Changes of vitamin D levels and bone turnover markers after CPAP therapy: a randomized sham-controlled trial

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SUMMARY

The aim was to investigate whether continuous positive airway pressure treatment could modulate serum vitamin D (25-hydroxyvitamin D) and bone turnover markers (collagen-type 1 cross-linked C-telopeptide, osteocalcin and N-terminal propeptide of type 1 collagen) in secondary analysis from a randomized controlled trial. Sixty-five continuous positive airway pressure-naïve male patients with obstructive sleep apnea (age = 49 ± 12 years, apnea–hypopnea index = 39.9 ± 17.7 events h^{-1}, body mass index = 31.3 ± 5.2 kg m^{-2}) were randomized to receive either real (n = 34) or sham (n = 31) continuous positive airway pressure for 12 weeks. At 12 weeks, all participants received real continuous positive airway pressure for an additional 12 weeks. After 12 weeks of continuous positive airway pressure (real versus sham), there were no between-group differences for any of the main outcomes [Δ25-hydroxyvitamin D: −0.80 ± 5.28 ng mL^{-1} (mean ± SE) versus 3.08 ± 3.66 ng mL^{-1}, P = 0.42; Δcollagen-type 1 cross-linked C-telopeptide: 0.011 ± 0.014 ng mL^{-1} versus −0.004 ± 0.009 ng mL^{-1}, P = 0.48; Δosteocalcin: 1.13 ± 1.12 ng mL^{-1} versus 0.46 ± 0.75 ng mL^{-1}, P = 0.80; ΔN-terminal propeptide of type 1 collagen: 2.07 ± 3.05 μg L^{-1} versus −1.05 ± 2.13 μg L^{-1}, P = 0.48]. There were no further differences in subgroup analyses (continuous positive airway pressure-compliant patients, patients with severe obstructive sleep apnea or sleepy patients). However, after 24 weeks irrespective of initial randomization, vitamin D increased in patients with severe obstructive sleep apnea (9.56 ± 5.51 ng mL^{-1}, P = 0.045) and in sleepy patients (14.0 ± 4.69 ng mL^{-1}, P = 0.007). Also, there was a significant increase in osteocalcin at 24 weeks (3.27 ± 1.06 ng mL^{-1}, P = 0.01) in compliant patients. We conclude that 12 weeks of continuous positive airway pressure did not modulate vitamin D or modulate any of the bone turnover markers compared with sham. However, it is plausible that continuous positive airway pressure may have late beneficial effects on vitamin D levels and bone turnover markers in selected groups of patients with obstructive sleep apnea.

INTRODUCTION

Despite its inanimate appearance, bone is a dynamic and living tissue that undergoes bone remodelling throughout life whereby bone resorption and bone formation are controlled by the osteoclast and osteoblast cells, respectively, to replace old and brittle bone with strong, but tensile, new bone. Biochemical products of the bone remodelling processes are termed bone turnover markers, which can be detected in the serum using specific assays. These include bone formation markers, such as osteocalcin and propeptide of type 1 collagen (P1NP), as well as bone resorption markers, such as collagen-type 1 cross-linked C-telopeptide (β-CTx) altogether reflecting current bone turnover (Biver, 2012). Vitamin D facilitates the absorption of calcium across the gut and thus plays an important role in bone metabolism, although it has also been shown to be involved in several metabolic processes (Takiishi *et al.*, 2010). In addition, many cells in the body have vitamin D receptors as well as the ability to convert vitamin D into its active form, 1,25-dihydroxyvitamin D_3 (Holick, 2007; McCarty *et al.*, 2014). In previous studies, vitamin D deficiency has been associated with diabetes, hypertension and stroke (Holick, 2007), all of which are overrepresented in patients with obstructive sleep apnea (OSA; Herrmann *et al.*, 2015).

Obstructive sleep apnea in combination with excessive daytime sleepiness, known as obstructive sleep apnea syndrome, affects approximately 6% of adult men and 4% of adult women (Arnardottir *et al.*, 2016; Franklin and Lindberg, 2015). Patients with OSA may present with multiple morbidities, and previous studies show OSA increases the risk of several metabolic disorders (Parish, 2012). The mechanisms are still not fully understood and cannot be entirely explained by concomitant obesity. Intermittent hypoxia and sleep fragmentation have been suggested as potential pathways by which OSA may promote metabolic dysfunction (Ip *et al.*, 2002; Peppard *et al.*, 2000).

