The role of the Wnt signalling pathway in the energy metabolism of bone remodelling

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Abstract
Objectives: Bone remodelling is necessary to repair old and impaired bone caused by aging and its effects. Injury in the process of bone remodelling generally leads to the development of various bone diseases. Energy metabolism plays crucial roles in bone cell formation and function, the disorder of which will disrupt the balance between bone formation and bone resorption.

Materials and Methods: Here, we review the intrinsic interactions between bone remodelling and energy metabolism and the role of the Wnt signalling pathway.

Results: We found a close interplay between metabolic pathways and bone homeostasis, demonstrating that bone plays an important role in the regulation of energy balance. We also discovered that Wnt signalling is associated with multiple biological processes regulating energy metabolism in bone cells.

Conclusions: Thus, targeted regulation of Wnt signalling and the recovery of the energy metabolism function of bone cells are key means for the treatment of metabolic bone diseases.

1 INTRODUCTION

Bone is a highly specialized and dynamic organ that regenerates constantly. The bone modelling process forms and maintains the shape of a bone, leading to the acquisition of maximum bone mass. Even after a bone is matured, the process of bone regeneration continues through periodic replacement of old bone with newly generated bone in the same place, which is known as bone remodelling. Bone remodelling is necessary to repair damaged bone due to daily physical load and to prevent the effects of ageing. The impairment of the bone remodelling process often accelerates the progression of osteoporosis, a severe health problem worldwide. The entire process of bone remodelling is a process regulated and coordinated by multiple cell types. The preservation of bone remodelling and whole-body mineral homeostasis requires a dynamic balance between bone resorption and bone formation.

Energy metabolism, like glucose, amino acid, and fatty acid metabolism, plays significant roles in the formation and function of bone cells, such as osteocytes, osteoblasts, and osteoclasts. Among the many sources of energy, glucose is the predominant source of energy and carbon for bone tissue. Previous studies using radiolabelled glucose analogues have confirmed a significant uptake of glucose by bone. Energy metabolism disorder will disrupt the balance between bone formation and bone resorption. Recent data have identified a close interplay between metabolic pathways and bone homeostasis, demonstrating that bone plays an important role in the regulation of energy balance. In addition, recent experiments have revealed that bone is associated with...
Several diseases share common pathophysiological features, including common regulating factors of energy expenditure and bone homeostasis, such as peroxisome proliferator-activated receptor gamma (PPARγ), which plays a prominent role in the control of energy metabolism in bone homeostasis as well as adipose tissue.

Anorexia nervosa is a psychological disease with a characteristic of a distorted body image and self-restricted caloric intake, and it primarily affects teenagers and young women. Patients with anorexia nervosa have less bone mass, one of the least desirable traits that threatens the health and quality of life while also decreasing whole-body fat accumulation. Obvious signs of corporal emaciation reflect a loss of adipose tissue and lean mass because of calorie restriction, which is often associated with high energy consumption during aerobic exercise. The persistence of nutrient restriction represents an unfavourable environment that prevents cell construction and eventually damages the growth and development of bone. Under malnourished conditions, the endocrine environment is extremely disadvantageous to bone anabolism. For example, the serum levels of growth hormone are elevated, indicating a condition of hormonal resistance, as confirmed by the decrease in circulating levels of insulin-like growth factor 1. Growth hormone resistance to promote insulin-like growth factor 1 synthesis does not extend to the direct mediating actions of growth hormone in intermediary metabolism, such as lipolysis and protein synthesis (Table 1). However, bone remodelling markers in patients with anorexia nervosa appear to be unresponsive to the use of recombinant human growth hormone administration.

Osteoporosis, being the most common metabolic bone disease, occurs mainly in postmenopausal women and elderly individuals and is characterized by low bone mass, bone microstructure degradation, and ultimately increased fracture susceptibility. The prevalence of osteoporosis in American adults is 10.3% and in women and men aged 50 years and older in China, it is 23.9% and 5.3%, respectively. Initial findings from human genetic research reported inactivating mutations in low-density lipoprotein receptor-related protein 5 (Lrp5), a Wnt coreceptor, leading to osteoporosis pseudoglioma syndrome, whereas gain-of-function mutations result in osteosclerosis. Recently, human exome sequencing found that multiple mutations in Wnt1 are related to early stage osteoporosis and osteogenesis imperfecta. Furthermore, Wnt16 missense mutations are related to reduced bone mineral density and incremental fracture risk in the forearm and hip. Glutamine metabolism is closely related to osteoporosis. Glutamine is critical for not only energy generation but also redox homeostasis in the homeostasis of bone, which may be a potential strategy for bone diseases like osteoporosis and osteoarthritis. Previous studies have reported that glutamine metabolism changes related to ageing in osteoporosis may disrupt the balance between osteogenesis and the adipogenesis of bone marrow mesenchymal stem cells (MSCs) through the destruction of pivotal enzymes in glutamine metabolism or the deterioration of mitochondrial metabolism (Table 1).

