Citation: BoneKEy Reports **2**, Article number: 361 (2013) | doi:10.1038/bonekey.2013.95

www.nature.com/bonekey

REVIEW

Clinical utility of serum sclerostin measurements

Bart L Clarke and Matthew T Drake

Endocrine Research Unit, Division of Endocrinology, Department of Medicine, College of Medicine, Mayo Clinic, Rochester, MN, USA.

Sclerostin is an osteocyte-secreted soluble antagonist of the Wnt/β-catenin signaling pathway requisite for osteoblast development and activity. Efforts over the past several years have focused on unraveling the role of sclerostin in both normal physiological and pathological conditions. Sclerostin levels are undetectable in the serum of patients with sclerosteosis. In normal individuals, serum sclerostin levels are higher in males and increase in both sexes across the adult lifespan. Some, but not other, studies have demonstrated that higher serum sclerostin levels are associated with increased fracture risk, particularly when paired with lower bone mineral density. Levels of circulating sclerostin are highly correlated with bone marrow sclerostin levels. Sclerostin levels are inversely related to parathyroid hormone levels. Clinical conditions in which serum sclerostin levels have been measured include ankylosing spondylitis, chronic kidney disease, diabetes, fractures, hypercortisolism, multiple myeloma and spinal cord injury. Even within clearly defined clinical conditions, however, consistent changes in serum sclerostin levels have not always been seen. This may reflect differences in currently available commercial assays or sample sources (serum versus plasma), and suggests further study is needed before sclerostin measurements are introduced into routine clinical practice. Until such issues are resolved, measurement of sclerostin levels appears to be most useful for understanding the mechanisms by which osteocytes regulate bone turnover through the integration of hormonal, physical and pharmacological stimuli, rather than to guide clinical-care decisions.

BoneKEy Reports **2**, Article number: 361 (2013) | doi:10.1038/bonekey.2013.95

Introduction

As a central mediator of osteoblast biology, the canonical Wnt/β-catenin signaling pathway directly affects osteoblast differentiation, proliferation, survival and ultimately bone formation. Much work has now carefully delineated the molecular hallmarks of this complex molecular signaling network, including the identification and characterization of endogenous soluble Wnt signaling pathway inhibitors that antagonize Wnt signaling and lead to pathway inactivation.[1] Among these secreted Wnt pathway antagonists is sclerostin, identified only a decade ago as a 213 amino-acid glycoprotein whose loss of function results in the autosomal recessive bone dysplasia sclerosteosis.[2,3] Sclerostin is secreted by osteocytes, and humans with inactivating sclerostin mutations have significantly increased bone formation rates. Interestingly, unlike subjects with sclerosteosis in whom serum sclerostin levels are undetectable even with the most sensitive commercially available assay,[4] subjects heterozygous for inactivating sclerostin mutations have serum sclerostin levels roughly half those of matched controls, but bone formation rates significantly increased relative to control subjects.[5] Such findings suggest that modulation of sclerostin levels may be a viable approach for increasing bone mass.

Yet while there is no question that sclerostin has a central role in bone biology, whether measurement of circulating sclerostin levels is of clinical value remains an open question. Herein, we review the recent plethora of studies in which serum sclerostin has been measured, and provide perspective on current deficits in human sclerostin biology that likely preclude its use in current clinical practice.

Measurement of Serum Sclerostin Levels Across the Lifespan

In a recent cross-sectional sample of boys and girls aged 6–21 years, Kirmani *et al.*[6] determined that serum sclerostin levels are significantly higher in boys than in girls ($P < 0.01$). Interestingly, the relationship between sclerostin levels and bone age was non-linear in both sexes. Thus, a split point in the sclerostin–bone age relationship occurred following pubertal onset, corresponding to bone ages of ~10 years in girls and 14 years in boys. In both sexes, serum sclerostin levels were positively

Correspondence: Dr MT Drake, Endocrine Research Unit, Division of Endocrinology, Department of Medicine, College of Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA.
E-mail: drake.matthew@mayo.edu

Received 13 March 2013; accepted 16 May 2013; published online 5 June 2013

correlated with bone age before the split point, but negatively associated with bone age after this point.

In a cross-sectional study of adults, Modder *et al.*[7] examined serum sclerostin levels in a population-based sample of 362 women and 318 men between the ages of 21 and 97 years. As was seen in the study of children/adolescents, sclerostin levels were again significantly higher in males than females ($P<0.001$). In pre- and postmenopausal women combined not maintained on estrogen therapy, and in men, serum sclerostin levels were positively correlated with age ($P<0.001$). Further, serum sclerostin levels increased 2.4-fold in women and 4.6-fold in men over the lifespan. Notably, when matched for total-body bone mineral content, subjects aged $\geqslant 60$ years had higher serum sclerostin levels than subjects aged 20–39 years. Using the same Biomedica assay as Modder *et al.*,[7] significantly higher sclerostin levels were also found in healthy adult men when compared with women ($P<0.001$); however, after adjustment for age, estimated glomerular filtration rate, bone mineral content, body mass index and physical activity, no difference was seen ($P=0.543$).[8]

The increase in sclerostin levels across the female lifespan was subsequently confirmed in an independent cohort of pre- and postmenopausal women.[9] Further direct human interventional studies in both postmenopausal woman and elderly men demonstrated that estrogen, but not testosterone, reduces the rise in sclerostin that occurs with aging.[10] Such results suggest that the estrogen decline with aging that occurs in both women and men (through reduced aromatization of testosterone to estradiol) may underlie the age-associated rise in circulating sclerostin levels.

