Curr Mol Bio Rep (2017) 3:71–78
DOI 10.1007/s40610-017-0061-y

Sclerostin and Adipose Tissue

Keertik Fulzele[1] · **Paola Divieti Pajevic**[1,2]

Published online: 2 May 2017

Abstract
Purpose of Review Since its discovery 15 years ago, inhibition of sclerostin has come to be identified as a potent anabolic agent. The sclerostin neutralizing antibody, romosozumab, is a promising treatment for postmenopausal osteoporosis. Animal studies also show a role of sclerostin in regulating hematopoietic cells and, more recently, adipocytes. This review will summarize the newly discovered effects of sclerostin on white, beige, and bone marrow adipocytes.
Recent Findings Sclerostin treatment of a preadipocytic cell line shows dose-dependent increase in differentiation of white adipocytes. The supraphysiological increase in circulating sclerostin in mice shows an increase in beige adipocytes, i.e., brown adipocyte-like cells in white adipose tissue depots. Sclerostin treatment also increases bone marrow adipogenesis. Moreover, sclerostin-deficient mice show decreased whole body fat due to increased fatty acid oxidation.
Summary These recent findings show that circulating sclerostin could affect the development of all types of adipocytes. However, sclerostin levels vary significantly depending on age, gender, and disease conditions. Moreover, significant variation is present among adipocytes depending on developmental stage and tissue location. Further studies are needed to determine the more precise role of sclerostin on adipocytes.

Keywords Sclerostin · White adipocytes · Beige adipocytes · Osteocytes · Bone marrow adipocytes

This article is part of the Topical Collection on *Molecular Biology of Skeletal Development*

✉ Keertik Fulzele
kfulzele@bu.edu

Paola Divieti Pajevic
pdivieti@bu.edu

[1] Molecular and Cell Biology, Boston University Henry M. Goldman School of Dental Medicine, 700 Albany Street, W201C, Boston, MA 02118, USA

[2] Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Introduction

Sclerostin is a 213 amino acid protein encoded by the gene SOST localized, in human, on chromosome 17 g12-q21. This cysteine-rich protein, with homology to gremlin, is primarily secreted by osteocytes, the bone cells deeply encased into the hard tissue of bone [1, 2]. Sclerostin was first identified in 2001–2002 as the factor responsible for two rare skeletal disorders, sclerosteosis and van Buchem disease, characterized by generalized osteosclerosis [3–5]. Consequently, most of the studies on sclerostin have focused on its role in bone homeostasis. Over the past decade SOST/sclerostin has emerged as a potent regulator of bone formation and a very promising therapeutic target. Phase 2 clinical trial of anti-sclerostin antibody (romosozumab) shows promising potential for osteoporosis treatment [6].

Sclerostin exerts its effect by binding to co-receptors of Wnt-signaling LRP4, LRP5, and LRP6 [7, 8]. Upon binding to these receptors, it inhibits Wnt ligand-receptor binding and subsequent activation of the canonical β-catenin signaling [7]. Mutational analysis of the protein identified a flexible loop and the cysteine knot that are important for the Wnt-signaling inhibition. Asn92 and Ile94 amino acids reside in the second loop and are key residues for binding to LRP5 and LRP6 [9]. LRP4, which acts as chaperone, is also required for the full inhibitory effects of sclerostin [10].

Several factors influence sclerostin circulating levels, including vitamin D, parathyroid state, myeloma, kidney diseases, and arthritis [11–15]. Serum sclerostin is elevated in patients with early chronic kidney disease (CKD) and is negatively correlated with CKD progression [16]. In vitro and in vivo studies, both in humans and in animal models, have demonstrated that parathyroid hormone (PTH) is a potent suppressor of SOST expression and sclerostin synthesis [17–19]. For example, intermittent PTH treatment to female volunteers significantly reduced their circulating sclerostin levels [20] and serum sclerostin is negatively correlated with PTH levels in postmenopausal women [21]. Moreover, serum sclerostin was significantly suppressed in patients with hyperparathyroidism and significantly elevated in hypoparathyroid patients [22].

Given that sclerostin is detected in the circulation [11] and that it targets ubiquitous Wnt-signaling pathway, it raises the possibility that it may exert biological effects in distant cell types and organs. In this review, we summarize the role of sclerostin in bone cells, its paracrine effects on hematopoietic cells in bone marrow microenvironment, and recently identified endocrine effects on peripheral adipose tissues.

Effects of Sclerostin on Bone

As stated above, sclerostin was initially discovered as the causative factor for two rare skeletal disorders: sclerosteosis and van Buchem disease. Since then, sclerostin-neutralizing antibodies have been developed as potent therapy for osteoporosis and osteopenias. Dramatic osteoanabolic effects were observed after antibody-mediated suppression of sclerostin in rat models of ovariectomy-induced osteoporosis [23] and age-based model of osteoporosis [24]. Mice lacking sclerostin (SostKO mice) displayed markedly increased bone formation and bone strength [25]. Loss of sclerostin-binding partners, the Wnt co-receptors LRP5 and LRP6, in mature osteoblasts resulted in loss of trabecular and cortical bone [26]. Similarly, ablation of β-catenin in osteoblasts and/or osteocytes resulted in marked osteopenia due to increased bone resorption [27, 28]. These studies indicate that sclerostin is a potent negative regulator of bone mass via its binding partner Wnt co-receptors on mature osteoblasts. However, mice with deletion of LRP4 have high bone mass despite high circulating level of sclerostin indicating that LRP4 is needed to "present" sclerostin protein to LRP5 and LRP6. Indeed, neutralizing Ab against LRP4 was also effective in increasing bone formation in mature animals [29].