| Metabolic diseases | Vulnerable population | Symptoms of diseases | Energy metabolism pathway | Pathogenesis | References |
|--------------------|-----------------------|----------------------|--------------------------|--------------|------------|
| Anorexia nervosa   | Teenagers and young women | Obvious signs of corporal emaciation | Suppressed glycolysis | Suppressed bone formation and increased bone resorption | de Paula et al.11; Yao et al.12; Pando et al.13 |
| Osteoporosis       | Postmenopausal women and elderly | Low bone mass, degradation of the bone microstructure | Changed glutamine metabolism | Destruction of pivotal enzymes in glutamine metabolism or deterioration of mitochondria metabolism | Zhou et al.14; Huang et al.15 |
| Type 2 Diabetes mellitus | Elderly people with obesity | Sudden weight loss | Impaired glucose metabolism | Affects osteoblastogenesis and disrupts osteoblastic adhesion | Li et al.16; Hie et al.17; Yang et al.18 |

These complex interactions may originate partially from the lineage differentiation of MSCs, and signalling pathways that influence the commitment to osteoblastogenic or adipogenic differentiation could play a crucial role in the regulation of bone and energy metabolism as well as their interactions. Wnt signalling is one of the conserved pathways involved in tissue repair, organ development, and homeostasis. Initiated by Wnt ligands, Wnt signalling can activate multiple intracellular signalling pathways, such as the canonical Wnt/β-catenin signalling pathway and non-canonical Wnt signalling pathways. Many lines of evidence indicate that Wnt signalling activation plays a significant role in the regulation of cellular bioenergetics. Here, we focused on the role and mechanism of the Wnt signalling pathway in regulating energy metabolism in bone remodelling with the goal of providing a theoretical basis for the treatment of related metabolic bone diseases.
Glucose serves as an alternative for fatty acids. Indeed, ACC is a rate-limiting enzyme for fatty acid synthesis. The genes of Wnt signaling pathway in bone remodeling. MSC can differentiate into adipocytes and osteoblasts, which can further differentiate into mature osteocytes. The balance between Wnt10b-mediated osteogenesis and PPARγ-mediated adipogenesis of MSC is disrupted when key enzymes of glutamine metabolism are impaired or mitochondrial metabolism is abnormal. Wnt7b disrupts the formation and activity of osteoclasts derived from HSC, leading to an increase in bone mass. HSC, haematopoietic stem cells; MSC, mesenchymal stem cells.

2 | WNT SIGNALLING IN ENERGY METABOLISM IN BONE TISSUE

2.1 | Energy metabolism in bone tissue

Cells take in energy from ingested or stored carbohydrates, proteins, and fats to generate adenosine 5'-triphosphate (ATP) through a series of enzyme-catalyzed reactions. Generally, energy metabolism includes glucose metabolism, amino acid metabolism, and fatty acid metabolism.

First, glucose from carbohydrates is the primary source of energy and carbon for mammalian cells. Intracellular glucose is phosphorylated to glucose-6-phosphate by hexokinase, and glucose-6-phosphate can be further transformed to glycogen or then converted to generate energy and a basis for biosynthesis. For most cell types (including bone cells), most glucose-6-phosphate enters the key glycolysis pathway to produce pyruvate, which then enters the mitochondria to be metabolized or remains in the cytoplasm to be converted to lactate. In mitochondria, pyruvate is completely oxidized via the tricarboxylic acid (TCA) cycle (also called the Krebs cycle) and is combined with oxidative phosphorylation (OXPHOS). In addition to the key glycolysis pathway, some glycolytic intermediates, such as glucose-6-phosphate, could be metabolized through other mechanisms, shunted via the pentose phosphate pathway (which is important for the synthesis of nucleotides and lipids), and converted to fructose-6-P through the hexosamine biosynthetic pathway for protein glycosylation. Gluconeogenesis serves as an alternative source of glucose when endogenous supplies are limited (in size and rate of availability) or when the exogenous supply of carbohydrates is too low or absent. The gluconeogenesis pathway is not simply a reversal of the glycolytic pathway: the irreversible steps of glycolysis are bypassed. The process of gluconeogenesis ultimately leads to the mobilization of muscle tissue to produce glucose. The energy cost of gluconeogenesis remains a matter of debate. Although glycolysis occurs universally, gluconeogenesis is confined to the liver and kidneys and has not been reported in bone tissue; therefore, we do not discuss gluconeogenesis in detail. In general, glucose is metabolized through a variety of pathways, yet the relative proportion of each metabolic fate may depend on the respective energy and biosynthesis demand in the cell (Figure 2).

Amino acids are another source of energy for bone tissue. Glutamine is the most abundant nonessential amino acid in circulation and has multiple metabolic uses in bone cells. Glutamine metabolism is initiated by the enzyme glutaminase, which deaminates glutamine to form glutamate, an important intermediate metabolite that has many biosynthetic uses in the cell. Glutamine is transformed into α-ketoglutarate through glutaminolysis and enters the Krebs cycle to be utilized as an alternative source of energy (Figure 2). Glutamine has been shown to be necessary for bone matrix mineralization.