Serum Sclerostin Levels May Predict Bone Mineral Density Changes and Fractures

Given that sclerostin regulates bone formation through inhibition of Wnt signaling, one might anticipate that low circulating sclerostin levels are correlated with higher bone mineral density (BMD) and reduced fracture risk. Conversely, high serum sclerostin levels might be expected to be associated with lower BMD and increased fracture risk. However, recent data suggest these hypotheses may be incorrect.

In a recent case-cohort study drawn from a community-based cohort of 9704 Caucasian women aged $\geqslant 65$ years as part of the prospective Study of Osteoporotic Fractures, Arasu *et al.*[11] examined whether fracture risk was correlated with sclerostin levels. Using a non-commercially available electrochemiluminescence-based enzyme-linked immunosorbent assay, sclerostin levels were measured in serum collected in 1989–1990 in 228 women with incident hip fractures, and in a randomly selected sample of 227 women from within the cohort; average follow-up time was 9.8 years. Sclerostin levels were positively correlated with total-hip BMD ($P<0.001$); further, hip fracture risk was increased when serum sclerostin levels were divided into quartiles ($P<0.001$ for trend). After adjustment for potential confounders, comparison of women in the highest sclerostin quartile to women in the lowest quartile was associated with a marked increased hip fracture risk (hazard ratio $=3.4$, 95% confidence interval, 1.7–7.0). Further division of the cohort into eight groups based on sclerostin quartile and median hip BMD demonstrated that women in the highest sclerostin quartile with lower total-hip BMD had a 22.3-fold (95% confidence interval, 5.8–86.3) increased fracture risk relative to women in the lowest sclerostin quartile with higher total-hip BMD.

In a similarly designed study, Ardawi *et al.*[12] examined the association between bone turnover markers, circulating sclerostin levels measured using a commercially available assay from Biomedica (**Table 1**) and osteoporosis-related fracture risk in a population-based study of 707 postmenopausal women from Saudi Arabia with a mean follow-up of 5.2 ± 1.3 years. In multivariate Cox proportional hazard regression models adjusted for confounding risk factors, relative fracture risk in postmenopausal women was increased >7-fold for each 1-s.d. increase in sclerostin level, with women in the highest sclerostin level quartile having a nearly 15-fold increased fracture risk. Similar to the results seen in the study of Arasu *et al.*,[11] serum sclerostin levels also correlated with BMD, and subjects with the highest sclerostin levels and lowest BMD were at greatest fracture risk.

Together, these two studies strongly suggest that serum sclerostin levels are predictive of both BMD and fractures in older postmenopausal women, and that fracture risk is amplified when high serum sclerostin levels are associated with low BMD. Thus perhaps in contrast to expectations, it appears that serum sclerostin levels may be positively associated with bone mineral mass, perhaps reflecting the presence of more sclerostin-synthesizing osteocytes. For a defined bone mass, however, the above studies in postmenopausal women suggest that higher sclerostin levels are associated with increased fracture risk. Reasons for this are unclear, but may be mediated by changes in bone quality, impairment of bone formation or micro-crack repair, or perhaps yet unrecognized mechanisms.

In contrast to the previous two studies, however, are results reported by Garnero *et al.*[13] from the population-based OFELY cohort, in which serum sclerostin levels were assessed using an enzyme-linked immunosorbent assay from TECO. In this study of 572 postmenopausal women with a mean age 67 years followed prospectively for a median of 6 years, there was no

Table 1 Comparison of commercially available sclerostin assays

Assay source	*Assay type*	*Primary antibody*	*Secondary antibody*	*Intra-assay CV (%)*	*Inter-assay CV (%)*	*Detection limit (pmol/l)*	*Detection range (pmol/l)*
Biomedica	ELISA	Polyclonal	Monoclonal	5	6	2.6	0–240
TECO	ELISA	Polyclonal	Monoclonal	<10	<10	5.7	11–176
MSD	ECL	Polyclonal	Polyclonal	6	10	0.1	1–440

Abbreviations: CV, coefficient of variation; ECL, electro-chemiluminescence assay; ELISA, enzyme-linked immunosorbent assay; MSD, Meso Scale Discovery; TECO, TECO Medical Group.

significant association between baseline serum sclerostin levels and fracture incidence. Rather, serum sclerostin levels were found to correlate positively with BMD at both the spine and total hip ($P<0.0001$, for each site). In partial contrast to the interventional study of Modder *et al.*[10] in which estrogen replacement reduced sclerostin levels in postmenopausal women, serum sclerostin levels were not found to be associated with estradiol levels. Further and in contrast to the studies described above,[11,12] serum sclerostin levels considered as either a continuous variable or when divided into quartiles were not significantly associated with prevalent or incident fracture risk, although the authors noted that as the number of incident fractures was small, a modest association may have been missed.