Secreted from osteocytes, sclerostin acts on nearby osteoblasts and bone lining cells to block their proliferation and function [7]. The osteoanabolic effect of sclerostin reduction by neutralizing antibody is associated with new bone formation on quiescent bone surfaces by increasing osteoblast numbers and activity [30]. However, how sclerostin treatment recruits new osteoblasts to the bone surface remains largely unknown. The Wnt-signaling pathway targeted by sclerostin plays essential role in osteoblast biology, and it has been implicated in osteolineage commitment, osteoblast proliferation, and function [7]. Sclerostin has been shown to inhibit osteogenic commitment of mesenchymal cells [31] and the progression of late osteoblasts to mature osteocytes in in vitro cultures [32]. Sclerostin is also elevated during unloading or reduced gravity (as during human space flights) and recent animal data suggests that sclerostin neutralizing antibody may be an effective countermeasure to unload-induced bone loss [33–35].

Effect of Sclerostin on Hematopoietic Compartment

Sclerostin also affects hematopoiesis and B cells survival. Animals lacking sclerostin (SostKO) have generalized hyperostosis associated with B cell defect due to altered bone microenvironment. The importance of the bone marrow microenvironment in maintaining the hematopoietic stem cells (HSCs) niche, a specialized stromal environment that protect and support the HSCs, was first described in 2003 [36, 37], when two independent groups demonstrated that endosteal osteoblasts were needed to maintain the niche. Over the last decade, the complexity and identity of the niche-supporting cells has expanded to delineate an intricate and sophisticated network between different bone marrow cells (such as endothelial, perivascular, and CAR cells) and different hematopoietic cells subtypes. The emerging concept is that the bone marrow is a dynamic entity comprising multiple microniches supporting different hematopoietic subtypes. Myeloid and lymphoid cells are also influenced by osteoblasts, but the exact mechanisms that regulate these cells fate are still unclear. In SostKO animals, HSCs were unchanged, whereas lymphoid and myeloid lineages were significantly reduced due to increased apoptosis. Bone marrow transplants studies in these animals demonstrated that the B cell defect in these animals was non-cell autonomous, but dependent of the bone marrow microenvironment [38]. Interestingly, no hematologic abnormalities have been reported in patients affected by sclerosteosis or van Buchem disease, indicating that in human other compensatory mechanisms might be in place. Opposite to SostKO animals, when SOST/sclerostin expression is increased, there is an increase in myeloid cell proliferation that is not rescued by treatment with Scl-Ab suggesting a possible paracrine or intracrine effect of the protein [39].

The Adipose Tissue

Traditionally, fat is known to function as storage for excess energy in the form of triglycerides that can be reused when needed. Adipocytes are the major building blocks of adipose

tissue along with other supporting cells such as adipocyte precursors, vascular endothelial cells, fibroblasts, and tissue resident macrophages. These supporting cells, along with signals from insulin and other endocrine hormones, guide the development of adipocyte precursors into mature adipocytes. It is much evident now that fat tissue also acts as an endocrine organ by secreting several endocrine factors including leptin, adiponectin, resistin, tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6), and others [40]. Thus, the adipose tissue plays multi-faceted role in nutrient homeostasis.

Distinct specialized adipocyte cells are characteristic of vertebrates compared to other lower organisms. There are multiple fat depots in vertebrates. In mice, there are eight major fat depots: gonadal, inguinal, retroperitoneal, mesenteric, omental, pericardial, popliteal, and brown adipose tissue (BAT). Gonadal white adipose tissues (GWAT) are the largest fat depots in mice, comprising about 30% of dissectible fat. The next largest fat depot is the pair of inguinal white fat tissues (InWAT). These are found subcutaneous anterior to the upper segment of the hind limbs. Surrounding the kidney consists of the paired retroperitoneal white adipose tissue depots along the dorsal wall of the abdomen. The mesenteric white adipose tissue supports the intestines, the stomach, and the omental depot by forming a web-like structure. Minor depots include the pericardial and the paired popliteal depots. In mice, BAT is located subscapular between the dorsal crests of the scapulae and is often covered by "infused" white adipose tissue. In addition to these traditionally recognized eight fat depots, recently bone marrow adipocytes have also been proposed as a new adipose tissue due to its distinct developmental pattern and endocrine activities [41]. Although similar in composition, the response of different adipose depots to pathophysiological conditions varies in gender dependent manner. In humans, adipose tissue depots develop in distinct regions and do not even have precise correlation with fat depots in mice. For example, large visceral fat in omentum in humans is barely present in mice; conversely large gonadal fat in male mice do not exist in humans [42]. Moreover, adipocytes from different depots display distinct gene signatures [43]. These considerations should be taken into account when interpreting data from mice experiments.