Lipid metabolism starts with the entry of fatty acids into bone cells. Depending on the cellular energy status, fatty acids taken up across the plasma membrane are converted into fatty acyl-CoAs and are either directed to mitochondria for β-oxidation or stored as lipids in lipogenic tissues such as adipose tissue and liver. The transportation of long-chain fatty acids (LCFA) into cells requires protein-type transporters. Fatty acid transport proteins (FATP1-6), plasma membrane-associated fatty-acid binding protein (FABPpm), and fatty acid translocase CD36 are the predominantly expressed fatty acid transporters in different types of tissues. LCFA uptake is mediated either by one or by the cooperation of multiple fatty acid transporters. Acetyl-CoA carboxylase (ACC) is a rate-limiting enzyme for fatty acid synthesis. Indeed, ACC carboxylates acetyl-CoA into malonyl-CoA, the first and regulatory step in fatty acid synthesis. Similar findings were simultaneously obtained with HMG-CoA reductase (HMGR), the rate-limiting enzyme of the cholesterol biosynthesis pathway. The inactivation of ACC and HMGR by their respective associated protein kinases and their reactivation by phosphatase treatment were confirmed later. For fatty acid β-oxidation, malonyl-CoA is a rate-limiting enzyme for mitochondrial fatty acid β-oxidation and acts as an inhibitor of carnitine palmitoyl-CoA transferase-1 (CPT-1). CPT-1 catalyses the transfer of the acyl moiety of LCFA-CoAs to carnitine and facilitates the transport of LCFA-CoAs across the inner mitochondrial membrane (Figure 2).
2.2 Role of Wnt signalling in energy metabolism in bone tissue

2.2.1 Wnt signalling in glucose metabolism in bone tissue

Glucose taken up by cells can be channelled catabolically into a bifurcating ATP-generating glycolysis pathway leading to the formation of pyruvate, or shunted to the NADPH-producing pentose phosphate pathway. Glycolysis is the main physiological way to obtain energy when the body is in a state of relative hypoxia. However, a similar phenomenon of heightened glycolysis even under aerobic conditions also occurs with cancer cells and tissues, often referred to as the Warburg effect, as glycolysis proceeds to pyruvate with an accumulation of the latter despite an aerobic condition. Aerobic glycolysis could have different pathophysiological effects in different cells; for instance, in macrophages, the apparent shift from ATP production via the TCA cycle and OXPHOS to that by glycolysis might contribute to the establishment of effective antimicrobial defences. As in cancer cells, the switch to aerobic glycolysis in neurons and glia in diseased states may be an adaptive mechanism to preserve survival under conditions of stress and energy deficiencies. The activation of Wnt signalling promotes the expression and/or the activity of hexokinase and PFK1, important glycolytic enzymes, as well as AMP-activated protein kinase (AMPK). The advantageous influence of the activation of Wnt signalling is partly disappears when glucose uptake is restrained.

Preclinical studies further showed that the Wnt signalling pathway participates in glucose metabolism. Mice with an Lrp5 gene null mutation develop osteopenic and glucose intolerance. Other mouse genetic models proved that Wnt signalling plays an active role in glucose metabolism. Sclerostin knockout mice have increased glucose tolerance with insulin sensitivity and decreased white adipose reserves, suggesting an endocrine role of sclerostin, which is thought to be produced exclusively in bone (Table 2). In contrast, Lrp5 knockout mice have significantly impaired glucose tolerance and suppressed glucose-induced insulin secretion.

In addition to the role Wnt signalling plays in bone homeostasis, it is also important in glucose and lipid metabolism. In fact, a mutation in
the coreceptor LRP6 of the Wnt signalling pathway was genetically linked to hyperlipidaemia, hypertension, diabetes, and osteoporosis. As a transcription factor, TCF7L2 (commonly referred to as TCF4) binds with β-catenin of the Wnt/β-catenin signalling pathway. Recently, owing to its non-coding variants, the TCF4 gene has become by far the strongest susceptibility gene for Type 2 diabetes, which further proves the role that the canonical Wnt signalling pathway plays in glucose homeostasis.

2.2.2 | Wnt signalling in glutamine metabolism in bone tissue

Pharmacological inhibition of glutaminase can ameliorate the excessive bone formation induced by the hyperactivity of Wnt signalling in mice.7 In the process of osteoblast differentiation, glutamine anaplerosis partly meets the energy requirements of bone formation by responding to Wnt signalling.21 Wnt promotes the anaplerotic flux of glutamine into the TCA cycle by promptly increasing the level and activity of the glutaminase protein.21 The enzyme glutaminase catabolizes glutamine into glutamate, which is the first step of glutamine-dependent anaplerosis. Since glutamine plays an important role in the replenishment of TCA intermediates to support energy production, it may be the main energy source activated during osteoblastogenesis. These data indicate that glutamine catabolism is an important regulatory step in achieving both the energetic and synthetic demands related to Wnt-induced bone anabolism (Figure 3).2