Consistent with their findings in the OFELY cohort of postmenopausal women is another recent prospective study in which the same investigators measured serum sclerostin levels using the same method in 710 men aged 50 and older, and correlated these levels with BMD measured at the lumbar spine, hip and distal forearm.[14] Consistent with previous cross-sectional data,[7] serum sclerostin levels increased with age ($P<0.001$). In direct contrast to the results from the earlier studies of postmenopausal women,[11,12] however, fracture risk was reduced when subjects in the upper two quintiles of serum sclerostin levels combined were compared with subjects in the lower three quintiles combined (6.1 versus 13.5%, $P<0.01$) in a Cox model adjusted for potential confounders. Stratification of the men by both serum sclerostin level and BMD showed that men with serum sclerostin levels and BMD in the two highest quintiles had a lower fracture risk than men who had BMD and circulating sclerostin levels in the three lower quintiles (hazard ratio = 0.24, 95% confidence interval, 0.10–0.62, $P<0.005$). Thus in this cohort of older men, higher serum sclerostin levels were associated with lower fracture risk and higher BMD.

Reasons for the differences reported between the three studies of postmenopausal women detailed above are unclear, although it is notable that each study used a different method to assay serum sclerostin levels. Also noteworthy is that when the studies involving postmenopausal woman are compared, measured serum sclerostin levels were ~4.5-fold higher in the study in which the Biomedica assay was used when compared with the non-commercially available assay, although perhaps somewhat reassuringly, this 4.5-fold relationship in measured sclerostin values appears to track fairly linearly across quartiles between the two studies. Unfortunately, no direct comparison with the TECO assay is possible here, however, as the OFELY postmenopausal cohort study did not report sclerostin values. Potential reasons why the findings in older men do not correlate with the findings in older women are also unknown. As such, measurement of serum sclerostin levels in older men and women, even in combination with BMD results, does not appear to be of clinical utility at present, although further characterization of these associations may make measurement of serum sclerostin levels for assessment of fracture risk of future clinical value.

Sclerostin May Mediate the Skeletal Response to Parathyroid Hormone

Intermittent parathyroid hormone (PTH) treatment stimulates bone formation, but the mechanisms underlying this effect remain unclear. Sclerostin inhibits Wnt signaling, and animal studies have demonstrated that PTH suppresses sclerostin production. A recent prospective study of 27 postmenopausal women treated with PTH 1–34 for 14 days versus 28 control women without treatment demonstrated that intermittent PTH treatment reduces circulating sclerostin levels,[15] with levels decreasing by 12.7% in PTH-treated subjects ($P<0.0001$) while remaining unchanged in the control women ($P<0.02$ for the change difference between groups). Notably, bone marrow plasma obtained from a subset of 19 control and 19 PTH-treated subjects at the end of the treatment period demonstrated that sclerostin levels in bone marrow plasma and serum were highly correlated ($r=0.64$, $P<0.0001$), with bone marrow plasma sclerostin levels 24% lower in PTH-treated compared with control women.

Given these findings, serum sclerostin could potentially be used as an early marker of therapeutic response to PTH 1–34, with reductions in serum sclerostin levels perhaps predictive of an early anabolic skeletal response. Although sclerostin is released predominantly by osteocytes and thought to act locally to suppress bone formation by osteoblasts, the strong correlation between bone marrow plasma and circulating sclerostin levels suggests that serum sclerostin may be useful as a marker of skeletal PTH treatment effect. Based on the results of a single short-term study, however, assessing changes in serum sclerostin levels in patients receiving PTH 1–34 treatment remains premature.

Because of the PTH effect on sclerostin, parathyroid disorders may be useful clinical settings to study this relationship. Patients with primary hyperparathyroidism have continuous exposure to elevated circulating PTH levels. Despite using different commercially available assays that provided serum sclerostin values that differed by ~25-fold, several studies have now demonstrated that patients with primary hyperparathyroidism have lower serum sclerostin levels relative to both matched controls and to patients following parathyroidectomy.[16,17] Interestingly, serum sclerostin levels have also been shown to normalize more rapidly than biochemical markers of bone turnover post-parathyroidectomy.[17] In conjunction with the results described previously in postmenopausal women treated with intermittent PTH, the inverse relationship between serum PTH and serum sclerostin levels also seen in primary hyperparathyroidism indicates that either intermittent or continuous PTH exposure results in down-regulation of sclerostin levels in humans.