Currently, three distinct types of adipocytes are recognized in peripheral fat depots: white, brown, and beige adipocytes. The unilocular white adipocytes are the most abundant adipocytes and constitute the bulk of fatty tissue. They specialize in storing excess glucose as triglycerides in large lipid droplets. During low-glucose stages, this triglyceride is used to generate energy. Brown adipocytes, specifically localized in BAT, are highly specialized cells that dissipate excess chemical energy in the form of heat via the actions of a mitochondrial protein, uncoupling-protein 1 (UCP1). Via this action, the BAT acts as a thermoregulatory organ in addition to shivering-induced thermogenesis in muscle. It should be noted that although BAT is present in mice throughout the life-span; in humans, only newborns have distinct and significant BAT and it is gradually lost with age. Instead, the third type of adipocytes, the beige adipocytes, were observed in human adults by [18F]-2-fluoro-D-2-deoxy-D-glucose (FDG) positron emission tomography (PET) which showed UCP1-postive loci in the supraclavicular and spinal regions [44]. In rodents, prolonged cold exposure or adrenergic signaling can induce UCP1-positive brown adipocyte-like cells in white adipose depots, especially in inguinal and gonadal depots [45]. These inducible UCP1-positive cells within the white adipose depots are now recognized as beige adipocytes. Because UCP1-positive cells facilitate excess energy expenditure, confirmations of these cells in human adults have opened new therapeutic investigations into targeting these cells to tackle obesity and better metabolic controls.

The cellular-lineage origins of white, brown, and beige adipocytes are quite distinct. White adipocytes are mostly derived from mesenchymal lineage cells primary guided by the action of the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ). For a long time, white and brown adipocytes were thought to originate from same common precursor. However, recent studies unambiguously showed that brown adipocytes originate from the same precursors of muscle cells. Interestingly, the beige adipocytes do not share the same origins as the classical brown adipocytes of BAT. Indeed, lineage-tracing studies demonstrated that brown adipocytes and muscle cells derive from Pax7+/Myf5+ progenitors; whereas, white and beige adipocytes derive from Pax7−/Myf5 stem cells [45].

Effect of Sclerostin on Adipocytes

A number of studies have reported altered body metabolism and composition in mice with genetic manipulation of osteoblast lineage cells or their intracellular signaling [46–54]. At least two different mechanisms have been shown to contribute to these effects: (i) regulation of insulin and glucose metabolism via secretion of undercarboxylated osteocalcin (ucOCN) by mature osteoblasts [55] and (ii) ucOCN independent mechanisms regulating body fat [56]. This is evident from studies of mice with induced osteoblast or osteocyte deficiency which show lean phenotypes and/or abnormal glucose metabolism [53, 54]. Mice with induced osteoblast cellular deficiency develop hyperglycemia, hypoinsulinemia (due to decreased pancreatic β-cell proliferation), decreased glucose tolerance and insulin sensitivity [53]. On the other hand, mice with osteocyte-deficiency do not show any changes in glucose or insulin metabolism [54]. Interestingly, both the osteoblast and osteocyte deficient mice have decreased white adipose depots [53, 54]. Many studies showed that osteolineage cells regulate insulin-glucose metabolism via the osteoblast secreted ucOCN [46, 57]. The target of ucOCN action

is pancreatic β-cells where ucOCN binds to its receptor GPCR6A to induce insulin secretion [51, 58]. In osteoblasts, components of insulin receptor signaling, including insulin receptor, mTORC2, and Foxo1, are involved in regulation of ucOCN [48, 49, 52, 59]. Treatment of the osteoblast-deficient mice with ucOCN completely normalized the glucose and insulin metabolism abnormalities; however, it could not rescue the lean phenotype [53]. These data suggests presence of other, ucOCN-independent mechanisms, through which osteolineage cells could directly regulate body adiposity. Indeed, mice lacking LRP5 in osteoblast have increased body adiposity without changes in glucose metabolism or ucOCN levels [56].

Several studies have shown that Wnt signaling is an important determinant of both white and brown adipogenesis. Activated Wnt signaling inhibits white adipogenic differentiation in favor of osteoblastogenesis [7, 60]. In brown adipocytes Wnt signaling has biphasic effects. Activated Wnt signaling inhibits brown adipogenesis in pre-adipocytes and inhibits UCP1 in differentiated brown adipocytes [60, 61]. This raises the possibility that the osteocytes-secreted factor sclerostin, which inhibits Wnt signaling by binding to the LRP5/LRP6 co-receptors, could also alter adipogenesis. Sclerostin is secreted into the circulation and is detectable in serum of healthy humans. In humans, LRP6 inactivating mutations and circulating sclerostin are associated with metabolic syndrome [62, 63] whereas LRP6 haploinsufficient mice show reduced body fat with increased PGC1α and UCP1 expression in white and brown fat [64]. In both men and postmenopausal women, circulating sclerostin levels increase with age with men having significantly increased sclerostin compared to women [65]. Circulating sclerostin levels are also positively correlated with bone mineral density, body mass index, and gonadal fat after partial correlation analysis adjusted for age, gender, and kidney function [65, 66]. Paradoxically, despite being a negative regulator of bone formation, high circulating sclerostin levels are positively associated with higher bone density [63, 65]. This suggests that the effects of sclerostin are more complex or that the circulating levels of this protein merely reflect bone mass rather than its biological effect. In other words, bigger bone, with more osteocytes, will produce more sclerostin compared to smaller bone with less osteocytes.