2.2.3 | Wnt signalling in fatty acid metabolism in bone tissue

Growing experimental evidence suggests that regulating cellular and systemic metabolism is a primary role of Wnt signalling mediated by Lrp5.55 The effects of Wnt-Lrp5 signalling on systemic metabolism may be mediated in part by actions in bone.55 Researchers have proposed a model in which Wnt-Lrp5 signalling maintains insulin signalling in

| TABLE 2 The genes of the Wnt signalling pathway involved in energy metabolism in bone remodelling and bone metabolic diseases |
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| **Genes** | **Cell type** | **Metabolic processes** | **Biological functions** | **Diseases** | **References** |
| Lgr4 | Osteoblasts lineage cells, MC3T3-E1 preosteoblast cells | Glycolysis | Activates canonical Wnt/β-catenin signalling pathway | Osteoporosis | Yang et al.18; Carmon et al.48 |
| Lgr5 | MSCs | Mitochondrial fragmentation and fission | Promotes osteogenic differentiation | Osteoporosis | An et al.49 |
| Lgr5 | BMSCs | Glutamine metabolism and mitochondrial metabolism | Affects key enzyme in glutamine metabolism and mitochondrial metabolism | Osteoporosis | Huang et al.14; Singh et al.15 |
| Sclerostin | Adipocytes | Glucose metabolism | Inhibits the Wnt/β-catenin signalling pathway | Type 2 diabetes, osteoporosis and obesity | Kim et al.50 |
| Wnt3a | Osteoprogenitor cells | Mitochondrial oxidative phosphorylation (OxPhos) | Acute stimulation of mitochondrial oxygen consumption | | Smith et al.51 |
| Wnt3a | ST2 cells | Aerobic glycolysis | Activates mTORC2 and AKT, resulting in upregulation of key glycolytic enzymes | Osteopenia | Esen et al.29 |
| Wnt3a | ST2 cells | Glutamine oxidation | Stimulates energy production through increased glutamine utilization via the TCA cycle | | Karner et al.34 |
| Wnt7b | Bone marrow macrophages (BMMs) | Glucose metabolism | Affects glucose consumption and the expression of glucose transporters (GLUTs); impacts AKT activation during osteoclastogenesis | Osteoporosis | Wu et al.52 |
| Wnt10b | MSCs | Glucose metabolism | Induces osteoblast gene expression and inhibits PPARγ2 expression | Obesity | Cawthorn et al.53 |
osteoblasts in the setting of high-fat diet feeding and consequently affects systemic metabolism by regulating fatty acid catabolism.\(^5\)

Sclerostin, a Wnt signalling antagonist, is produced mostly by osteocytes embedded in the bone matrix, and its ability to profoundly control bone formation has been preliminarily investigated.\(^56,57\) After combining with low-density lipoprotein receptor-related protein 4 (LRP4), a sclerostin receptor, sclerostin interacts with the specific structural domain of the Wnt coreceptor LRP5/6, thus inhibiting osteoanabolic Wnt/β-catenin signalling.\(^58–60\) Additionally, according to a report, Lrp5 deletion leads to a decrease in fatty acid metabolism in osteoblasts, whereas an increase is induced by the expression of the Lrp5 high-bone-mass variant.\(^61\) Previous studies have shown that sclerostin can also regulate adipose physiology.\(^50,62\) Under each circumstance, the alteration in adipose tissue mass was related to a corresponding change in markers of Wnt/β-catenin signalling together with the ratio of catabolism to anabolism, indicating that circulating sclerostin allows communication between bone and adipose tissue to coordinate their activity (Figure 3).\(^63\)

Furthermore, the findings of an in vivo study suggest that sclerostin regulates not only anabolic but also catabolic metabolism, resulting in an increased fat mass and adipocyte diameter.\(^50\) For the in vitro study, after recombinant sclerostin treatment, the oxidation of long-chain fatty acids was repressed, and fatty acid synthesis was enhanced, which was entirely consistent with the findings of a previous study.\(^50\) In addition, these data support recent findings showing that Wnt signalling could regulate metabolic ability in organs such as the liver and bone and is analogous to the phenotype of mice that lack secreted frizzled-related protein 5, which is a secreted Wnt signalling inhibitor, generated by adipocytes.\(^61,64\) Similar to sclerostin knockout mice, genetic ablation of secreted frizzled-related protein 5 expression inhibits adipocyte hypertrophy and increases adipocyte metabolic capacity.\(^65\)

3 | WNT SIGNALLING PATHWAY AND BONE CELLS

3.1 | Energy metabolism of bone marrow mesenchymal stem cells (BMSCs)

BMSCs, known as non-haemopoietic multipotent mesenchymal cells, are traditionally capable of differentiating into osteoblasts, adipocytes,
and chondrocytes, thereby regulating bone homeostasis.\(^ {64,67}\) Recently, energy metabolisms, including glucose metabolism, glutamine metabolism, and fatty acid metabolism, in BMSCs in various contexts have been consistently reported.\(^ {68-70}\) Glucose is a major nutrient for BMSCs.\(^ {71}\) Glutamine, as the second critical regulator after glucose, exerts an essential modulatory effect on BMSCs proliferation, lineage allocation, osteoblast specification, and even immunomodulatory properties.\(^ {3}\) Fatty acids, generated from stored triacylglycerides or fat depots and released into the circulation, are degraded in the mitochondria for the generation of ATP in bone cells, while the amount that is utilized for ATP production is currently unknown.\(^ {7}\)

More recent evidence has shown that altered glucose uptake affects BMSCs differentiation.\(^ {9,72}\) Glycolysis is thought to preserve the ‘stemness’ of proliferating BMSCs.\(^ {73}\) However, some recent findings highlight that aerobic glycolysis is the predominant source of energy that promotes the differentiation of BMSCs.\(^ {74-76}\) Conversely, another study implied that osteogenic differentiation of BMSCs requires a metabolic switch from glycolysis to increased mitochondrial OXPHOS to ensure a sufficient energy supply.\(^ {77}\) OXPHOS is not necessary for the osteogenic differentiation of human BMSCs and ST2 cells but contributes to adipogenic differentiation in human BMSCs.\(^ {29,78,79}\)

