Assessment of Sclerostin as a Bone Metabolism Marker in Egyptian Children with Nephrotic Syndrome

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Abstract

Background: Nephrotic syndrome (NS) is one of the common chronic diseases observed in the childhood. Idiopathic nephrotic syndrome (INS)is the most common type , it constitutes about 90% of NS in the childhood. In children, nephrotic syndrome (NS) is defined as protein excretion of more than 40 mg/m2/h or a first-morning urine protein/creatinine of 2-3 mg/mg creatinine or greater. it is composed of NS and primary glomerular disease without an identifiable causative disease or infection, up to 85%–90% of children with INS are steroid sensitive, but follow a relapsing and remitting course in the majority of cases. Sclerostin (SOST) is a glycoprotein and a product of the SOST gene located on chromosome 17q12-q21 , It is not only secreted from osteocytes, but also by other cells such as chondrocytes, cementocytes as well as from the kidney and liver. Sclerostin negative regulator of bone formation, has been originally known as an osteocyte product. Recently, it has been also detected in hypertrophic chondrocytes, distinctive cells of avascular cartilage which is invaded by capillaries and then replaced by vascularized bone. In the treatment of idiopathic nephrotic syndrome (INS) in children, glucocorticosteroids (GCSs) remain the main medication. Recurrent nature of the disease and steroid dependence imply a long-term therapy and probably increa sed risk of bone metabolism disorders. To this time, pediatrics population has not been analyzed in the context of changes in bone parameters during steroid therapy.

Keywords: Sclerostin, Bone Metabolism, Nephrotic Syndrome

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Nephrotic syndrome (NS) is one of the common chronic diseases observed in the childhood. Idiopathic nephrotic syndrome (INS)is the most common type , it constitutes about 90% of NS in the childhood **.(1)** (INS) describes a group of pathologies of the renal glomerulus that result in the classic triad of heavy proteinuria, oedema and hypoalbuminemia**.** Nephrotic syndrome is defined as urine protein excretion (urine protein-to creatinine ratio ≥2000 mg/g or ≥300 mg/dL on urine dipstick), hypoalbuminemia (serum albumin ≤2.5 g/dL), and edema. Nephrotic syndrome may be accompanied by hypercholesterolemia and hypertriglyceridemia, which usually improves as proteinuria improves**.** In children, nephrotic syndrome (NS) is defined as protein excretion of more than 40 mg/m2/h or a first-morning urine protein/creatinine of 2-3 mg/mg creatinine or greater**.** It is composed of NS and primary glomerular disease without an identifiable causative disease or infection, up to 85%–90% of children with INS are steroid sensitive, but follow a relapsing and remitting course in the majority of cases**. (2)**

The incidence of idiopathic nephrotic syndrome varies with age and other ecological factors, the peak age of presentation of nephrotic syndrome is 2 years, and 70–80% of cases of nephrotic syndrome occur in children less than 6 years of age. Patients' characteristics vary according to clinical and histopathological presentation and response to therapy. These changes may be owing to differences in genetic, geographic, and environmental factors **(3).**

The mean age of onset was 4.43 ± 2.7 years in Egypt . Thirty-four percent of patients were steroid resistant, and 66% showed initial steroid response; 46 of the latter were steroid dependent. Forty patients underwent a renal biopsy with minimal change nephrotic syndrome occurring in 30%, mesangioproliferative glomerulonephritis in 37.5% and focal segmental glomerulosclerosis in 30%. Nine percent of cases developed chronic renal insufficiency. Response to cyclophosphamide and cyclosporine occurred in 37.5% and 33.3% of steroid-resistant nephrotic syndrome patients, respectively **(4).**

Sclerostin (SOST) is a glycoprotein and a product of the SOST gene located on chromosome 17q12-q21 , It is not only secreted from osteocytes, but also by other cells such as chondrocytes, cementocytes as well as from the kidney and liver . Sclerostin, the product of the *SOST* gene has primarily been studied for its profound impact on bone mass. By interacting with LRP5 and LRP6, the glycoprotein suppresses the propagation of Wnt signals to β-catenin and thereby suppresses new bone formation. **(5)**

sclerostin has been identified as an important regulator of bone homeostasis through inhibition of the canonical Wnt-signaling pathway, and it is involved in the pathogenesis of many different skeletal diseases. **(5)**