To examine the direct effects of sclerostin on white adipogenesis, Ukita et al. tested the effects of sclerostin treatment on differentiation of 3 T3-L1 pre-adipocyte cell line [67••]. Treatment of 3 T3-L1 cell with 5–10 ng/ml recombinant sclerostin for the whole differentiation period significantly and dose dependently increased white adipogenesis as assessed by oil red O staining and gene expression of PPARγ. Sclerostin treatment also rescued inhibition of adipogenesis by Wnt3a treatment. Sclerostin achieved this effect, at least in part, by activation of C/EBPβ protein levels and suppression of TAZ (Transcriptional

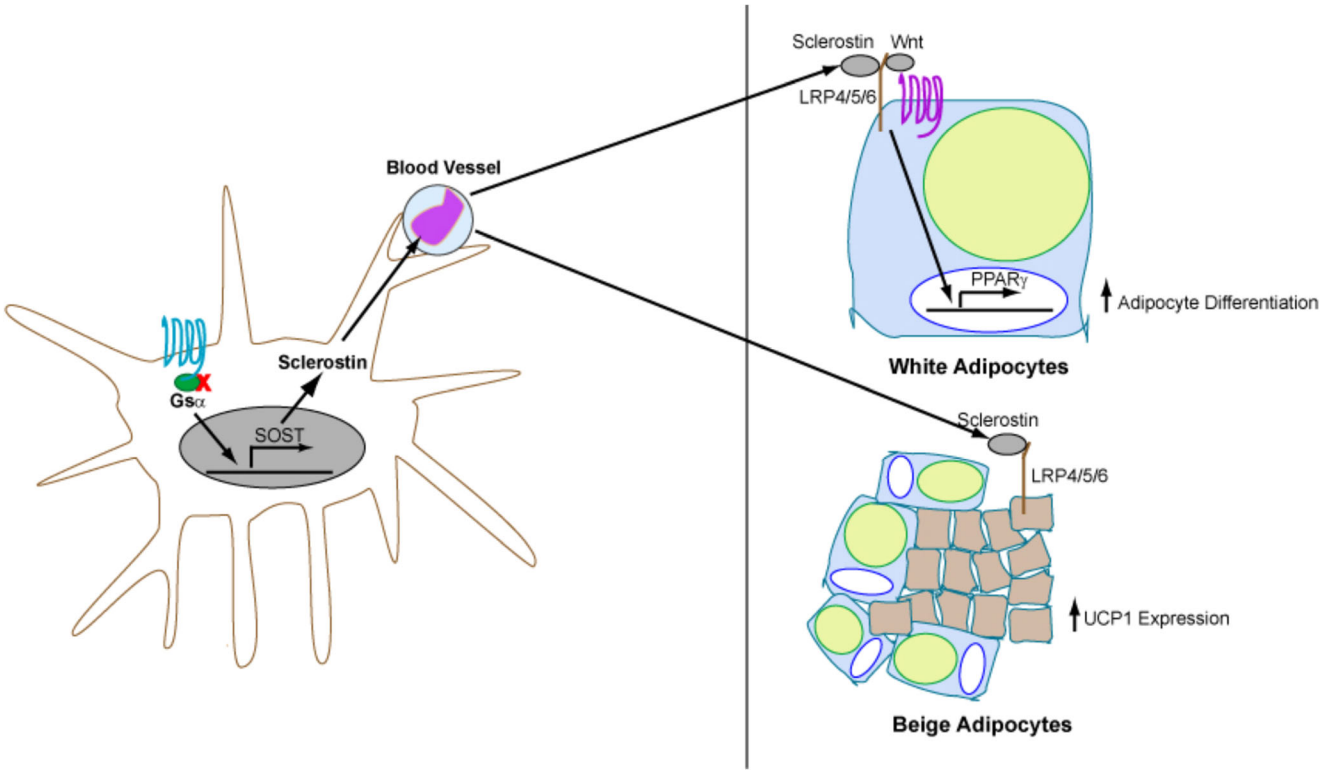

Fig. 1 Effects of sclerostin on peripheral adipocytes. Sclerostin is highly upregulated by deletion of Gsα signaling in osteocytes. Sclerostin is secreted into circulation via blood vessels in close proximity to osteocytes. In *white adipocytes*, sclerostin acts to increase expression of PPARg and adipocyte differentiation. Sclerostin also increases *beige* adipogenesis and expression of UCP1

coactivator with PDZ binding domain) [67••]. This data demonstrates that sclerostin directly enhances white adipogenic differentiation of 3 T3-L1 cells (Fig. 1). Further studies are needed to examine role of sclerostin in primary adipocytes and determine at which stage of adipocyte differentiation and maturation the protein exerts its effect.