BMSCs consume and metabolize a significant amount of glutamine as they undergo differentiation into osteoblasts. As BMSCs differentiate towards osteoblasts, glutamine metabolism provides ATP through the TCA cycle.\(^ {31}\) Furthermore, an integrated mechanism in a glutamine-dependent pattern is involved in meeting energetic and synthetic demands during BMSCs differentiation.\(^ {3}\) In addition to glutamine, glutaminase also promotes osteogenic differentiation of BMSCs.\(^ {90}\) Wang et al.\(^ {81}\) found that the glutamine metabolite \(\alpha\)-ketoglutarate (\(\alpha\)-KG) promotes the osteogenic potential of BMSCs by decreasing the accumulation of histone methylation. Taken together, these findings show that glutamine itself, glutaminase, and the glutamine metabolites \(\alpha\)-KG can promote the osteogenic differentiation of BMSCs.

Studies have revealed that fatty acids are second only to glucose as a main nutritional determinant for BMSCs and that fatty acid metabolism can also promote BMSCs osteogenic differentiation.\(^ {82,83}\) An in vitro study demonstrated that long-chain saturated fatty acids such as palmitate can inhibit the osteogenic differentiation of BMSCs, which can be mitigated by oleate.\(^ {84-86}\) Fatty acids function as an energy source to facilitate bone formation, and fatty acid deprivation suppresses osteogenic differentiation possibly by inhibiting fatty acid oxidation and the expression of specific receptors.\(^ {87}\)

### 3.2 Energy metabolism of osteocytes

With 90%–95% of the overall cell number in bone, osteocytes can live for more than 20 years. However, there are few data on the bioenergetics of osteocytes. Recently, an increasing number of endocrine functions have been attributed to osteocytes. By using osteocyte-depleted mice, researchers demonstrated that osteocytes help regulate the immune system and energy metabolism.\(^ {88}\) Furthermore, recent studies found that oxygen sensing downregulates bone mass in osteocytes.\(^ {89}\) In addition, there is evidence that the content of glycolysis-related proteins and mitochondria in osteocytes is highly related to oxygen-regulated protein 150 expression.\(^ {90,91}\) These facts indicated that osteocytes have a high degree of glycolysis in producing energy. These results imply that osteocytes should have metabolic activity in the process of bone remodelling.

### 3.3 Energy metabolism of osteoblasts

Due to their biomass and energy consumption during the process of bone remodelling, bones are important places for nutrient absorption.\(^ {8,89,92}\) The balance between bone formation and resorption is essential for bone homeostasis. In addition, osteoblast differentiation and its function require the integrity of nutrient utilization.\(^ {7}\) Glucose has long been considered a major nutrient of osteoblasts, of which the main product is lactate. Osteoblasts consume glucose in the process of glycolysis, even the oxygen is sufficient.\(^ {93}\) Aerobic glycolysis is an osteoblast metabolic characteristic as well as an indispensable function of osteoblasts. The aerobic glycolysis stimulation of preosteoblasts stabilized by HIF1\(\alpha\) results in increased formation of bone and is alleviated through the suppression of glycolysis.\(^ {7}\) Parathyroid hormone is a typical osteogenic-promoting factor, as is Wnt signalling, and has recently been identified to promote osteoblast aerobic glycolysis.\(^ {29,94-96}\)

It has been shown that amino acid metabolism is also significant for osteoblast differentiation.\(^ {4}\) Among amino acids in circulation, glutamine is the most plentiful source of energy.\(^ {97}\) There is evidence that in the TCA cycle, glutamine can be oxidized, thereby promoting energy generation in mitochondria. Besides, glutamine is a favourable replaceable fuel for osteoblasts since its metabolism promotes osteoblast capability when glucose is lacking.\(^ {2,32}\) Similarly, glutamine is critical in the maintenance of redox homeostasis, further improving osteoblast capability.\(^ {3}\)

In addition, fatty acid metabolism in osteoblasts is needed for bone acquisition depending on sex and diet.\(^ {6}\) Fatty acids circulate throughout the body and exist in bone marrow sera as well.\(^ {98}\) Besides, some kinds of long-chain fatty acids can be increased and processed during osteoblast differentiation, indicating greater use of fatty acids as substrates for energy production.\(^ {7}\) However, some lipids have deleterious effects on osteoblasts. Therefore, the roles of fatty acids are (1) a source of energy to promote bone formation and (2) the inhibition of deleterious effects of osteogenic differentiation, possibly through specific receptors.

### 3.4 Energy metabolism of osteoclasts

Glucose is of great importance for supporting osteoclast energy needs.\(^ {4}\) Osteoclasts are reported to require a large amount of energy because of their specific performance and motility associated with bone resorption.\(^ {6}\) Not only glycolysis in the cytoplasm but also the
TCA cycle in mitochondria is significant for osteoclast differentiation and functions. Reactive oxygen species (ROS), which are radical forms of oxygen, occur as outcomes of mitochondrial respiration and oxidase activity. Elevated levels of reactive oxygen species have two important consequences: one is the destruction of DNA, lipids, and proteins, resulting in cell death, and the other is that they can activate specific signalling pathways. Reactive oxygen species have been proven to play significant roles in stimulating osteoclast formation and resorption.