Sclerostin negative regulator of bone formation, has been originally known as an osteocyte product. Recently, it has been also detected in hypertrophic chondrocytes, distinctive cells of avascular cartilage which is invaded by capillaries and then replaced by vascularized bone.Sclerostin is a key molecular coordinator of both bone formation and bone resorption **(6).**

sclerostin expression is regulated by complex mechanisms that involve crosstalk between hormones and mechanical stimuli, through activation of transcription factors and chromatin epigenetic modifications**(6).**

sclerostin inhibits Wnt/β-catenin signaling in osteoblasts to regulate bone mass.(Riddle, 2023)

sclerostin inhibits the canonical Wnt-signaling pathway, and through this action, it controls bone formation by osteoblasts. Numerous research papers underline sclerostin’s involvement in the pathogenesis of many skeletal disorders. **(5)**

Genetic studies in humans and mice have revealed that Wnt signaling pathway plays a crucial role in skeletal development and homeostasis, especially by modulating bone formation through the control of progenitor cells proliferation, commitment, and differentiation. Wnt pathway inactivation may lead to bone related disorders like osteoporosis**(7).**

Genetic and pharmacologic inhibition of Sost/sclerostin expression markedly affects skeletal homeostasis resulting from potent stimulation of bone formation and inhibition of bone resorption. **(6).**

sclerostin increases osteoclastogenesis by stimulating the expression of RANK-L. Based on the adverse effects of sclerostin on bone, monoclonal antisclerostin antibodies have been developed for osteoporosis treatment. Studies support that inhibition of sclerostin action results in increased bone strength and resistance to fractures. Besides, Wnt activation by the blocking effect of sclerostin leads to decreased bone resorption **(8)**

Although sclerostin is mainly secreted by osteocytes, it has also been found in chondrocytes and in osteoclasts. With the exception of bone, sclerostin has been found in the lungs, kidneys, and liver as well as in the epididymis, pyloric sphincter, cerebellum, and the embryonic hand . Elevated serum sclerostin levels were found in vascular calcifications in humans with and without renal disease **. (5)**

A critical advance in our understanding of skeletal biology of the last few years was the discovery of the role of Wnt/βcatenin signaling in bone. Wnt/βcatenin signaling is activated by binding of Wnt proteins to receptor complexes composed of frizzled receptors and co-receptors of the low density lipoprotein receptor-related protein (LRP) family, LRP5 and 6 **(6).**

Skeletal remodeling is driven in part by the osteocyte's ability to respond to its mechanical environment by regulating the abundance of sclerostin, a negative regulator of bone mass. **(9**)
Osteocytes are key players in the regulation of the canonical Wnt signaling pathway as producers and targets of Wnt ligands and as secretors of molecules that modulate Wnt actions. A potent antagonist of Wnt signaling secreted by osteocytes is sclerostin, a protein encoded by the Sost gene primarily expressed by mature osteocytes but not by early osteocytes or osteoblasts. Sclerostin binds to the Wnt co-receptors LRP5/6 antagonizing downstream signaling. Sclerostin also interacts with LRP4, another member of the LRP family of proteins, which acts as a chaperone and is required for the inhibitory action of sclerostin on Wnt/βcatenin signaling. Osteoblast cells are energetic in protein synthesis and matrix secretion to preserve and form new healthy bones. Following mineralization of bone matrix, fully differentiated and matured osteoblasts become osteocytes and are implanted in the bone matrix. **(10)**

genetic deletion of Sost or LRP4 in mice or neutralizing antibodies for sclerostin or LRP4 reproduce the high bone mass phenotype found in humans lacking sclerostin or LRP4 activity. In contrast, overexpression of Sost/sclerostin decreases bone mass (**10**).