Recent evidence has identified a link between OSA and vitamin D deficiency (Archontogeorgis *et al.*, 2017; Barcelo *et al.*, 2013; Bozkurt *et al.*, 2012; Erden *et al.*, 2014; Mete *et al.*, 2013; Salepci *et al.*, 2017; Toujani *et al.*, 2017), as well as shown potential associations between OSA and osteoporosis (Upala *et al.*, 2016). Furthermore, the bone turnover markers osteocalcin and β-CTx have also been shown to be reduced in OSA compared with controls (Terzi and Yilmaz, 2016; Tomiyama *et al.*, 2008). Previous studies have considered whether OSA can affect bone turnover and lead to bone loss through various mechanisms, including hypoxia (Chakhtoura *et al.*, 2015). In addition, the bone formation marker, osteocalcin, has been correlated with glucose metabolism (Brennan-Speranza and Conigrave, 2015). Altogether, these studies have shown associations between bone turnover markers, vitamin D and OSA or features of OSA, as well as energy metabolism. These correlations indicate the novel possibility of a link between OSA and metabolic disease (Swanson *et al.*, 2015).

Previous observational studies have shown continuous positive airway pressure (CPAP) increases vitamin D levels (Liguori *et al.*, 2015, 2017) and decreases markers of bone resorption (Tomiyama *et al.*, 2008). It should be noted, however, that most of these studies have relied on short follow-up times (Liguori *et al.*, 2015; Tomiyama *et al.*, 2008) and, to date, no randomized controlled trials (RCTs) have investigated the long-term effects of CPAP on both vitamin D levels and bone turnover markers. This study therefore investigated the effects of CPAP treatment on serum vitamin D and bone turnover markers using a randomized placebo-controlled design. A secondary explorative aim was to assess correlations between vitamin D, bone turnover markers and some measures of glucose metabolism. The previously published primary aim of the study was to determine the effects of CPAP on markers of cardio-metabolic function (Hoyos *et al.*, 2012).

MATERIALS AND METHODS

Participants and setting

The study methods have been described in detail previously (Hoyos *et al.*, 2012). In short, all participants were recruited from tertiary referral sleep clinics at Royal Prince Alfred Hospital and the Woolcock Institute of Medical Research, Sydney, Australia. Eligible participants were adult men (aged $\geq$ 18 years) with moderate to severe OSA, defined as an apnea–hypopnea index (AHI) $\geq$ 20 events h^{-1} and an oxygen desaturation index (ODI) 3% $\geq$ 15 events h^{-1} measured by in-laboratory polysomnography (PSG). Exclusion criteria were: type II diabetes mellitus; previous CPAP treatment; minimum oxygen saturation $<$ 65%; or an AHI $>$ 80 events h^{-1}. Participants were also excluded if they had an uncontrolled concurrent medical illness, drug abuse or psychiatric illness, or irregular sleep patterns (for instance, shift workers).

Study design

This was a randomized, double-blind, sham-controlled, parallel group study. Eligible participants were randomized to receive either real or sham-CPAP for 12 weeks. Clinical assessments were performed before (week 0), midway at 6 weeks, and after treatment (week 12). At the end of the 12-week blinded period, all participants were provided with open-label real-CPAP for an additional 12 weeks and assessments were recollected at 24 weeks. The study flow of participants through the study has previously been reported in detail (Hoyos *et al.*, 2012).

Randomization, allocation concealment and blinding

Randomization was done using a computer program that produced randomized permuted blocks with a block size of four. Participants were assigned to real- or sham-CPAP in a

1 : 1 ratio. At baseline, each participant was assigned a unique number in sequential, ascending, chronological order that corresponded to the treatment allocation. The study investigators were blinded to treatment allocation for the duration of the study. Also during the open-label period both the participants and study investigators remained blinded to the initial treatment allocation. All machine preparation was performed by a person separate to the study investigators and not involved in participant assessments.

CPAP machines and titration

The real- and sham-CPAP machines (Remstar Auto, Philips Respironics, Murrysville, Pennsylvania, USA) used in the study were identical in appearance to each other and have been used previously (Kushida *et al.*, 2006; Phillips *et al.*, 2011). The sham device was constructed to deliver airflow with minimal pressure (0.5 cm H_2O). Prior to randomization, every participant received a standard CPAP education program and a mask was fitted. Compliance data were recorded by an internal clock within all real- and sham-CPAP machines, and data were downloaded after the home titration and at each study visit. Further details have been reported previously (Hoyos *et al.*, 2012).

PSG and measures of OSA and sleepiness

Sleep and breathing variables in all participants were assessed using an attended overnight, in-laboratory PSG device (Sandman Elite V.9.2; Tyco Healthcare, Denver, Colorado, USA). PSGs were recorded at week 0 (standard diagnostic PSG) and also at weeks 12 and 24, in all participants while using their allocated CPAP machines. The Epworth Sleepiness Scale (ESS; Johns, 1991) was used at all visits to assess subjective sleepiness. Additional details have been reported elsewhere (Tomiyama *et al.*, 2008).

