Alcohol and Alcoholism Vol. 48, No. 3, pp. 278–282, 2013
Advance Access Publication 7 January 2013
doi: 10.1093/alcalc/ags136

ORIGINAL ARTICLE

Serum Sclerostin in Alcoholics: A Pilot Study

E. González-Reimers[1,*], C. Martín-González[1], M.J. de la Vega-Prieto[2], R. Pelazas-González[1], C. Fernández-Rodríguez[1], J. López-Prieto[1], J. Alvisa-Negrín[1] and F. Santolaria-Fernández[1]

[1]Servicio de Medicina Interna and [2]Servicio de Laboratorio, Hospital Universitario de Canarias, Universidad de La Laguna, Tenerife, Canary Islands, Spain
*Corresponding author: Servicio de Medicina Interna, Hospital Universitario de Canarias, Ofra s/n, Tenerife, Canary Islands, Spain; Tel.: +34 922-678600; E-mail: egonrey@ull.es

(*Received* 2 *September* 2012; *first review notified* 25 *October* 2012; *in revised form* 7 *November* 2012; *accepted* 30 *November* 2012)

Abstract — Aims: Sclerostin is an endogenous inhibitor of the Wnt/β-catenin pathway secreted by osteocytes, which inhibits osteoblast function, differentiation and survival. As a consequence, sclerostin tends to decrease bone mass. Alcoholics frequently present osteoporosis, mainly due to decreased bone synthesis. The behaviour of sclerostin in these patients is unknown. The aim of this work was to analyse the relationship between serum sclerostin levels and bone mineral density (BMD), ethanol consumption, nutritional status, liver function derangement and biomarkers of bone homeostasis in alcoholic patients. **Methods:** We included 31 alcoholic patients, of whom 11 were infected with Hepatitis C virus (HCV) and 7 age and sex-matched controls. All underwent densitometry, and serum sclerostin, osteocalcin, collagen telopeptide, parathyroid hormone (PTH), vitamin D, cortisol and testosterone were determined. **Results:** Sclerostin levels were significantly higher in patients (30.95 ± 18.91 pmol/l) than controls ($t = 4.4$; $P < 0.001$), especially in non-HCV patients; they showed an inverse correlation with osteocalcin, prothrombin activity and serum albumin, and a direct correlation with bilirubin and telopeptide, but not with BMD, nutritional status or ethanol intake. **Conclusions:** Serum sclerostin was raised in alcoholic patients, and it correlated with decreased markers of bone synthesis and increased markers of bone breakdown. The elevation in sclerostin levels was clearly related with liver function, but not with ethanol intake, nutritional status or concomitant HCV infection.

INTRODUCTION

Current knowledge supports the view that sclerostin is a key signalling protein involved in the mechanosensing function of osteocytes, modulating changes in bone synthesis after bone loading (Robling *et al*., 2008; Lin *et al*., 2009). Activation of the Wnt/β-catenin pathway in bones leads to increased bone formation and osteoblast proliferation (Hoeppner *et al*., 2009), and to decreased bone resorption, because it also blocks osteoclastogenesis by increasing the OPG/RANKL ratio (Kubota *et al*., 2009). Sclerostin is an endogenous inhibitor of the Wnt/β-catenin pathway (Williams and Isogna, 2009) secreted by osteocytes (Van Bezooijen *et al*., 2005). It antagonizes Wnt/β-catenin signalling (Li *et al*., 2005) by binding to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6), thereby inhibiting osteoblast function, differentiation and survival (Baron and Rawadi, 2007; Li *et al*., 2008). As a consequence, sclerostin tends to decrease bone mass (Van Bezooijen *et al*., 2004; Poole *et al*., 2005) and bone turnover (Ardawi *et al*., 2012; Gaudio *et al*., 2012). Indeed, high sclerostin levels have been reported for Type II diabetic patients with osteoporosis (Gaudio *et al*., 2012), although it is noteworthy that low sclerostin levels have been found in osteoporotic women (Sheng *et al*., 2012). No changes in sclerostin levels were observed in girls with anorexia nervosa (Faje *et al*., 2012), but in other diseases characterized by increased bone turnover, such as Paget disease or metastatic prostate cancer, serum sclerostin levels were significantly increased (Yavropoulou *et al*., 2012), as in other forms of osteoporosis (Voskaridou *et al*., 2012).