We have recently investigated body composition and metabolism in mice with high circulating sclerostin [68••]. We have focused our studies on Gsα signaling in osteolineage cells. Gsα is the cAMP stimulatory secondary messenger of G-protein coupled receptors. We have generated two constitutive and one inducible mouse models of Gsα-disruption specifically in osteolineage cells. Mice lacking Gsα in osteocytes were generated using the Dmp1-Cre promoter (Dmp1-Gsα^{KO}) whereas mice lacking Gsα in both mature osteoblasts and osteocytes were generated using the Osteocalcin (Oc)-Cre promoter (Oc-Gsα^{KO}). In addition, a tamoxifen-inducible postnatal loss of Gsα in osteocytes was also generated by using the Dmp1-Cre-^{ERT2} promoter (Dmp1-^{ERT2}Gsα^{KO}). All three mouse models had significant increase in serum sclerostin and increased sclerostin expression in osteocytes. We observed that these mice become progressively lean with age with reduction in gonadal and inguinal fat pad weights. Serum insulin levels were unchanged indicating that the decreased body fat mass was not due to altered levels of ucOCN levels. Interestingly, the lean phenotypes were associated with a dramatic increase in UCP1-positive beige adipocytes in both the inguinal and gonadal white adipose tissues. Because activation of Wnt signaling in differentiated brown adipocytes suppresses UCP1 expression, we hypothesize that the inhibition of Wnt signaling by sclerostin could lead to increased UCP1 expression and beige adipogenesis in these mice. Treatment of the Dmp1-Gsα^{KO} mice with anti-sclerostin neutralizing antibody significantly reduced the increased expression of beige adipogenic markers in WAT depots. Conversely, treatment of wild-type mice with sclerostin significantly increased UCP1-expression in WAT of these animals. Sclerostin also dose dependently increased UCP1 expression in primary white adipocytes. Altogether these data show that the increased sclerostin in vivo, at least in part, contribute to increased beige adipogenesis (Fig. 1).

By contrast, in a converse in vivo model, Frey et al. recently examined body composition and metabolism changes in sclerostin-null (SostKO) mice [69••]. Whole-body fat mass and fat pad weights were significantly decreased in SostKO mice whereas glucose tolerance and insulin sensitivity were significantly increased. UcOCN levels were not changed in these mice. Overexpression of sclerostin by adeno-associated virus gene transfer to liver led to increased fat pad weights with impaired glucose tolerance. Examination of white adipose depots from SostKO mice showed enhanced ability to oxidize fatty acids, increased in the gene expression of UCP1 and Ppargc1α, and a reduction in de novo fatty acid synthesis. Therefore, significant decrease in fat pad weights in SostKO mice appears to be the result of cell non-autonomous enhancements in Wnt signaling in white adipocytes and a shift towards anabolic metabolism in this tissue. This paradoxically similar phenotypes in mouse models of increased and decreased sclerostin demonstrate the need for additional investigation to unravel the precise effect of sclerostin on adipocytes.

In addition to the effect of sclerostin on white and beige adipocytes, circulating sclerostin levels are also associated with higher vertebral marrow fat in men [70]. Recently Reagan et al. showed that sclerostin increases bone marrow adipogenesis [71••]. The authors also reported that sclerostin increases adipogenesis of 3 T3-L1 cells, as previously observed [67••] and also in bone marrow mesenchymal stem cells from both mouse and human.

Conclusion and Future Directions

Taken together, these recent in vivo and in vitro studies demonstrate a role for sclerostin in the development and function of white and beige adipocytes. It should be noted that serum sclerostin levels are influenced by gender, age, and disease conditions. To complicate the matter further, energy metabolism and body composition are also highly influenced by gender, age, and disease conditions. Therefore, determining precise effects of sclerostin on adipocytes or energy metabolism is fairly complicated and additional studies, both in vitro and in vivo, are needed to elucidate the effects of this protein on adipose tissue. Population-based studies show that the serum sclerostin levels are higher in men compared to women, it increases markedly with age, and it is surprisingly higher in older individuals [72, 73]. Circulating sclerostin levels are also increased in patients with Type 2 diabetes mellitus [73] and in patients with early chronic kidney disease (CKD) where they negatively correlated with CKD progression [16]. Interestingly, these disease conditions also affect adipocytes and energy metabolism. Therefore, further studies are needed to determine precise effects of sclerostin on adipocytes, fat accumulation, and energy metabolism.

Compliance with Ethical Standards

Conflict of Interest Keertik Fulzele and Paola Divieti Pajevic each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Grant Supporters This work was supported by the National Institutes of Health grants AR060221, AR059655, and DK079161 to PDP. This work was partially supported by GAP award from ASBMR, and grants from Boston University Clinical and Translational Science Institute (CTSI) (NIH 1UL1TR001430), and from Center for Skeletal Research (CSR) of Massachusetts General Hospital (NIH NIAMS P30 AR066261) to KF.