Blood sampling

Fasting morning blood samples were drawn when patients came to the clinic at baseline and again at weeks 6, 12 and 24. Vitamin D {measured as 25-hydroxyvitamin D3 [25 (OHD)]; ng mL^{-1}} was chromatographically measured using the Roche Modular P device (Roche, Sydney, Australia). The bone turnover markers: plasma osteocalcin (ng mL^{-1}); β-CTx (ng mL^{-1}); and P1NP (μg L^{-1}) were measured via electrochemiluminescence method using the Cobas® kit (Roche) on the Roche Modular P machine (Roche Diagnostics). In addition, parathyroid hormone (PTH), thyroid-stimulating hormone (TSH), calcium, phosphorus levels, glucose, insulin and insulin-like growth factor-1 (IGF-1) concentrations were all measured using commercially available assays. All samples were stored at −80 °C for subsequent batched analysis, and all samples from an individual patient were run within a single assay.

Ethics

The study complied with Good Clinical Practice guidelines, applicable regulatory requirements and the Declaration of Helsinki. All participants provided written informed consent to participate in the study, which was approved by the Sydney South West Area Health Service Human Research and Ethics Committee (RPAH Zone). The study is registered with the Australia New Zealand Clinical Trials Network, http://www.anzctr.org.au, number ACTRN12608000301369.

Statistical analysis

The outcome variables were the calculated differences in vitamin D and the bone turnover markers, from baseline to 12 and 24 weeks, respectively. Mixed model analysis was used to determine between-treatment group differences from baseline during the blinded period (0–12 weeks). Further analyses explored the influence of treatment compliance, baseline severity (AHI) and sleepiness (ESS) with predefined cut-off points (compliance 4 h $night^{-1}$; AHI 30 events h^{-1}; ESS > 10). These analyses were performed to assess whether the effect of CPAP was influenced by compliance, baseline AHI, obesity or sleepiness.

In addition, we also performed multiple regression analysis to assess the effect of CPAP treatment on vitamin D and bone turnover markers at week 12 using the 12-week measurements as dependent variables adjusting for baseline measurements and potential confounders (PTH, TSH, calcium and phosphorus levels).

Second, pooled mean changes and the standard error of the mean from baseline to week 24 were determined regardless of initial treatment allocation, and both the Student's *t*-test and the Wilcoxon signed-rank test were performed to test differences between groups between baseline and 24 weeks in the whole group and in subgroups.

Finally, correlations among vitamin D, bone turnover markers and IGF-1, glucose and insulin were assessed using Pearson's correlation for measures at each time point, and also for changes between time points [baseline, week 12 (all participants) and week 24 (all participants)]. Analyses were performed using Stata 13 (Stata, College Station, TX, USA). All participants with available data, regardless of CPAP use, were included in all analyses. Data were considered significantly different at $P < 0.05$ (two-sided).

RESULTS

The RCT flow has been presented previously (Hoyos *et al.*, 2012); however, in short: 65 men were randomized to receive either real- ($n = 34$) or sham- ($n = 31$) CPAP treatment. Outcome data were available for 48 men at week 12 and 44 men at week 24. Baseline characteristics were comparable in the two treatment groups (Table 1).

Table 1 Baseline characteristics of the study population

	CPAP *Mean ± SD or n (%)* N = *34*	*Sham* *Mean ± SD or* n *(%)* N = *31*	P-*value*
Age (years)	51 ± 12.3	46.4 ± 10.4	0.11
AHI (events h^{-1})*	36.1 (31.7–41.0)	37.1 (31.2–44.1)	0.78
Severe OSA (AHI ≥ 30 per h)	24 (70.6)	18 (58.1)	0.29
CPAP use by week 12 (h $night^{-1}$)	3.6 ± 1.9	2.8 ± 2.1	0.12
ESS (score; 0–24)	10.0 ± 4.0	10.2 ± 4.8	0.82
Total sleep time (min)*	341.7 (318.2–366.9)	361.0 (336.3–387.6)	0.27
Short sleep (< 6 h)	17 (50.0)	12 (38.7)	0.36
BMI (kg m^{-2})	31.6 ± 5.3	31.0 ± 5.1	0.65
β-CTx* (ng mL^{-1})	0.28 (0.25–0.32)	0.28 (0.24–0.33)	0.96
Osteocalcin* (ng mL^{-1})	19.8 (17.9–21.8)	19.3 (17.4–21.5)	0.75
P1NP* (μg L^{-1})	37.4 (33.1–42.3)	39.6 (34–46.1)	0.55
25(OHD)* (ng mL^{-1})	50.9 (43.2–60.1)	55.7 (48.6–63.9)	0.40
IGF-1 (nmol L^{-1})	19.3 ± 5.7	21.5 ± 6.8	0.15
Corrected calcium (mmol L^{-1})	2.33 ± 0.13	2.31 ± 0.09	0.41
Phosphate (mmol L^{-1})	1.13 ± 0.14	1.16 ± 0.13	0.35
Creatinine (μmol L^{-1})	93.1 ± 11.9	93.7 ± 14.8	0.86
PTH (pmol L^{-1})	4.29 ± 1.90	3.61 ± 1.57	0.13
HOMA index* (unit)	2.44 (1.98–3.00)	2.28 (1.68–3.08)	0.70
Fasting glucose* (mmol L^{-1})	5.26 (5.04–5.50)	5.19 (5.03–5.35)	0.57
Fasting insulin* (μIU mL^{-1})	10.37 (8.62–12.48)	9.91 (7.36–13.34)	0.79