Cellular sources of Sost/sclerostin
Osteocytes, osteoblasts (bone-forming cells), and osteoclasts (bone-resorbing cells) are the primary types of cells that regulate bone metabolism in mammals. Sclerostin produced in bone cells activates osteoclasts, inhibiting bone formation; excess production of sclerostin, therefore, leads to the loss of bone mass.**(11)**
Sost/sclerostin is a marker of mature osteocytes and its expression progressively increases as osteocytes mature and acquire their full molecular signature. Thus, sclerostin is rarely detected in recently embedded osteocytes (osteoid osteocytes) or in osteocytes close to bone forming surfaces. In contrast, high levels of sclerostin are found in osteocytes surrounded by mineralized bone and distant from active bone surfaces (**11).**
Sost/Sclerostin expression has been also detected in bone marrow derived-osteoclast precursors and found to decrease as osteoclasts are formed in vitro. In addition, higher levels of sclerostin are produced by osteoclast cultures established from bone marrow of old compared to young mice, suggesting that osteoclast-derived sclerostin may contribute to the decrease in bone formation that ensues with aging. (**11**).
Regulation of Sost expression :
Sclerostin expression progressively increases during osteocyte maturation, and higher levels were reported in osteocytes surrounded by mineralized bone.**(12)**
In vitro studies using a reporter construct carrying the proximal Sost promoter also identified forskolin and oncostatin M as potential regulators of Sost expression. In addition to PTH, other systemic hormones have a strong impact on Sost regulation. Androgens and estrogens inhibit Sost expression, although the mechanisms are still unclear. In contrast, excess of glucocorticoids increases Sost and sclerostin production in osteocytes. Vitamin D can also regulate Sost expression, although contradictory data has been published. Thus, using Saos-2 cells, It was showed that 1α,25-dihydroxyvitamin D3 induces the expression of Sost/Sclerostin, whereas It was found that it decreases the transcriptional activity of the Sos t promoter. Similarly conflicting clinical data on the effects of vitamin D status on circulating sclerostin levels have been reported. Furthermore, tumor necrosis factor alpha and Tnf-related weak inducer of apoptosis increase Sost expression. In addition, glucose and advanced glycation end products increase Sost expression in vitro, and Sost/sclerostin expression is increased in mice with diabetes type 1 **(13)**.
The anabolic effects of PTH on bone are exerted in part by inhibiting the expression of sclerostin**.** Sclerostin is a key osteocyte-derived factor that suppresses bone formation and promotes bone resorption, therefore regulators of sclerostin secretion are a likely source of new therapeutic strategies for treatment of skeletal disorders. Deletion of CK2 regulatory subunit, *Csnk2b*, in osteocytes ($Csnk2b^{Dmp1}$) results in low bone mass due to elevated levels of sclerostin.**(14)**
SOST/SCLEROSTIN FUNCTION

Sclerostin has been identified as an important regulator of bone homeostasis through inhibition of the canonical Wnt-signaling pathway, and it is involved in the pathogenesis of many different skeletal diseases. **(5)**

Studies with genetically modified mice provided insights into the mechanism of action of Sost/sclerostin in bone. Deletion of the Sost gene from the murine genome reproduced the high bone mass hallmark of humans with inheritable sclerostin deficiency as in cases of sclerosteosis or Van Buchem disease. This phenotype is driven by increased bone formation on all bone surfaces (**14).**

In the 1950s, sclerosteosis and van Buchem disease, two rare autosomal recessive disorders characterized by massive and progressive bone overgrowth, were described for the first time , Since both diseases are clinically and radiographically very similar, it was speculated that these two conditions result from mutations in the same gene. Sclerosteosis and van Buchem disease are characterized by a marked increase in bone formation that cannot be compensated by bone resorption by osteoclasts; therefore, it was hypothesized that sclerostin negatively regulates osteoblast differentiation and/or function**.(15)**

- **Roles of Sclerostin in Bone Development and Bone Formation**

Sclerostin was initially considered to be an inhibitor of bone morphogenetic protein (BMP) because of its homology to the differential screening-selected gene in neuroblastoma family members, such as the BMP antagonist noggin. Immunoprecipitation assays showed that sclerostin bound to BMP6 and that this binding was competitively inhibited by the BMP receptor BMPRIA. Sclerostin mildly suppressed the phosphorylation of SMAD by BMP6 and the mineralization of human mesenchymal cells.**(16)**

Sost/sclerostin stimulates bone resorption

Wnt/βcatenin signaling inhibits osteoclast formation directly by acting on osteoclast precursors, as knockout of βcatenin in these cells increases osteoclast number and enhances resorption**. (6).**

Research has shown that the canonical Wnt/β-catenin signaling is a critical regulator of osteoclastogenesis . During the inhibition of canonical Wnt signaling, the expression of OPG is decreased, thereby increasing the RANKL/OPG ratio, and, thus, bone resorption . It was showed that, in response to RANKL, β-catenin in osteoclast precursors is downregulated, which is needed to allow the differentiation of osteoclast precursors towards mature osteoclasts. Furthermore, It was demonstrated that osteoclast lineage cells express canonical Wnt receptors , indicating that sclerostin might have direct0 0001effects on osteoclast formation and maturation.**(17)**