References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J. 2003;22(23):6267–76.
2. van Bezooijen RL, Roelen BA, Visser A, van der Wee-Pals L, de Wilt E, Karperien M, et al. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med. 2004;199(6):805–14.
3. Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, et al. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet. 2001;10(5):537–43.
4. Staehling-Hampton K, Proll S, Paeper BW, Zhao L, Charmley P, Brown A, et al. A 52-kb deletion in the SOST-MEOX1 intergenic region on 17q12-q21 is associated with van Buchem disease in the Dutch population. Am J Med Genet. 2002;110(2):144–52.
5. Balemans W, Patel N, Ebeling M, Van Hul E, Wuyts W, Lacza C, et al. Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. J Med Genet. 2002;39(2):91–7.
6. McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, et al. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med. 2014;370(5):412–20.
7. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med. 2013;19(2):179–92.
8. Cui Y, Niziolek PJ, Macdonald BT, Zylstra CR, Alenina N, Robinson DR, et al. Lrp5 functions in bone to regulate bone mass. Nat Med. 2011;17(6):684–91.
9. Boschert V, van Dinther M, Weidauer S, van Pee K, Muth EM, Ten Dijke P, et al. Mutational analysis of sclerostin shows importance of the flexible loop and the cystine-knot for Wnt-signaling inhibition. PLoS One. 2013;8(11):e81710. doi:10.1371/journal.pone.0081710.
10. Fijalkowski I, Geets E, Steenackers E, Van Hoof V, Ramos FJ, Mortier G, et al. A novel domain-specific mutation in a sclerosteosis patient suggests a role of LRP4 as an anchor for sclerostin in human bone. J Bone Miner Res. 2016; doi:10.1002/jbmr.2782.
11. Clarke BL, Drake MT. Clinical utility of serum sclerostin measurements. Bonekey Rep. 2013;2:361. doi:10.1038/bonekey.2013.95.
12. Fernandez-Roldan C, Genre F, Lopez-Mejias R, Ubilla B, Mijares V, Cano DS, et al. Sclerostin serum levels in patients with systemic autoimmune diseases. Bonekey Rep. 2016;5:775. doi:10.1038/bonekey.2016.2.
13. Garcia-Martin A, Rozas-Moreno P, Reyes-Garcia R, Morales-Santana S, Garcia-Fontana B, Garcia-Salcedo JA, et al. Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2012;97(1):234–41. doi:10.1210/jc.2011-2186.
14. Eda H, Santo L, Wein MN, Hu DZ, Cirstea DD, Nemani N, et al. Regulation of sclerostin expression in multiple myeloma by Dkk-1: a potential therapeutic strategy for myeloma bone disease. J Bone Miner Res. 2016; doi:10.1002/jbmr.2789.
15. Acibucu F, Dokmetas HS, Acibucu DO, Kilicli F, Aydemir M, Cakmak E. Effect of vitamin D treatment on serum sclerostin level. Experimental and clinical endocrinology & diabetes: official journal, German Society of Endocrinology [and] German Diabetes Association. 2016; doi:10.1055/s-0035-1559790.
16. Pelletier S, Dubourg L, Carlier MC, Hadj-Aissa A, Fouque D. The relation between renal function and serum sclerostin in adult patients with CKD. Clinical journal of the American Society of Nephrology : CJASN. 2013;8(5):819–23. doi:10.2215/CJN.07670712.
17. Keller H, Kneissel M. SOST is a target gene for PTH in bone. Bone. 2005;37(2):148–58.
18. Leupin O, Kramer I, Collette NM, Loots GG, Natt F, Kneissel M, et al. Control of the SOST bone enhancer by PTH using MEF2 transcription factors. J Bone Miner Res. 2007;22(12):1957–67. doi:10.1359/jbmr.070804.
19. Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA, et al. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. Endocrinology. 2005;146(11):4577–83. doi:10.1210/en.2005-0239.
20. Drake MT, Srinivasan B, Modder UI, Peterson JM, McCready LK, Riggs BL, et al. Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. J Clin Endocrinol Metab. 2010;95(11):5056–62. doi:10.1210/jc.2010-0720.
21. Mirza FS, Padhi ID, Raisz LG, Lorenzo JA. Serum sclerostin levels negatively correlate with parathyroid hormone levels and free estrogen index in postmenopausal women. J Clin Endocrinol Metab. 2010;95(4):1991–7. doi:10.1210/jc.2009-2283.
22. Costa AG, Cremers S, Rubin MR, McMahon DJ, Sliney Jr J, Lazaretti-Castro M, et al. Circulating sclerostin in disorders of parathyroid gland function. J Clin Endocrinol Metab. 2011;96(12):3804–10. doi:10.1210/jc.2011-0566.
23. Li X, Ominsky MS, Warmington KS, Morony S, Gong J, Cao J, et al. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. J Bone Miner Res. 2009;24(4):578–88.
24. Li X, Warmington KS, Niu QT, Asuncion FJ, Barrero M, Grisanti M, et al. Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass, and bone strength in aged male rats. J Bone Miner Res. 2010;25(12):2647–56.
25. Li X, Ominsky MS, Niu QT, Sun N, Daugherty B, D'Agostin D, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. J Bone Miner Res. 2008;23(6):860–9.
26. Riddle RC, Diegel CR, Leslie JM, Van Koevering KK, Faugere MC, Clemens TL, et al. Lrp5 and Lrp6 exert overlapping functions in osteoblasts during postnatal bone acquisition. PLoS One. 2013;8(5):e63323. doi:10.1371/journal.pone.0063323.
27. Kramer I, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB, et al. Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. Mol Cell Biol. 2010;30(12):3071–85.
28. Glass 2nd DA, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell. 2005;8(5):751–64.
29. Chang MK, Kramer I, Huber T, Kinzel B, Guth-Gundel S, Leupin O, et al. Disruption of Lrp4 function by genetic deletion or pharmacological blockade increases bone mass and serum sclerostin levels. Proc Natl Acad Sci U S A. 2014;111(48):E5187–95. doi:10.1073/pnas.1413828111.
30. Padhi D, Jang G, Stouch B, Fang L, Posvar E. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. J Bone Miner Res. 2011;26(1):19–26.
31. Wu JY, Aarnisalo P, Bastepe M, Sinha P, Fulzele K, Selig MK, et al. Gsalpha enhances commitment of mesenchymal progenitors to the osteoblast lineage but restrains osteoblast differentiation in mice. J Clin Invest. 2011;121(9):3492–504.