*Geometric mean (95% CI).
β-CTx, collagen-type 1 cross-linked C-telopeptide; 25(OHD), 25-hydroxyvitamin D; AHI, apnea–hypopnea index; BMI, body mass index; CPAP, continuous positive airway pressure; ESS, Epworth Sleepiness Scale; IGF-1, insulin-like growth factor-1; OSA, obstructive sleep apnea; P1NP, N-terminal propeptide of type 1 collagen; PTH, parathyroid hormone.

Blinded period (12 weeks)

During the blinded period, real-CPAP use was 3.6 h per night and sham-CPAP use was 2.8 h per night ($P = 0.07$), and at 12 weeks the CPAP group showed significantly greater change in all OSA variables compared with the sham group (ΔAHI: −35.0 versus −1.95 per h, $P < 0.0001$; ΔODI: −28.6 versus 2.37 per h, $P < 0.0001$; and Δtime during night with oxygen saturation < 90% (ΔT90): −4.24 versus 0.029 min, $P = 0.02$).

No significant differences in serum vitamin D levels or any of the bone turnover markers were observed between the CPAP and the sham group at 12 weeks [Δ25(OHD): -0.80 ± 5.28 ng mL^{-1} (mean ± SE) versus 3.08 ± 3.66 ng mL^{-1}, $P = 0.42$; Δβ-CTx: 0.011 ± 0.014 ng mL^{-1} versus -0.004 ± 0.009 ng mL^{-1}, $P = 0.48$; Δosteocalcin: 1.13 ± 1.12 ng mL^{-1} versus 0.46 ± 0.75 ng mL^{-1}, $P = 0.80$; ΔP1NP: 2.07 ± 3.05 μg L^{-1} versus -1.05 ± 2.13 μg L^{-1}, $P = 0.48$; Fig. 1].

We further divided the group by CPAP compliance, severity of OSA at baseline and excessive daytime sleepiness at baseline to assess if there were any changes in vitamin D or bone turnover markers at 12 weeks in any subgroup. As in the whole group, we could not detect any significant differences between groups for vitamin D or the bone turnover makers in any of the subgroups (data not shown).

Results from the multiple regression analysis to assess the effect of CPAP treatment on vitamin D and bone turnover markers at week 12 showed similar results to the mixed models analysis (Table S1a–d). In addition, the multiple regression model with 25(OHD) as the dependent variable was also performed adjusting for season, but this did not change the results (data not shown).

Open-label period (24 weeks)

During the open-label period, CPAP use was 4 h per night for both groups ($P = 0.72$), and there were no significant differences from baseline for AHI, ODI and T90 compared with the sham group (ΔAHI: −36.9 versus −40.6, $P = 0.51$; ΔODI: −29.9 versus −33.6, $P = 0.49$; ΔT90: −3.2 versus −5.9, $P = 0.27$). Nonetheless, in the analysis of 24-week data, all analyses were performed within the whole study group.

Table 2 shows the results from the analysis at 24 weeks, and showed a trend for an increase in both vitamin D and osteocalcin in the whole group. There was also a significant increase in vitamin D levels in patients with severe sleep apnea and also patients with excessive daytime sleepiness at 24 weeks. In addition, osteocalcin significantly increased in the CPAP-compliant patients at 24 weeks.

As in the 12-week data, a multiple regression model was performed with 25(OHD) at 24 weeks as the dependent variable and adjusting for confounders including season. However, this did not change the results (data not shown).