Serum sclerostin levels have also been assessed in hypoparathyroidism, a condition marked by continuously decreased circulating PTH levels. In a cross-sectional study, Costa *et al.*[18] evaluated 20 patients with hypoparathyroidism and analyzed the relationship between serum sclerostin levels, PTH, bone turnover markers and BMD relative to a control group. In contrast to the results seen in patients with primary hyperparathyroidism in which sclerostin levels were reduced, sclerostin was significantly higher in hypoparathyroid subjects relative to controls ($P<0.0001$). Markers of both bone formation (amino-terminal propeptide of type I collagen—P1NP) and bone resorption (carboxyl-terminal peptide of type I collagen—CTX) were positively correlated with serum sclerostin levels, although only the relationship between sclerostin and CTX reached statistical significance ($P=0.01$). No significant correlation between sclerostin levels and BMD was identified. Interestingly,

however, there was a significant positive relationship between serum sclerostin levels and bone mineral content at all sites assessed (lumbar spine, hip and distal radius) in hypoparathyroid patients.

When viewed in context with the decreased sclerostin levels seen with either intermittent or continuous PTH, the finding of increased circulating sclerostin levels in hypoparathyroidism further solidifies the PTH–sclerostin inverse relationship. Despite recognition of this inverse relationship, however, it remains unclear how sclerostin measurement might be clinically useful in patients with parathyroid gland disorders, with the possible exception of measuring sclerostin to assess surgical success following parathyroidectomy, a situation where direct measurement of serum PTH levels provides a far more rapid and direct assessment of surgical cure.

Measurement of Serum Sclerostin Levels in Other Clinical Conditions

In addition to the studies already detailed, serum sclerostin levels have been measured across a variety of other conditions. Results from several of these studies, and potential clinical implications, are described below.

Ankylosing spondylitis. Ankylosing spondylitis (AS) is a chronic inflammatory disease of the spine and sacroiliac joints characterized by new bone formation. In a study of 50 healthy control subjects and 46 AS patients, sclerostin levels were significantly lower ($P<0.01$) in patients with AS than in controls.[19] Intriguingly, 2-year longitudinal sclerostin level measurements demonstrated significantly lower sclerostin levels in AS patients with syndesmophyte growth compared with AS patients without ($P<0.01$), emphasizing the role of sclerostin in suppressing bone formation in AS. Accordingly, longitudinal assessment of serum sclerostin levels in AS might be of clinical utility to assess response to pharmacological intervention or to correlate with skeletal radiographic changes.

Bed rest/immobilization/spinal cord injury. As osteocytes are integral to regulation of the skeletal response to mechanical loading, several studies have examined sclerostin levels in conditions of reduced mechanical loading, both experimental and in patients with immobilization following spinal cord injury (SCI). In young healthy males subjected to bed rest, sclerostin levels significantly increased from baseline within 14 days ($P<0.02$),[20] and remained elevated at both 60 ($P<0.001$) and 90 ($P=0.07$) days.[21] In patients with SCI, increased serum sclerostin levels were seen in subjects with SCI $\leqslant$5 years post injury, with levels decreasing by the 5-year period such that subjects injured $<$3 years have significantly higher sclerostin levels than those injured 3–5 years.[22] In subjects with chronic SCI of $>$5 years, lower sclerostin levels were significantly associated with bone mineral content and BMD, suggesting that in subjects with chronic SCI, circulating sclerostin levels may be a biomarker of osteoporosis severity rather than as a mediator of on-going bone loss.[23,24] Given that dual energy X-ray absorptiometry imaging can provide accurate assessment of changes in BMD and bone mineral content, the clinical utility of measuring sclerostin levels following SCI remains unclear.

Chronic kidney disease. Chronic kidney disease (CKD) is associated with a significant skeletal pathology and disordered mineral homeostasis. Consistent with previous population-based studies,[7] serum sclerostin levels were significantly correlated with age and were higher in male than female patients with stage 3B and 4 CKD.[25] After correction for age and gender, an inverse relationship between serum sclerostin and glomerular filtration rate was identified ($P<0.01$). The inverse relationship between declining glomerular filtration rate and increasing serum sclerostin levels has now been independently confirmed in a separate cohort, and raises the possibility that the positive correlation between age and sclerostin levels noted previously may be partially explained by the age-associated decline in renal function.[26] How measurement of serum sclerostin levels in patients with CKD might be used to guide clinical care, however, is not evident at present.

Diabetes. Diabetes mellitus type 2 (DM2) is associated with reduced bone quality, low bone turnover and increased fracture risk. In a cross-sectional study, serum sclerostin levels were significantly increased ($P<0.001$) in 74 subjects with DM2 compared with 50 control subjects, positively associated with DM2 duration ($P=0.06$), and significantly reduced in osteoporotic compared with non-osteoporotic DM2 subjects ($P<0.05$).[27] This increase in sclerostin levels in DM2 patients relative to control subjects has been independently confirmed.[28–30] Interestingly, whereas thiazolidinedione treatment for 24 weeks further augmented sclerostin levels, metformin treatment led to a slight reduction ($P<0.02$ for the difference between treatments), suggesting that while increased serum sclerostin levels in DM2 may contribute to increased skeletal fragility, this may be further exacerbated by thiazolidinedione treatment.[30] How measurement of sclerostin levels in patients with DM2 might guide clinical care, however, is not currently evident.