- **Role of Sclerostin in Skeletal Diseases**
  - **Osteoporosis:**

Osteoporosis is characterized by lower bone mineral density due to an imbalance between bone formation and bone resorption**. (5)**

Sclerostin, as an inhibitor of the Wnt-signaling pathway, prevents osteoblastogenesis and OPG production, suppresses osteoclast-mediated bone formation, and increases bone resorption by stimulating RANKL expression from osteocytes. Considering its role in bone homeostasis, targeting sclerostin with the antisclerostin antibody was evidenced as an effective treatment of osteoporosis **(5)**

  - **Osteonecrosis:**

In patients with nontraumatic osteonecrosis of the femoral head, serum sclerostin levels were lower than controls, and the decrease was related to the stage of osteonecrosis, meaning the higher the stage, the lower the levels of serum sclerostin that were measured . Additionally, sclerostin expression in the bone sections of femoral heads with osteonecrosis was significantly reduced compared to controls. **(5)**

  - **Osteoarthritis:**

SOST-gene expression has been detected in hypertrophic chondrocytes and was found elevated in degenerative cartilage . Interestingly, sclerotic subchondral bone in osteoarthritis exhibits decreased sclerostin expression, possibly because of increased mechanical forces. These findings suggest that sclerostin is involved in osteoarthritis pathogenesis. **(5)**

  - **Rheumatoid Arthritis:**

Rheumatoid arthritis is characterized by decreased bone mass, bone and cartilage erosions, and systemic inflammation and is associated with increased serum sclerostin levels and reduced Wnt signaling **(5).**

- **ROLE OF SCLEROTIN IN THE BONE LOSS INDUCED BY GLUCOCORTICOIDS**

the synthetic glucocorticoid dexamethasone alters the anabolic Wnt signaling pathway. The Wnt pathway inhibitor sclerostin has several glucocorticoid response elements, and dexamethasone administration to osteoblastic cells induces sclerostin expression. **(18)**
Glucocorticoid excess is a leading cause of bone fragility worldwide, and the mechanisms activated by the hormone and how to interfere with its actions in bone are a matter of intense investigation**. (6).** Recent findings demonstrate that glucocorticoids hinder the expression of target genes of the Wnt/βcatenin pathway, regardless of whether they are associated with bone anabolism (i.e. bone gain) or anti-catabolism (i.e. inhibition of bone loss). This action of the hormone is exerted, at least in part, by increasing the expression of Sost/sclerostin**. (6).**
Chronic GC therapy leads to decreased bone mineral density, and up to 50% of treated patients experience vertebral fractures**.** Sclerostin-deficient mice display increased bone formation, bone mass and strength, underlining its negative regulation of bone homeostasis , Blocking sclerostin using specific antibodies has already been shown to restore bone mass**(19)**

Bone mineralization is a complex multifactorial process, influenced by genetic, hormonal, and nutritional factors during the entire lifespan. Different studies highlighted a deleterious impact of corticosteroids on bone metabolism. Biochemical markers of bone metabolism and densitometry are useful to trace the bone turnover; however, the interpretation of the results in children is difficult as many factors like age, gender, pubertal stage, race, nutritional, and health status may weigh in **(19)**

. However, it is unclear whether this phenomenon is due to direct regulation of transcription of the Sost gene or to indirect mechanisms. Nevertheless, Sost/sclerostin deficiency protects against the loss of bone mass, deterioration of microarchitecture, and reduction of extrinsic/structural and intrinsic/material mechanical properties induced by glucocorticoids. Remarkably, however, the bone protective effect of Sost/sclerostin deficiency against glucocorticoids is not due to an opposing action to increase bone formation and maintain anabolic signaling. Instead, it is due to preservation of the Wnt/βcatenin anti-catabolic cellular and molecular signature. Therefore, this pathway which is predominantly anabolic for bone is switched to anti-catabolic in the frame of glucocorticoid excess. These findings suggest that therapeutic interventions targeting sclerostin and activating Wnt/βcatenin signaling could effectively halt the high bone resorption responsible for the profound and rapid bone loss induced by glucocorticoids, which in humans can reach up to 12% during the first year of treatment **(13)**.
Consistent with these findings in skeletally mature Sost/sclerostin deficient mice, pharmacologic inhibition of sclerostin with a neutralizing antibody opposed the lack of bone gain and the loss of strength induced by glucocorticoids in growing mice, Likewise, sustained activation of the Wnt/βcatenin signaling in Sost deficient mice abolished the increase in resorption induced by glucocorticoids but not the decreased bone formation (**20**).
Moreover, the decrease in Opg and increase the Rankl/Opg ratio induced by glucocorticoids is abolished in bones from Sost−/− mice or in WT bones treated with an anti-sclerostin antibody. Taking together, these findings demonstrate that Sost/sclerostin deficiency, either genetic or pharmacologically achieved, maintains bone mass and strength in conditions of glucocorticoid excess by inhibiting bone resorption, through sustained anti-catabolic signaling driven by Opg.