Alcoholics frequently present osteoporosis (Diamond *et al*., 1989). These patients show decreased osteocalcin levels, suggesting decreased bone synthesis (Farley *et al*., 1985), whereas normal, decreased or increased bone breakdown parameters have been reported. Therefore, the intensity of bone breakdown is unclear, although in many cases it seems that a low-turnover osteoporosis ensues. Since sclerostin is related to low-turnover osteoporosis, it is important to analyse the behaviour of this protein in alcoholics. This was the aim of the present study, in which we compared serum sclerostin levels in alcoholic patients with bone mineral density (BMD), nutritional status, alcohol intake and liver function.

PATIENTS AND METHODS

We included 31 alcoholic patients, drinkers of >150 g ethanol/day during a protracted period (Table 1), 26 men and 5 women, aged 49.77 ± 10.83 and 46.60 ± 10.21 years, respectively. They were compared with 7 age and sex-matched controls (2 women; Table 1), drinkers of <10 g ethanol/day. Eleven patients were also positive for hepatitis C virus (HCV), assessed either by the presence of anti-HCV and/or HCV RNA by reverse transcriptase polymerase chain reaction (PCR; genotype 1 in 7 cases, 3 in 2 cases; unknown in 2 cases).

Bone densitometry and nutritional evaluation

After informed consent, patients and controls underwent densitometric evaluation with a Lunar Prodigy Advance device (General Electric, Piscataway, NJ, USA). Two kinds of evaluation were performed: a specific bone densitometric study of hip and lumbar spine, in order to record Z and T scores, following standard criteria (Cummings *et al*., 2002), and a whole body densitometric analysis (only in patients), recording BMD, fat and lean mass at different parts of the body, such as upper limbs, ribs, pelvis, lower limbs, spine and total body. Total lean mass and total fat mass were used in the assessment of nutritional status. Body mass index (BMI, as weight (in kg)/height^2 (in m)) was also recorded.

Table 1. Some biological parameters of patients and controls ($x \pm$ SD are shown)

	Non-HCV alcoholics	HCV alcoholics	Controls	T (Z),; *P*
Age (years)	52.40 ± 8.33	43.55 ± 12.32	50.43 ± 6.19	$F = 3.22$; NS
Body mass index (kg/m^2)	26.7 ± 5.3	25.4 ± 3.2	27.9 ± 5.00	$F = 0.65$; NS
Total hip T-score	−0.82 ± 1.08	−0.16 ± 1.24	0.45 ± 1.19	$F = 3.36$; $P = 0.047$
Lumbar spine T-score	−0.56 ± 1.46	−0.31 ± 1.06	−0.01 ± 1.91	$F = 0.38$; NS
Ethanol consumption (daily amount (g))	207 ± 75	167 ± 54	<10	$T = 1.79$; NS
Years of consumption	26 ± 7	16 ± 12	—	$T = 2.8$; $P = 0.008$
Serum sclerostin (pmol/L)	36.33 ± 20.48	21.15 ± 6.84	15.06 ± 3.93	$F = 6.31$; $P = 0.005$
Serum osteocalcin (ng/ml)	3.37 ± 2.51	5.27 ± 2.95	7.56 ± 3.30	KW = 12.52; $P = 0.002$
Serum telopeptide (nmol/L)	0.55 ± 0.50	0.36 ± 0.21	0.20 ± 0.11	$F = 1.89$; NS
Serum IGF-1 (ng/ml)	79.72 ± 74.02	122.88 ± 76.82	214.71 ± 78.20	$F = 7$; $P = 0.004$
Serum PTH (pg/ml)	42.80 ± 28.82	36.22 ± 24.86	62.07 ± 54.01	$F = 1.16$; NS
Serum calcitriol (pg/ml)	45.98 ± 25.85	50.50 ± 16.66	100.53 ± 25.92	$F = 15$; $P < 0.001$
Serum cortisol (μg/dl)	22.35 ± 7.01	13.87 ± 5.58	17.82 ± 7.07	$F = 4.36$; $P = 0.025$
Serum testosterone (pg/ml)	6.15 ± 5.73	4.36 ± 5.92	18.55 ± 3.60	$F = 15.27$; $P < 0.001$

Biochemical assessment

In addition to routine laboratory tests (which included creatinine, bilirubin, prothrombin activity and serum albumin), we performed the following biochemical determinations:

Serum sclerostin, for all the patients and controls, by one step enzyme-linked immunosorbent assay (ELISA) (Biomedica Gruppe, Wien, Austria; interassay variation coefficient 4–6%; intra-assay variation coefficient 5%); serum osteocalcin, for all the patients and controls, by immunometric chemiluminiscent assay (recovery = 97–121%; variation coefficients of assays ranging from 3.5% to 7.1%; DPC, Los Angeles, CA, USA), as a marker of bone synthesis and C-terminal telopeptide of Type I collagen (CrossLaps), by one step ELISA, with a recovery ranging from 94 to 107% and an intra- and interassay variation coefficient of 4.7–4.9% and 5.4–8.1%, respectively (Osteometer Bio Tech A/S, Herlev, Denmark), as a marker of bone breakdown (performed in all the controls and 25 patients). We also determined serum insulin-like growth factor 1 (IGF-1) (Chemiluminiscent assay, DPC, Los Angeles, CA, USA), in 23 patients and all the controls; 1,25 dihydroxyvitamin D3 in 19 patients and all the controls (radioimmunoassay (RIA), Nichols, San Juan Capistrano, CA, USA), parathyroid hormone (PTH), in 22 patients and all the controls; serum testosterone in 14 patients and all the controls (solid phase RIA).

The study protocol was approved by the local ethics committee of our Hospital (PI 25/2009) and was carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Statistics

The Kolmogorov–Smirnov test was used to test for normal distribution. ANOVA and Student's *t* test or Kruskall–Wallis and Mann–Whytney's *U*-test for variables with non-parametric distribution) were used to compare mean values between three or two different groups, respectively. Spearman's ρ and Pearson's correlation were used to compare quantitative variables, and χ^2 test was used to compare qualitative variables. Multiple linear regression analyses were used when necessary, to discern if correlations observed in the univariate analysis between sclerostin and different variables were independent or not.

All these analyses were performed using the SPSS software (Chicago, IL, USA). A *post-hoc* statistical power analysis showed that the sample size necessary to detect differences between patients and controls was 38 cases, and to detect differences among advanced (Child's B and C patients) and less advanced ones (Child's A patients) it was 22 (http://www.danielsoper.com/statcalc).

RESULTS

Sclerostin levels were significantly higher in patients (30.95 ± 18.91 pmol/l) than controls ($t = 4.4$; $P < 0.001$). On comparing patients with concomitant HCV infection versus those without, differences were also significant (Table 1). A direct correlation was observed between age and sclerostin levels, both when patients and controls were pooled together ($r = 0.40$; $P = 0.012$) and when only patients were included ($r = 0.47$; $P = 0.007$). Considering only patients, there was a nearly significant trend to higher values of sclerostin in men (33.65 ± 18.76 pmol/l) than in women (16.91 ± 5.01 pmol/l; $t = 1.96$; $P = 0.06$). However multiple correlation analysis with stepwise entry of the variables age, sex and group, showed that age was the only independent variable associated with sclerostin levels.

Relationship with bone alterations

All patients had normal serum creatinine values (range = 0.30–1.10 mg/dl; median (interquartile range) = 0.70 (0.5–0.83) mg/dl. Despite preserved renal function, 14 patients were osteopenic according to total hip T-score (T-score range = −1.10 to −2.40), and the remaining 17 were normal (T-score range = −0.90 to +1.60). According to lumbar spine T-score, 2 patients were osteoporotic (T-score −2.60 and −2.80), 9 were osteopenic (range = −1.20 to −1.60) and the others were normal (range = −0.80 to +3.70). As with sclerostin, patients showed lower total hip T-score than controls ($t = 2.1$; $P = 0.043$), but no differences in lumbar spine T-score ($T = 0.77$; NS). No correlations were observed, however, between sclerostin and BMD or T-score for any of the parts of the skeleton analysed.

Relationship with biochemical markers of bone homeostasis and hormones

Alcoholic patients showed lower osteocalcin, and also a trend to higher telopeptide levels than controls (Table 1). This trend was more pronounced in alcoholics without HCV

infection than in those with HCV infection. Sclerostin showed a direct correlation with serum telopeptide ($r = 0.57$; $P = 0.003$), and an inverse correlation with osteocalcin ($\rho = -0.46$; $P = 0.009$). Several hormones related with bone homeostasis showed significant differences between patients and controls, as shown in Table 1. This was the case for IGF-1 ($T = 3.43$; $P = 0.002$), testosterone ($T = 5.68$; $P < 0.001$) and calcitriol ($t = 4.91$, $P < 0.001$), which were always lower in patients than in controls. However, no correlations were observed between sclerostin and any of these hormones.