Fractures. Fracture healing in humans is a complex process involving formation and maturation of a fracture callus composed of bone cells, cartilage cells and connective tissue under the guidance of an array of factors including hormones, cytokines, extracellular matrix proteins and growth factors. In a recent study of adults with meta-/diaphyseal long-bone fractures (humerus, femur, lower leg and forearm), Sarahrudi *et al.*[31] studied 69 subjects with normal fracture healing, 6 subjects with impaired (atrophic non-union) healing requiring surgical revision and 34 healthy volunteers without fractures. Fracture subjects had serum samples obtained 0, 1, 2, 4, 6, 8, 12, 24 and 48 weeks following fracture, while control subjects provided one sample. Immediately after fracture, sclerostin levels were significantly higher in fracture subjects relative to controls, and remained significantly elevated throughout the 48-week period. Subjects with atrophic non-union had increased sclerostin levels slightly lower, but not significantly different, than subjects who healed normally. Thus although serum sclerostin levels are likely to be increased following fracture, their measurement does not appear to differentiate between subjects likely to develop non-union from those who heal normally. Accordingly, routine measurement of sclerostin levels following fracture is not currently justified.

Hypercortisolism. Supraphysiologically circulating corticosteroid levels from endogenous (as occurs in Cushing's syndrome) or exogenous (as occurs with pharmacological intervention) sources suppresses bone formation and increases skeletal fragility. As recently shown,[4] serum sclerostin levels when measured by the commercially available Meso Scale Discovery (MSD) assay were significantly decreased ($P<0.001$) in patients with endogenous hypercortisolism relative to matched controls. Further, surgical resolution of hypercortisolism resulted in significant increases in sclerostin levels ($P<0.01$) and were associated with marked increases in bone turnover markers (P1NP levels, $P<0.05$; and CTX, $P<0.001$). In direct contrast, however, is a study that used the Biomedica assay and determined that serum sclerostin levels were increased in patients with endogenous hypercortisolism relative to matched controls.[32] Although it is notable that different commercially available assays were used in the studies, other potential reasons for the opposing findings are not readily evident. Given the contradictory data, however, measurement of serum sclerostin levels in patients with hypercortisolism is not likely to be of current clinical use.

Monoclonal gammopathies. Monoclonal gammopathy of undetermined significance (MGUS) is a requisite precursor condition for the plasma cell malignancy multiple myeloma. While serum sclerostin levels in patients with MGUS were not different than matched controls,[33] sclerostin levels in patients with myeloma were elevated ($P<0.01$) relative to both MGUS subjects and healthy controls.[34] Among myeloma patients, increased sclerostin levels correlated with advanced diseases, including increased fracture risk at diagnosis ($P<0.01$), shorter median survival ($P<0.05$) and worse clinical staging ($P<0.001$). Whether the renal dysfunction so common in patients with multiple myeloma has a role in these increased sclerostin levels is unknown. Despite this prognostic relationship, however, it is presently unclear how serum sclerostin measurement may be of clinical use, although sclerostin may represent a target for novel therapies to combat the osteoblast suppression that occurs in myeloma.

Current Deficits in our Understanding of Sclerostin Biology in Humans

Although much has been learned about human sclerostin biology, fundamental gaps in our knowledge must be resolved before serum sclerostin measurements are incorporated into clinical practice. Important potential issues are briefly detailed below.

Sources of biological variability. Human bone metabolism reflects a complex process involving the integration of a wide array of local and systemic signals. Though current evidence is consistent with sclerostin serving as an integral regulator of bone metabolism owing to actions that are likely primarily local, serum sclerostin levels do reflect those in bone marrow.[15] While sex and age both clearly impact serum sclerostin levels, significant questions remain about the potential influence of other factors. These include, but are not limited to the following: (a) day-to-day, seasonal and circadian variability; (b) the proportion of osteocyte-produced sclerostin released into the systemic circulation; (c) the mechanism by which sclerostin is cleared (perhaps via liver or kidney) from the circulation, which might affect sclerostin level interpretation in subjects with organ impairment; (d) the relationship between sclerostin levels and total-body bone mineral content; (e) how physical activity and oral intake affect sclerostin levels; and (f) how sclerostin levels are impacted by pharmacological intervention.[35,36] A better understanding of adjustments necessary to account for such potential confounding variables will be crucial for proper interpretation of sclerostin levels in future clinical practice.