Role of the Wnt signalling pathway in the energy metabolism of bone cells

Wnt/β-catenin signalling regulates numerous osteoblast functions, including the initiation of fate determination and maturation. β-catenin is an important factor in the canonical Wnt signalling pathway. When the pathway is activated by any Wnt ligand, it can regulate the transcription of downstream Wnt target genes. Thus, β-catenin is a critical target for exploring the function of this pathway.

Diabetes mellitus affects osteoblastogenesis and disrupts osteoblastic adhesion related to Wnt signalling by inhibiting AKT signalling. By introducing PPARγ in MC-3 T3 cells, β-catenin, p-AMPK, and p-AKT signalling were activated, but p-glycogen synthase kinase 3β (p-GSK3β) signalling was downregulated. Meanwhile, PPARγ can significantly enhance mitochondrial biosynthesis and counteract glucose oxidation. The inhibition of GSK3β activates β-catenin, which then promotes the differentiation of osteoblasts and MSCs.

As a significant factor of energy metabolism, p-AMPK promotes cell differentiation and is involved in insulin sensitivity through crosstalk with Wnt signalling. In the latest research, the activation of canonical Wnt signalling ameliorated impaired glycolysis in osteoblast cells, whereas blocking Wnt/β-catenin signalling had the opposite effect. In addition, studies have found that Wnt signalling can promote fatty acid oxidation via β-catenin in osteoblasts. The Wnt coreceptors Lrp5/6 are important for postpartum bone recovery and osteoblast function. Recently, researchers discovered a unique function of Lrp5-facilitating the oxidation of fatty acids in osteoblasts. Lrp5-deficient mice have reduced bone mass after birth but increased body weight and reduced energy expenditure. In contrast, mice with a mutant Lrp5 allele of high bone mass had reduced fat content. In this case, Wnt initiates signalling downstream of Lrp5 (not Lrp6) to induce β-catenin activation, which thereby regulates key enzyme expression in fatty acid β-oxidation (Figure 3). Thus, Wnt-Lrp5 signalling, apart from being associated with fundamental cellular activities related to bone fate and differentiation, participates in bone regulation and affects whole-body energy homeostasis through pathways distinct from osteocalcin and glucose metabolism.

4 | THE GENES IN THE WNT SIGNALLING PATHWAY INVOLVED IN THE ENERGY METABOLISM OF BONE REMODELLING

4.1 Lgr4

Leucine-rich repeat-containing G protein-coupled receptor (GPCR) 4 (Lgr4), also known as G protein-coupled receptor 48, is involved in many physiological and pathological reactions, such as bone remodelling and energy metabolism. Wnt/β-catenin signalling is reported to activate glycolysis in a variety of tumorigenic processes. Once Lgr4 is deficient, the function of Wnt/β-catenin signalling is attenuated. Lgr4 promotes osteoblast glycolysis via the Wnt/β-catenin signalling pathway. In MC3T3-E1 preosteoblasts, Lgr4 is required for osteoblastogenesis, and its low expression results in a decreased level of aerobic glycolysis in osteoblast cell lines (Table 2). Besides, Lgr4 is proved to mediate the amplification of Wnt/β-catenin signalling. Lgr4 ablation harms aerobic glycolysis and osteogenic capacity, and this can be restored by activating canonical Wnt signalling in preosteoblasts. In addition, mice lacking osteoblast Lgr4 specifically (Lgr4<sup>−/−</sup>) appear to have reduced bone mass and reduced bone-forming ability. The down-regulation of osteoblast differentiation and glycolysis markers in Lgr4<sup>−/−</sup> mice reveal the energy metabolic function of Lgr4, affording a new target for the treatment of osteoporosis. In osteoclasts, Lgr4 serves as a decoy receptor for RANKL, inhibiting osteoclast differentiation. These studies emphasize the critical role of Lgr4 during bone remodelling. Furthermore, it was found that Lgr4 is a regulator of cell metabolism, as Lgr4 ablation increases energy consumption by promoting white-to-brown fat conversion.
4.2 | Lgr5

Leucine-rich repeat-containing GPCR 5 (Lgr5), also known as G protein-coupled receptor 49, is another target gene of Wnt, and combined with Wnt ligand R-spondin can regulate Wnt signalling strength. In recent years, Lgr5 has been found to be a self-renewal molecular marker in multiple organs, such as the gut, hair follicles, kidneys, and ovaries.\(^{52,117-120}\) Furthermore, Wnt signalling could be a connection between energy metabolism and bone.\(^{45}\) Canonical Wnt signalling is of great importance to the maintenance of bone homeostasis. The gain of bone mass is closely related to functional acquisition mutations in human LRP5.\(^{121,122}\)

Mitochondria are complex reticular organelles critical for maintaining stem cell pluripotency and differentiation capacity.\(^{123-125}\) Mitochondrial morphology is a significant regulator and indicator to judge cell function and fate.\(^{53,126-128}\) A study showed that inhibiting Lgr5 expression may weaken Wnt signalling, stimulate mitochondrial fragmentation and fission in mouse bone marrow MSCs, and suppress osteogenic differentiation (Table 2).\(^{49}\) In addition, there seems to be a crosstalk between ERK signalling and Wnt signalling.\(^{129,130}\) The dependence of MSCs on osteoblasts requires the mediation of ERK signalling.\(^{131}\) Furthermore, studies have shown that ERK signalling promotes osteogenesis and bone growth by activating the phosphorylation level of RUNX2 and regulating transcriptional activity.\(^{132}\) Lgr5 was also found to be involved in MSCs osteogenic differentiation by regulating Wnt and ERK signalling as well as mitochondrial fusion and fission.