Relationship with liver function

Sclerostin was significantly directly correlated with serum bilirubin ($r = 0.73$; $P < 0.0001$), and inversely with albumin ($r = -0.54$, $P = 0.001$) and prothrombin activity ($r = -0.80$; $P < 0.0001$). Indeed, by multiple regression analysis, prothrombin activity and bilirubin, and age in the third place, were independently correlated with sclerostin. According to the Child-Pugh score (based on the presence and characteristics of ascites, encephalopathy, and serum albumin, bilirubin and prothrombin activity), 6 patients were classified as Child C, 7 Child B and 18 Child A. Sclerostin was significantly higher in Child C and Child B patients than in Child A patients (KW = 11.74; $P = 0.003$, Fig. 1). A similar difference was observed when Child B and C patients were pooled together (13) and compared with Child A ones (18) ($Z = 3.12$; $P = 0.002$). Statistical power analysed revealed that the minimum size required for this analysis is 22 cases, at least 11 for each group.

Relationship with ethanol intake and nutritional status

Sclerostin levels were unrelated to ethanol intake ($r = 0.25$) or viral load ($\rho = -0.21$; $P > 0.1$ in both cases), but were significantly related with the duration of the drinking habit ($r = 0.51$, $P = 0.004$). However, this relation was displaced by prothrombin activity and age in the multiple regression analysis (i.e. those who had been drinking for longer periods of time were older and showed lower prothrombin activity). We also failed to find any relationship between sclerostin levels and lean mass or fat mass-related variables or with BMI.

DISCUSSION

This study showed that sclerostin levels were raised in alcoholics compared with a control group. Alcoholics showed a lower T-score than controls, and, in accordance with other studies, this bone alteration is probably related to decreased bone formation, given the finding of low osteocalcin levels in these patients. Decreased bone formation as a major feature of alcohol-mediated bone loss has been reported by many authors, in studies performed decades ago (Crilly *et al.*, 1988; Diamond *et al.*, 1989) as well as in recent ones (Santori *et al.*, 2008). Some controversy exists regarding bone breakdown (Preedy *et al.*, 1991; Dai *et al.*, 2000). We observed a non-significant trend to increased bone breakdown in alcoholics, according to serum telopeptide levels, but serum telopeptide may be influenced by liver collagen metabolism (Ricard-Blum *et al.*, 1996); moreover, the patients included here were heavy drinkers and most had advanced liver disease. Indeed, experimental data suggest that bone breakdown is also decreased in alcoholics (Preedy *et al.*, 1991; Turner *et al.*, 2001), so low bone turnover osteoporosis seems to be the characteristic feature of these patients. Therefore, the finding of raised sclerostin levels reported in this study is fully in

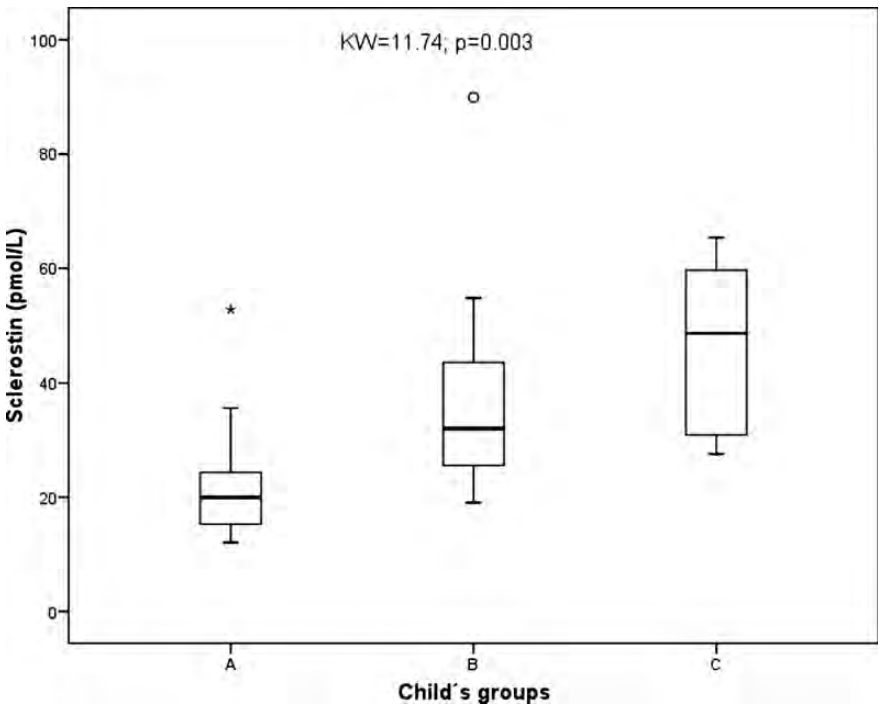

Fig. 1. Serum sclerostin levels in alcoholics, classified according to Child's groups. Circles correspond to outliers, and asterisks, to extreme values.

accordance with the observations of raised sclerostin levels in other forms of osteoporosis characterized by low bone turnover.