A clinical case reported that glucocorticoids stop the exaggerated bone gain and reduced the high circulating P1NP in a patient with Van Buchem disease, in which impaired production of sclerostin expression leads to continuous bone anabolism causing life-threatening increased intracranial pressure. The findings that bone formation and Wnt/βcatenin anabolic signaling is still decreased in Sost/sclerostin deficient mice treated with glucocorticoids provide a mechanistic explanation for these clinical findings. Taken together, these pieces of evidence demonstrate that glucocorticoids oppose the effects of Sost/sclerostin deficiency on bone formation in both humans and mice.

Another unwanted consequence of glucocorticoid excess, either endogenous or iatrogenic, is muscle weakness; which reduces body balance and, when combined with lower bone mass, increases the risk of bone fractures. Due to their intimate association as a mechanical unit, changes in bone could potentially impact skeletal muscle and vice versa. The mouse model of glucocorticoid excess faithfully reproduces the bone and skeletal muscle atrophy exhibited by humans **(13)**. However, the bone preservation resulting from Sost/sclerostin deficiency did not protect from muscle atrophy; and conversely, the marked loss of muscle mass experienced by the Sost deficient mice did not translate into apparent detrimental effects on bone volume or mechanical properties. These findings demonstrate that Sost/sclerostin deficiency protects exclusively bone, but not muscle, from the action of glucocorticoids, and show a lack of crosstalk between these two tissues in the frame of glucocorticoid-induced musculoskeletal atrophy. Future studies are warranted to investigate whether muscle-derived factors contribute to the low bone formation and high prevalence of osteoblast and osteocyte apoptosis still exhibited by Sost/sclerostin deficient mice treated with glucocorticoids

1. ROLE OF SCLEROSTIN (AND DKK1) AS NEGATIVE FEEDBACK INHIBITORS OF WNT SIGNALING-DRIVEN BONE FORMATION

We recently showed that genetic activation of βcatenin signaling in osteocytes increases bone formation and resorption leading to bone gain. Bone anabolism is achieved by enhancing osteoblast to osteocyte differentiation, and, consequently, the expression of osteocyte markers, including Sost/sclerostin and Dkk1, is increased. Similarly, the activation of Wnt/βcatenin signaling and increased bone formation exhibited by rodents receiving neutralizing antibodies against sclerostin is also accompanied by increased expression of both Sost and Dkk1. Upregulation of the Wnt antagonists might temper the anabolic response triggered by activation of the pathway to protect from excessive bone gain and could explain at least partially the reduced anabolic potency that presents with prolonged sclerostin inhibition and the decline of bone formation makers after each dose of anti-sclerostin antibody (**17).** Consistent with this notion, dual inhibition of sclerostin and Dkk-1 with a novel bispecific antibody result in greater increases bone formation when compared to neutralization of each of the Wnt antagonists alone. Taken together, these findings suggest that Sost and Dkk1 act as a negative feedback mechanism that limits Wnt-driven bone formation.

2. ROLE OF SOST/SCLEROSTIN IN MULTIPLE MYELOMA BONE DISEASE

In multiple myeloma (MM) increased numbers of monoclonal plasma cells in the bone marrow induce localized osteolytic lesions that rarely heal, due to increased bone resorption and suppressed bone formation. Serum sclerostin levels are elevated in MM patients, which correlate with reduced osteoblast function and poor survival. Recent studies demonstrate that osteocytes contribute to MM generating a microenvironoment that is conducive to enhanced bone resorption and suppressed bone formation, and that osteocytes in mice bearing MM tumors express elevated Sost/sclerostin. Further, direct cell-to-cell contact with MM cells increases Sost/sclerostin expression in osteocytes and sclerostin accumulated in culture media decreases Wnt signaling and inhibits osteoblastic gene expression. Sost/sclerostin expression has also been detected in CD138+ cells from MM patients; and more recently it has been suggested that osteoblasts could also be a potential source of this protein in MM. Regardless of the cell source, recent findings suggest that blockade of Sost/sclerostin action could ameliorate MM bone disease. Thus, genetic deletion of the Sost gene prevented the bone loss and decreased osteocytic lesions in an immunodeficient/SCID MM model of MM. Moreover, administration of a neutralizing anti-sclerostin antibody reduced the development of osteolytic lesions and the decrease in circulating bone formation markers exhibited by immunocompetent mice with established MM. In these studies, Sost deficiency or pharmacological inhibition of sclerostin did not alter tumor burden.