32. Atkins GJ, Rowe PS, Lim HP, Welldon KJ, Ormsby R, Wijenayaka AR, et al. Sclerostin is a locally acting regulator of late-osteoblast/preosteocyte differentiation and regulates mineralization through a MEPE-ASARM-dependent mechanism. J Bone Miner Res. 2011;26(7):1425–36.
33. Pajevic PD, Spatz JM, Garr J, Adamson C, Misener L. Osteocyte biology and space flight. Current biotechnology. 2013;2(3):179–83. doi:10.2174/22115501113029990017.
34. Spatz JM, Ellman R, Cloutier AM, Louis L, van Vliet M, Suva LJ, et al. Sclerostin antibody inhibits skeletal deterioration due to reduced mechanical loading. J Bone Miner Res. 2013;28(4):865–74. doi:10.1002/jbmr.1807.
35. Robling AG, Niziolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I, et al. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem. 2008;283(9):5866–75. doi:10.1074/jbc.M705092200.
36. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003;425(6960):841–6.
37. Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003;425(6960):836–41.
38. Cain CJ, Rueda R, McLelland B, Collette NM, Loots GG, Manilay JO. Absence of sclerostin adversely affects B-cell survival. J Bone Miner Res. 2012;27(7):1451–61. doi:10.1002/jbmr.1608.
39. Fulzele K, Krause DS, Panaroni C, Saini V, Barry KJ, Liu X, et al. Myelopoiesis is regulated by osteocytes through Gsα-dependent signaling. Blood. 2013;121(6):930–9.
40. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature Reviews Immunology. 2011;11(2):85–97
41. Scheller EL, Doucette CR, Learman BS, Cawthorn WP, Khandaker S, Schell B, et al. Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues. Nat Commun. 2015;6:7808. doi:10.1038/ncomms8808.
42. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol Asp Med. 2013;34(1):1–11. doi:10.1016/j.mam.2012.10.001.
43. Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, et al. Mechanisms and metabolic implications of regional differences among fat depots. Cell Metab. 2013;17(5):644–56. doi:10.1016/j.cmet.2013.03.008.
44. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB et al. Identification and Importance of Brown Adipose Tissue in Adult Humans. New England Journal of Medicine 2009;360(15):1509–1517
45. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nat Med. 2013;19(10):1252–1263
46. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. Cell. 2007;130(3):456–69.
47. Yoshizawa T, Hinoi E, Jung DY, Kajimura D, Ferron M, Seo J, et al. The transcription factor ATF4 regulates glucose metabolism in mice through its expression in osteoblasts. J Clin Invest. 2009;119(9):2807–17.
48. Rached MT, Kode A, Silva BC, Jung DY, Gray S, Ong H, et al. FoxO1 expression in osteoblasts regulates glucose homeostasis through regulation of osteocalcin in mice. J Clin Invest. 2010;120(1):357–68.
49. Riddle RC, Frey JL, Tomlinson RE, Ferron M, Li Y, DiGirolamo DJ, et al. Tsc2 is a molecular checkpoint controlling osteoblast development and glucose homeostasis. Mol Cell Biol. 2014;34(10):1850–62.
50. Wei J, Hanna T, Suda N, Karsenty G, Ducy P. Osteocalcin promotes beta-cell proliferation during development and adulthood through Gprc6a. Diabetes. 2014;63(3):1021–31.
51. Ferron M, Lacombe J. Regulation of energy metabolism by the skeleton: osteocalcin and beyond. Arch Biochem Biophys. 2014;
52. Fulzele K, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D, et al. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. Cell. 2010;142(2):309–19.
53. Yoshikawa Y, Kode A, Xu L, Mosialou I, Silva BC, Ferron M, et al. Genetic evidence points to an osteocalcin-independent influence of osteoblasts on energy metabolism. J Bone Miner Res. 2011;26(9):2012–25.
54. Sato M, Asada N, Kawano Y, Wakahashi K, Minagawa K, Kawano H, et al. Osteocytes regulate primary lymphoid organs and fat metabolism. Cell Metab. 2013;18(5):749–58.
55. Karsenty G, Oury F. Biology without walls: the novel endocrinology of bone. Annu Rev Physiol. 2011.
56. Frey JL, Li Z, Ellis JM, Zhang Q, Farber CR, Aja S, et al. Wnt-Lrp5 signaling regulates fatty acid metabolism in the osteoblast. Mol Cell Biol. 2015;35(11):1979–91. doi:10.1128/MCB.01343-14.
57. Hinoi E, Gao N, Jung DY, Yadav V, Yoshizawa T, Myers Jr MG, et al. The sympathetic tone mediates leptin's inhibition of insulin secretion by modulating osteocalcin bioactivity. J Cell Biol. 2008;183(7):1235–42.
58. Oldknow KJ, MacRae VE, Farquharson C. Endocrine role of bone: recent and emerging perspectives beyond osteocalcin. J Endocrinol. 2015;225(1):R1–19. doi:10.1530/JOE-14-0584.
59. Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell. 2010;142(2):296–308.
60. Kang S, Bennett CN, Gerin I, Rapp LA, Hankenson KD, Macdougald OA. Wnt signaling stimulates osteoblastogenesis of mesenchymal precursors by suppressing CCAAT/enhancer-binding protein α and peroxisome proliferator-activated receptor γ. J Biol Chem. 2007;282(19):14515–24.
61. Kang S, Bajnok L, Longo KA, Petersen RK, Hansen JB, Kristiansen K, et al. Effects of Wnt signaling on brown adipocyte differentiation and metabolism mediated by PGC-1α. Mol Cell Biol. 2005;25(4):1272–82.
62. Mani A, Radhakrishnan J, Wang H, Mani A, Mani MA, Nelson-Williams C, et al. LRP6 mutation in a family with early coronary disease and metabolic risk factors. Science. 2007;315(5816):1278–82. doi:10.1126/science.1136370.
63. Sheng Z, Tong D, Ou Y, Zhang H, Zhang Z, Li S, et al. Serum sclerostin levels were positively correlated with fat mass and bone mineral density in central south Chinese postmenopausal women. Clin Endocrinol. 2012;76(6):797–801. doi:10.1111/j.1365-2265.2011.04315.x.
64. Liu W, Singh R, Choi CS, Lee HY, Keramati AR, Samuel VT, et al. Low density lipoprotein (LDL) receptor-related protein 6 (LRP6) regulates body fat and glucose homeostasis by modulating nutrient sensing pathways and mitochondrial energy expenditure. J Biol Chem. 2012;287(10):7213–23. doi:10.1074/jbc.M111.286724.
65. Amrein K, Amrein S, Drexler C, Dimai HP, Dobnig H, Pfeifer K, et al. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. J Clin Endocrinol Metab. 2012;97(1):148–54. doi:10.1210/jc.2011-2152.
66. Urano T, Shiraki M, Ouchi Y, Inoue S. Association of circulating sclerostin levels with fat mass and metabolic disease—related markers in Japanese postmenopausal women. J Clin Endocrinol Metab. 2012;97(8):E1473–7. doi:10.1210/jc.2012-1218.
67.•• Ukita M, Yamaguchi T, Ohata N, Tamura M. Sclerostin enhances adipocyte differentiation in 3T3-L1 cells. J Cell Biochem. 2015;