Immunoassay variability. The introduction of any assay for clinical use requires rigorous validation. As has been noted in multiple locations throughout the text, substantial differences in serum sclerostin levels measured using the three commercially available sclerostin assays may exist, which may be still different from levels measured using non-commercially available[11,15] or laboratory-generated 'in-house'[19] assays. As shown in **Table 1**, differences between the assays exist. Whereas both the Biomedica and TECO assays use a monoclonal anti-sclerostin secondary antibody, the MSD assay uses a polyclonal secondary antibody. To our knowledge and review of the literature, rigorous epitope mapping has only been described for the antibodies used in the MSD assay, which appears to detect the intact sclerostin molecule.[5]

When two (Biomedica and TECO) of the three different commercially available sclerostin immunoassays were used to directly compare measurements of sclerostin levels in peripheral blood serum and plasma from healthy control subjects, differences in measured sclerostin levels were seen, with variability occurring both between the different assays as well as between serum and plasma samples within the same assay.[37] Even larger differences in serum and plasma sclerostin levels were subsequently reported in a study that used the TECO assay.[4] Such results suggest that sclerostin in serum may partially exist as a protein-bound complex, with free and bound forms recognized differentially by the antibodies used in the assays. Overall, however, differences in the sclerostin levels determined by the Biomedica and TECO assays appear to be less than between these two assays and the MSD assay. Reasons for differences between the assays remain unclear at this time, but might include differences in the epitopes recognized (such that proteolytic fragments are measured by some but not other of the assays), cross-reactivity with the related sclerostin domain-containing protein 1 (SOST-DC1)[38] that shares $\sim$40% homology at the protein level, or other as yet unknown reasons. A summary of the studies included in this review, noting the source of the sclerostin assay, the cohort studied and the sclerostin-associated results, is provided in **Table 2**.

Given the above potential assay-associated concerns, however, it appears prudent for research purposes to use the same sclerostin assay to compare subject groups or individual subjects followed longitudinally. Sclerostin assay standardization would be necessary before introduction into general clinical laboratory use.

Summary and Conclusions

Serum sclerostin is secreted locally from bone matrix-embedded osteocytes, where it antagonizes Wnt/β-catenin signaling in osteoblasts. Despite the local skeletal actions of

Table 2 Summary of sclerostin assays, and outcomes observed, in the reviewed studies

Assay source	*Group studied*	*Sclerostin outcome*	*Ref.*
Biomedica	Children aged 6–21 years	Higher in boys; split point at puberty in both sexes	6
Biomedica	Men/women aged 21–97 years	Higher in men; increased in both sexes with aging	7
Biomedica	Adult men/premenopausal women	Increased with age; multiple adjustments eliminated sex difference	8
Biomedica	Women aged 20–79 years	Increased with age	9
Biomedica	Postmenopausal women/elderly men	Estrogen regulates levels in both sexes	10
Biomedica	Postmenopausal women	Positive association with fracture risk	12
Biomedica	Hyperparathyroidism/post-parathyroidectomy	Decreased in hyperparathyroidism compared with controls and post-parathyroidectomy patients	17
Biomedica	Young men on bed rest	Increased by 28 days; elevated to 90 days	21
Biomedica	Men with spinal cord injury	Increased $\leqslant$5 years; decreased $>$5 years post spinal cord injury	22
Biomedica	Men with chronic spinal cord injury	Positively associated with bone mineral content and density	24
Biomedica	Stage 3B and 4 chronic kidney disease	Negative association with glomerular filtration rate	25
Biomedica	Chronic kidney disease	Negative association with glomerular filtration rate	26
Biomedica	Adults with type 2 diabetes	Increased; associated with diabetes duration and HbA1c values	27
Biomedica	Women with type 2 diabetes	Increased; negative association with β-catenin levels	28
Biomedica	Adults with type 1 and 2 diabetes	Increased in type 2 diabetes versus type 1 diabetes and controls	29
Biomedica	Adults with fractures	Increased during fracture healing	31
Biomedica	Primary hypercortisolism	Increased versus controls	32
Biomedica	MGUS	Unchanged versus controls	33
Biomedica	Multiple myeloma	Increased; associated with bone disease/overall survival	34
Biomedica	Bisphosphonate treatment	Increased following bisphosphonate treatment	36
Biomedica/ TECO	Assay comparison	Biomedica values lower; plasma values higher than serum values	37
TECO	Postmenopausal women	Positive association with BMD; no association with fracture risk	13
TECO	Men aged $\geqslant$50 years	Positive association with age/BMD; negative association with fracture	14
TECO	Hyper- and hypoparathyroidism	Decreased in hyperparathyroidism; increased in hypoparathyroidism	18
TECO	Young men on bed rest	Increased by 14 days; elevated to 90 days	20
MSD	Sclerosteosis patients	Undetectable in sclerosteosis	5
MSD	Hyperparathyroidism/post-parathyroidectomy	Decreased in hyperparathyroidism compared with controls and post-parathyroidectomy patients	16
MSD	Men with type 2 diabetes	Increased; further increased with thiazolidinedione therapy	30
MSD	Primary hypercortisolism	Decreased; increased following surgical cure	4
Amgen[a]	Women aged $\geqslant$65 years	Positively associated with hip BMD and increased fracture risk	11
Amgen[a]	PTH-treated women	Decreased by intermittent PTH; serum and plasma levels similar	15
'In-house'/ R&D[b]	Ankylosing spondylitis	Decreased versus controls; lower with new syndesmophyte formation	19