4.3 | Sclerostin

Sclerostin is a Wnt inhibitor that participates in regulating bone remodelling, and it is one of the markers for bone metabolism in clinical research.\(^{133-138}\) Specifically, sclerostin, a glycoprotein mainly secreted in the mineralized bone matrix, inhibits the Wnt/β-catenin signalling pathway, leading to the suppression of bone formation, which is important for osteoblast development and function.\(^{137,138}\) Additionally, the role of sclerostin in glucose metabolism has been proven to regulate insulin secretion and sensitivity and increase energy metabolism.\(^{133,136,139}\)

Another research reported that sclerostin can regulate both catabolism and anabolism in adipocytes and inhibit the Wnt/β-catenin signalling pathway, showing an endocrine function.\(^{50}\) In addition, mice lacking sclerostin have increased insulin sensitivity together with lower adipose tissue accumulation.\(^{50}\) Therefore, as a regulator of the Wnt/β-catenin signalling pathway, sclerostin can regulate not only bone metabolism but also adipose tissue metabolism.\(^{134,140}\) Since adipocytes cannot produce sclerostin, the studies suggest that sclerostin promotes communication between the bone and adipose tissue.\(^{134,140}\)

4.4 | Wnt proteins

Wnt proteins are members of the secreted glycoprotein family, which regulates multiple cell functions.\(^{141}\) Wnt proteins function through canonical and non-canonical pathway. The former depends on the stability of β-catenin and is significant for the maintenance of bone mass. In particular, Wnt proteins integrate with Frizzled and LRP5/6 receptors, thereby stimulating downstream signalling and inhibiting the activity of GSK3β, which consequently prevents β-catenin phosphorylation. In contrast, when lacking Wnt ligands, GSK3β phosphorylates β-catenin, ultimately leading to its ubiquitination.\(^{145}\) Wnt pathways can be regulated by several families of secretory antagonists and regulators, such as secreted frizzled-related proteins, which antagonize both the canonical and non-canonical pathways as decoy receptors, and Dickkopfs and sclerostin, which can combine with LRP5/6 receptors through various mechanisms and specifically inhibit canonical Wnt signalling.\(^{142,143}\)

4.4.1 | Wnt3a

Many signalling pathways, such as Wnt3a and BMP2, can regulate the osteogenic differentiation of MSCs and stromal cells.\(^{144}\) Wnt3a and BMP2 have little effect on glycolysis but promote mitochondrial OCRPHOS in osteogenic media.\(^{51}\) A dramatic increase in mitochondrial oxygen consumption in long bone and skull-derived osteoprogenitors during osteogenic induction caused by Wnt3a or BMP2 is a mutual characteristic (Table 2).\(^{51}\)

Currently, Wnt signalling is independent of β-catenin and directly regulates glucose metabolism.\(^{29}\) In addition, Wnt3a activates downstream mammalian/mechanistic target of rapamycin complex (mTORC) 2 and AKT via LRP5 and RAC1 signalling, leading to the upregulation of key enzymes in glycolysis (Table 2).\(^{29}\) Functionally, in vitro, the regulation of metabolism is beneficial to Wnt-promoted osteogenic differentiation; in vivo, the regulation of metabolism is related to the bone formation through LRP5 signalling.\(^{29}\)

Wnt3a, Wnt7b, and Wnt10b are known to facilitate osteoblast differentiation of the ST2 cell line, and they activate glucose consumption and promote lactate production. Wnt3a could promote the expression of pyruvate dehydrogenase kinase 1, which negatively regulates pyruvate dehydrogenase activity, thereby reducing the amount of pyruvate derived from glucose entering the TCA cycle.\(^{145}\) Apart from glycolysis and fatty acid metabolism, Wnt3a also activates glucose metabolism in the TCA cycle through mTORC1 (Table 2).\(^{2}\) In osteoblasts, glycolysis and OCRPHOS can produce energy.\(^{73,93}\) Osteogenic drugs such as parathyroid hormone and Wnt3a exert therapeutic effects by increasing aerobic glycolysis in osteoblasts.\(^{29}\)

4.4.2 | Wnt7b

As a potent Wnt ligand, an in vitro study showed that Wnt7b promotes bone formation, increases bone mass, and inhibits osteoclastogenesis (Figure 1).\(^{146}\) Furthermore, increased Wnt levels in cells of the macrophage lineage markedly destroy osteoclast formation and activity, resulting in a rapid increase in bone mass.\(^{146}\) The mechanism is that Wnt7b affects the process of glucose metabolism and the activation of AKT during osteoclastogenesis (Table 2).\(^{146}\)
Wnt7b is generally expressed by the osteogenic perichondrium, which benefits the development of long bones in mice, and the deletion of Wnt7b results in delayed osteogenesis in mouse embryos.\(^{147,148}\) In contrast, the overexpression of Wnt7b in osteoblasts significantly promotes bone formation in mice.\(^{149}\) Genetic studies from humans and mice have provided strong evidence that Wnt signalling plays an important role in the control of bone mass. In osteoblast lineage cells, Wnt7b activates protein kinase C and mTORC1 instead of \(\beta\)-catenin signalling.\(^{147,149,150}\) Bone anabolism by Wnt signalling is closely associated with not only glucose metabolism but also fatty acid metabolism.\(^{21}\)