Similarly, pharmacologic inhibition of sclerostin reversed the osteolytic bone disease in a humanized MM xenograft mouse model bearing human MM cells, also without affecting tumor growth; and combination of anti-sclerostin therapy with the anti-tumor drug Bortezomib decreased tumor burden and improve bone disease. Further, simultaneous injection of MM cells together with an anti-sclerostin antibody prevented the development of MM bone disease and suppressed tumor growth **(6).** Future research is warranted to identify the factors involved in the dysregulation of Sost/sclerostin in MM and the mechanisms underlying the protective effects on bone of inhibition of sclerostin in these preclinical models of MM and to develop therapeutic approaches based on pharmacological inhibition of sclerostin in combination with other anti-tumor drugs to synergistically decrease MM growth and prevent bone disease.

In addition, sclerostin may play a role in other cancers that grow in bone, as patients with prostate cancer exhibit high serum sclerostin levels compared to healthy subjects; and breast cancer cells have been reported to produce sclerostin and inhibit osteoblast differentiation. Thus, future studies are also needed to investigate the contribution of sclerostin to other cancers that grown in bone and weaken the skeleton **(21).**

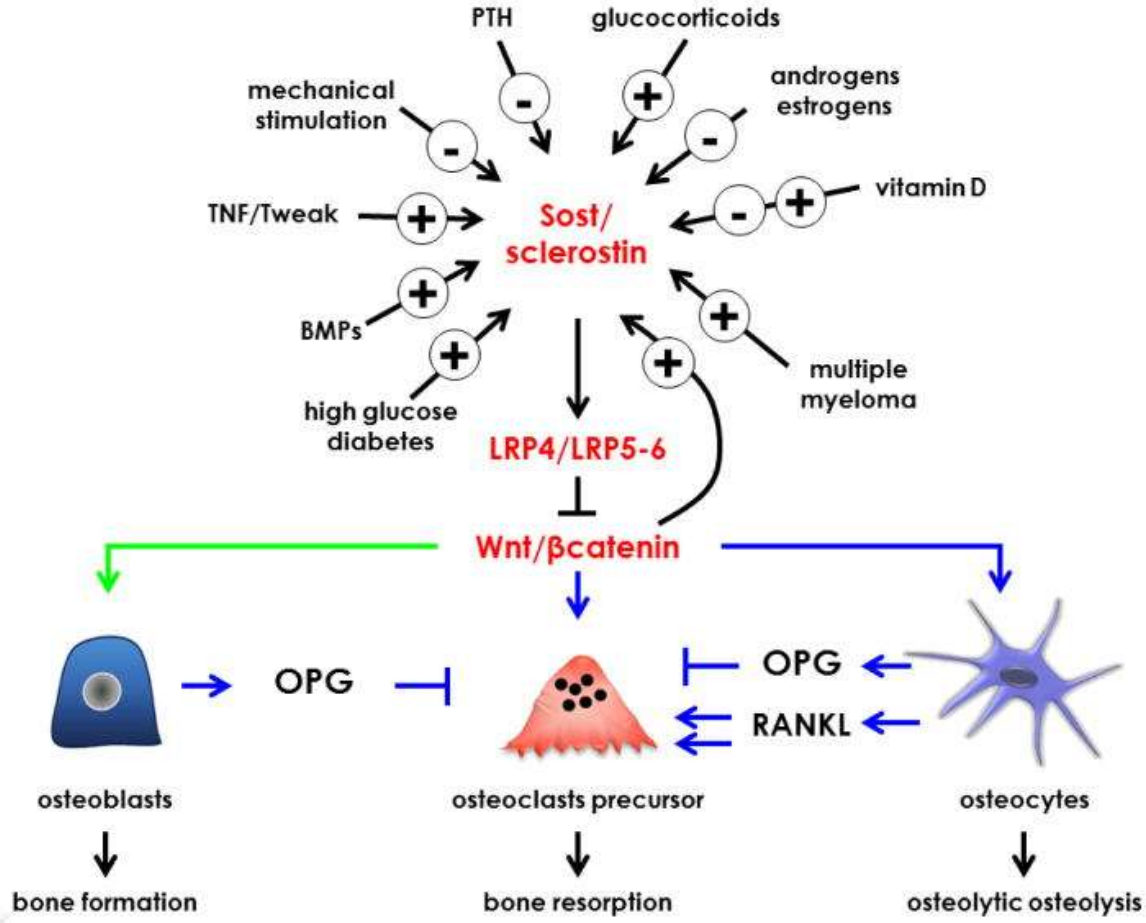

Figure 1 Role and mechanism of action of Sost/sclerostin in bone

The expression of Sost/sclerostin is tightly regulated by complex mechanisms involving crosstalk between systemic hormones, cytokines and mechanical stimuli (black lines). The chaperone LRP4 presents sclerostin to the Wnt co-receptors LRP5/6, thus facilitating sclerostin inhibition of Wnt/βcatenin signaling. The consequent inhibition of the Wnt/βcatenin pathway leads to decreased bone formation due to impaired osteoblastogenesis and decreased osteoblast survival (green line). Novel findings suggest that activation of osteocytic Wnt/βcatenin signaling itself increases the expression of Sost/sclerostin, which in turn acts in a negative feedback limiting bone formation driven by the pathway. Sclerostin not only regulates bone formation, but also bone resorption (blue lines). Inhibition of Wnt/βcatenin signaling in osteoblasts and osteocytes decreases the expression of Opg, and direct actions of sclerostin on osteocytes increase the expression of RANKL. In addition, inhibition of Wnt/βcatenin signaling in osteoclast precursors directly favors their differentiation. Thus, by antagonizing Wnt/βcatenin signaling, sclerostin has the potential to

stimulate osteoclast differentiation and enhance bone resorption. Further, sclerostin increases in osteocytes the expression and activity of enzymes that remodel perilacunar matrix leading to osteolytic osteolysis (**22).**