Abbreviations: BMD, bone mineral density; HbA1c, glycosylated hemoglobin A1c fraction; MGUS, monoclonal gammopathy of undetermined significance; MSD, Meso Scale Discovery; PTH, parathyroid hormone 1–34; R&D, R&D Systems; TECO, TECO Medical Group.
[a]Non-commercially available.
[b]Enzyme-linked immunosorbent assay developed in the study laboratory using primary and secondary anti-sclerostin antibodies from R&D Systems.

sclerostin, circulating sclerostin levels correlate closely with bone marrow levels. Scerostin levels appear to increase with age in both sexes, and decrease with pharmacological estrogen or PTH 1–34 treatment. Serum levels of sclerostin have now been assessed in many clinical conditions in which bone loss and fractures are increased. The association of sclerostin levels with BMD and fracture risk in some, but not all, studies suggests that sclerostin measurement may have a future role in clinical practice. However, sclerostin levels do not always correlate as expected with observed BMD or bone turnover markers, suggesting that in many clinical scenarios, confounding variables are likely present that must be taken into account before routine clinical implementation. In addition, there exists a current dearth of expertise and information related to multiple aspects of fundamental sclerostin biology including: (a) potential sources of biological variability; (b) mechanism (renal or hepatic) and kinetics of clearance from the circulation; (c) relationship to total-body bone mineral content; and (d) assay standardization. Until such issues are resolved, measurement of serum sclerostin levels appears most useful for understanding the fundamental mechanisms by which osteocytes integrate hormonal, physical and pharmacological stimuli to regulate bone health, rather than as a guide for clinical-care decisions.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by National Institutes of Health Grant K08-AR059138 (to MTD).

References

1. Lim V, Clarke BL. New therapeutic targets for osteoporosis: beyond denosumab. *Maturitas* 2012;**73**:269–272.
2. 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**:537–543.
3. Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S *et al.* Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. *Am J Hum Genet* 2001;**68**:577–589.
4. van Lierop AH, van der Eerden AW, Hamdy NA, Hermus AR, den Heijer M, Papapoulos SE. Circulating sclerostin levels are decreased in patients with endogenous hypercortisolism and increase after treatment. *J Clin Endocrinol Metab* 2012;**97**:E1953–E1957.