In vitro experiments in a study demonstrated that decreased glucose metabolism in osteoblast lineage cells is closely involved in the loss of glucose transporter 1 (Glut1), suggesting that other glucose transporters are unable to compensate for the function.\(^{151}\) Glut1 deletion might remarkably reduce ATP levels in cells and decrease the intermediate metabolites needed for energy metabolism in the process of bone formation. In addition, another in vitro study proved that Wnt7b significantly increased Glut1 protein production with little effect on mRNA levels, but the mechanism remained unclear.\(^{151}\) Wnt7b promoted Glut1 levels and increased glucose expenditure when osteoblast cell lines were cultured, and Glut1 deficiency inhibited osteoblast differentiation in vitro. As a result, part of the effect of Wnt7b in promoting bone formation is achieved by facilitating glucose metabolism in osteoblast lineage cells.

### 4.4.3 | Wnt10b

Wnt10b/\(\beta\)-catenin canonical signal transduction promotes the differentiation of MSCs into osteoblast cells, while PPAR\(\gamma\)2 promotes their differentiation into adipocytes (Figure 1).\(^{152-155}\) Functionally, PPAR\(\gamma\)2 and canonical Wnt signalling pathways are interconnected, since the Wnt10b/\(\beta\)-catenin signalling pathway could significantly inhibit PPAR\(\gamma\)2 activity and adipogenesis, and in comparison with this, PPAR\(\gamma\)2 restrains the Wnt10b/\(\beta\)-catenin signalling pathway as well as osteogenesis (Table 2).\(^{152,156}\) Surprisingly, selectively activating the anti-osteoblastic features of PPAR\(\gamma\)2 suppresses Wnt10b expression, whereas selectively activating the pro-adipocytic features of PPAR\(\gamma\)2 has little effect on Wnt10b expression, which suggests that the two pathways could not be completely reciprocal and may be partially dependent on other mechanisms.\(^{156}\)

The activities of Wnt10b in promoting osteogenic differentiation and inhibiting adipogenic differentiation have been demonstrated in numerous studies.\(^{152,153,157,158}\) Specifically, the overexpression of Wnt10b can induce MSCs' osteogenic differentiation and inhibit the expression of PPAR\(\gamma\)2, while ectopic expression of Wnt10b in adipocytes increases animal bone mass and compensates for bone loss with ageing.\(^{152,157}\) In contrast, Wnt10b-deficient mice showed reduced bone mass and limited proliferation and differentiation ability, with an accumulation of fat in muscle cells.\(^{153,159}\) Wnt10b overexpression in hyperinsulinaemia and insulin resistance models ameliorated insulin sensitivity resistance, which markedly decreased fat mass, which is beneficial to glycaemic homeostasis.

Recent studies have implicated Wnt signalling in the regulation of lipid metabolism in bone. Stimulation with the ligand Wnt10b increases fatty acid metabolism gene expression and stimulates lipid oxidation in bone. Mechanistically, the regulation by Wnt10b appears to be \(\beta\)-catenin dependent as GSK3\(^\beta\) inhibition or \(\beta\)-catenin overexpression is sufficient to stimulate fatty acid metabolism in osteoblasts.\(^{61}\)

### 5 | CONCLUSIONS

This article reviews the role of the Wnt signalling pathway in cellular energy metabolism in bone remodelling and the related molecular mechanisms. By summarizing the new evidence, we found that the Wnt signalling pathway controls glucose metabolism, glutamine metabolism, and fatty acid metabolism in bone in connection with other signalling pathways, and the bone anabolic function of Wnt signalling is associated with increased energy metabolism in osteoblast-lineage cells. Abnormal expression of genes related to the pathway will lead to several metabolic diseases such as osteoporosis. In addition, Wnt signalling is associated with multiple biological processes (such as osteoblast differentiation) regulating energy metabolism in bone cells, suggesting its important function in bone remodelling. Wnt molecules (Wnt3a, Wnt7b, and Wnt10b) are associated with both bone remodelling and energy metabolism, which are important bridges between these processes. However, the latest studies mostly concentrate on the impact of energy metabolism disorders on disease occurrence. Further research is still required to determine how metabolic diseases impair energy metabolism and the function of bone homeostasis regulation. Thus, targeted regulation of Wnt signalling and the recovery of the energy metabolism function of bone cells are key means for the treatment of metabolic bone diseases.

### AUTHOR CONTRIBUTIONS
Mengyuan Zhu: design and conception, search of literature, manuscript writing, creation of figure and table, and final approval of the manuscript. Zhipeng Fan: design and conception, manuscript revising, financial support, and final approval of the manuscript. All authors read and approved the final paper.

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### CONFLICT OF INTEREST
The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT
All data used to support the findings of this study are included within the article.
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