In the treatment of idiopathic nephrotic syndrome (INS) in children, glucocorticosteroids (GCSs) remain the main medication. Recurrent nature of the disease and steroid dependence imply a long-term therapy and probably increa sed risk of bone metabolism disorders. To this time, pediatrics population has not been analyzed in the context of changes in bone parameters during steroid therapy. **(23).**
The mechanism of GCS impact on bone tissue is complex and depends on the duration of therapy. In the early period of treatment, bone resorption may be observed as a result of increased number and prolonged survival time of osteoclasts. In the case of long-term steroid treatment, a reduction in the number and impairment of the function of osteoblasts occur, which results in the inhibition of bone formation process. GCS suppresses the differentiation of the bone marrow stromal cells in osteoblasts, through inhibiting the signaling pathway of Wnt/B-catenin and through promoting the transformation of stromal cells into adipocytes **(23).**
The changes in bone metabolism due to glucocorticosteroid treatment also result from inhibition of transcription of the insulin-like growth hormone (IGF-1), reduction of intestinal calcium absorption, renal calcium reabsorption, modulation of the secretion of parathyroid hormone (PTH), and reduction of the secretion of growth hormone and gonadotropins **(23).**
The nephrotic syndrome is a glomerular disease with massive proteinuria exceeding 50 mg/kg/24 h, hypoalbuminemia, hyperlipidemia, and edema, usually responding to corticosteroids. The idiopathic nephrotic syndrome is the most common form in children, with the incidence of 2–7/100,000 children aged below 16. Based on the response to corticosteroids, the disease is categorized as steroid-sensitive, steroid-dependent, and steroid-resistant. Long-term application of corticosteroids in children with nephrotic syndrome may lead to bone metabolism disorders, whose mechanisms are underlain by both steroid therapy and biochemical changes due to proteinuria that influences bone turnover and mineralization **(23).**
The onset and subsequent relapses of nephrotic syndrome, requiring steroid treatment for several months, frequently coincide with periods of maximal bone mineral accretion; thus bone mineral density can be impaired in the adulthood. Corticosteroids reduce bone formation by inhibition of bone matrix synthesis, induction of apoptosis of osteoblasts, and by promotion of osteoclastogenetic-related bone resorption. In effect, suppression of bone formation, osteoporosis, and impaired growth may develop. Corticosteroids also affect bone indirectly by reducing intestinal calcium absorption and increasing urinary calcium loss **(3).**