5. van Lierop AH, Hamdy NA, Hamersma H, van Bezooijen RL, Power J, Loveridge N *et al.* Patients with sclerosteosis and disease carriers: human models of the effect of sclerostin on bone turnover. *J Bone Miner Res* 2011;**26**:2804–2811.
6. Kirmani S, Amin S, McCready LK, Atkinson EJ, Melton 3rd LJ , Muller R *et al.* Sclerostin levels during growth in children. *Osteoporos Int* 2012;**23**:1123–1130.
7. 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**:373–379.
8. 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**:148–154.
9. Ardawi MS, Al-Kadi HA, Rouzi AA, Qari MH. Determinants of serum sclerostin in healthy pre- and postmenopausal women. *J Bone Miner Res* 2011;**26**:2812–2822.
10. Modder UI, Clowes JA, Hoey K, Peterson JM, McCready L, Oursler MJ *et al.* Regulation of circulating sclerostin levels by sex steroids in women and in men. *J Bone Miner Res* 2011;**26**:27–34.
11. Arasu A, Cawthon PM, Lui LY, Do TP, Arora PS, Cauley JA *et al.* Serum sclerostin and risk of hip fracture in older Caucasian women. *J Clin Endocrinol Metab* 2012;**97**:2027–2032.
12. Ardawi MS, Rouzi AA, Al-Sibiani SA, Al-Senani NS, Qari MH, Mousa SA. High serum sclerostin predicts the occurrence of osteoporotic fractures in postmenopausal women: the Center of Excellence for Osteoporosis Research Study. *J Bone Miner Res* 2012;**27**: 2592–2602.
13. Garnero P, Sornay-Rendu E, Munoz F, Borel O, Chapurlat RD. Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. *Osteoporos Int* 2013;**24**:489–494.
14. Szulc P, Bertholon C, Borel O, Marchand F, Chapurlat R. Lower fracture risk in older men with higher sclerostin concentration - A prospective analysis from the MINOS study. *J Bone Miner Res* 2012;**28**:855–864.
15. 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**:5056–5062.
16. van Lierop AH, Witteveen JE, Hamdy NA, Papapoulos SE. Patients with primary hyperparathyroidism have lower circulating sclerostin levels than euparathyroid controls. *Eur J Endocrinol* 2010;**163**:833–837.
17. Ardawi MS, Al-Sibiany AM, Bakhsh TM, Rouzi AA, Qari MH. Decreased serum sclerostin levels in patients with primary hyperparathyroidism: a cross-sectional and a longitudinal study. *Osteoporos Int* 2012;**23**:1789–1797.
18. 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**:3804–3810.
19. Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R *et al.* Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. *Arthritis Rheum* 2009;**60**:3257–3262.
20. Frings-Meuthen P, Boehme G, Liphardt AM, Baecker N, Heer M, Rittweger J. Sclerostin and DKK1 levels during 14 and 21 days of bed rest in healthy young men. *J Musculoskelet Neuronal Interact* 2013;**13**:45–52.
21. Spatz JM, Fields EE, Yu EW, Divieti Pajevic P, Bouxsein ML, Sibonga JD *et al.* Serum sclerostin increases in healthy adult men during bed rest. *J Clin Endocrinol Metab* 2012;**97**:E1736–E1740.
22. Battaglino RA, Sudhakar S, Lazzari AA, Garshick E, Zafonte R, Morse LR. Circulating sclerostin is elevated in short-term and reduced in long-term SCI. *Bone* 2012;**51**:600–605.
23. Morse LR, Sudhakar S, Lazzari AA, Tun C, Garshick E, Zafonte R *et al.* Sclerostin: a candidate biomarker of SCI-induced osteoporosis. *Osteoporos Int* 2013;**24**:961–968.
24. Morse LR, Sudhakar S, Danilack V, Tun C, Lazzari A, Gagnon DR *et al.* Association between sclerostin and bone density in chronic spinal cord injury. *J Bone Miner Res* 2012;**27**:352–359.
25. Thambiah S, Roplekar R, Manghat P, Fogelman I, Fraser WD, Goldsmith D *et al.* Circulating sclerostin and Dickkopf-1 (DKK1) in predialysis chronic kidney disease (CKD): relationship with bone density and arterial stiffness. *Calcif Tissue Int* 2012;**90**:473–480.
26. Pelletier S, Dubourg L, Carlier MC, Hadj-Aissa A, Fouque D. The relation between renal function and serum sclerostin in adult patients with CKD. *Clin J Am Soc Nephrol* 2013;**8**:819–823.
27. 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**:234–241.
28. Gaudio A, Privitera F, Battaglia K, Torrisi V, Sidoti MH, Pulvirenti I *et al.* Sclerostin levels associated with inhibition of the Wnt/beta-catenin signaling and reduced bone turnover in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2012;**97**:3744–3750.
29. 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**: 1737–1744.
30. van Lierop AH, Hamdy NA, van der Meer RW, Jonker JT, Lamb HJ, Rijzewijk LJ *et al.* Distinct effects of pioglitazone and metformin on circulating sclerostin and biochemical markers of bone turnover in men with type 2 diabetes mellitus. *Eur J Endocrinol* 2012;**166**:711–716.
31. Sarahrudi K, Thomas A, Albrecht C, Aharinejad S. Strongly enhanced levels of sclerostin during human fracture healing. *J Orthop Res* 2012;**30**:1549–1555.
32. Belaya ZE, Rozhinskaya LY, Melnichenko GA, Solodovnikov AG, Dragunova NV, Iljin AV *et al.* Serum extracellular secreted antagonists of the canonical Wnt/beta-catenin signaling pathway in patients with Cushing's syndrome. *Osteoporos Int* 2013; e-pub ahead of print 29 January 2013; doi:10.1007/s00198-013-2268-y.
33. Ng AC, Khosla S, Charatcharoenwitthaya N, Kumar SK, Achenbach SJ, Holets MF *et al.* Bone microstructural changes revealed by high-resolution peripheral quantitative computed tomography imaging and elevated DKK1 and MIP-1alpha levels in patients with MGUS. *Blood* 2011;**118**:6529–6534.
34. Terpos E, Christoulas D, Katodritou E, Bratengeier C, Gkotzamanidou M, Michalis E *et al.* Elevated circulating sclerostin correlates with advanced disease features and abnormal bone remodeling in symptomatic myeloma: reduction post-bortezomib monotherapy. *Int J Cancer* 2012;**131**:1466–1471.
35. 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**:19–26.
36. Gatti D, Viapiana O, Adami S, Idolazzi L, Fracassi E, Rossini M. Bisphosphonate treatment of postmenopausal osteoporosis is associated with a dose dependent increase in serum sclerostin. *Bone* 2012;**50**:739–742.
37. McNulty M, Singh RJ, Li X, Bergstralh EJ, Kumar R. Determination of serum and plasma sclerostin concentrations by enzyme-linked immunoassays. *J Clin Endocrinol Metab* 2011;**96**:E1159–E1162.
38. He JW, Yue H, Hu WW, Hu YQ, Zhang ZL. Contribution of the sclerostin domain-containing protein 1 (SOSTDC1) gene to normal variation of peak bone mineral density in Chinese women and men. *J Bone Miner Metab* 2011;**29**:571–581.