doi:10.1002/jcb.25432. **This study shows the effect of sclerostin on white adipocytes using a preadipocytic cell line**

68.•• Fulzele K, Lai F, Dedic C, Saini V, Uda Y, Shi C, et al. Osteocyte-secreted Wnt signaling inhibitor sclerostin contributes to beige adipogenesis in peripheral fat depots. J Bone Miner Res. 2016; doi:10.1002/jbmr.3001. **This study shows the effects of sclerostin on beige adipocytes using mouse model of high circuating sclerostin**

69.•• Frey J, Kim S, Li Z, Tomlinson R, Hussain M, Thorek D et al. Sclerostin influences body composition by regulating catabolic and anabolic metabolism in adipocytes. J Bone Miner Res. 2016;31(Suppl 1). **This study shows the effects of sclerostin on body composition and white adipose tissue using sclerostin-deficient mice**.

70. Ma YH, Schwartz AV, Sigurdsson S, Hue TF, Lang TF, Harris TB, et al. Circulating sclerostin associated with vertebral bone marrow fat in older men but not women. J Clin Endocrinol Metab. 2014;99(12):E2584–90. doi:10.1210/jc.2013-4493.

71.•• Reagan M, Fairfield H, Falank C, Rosen C. Bone marrow adiposity is induced by the osteocyte derived factor sclerostin. J Bone Miner Res. 2016;31(Suppl 1). **This study shows effect of sclerostin on bone marrow adipocytes using in vitro studies**.

72. Modder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res. 2011;26(2):373–9. doi:10.1002/jbmr.217.

73. Gennari L, Merlotti D, Valenti R, Ceccarelli E, Ruvio M, Pietrini MG, et al. Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. J Clin Endocrinol Metab. 2012;97(5):1737–44. doi:10.1210/jc.2011-2958.