To our knowledge, this is the first study to analyse sclerostin levels in alcoholics, although experimental data suggest that the expression of sclerostin was significantly increased after acute binge alcohol treatment (Callaci *et al*., 2009), and it seems that ethanol may induce osteocyte apoptosis (Maurel *et al*., 2012). Interestingly, sclerostin levels showed significant relationships with biomarkers of bone remodelling, in a way consistent with the physiological actions of this substance: a direct correlation with telopeptide, in accordance with the increase in osteoclastogenesis and an inverse correlation with osteocalcin, consistent with the inhibition of osteoblastic differentiation and function. These correlations were significant even in the absence of any relationship with BMD—although there was a non-significant trend to higher values among those classified as osteoporotic according to total hip T-score. Alcoholic patients also showed decreased levels of hormones involved in bone synthesis, such as IGF-1, testosterone and vitamin D. Although there were no significant correlations with sclerostin, low levels of these hormones strengthen the hypothesis that decreased bone synthesis plays a major role in bone alterations observed in alcoholics.

A close correlation was observed between sclerostin and liver function: the inverse correlation with prothrombin activity and the direct one with serum bilirubin underscore this assertion. Indeed, many studies have reported a relationship between bone loss and liver function (Jorge-Hernández *et al*., 1988; Guañabens and Parés, 2010); therefore, our findings are clearly related with the observed effect of impaired liver function on bone metabolism. Other studies have shown that osteoporosis in alcoholics depends on the amount of ethanol ingested and that bone alterations improve with abstinence (Peris *et al.,* 1994; Alvisa-Negrín *et al*., 2009), raising the possibility of a direct effect of ethanol on bone homeostasis, a link which has been convincingly demonstrated, at least regarding osteoblast function (Diamond *et al.,* 1989). However, in this study, no correlation was observed between sclerostin and the amount of ethanol ingested, and the initially observed correlation with the duration of ethanol intake was in fact due to the confounding effect of more severe liver disease in those who had been drinking for a longer time.

HCV infection is common in alcoholics (Mueller *et al*., 2009) and may lead to osteoporosis (Gallego-Rojo *et al*., 1998; Corazza *et al*., 2000), although it is debatable whether the effect is due to the virus itself, to liver function derangement or other factors such as impaired nutritional status. This study showed that HCV infected patients had slightly higher sclerostin values than controls, but clearly lower than those of the non-HCV alcoholics. However, better liver function was observed in patients with HCV infection than in those without. Indeed, multiple regression analysis showed that liver function displaced the variables HCV infection and viral load as correlates of serum sclerostin. The possibility that sclerostin is also raised in other forms of liver disease remains speculative, but it is seems that the degree of osteopenia in liver cirrhosis may be related to the severity and not the aetiology of the liver disease (Giouleme *et al*., 2006).

Finally, we found no correlation with nutritional status, evaluated both by BMI and densitometric body composition analysis. It is clear that malnutrition in alcoholic patients, especially if advanced, leads to osteoporosis (Santolaria *et al.,* 2000), but our patients were not malnourished since BMI and lean mass were similar to those of the controls. Possibly, this fact hampered our evaluation of the influence of malnutrition on sclerostin levels in alcoholics.

In summary, we report here high values of serum sclerostin in alcoholic patients, which correlated with decreased markers of bone synthesis and increased markers of bone breakdown. The elevation in sclerostin levels was clearly related with liver function, but not with ethanol intake, nutritional status or concomitant HCV infection. Sclerostin is secreted by osteocytes and exerts an inhibitory effect on osteoblasts via inhibition of the canonical Wnt signalling pathway, although it also increases osteoclast formation and activity (Wijenayaka *et al*., 2011). Callaci *et al.* (2009) showed that sclerostin increases after acute binge alcohol exposure. Our study strongly suggests that osteocytes and the canonical Wnt signalling pathway also play an important role in bone changes observed in chronic alcoholics, although it seems that the effect of deranged liver function is more important than that of ethanol itself.

Conflict of interest statement. None declared.

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