone, yet the Wnt4 effect from bone to muscle requires further studies.

Wnt10b was initially identified in the mouse mammary gland in 1995 [68] and then in human breast carcinoma in 1997 [69]. Wnt10b is of great importance in bone formation. Overexpression of *Wnt10b* in transgenic mice increased bone mass by enhancing osteoblastogenesis through the canonical Wnt/β-catenin pathway [70]. Yet, anti-WNT10B antibody increased RANKL expression in the goldfish model and activated more osteoclasts differentiation [71]. These studies support that WNT10B increases bone mineral density by participating in the RANK/RANKL signaling pathways and by activating the osteoblasts and osteoclasts. However, *Wnt10b* knock-out decreased serum osteocalcin and trabecular bone of mice, verifying its endogenous regulation in the

osteogenesis [72]. Furthermore, *Wnt10b* deficiency causes age-dependent loss of bone mass [73], making it necessary for maintaining bone density in aging adults.

Two studies related to Wnt10b in muscle-bone crosstalk are reviewed herein. Kawao et al. found that the *Wnt10b* gene expression decreased in soleus by hindlimb unloading in mice, yet a significant increase was observed with hyper-gravity with 3 g versus 1 g [42]. The inhibition of the Wnt10b/β-catenin signaling pathway in the skeletal muscles by *Dkk2 mRNA* was induced by disuse- and microgravity-induced bone loss. Muscle disuse or failure increased *Dkk2 mRNA* in the muscle [42], inhibiting the Wnt/β-catenin signaling pathway and eventually decreasing terminal osteoblast differentiation and mineralized matrix formation in bone [11]. They also indicated that DKK2 protein could bind to LRP5/6, suppressing the Wnt10b/β-catenin signaling pathway in muscle and leading to muscle impairment. This result was similar to the previous findings, where *Dkk3* accelerated age-related skeletal muscle atrophy by inhibiting the Wnt pathway [74]. Another study found that WNT10B proteins was significantly increased in osteoblasts by FGF-2 [46] by localizing myokine FGF-2 in the muscle-bone interface [75]. This localization restored the Wnt/β-catenin signaling pathway by inhibiting sclerostin expression in osteoblasts. Sclerostin proteins impeded Wnt signaling function by binding with LRP5/6 receptors in osteoblasts and further impaired RANK/RANKL signaling pathways, eventually leading to failed bone formation [76,77]. A study by Brent et al. also supported the mechanism of sclerostin, in which they found that anti-sclerostin antibodies prevented bone loss in the rat model with hindlimb unloading [78]. However, anti-sclerostin antibodies did not change muscle mass and muscle cross-sectional area of the right rectus femoris muscle, indicating that the sclerostin impeded Wnt10b signaling probably only in bone but not in muscle. These findings suggest that inactivation of Wnt10b in muscles can impair while activation can promote bone formation.

To further investigate the effects of Wnt10b on muscle-bone crosstalk, it is required to clarify the effect of Wnt10b on muscle function which is short of evidence. It was reported that inhibition of the Wnt10b/β-catenin signaling pathway induced myoblast differentiation to adipocytes [28]. In contrast, *Wnt10b* mRNA over-expression was found to inhibit adipogenesis of muscle satellite cells in flexor digitorum brevis muscle of rats by mediating Wnt receptor Frizzled 1/2/5 and co-receptor *Lrp5/6* [79]. The muscle-fat crosstalk with Wnts was also shown in obese rats [80] and muscle-detached rabbits [81]. A number of studies validated the role of the Wnt/β-catenin signaling pathway in myogenesis and muscle regeneration [54,61,82], yet the study of Wnt10b on muscle is scarce. Vertino et al. found that *Wnt10b* deficiency in myoblasts with aging might contribute to impaired muscle regenerative capacity and increased muscle adiposity [29]. These results reveal that the role of Wnt10b in muscle should be important, but its changes in aging need further experiments to confirm.

To date, there is not enough scientific evidence to draw solid conclusions for other Wnts including Wnt1, Wnt2, Wnt2a, Wnt5a, Wnt8a, Wnt8b, Wnt9a, Wnt10a and Wnt16. As for *Wnt1 gene*, its expression significantly increased in muscle at week-1 after arthrodesis [51]. In contrast, *Wnt1* gene expression was decreased in osteoblasts by muscle electrical stimulation [49], which these results were contradictory for interpretation. In the review, there is only one study reported to explore each of Wnt2, Wnt2a [48], Wnt8, Wnt8a and Wnt9a [42] for the muscle-bone crosstalk, hence the evidence is not adequate to conclude. *Wnt10a mRNA* was down-regulated in the soleus by hindlimb unloading [42], yet *Wnt10a mRNA* was not changed in the soleus by hypergravity, and there is insufficient evidence for Wnt10a to support muscle-bone crosstalk. *Wnt10a* deletion in mice affected both bone and muscle functions simultaneously, so it was inconclusive which one affected the other in the muscle-bone crosstalk [44]. For Wnt5a [42,49,51] and Wnt16 [48,49], some studies reported their changes in bones or muscles, yet their research in crosstalk between muscle and bone is scarce. All in all, there are too few studies for the other Wnts, and more high-quality studies are required to understand the roles of these Wnts in muscle-bone crosstalk.

In this review, the Wnt-related components identified include Frizzled, LRP5/6, DVL, AXIN, GSK3β, β-catenin, TCF/LEF. However, current evidence does not suggest direct interactions among these Wnt-related components within the context of muscle-bone crosstalk. Conversely, Wnt inhibitors such as DKK1/2, SFRP2 and sclerostin appear to mediate interactions between muscle and bone, as indicated in Table 1. Consequently, the role of these Wnt inhibitors warrants further investigation in the context of muscle-bone crosstalk in future studies.

There are some limitations in this systematic review. The heterogeneity and inconsistent methodologies in the included studies make it infeasible to conduct a meta-analysis. Also, there are not sufficient evidence on bone-derived factors decreasing myogenesis of muscle cells via Wnt/β-catenin signaling pathway. Based on the current evidence, it is still unclear how WNTs proteins are transmitted between muscle and bone and what other pathways are involved in this process. There was no clinical study to associate bone and muscle through Wnt signaling pathway.

In conclusion, cumulative evidence indicates that Wnt/β-catenin signaling pathways participate in and show their potential in regulating the interaction between skeletal muscles and bones. Based on the included papers, Wnt3a plays an important role in muscle-bone crosstalk. Wnt4 and Wnt10b also participate in muscle-to-bone crosstalk, yet the effect of Wnt4 from bone to muscle needs further studies. The roles of Wnt10b in muscle during aging also require more evidence. Moreover, sarcopenia and osteoporosis are recognized as progressive disorders that are commonly associated with aging and often develop concomitantly. However, current evidence is insufficient to clarify the specific role of Wnt/β-catenin signaling in the interplay between sarcopenia and osteoporosis. It is evident that future studies are required to investigate the definitive regulatory functions of Wnt proteins in the muscle-bone crosstalk pertinent to sarcopenia and osteoporosis.

Credit author statement

WJL, CC, CRL conceived the systematic review and developed the methodology. WHC, SKHC and WJL carried out the data curation, Formal analysis, and drafted the manuscript. NZ, SKHC, RMYW, WQJ and CSL contributedto the interpretation of the results and drafting of the discussion. WHC secured the funding. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors have no conflict of interest relevant to this review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jot.2024.06.003.

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