It affects bone formation by inhibiting the Wnt signaling Scl is a protein encoded by SOST gene, located on chromosome 17q12-q21, synthesized mainly by osteocytes.pathway in osteoblasts. In studies on mice with induced Scl deficiency, **Ryan et al.** observed the increase in bone mass due to increased activity of osteoblasts with simultaneous decrease in activity of osteoclasts. FGF-23 is a circulating secretory protein of molecular weight of 32 kDa, encoded by the FGF-23 gene, mainly expressed in osteocytes. The results of the conducted studies concerning the impact of FGF-23 factor on bone tissue are inconclusive; however, it has been shown that FGF-23 regulates osteoblast differentiation and its absence impairs skeletal mineralization, despite normal or increased levels of calcium and phosphorus. PTH and FGF-23, strongly inhibit proximal tubule phosphate reabsorption by stimulating NaPi-IIa endocytosis and its lysosomal degradation. The biologic activity of FGF-23 on the proximal tubule requires the presence of klotho, expressed mainly in the distal tubule. Klotho acts as an obligatory cofactor for binding between FGF-23 and the FGFR receptor downstream signaling **(3).**

Glucocorticoids

Glucocorticoids (GCs) are steroid hormones, which are essential for life. They are secreted by the adrenal cortex under the control of the hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoids are essential for the normal function of most organ systems and, in both, excess and deficiency can lead to significant adverse consequences.**(24).**

The signaling axis of GC consists of the hypothalamic-pituitary-adrenal (HPA) axis and is influenced by a multitude of factors including neuroinflammation, physical stress, circadian rhythm, and negative feedback. Self-attenuation of GC signaling on GC receptors in the hippocampus, hypothalamus and pituitary gland is designed to stabilize the response.**(18)**

Glucocorticoids (GCs) belong to the class of steroid hormones that are the most widely prescribed drugs to treat autoimmune and inflammatory disorders for many decades**.** Corticosteroids remain the mainstay for treatment of nephrotic syndrome.**(25)**

Excess glucocorticoids also causes muscle weakness with the consequent loss of body balance and increased falls, which in turn contribute to elevate the risk of bone fractures.**(13)**

High levels of GCs can lead to bone loss.**(26)**

Human skeleton during the growth period may be particularly vulnerable to adverse effects of long-term steroid therapy. The studies on children with INS showed that there is a reverse correlation between bone density and cumulative dose of GCS treatment. As a result, there is a need to assess new markers of mineral and bone metabolism in INS children treated with GCS **(23).**

Pukajło-Marczyk, et al., (23) revealed that the plasma concentration of Scl in patients with INS increases along with the duration of GCS therapy, the exponent of which was the number of disease relapses. That observation is consistent with results of study by Yao et al. In mice, the duration of the steroid treatment positively correlated with expression of Scl-encoding gene SOST, associated with the inhibition of activation and maturation of osteoblasts (**20**). Data concerning connection between Scl and GCS therapy are very scarce. On the other hand, the influence of Scl on bone metabolism has been the subject of various studies. In a rat model of postmenopausal osteoporosis due to ovariectomy, treatment with a Scl antibody (Scl-Ab) increased bone mass at all skeletal sites and completely prevented bone loss associated with estrogen deficiency (**25).**

It was conducted on monkeys with intact gonads also revealed clear anabolic effect of Scl-Ab, with marked dose-dependent increases in bone formation on trabecular, periosteal, endocortical, and intracortical surfaces, in a short period of time after the administration of Scl-Ab.

Those results stand in contradiction with the study of **Polyzos et al. (27**), in which the serum concentrations of Scl were lower in the group of postmenopausal women with osteoporosis than those in the group with normal bone density. Dutch authors, in turn, demonstrated that diseases characterized by high bone turnover, i.e., Paget's disease, or in metastatic prostate cancer, are associated with significantly increased Scl concentrations in the serum.

Cejka et al. (28) noted that in patients on hemodialysis, the serum concentrations of Scl were significantly higher compared to those observed in sex- and age-matched healthy controls. Additionally, the concentrations of Scl correlated positively with vitamin $25(OH)D_3$ and calcium and negatively with intact PTH concentrations. Based on the above results, the role of Scl in mineral and bone metabolism remains unclear.

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