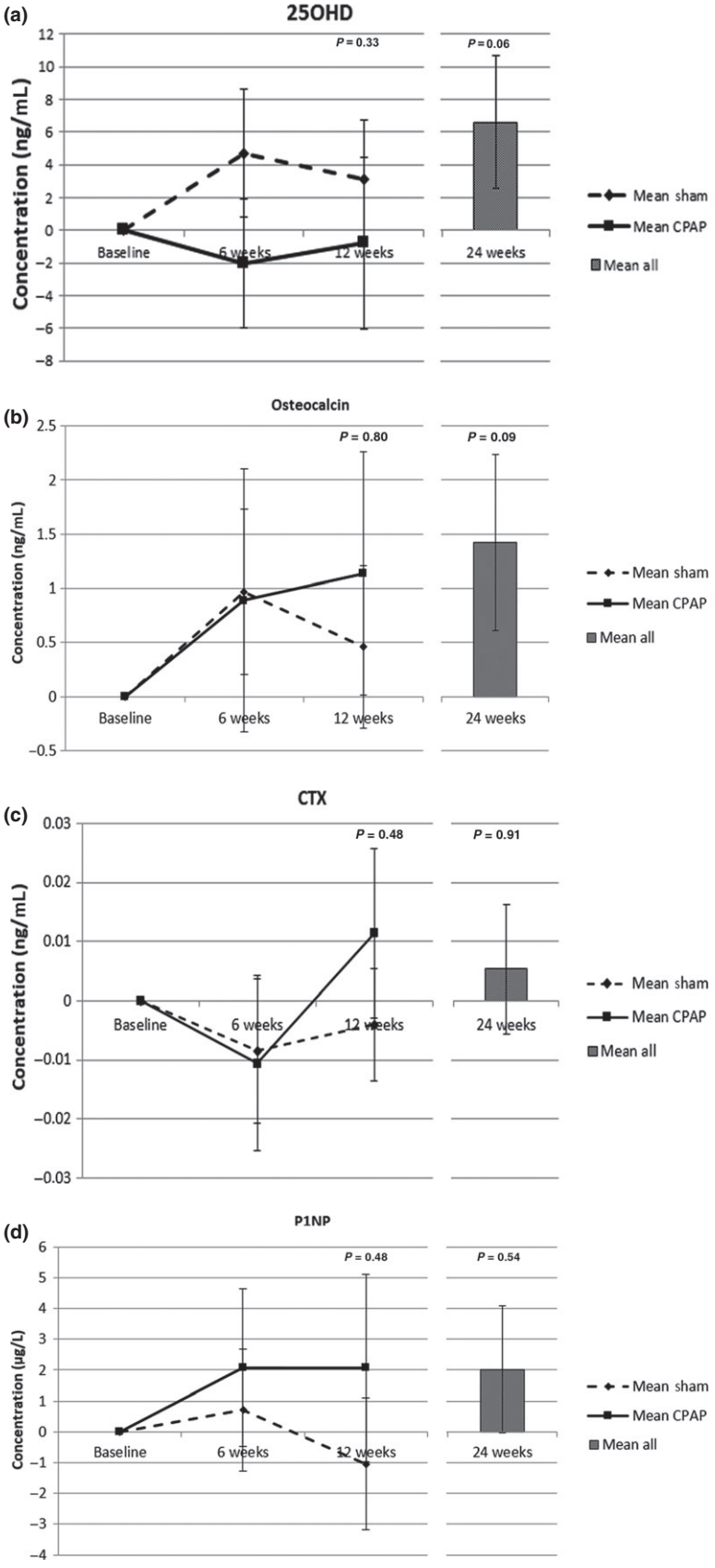

Figure 1. Mean change and SE of: (a) concentration in 25-hydroxyvitamin D [25 (OHD)]; (b) osteocalcin; (c) N-terminal propeptide of type 1 collagen (P1NP); and (d) collagen-type 1 cross-linked C-telopeptide (β-CTx) after 6, 12 [continuous positive airway pressure (CPAP) versus sham] and 24 weeks (all). The left-hand line graph shows the mean change at weeks 6 and 12 from baseline for the real-CPAP (filled line) and sham-CPAP (dashed line) groups. The *P*-value above this graph denotes the between-group difference as determined by mixed model analysis. The right-hand bar graph shows the pooled mean change at week 24 from baseline in all participants regardless of initial treatment allocation. The *P*-value above this graph denotes the significance of the change as determined by Wilcoxon rank-sum test.

Table 2 Whole and within initial treatment group changes from baseline to week 24 with results from subgroup analysis

	Overall mean change (95% CI)	P-*value*	*CPAP mean change (95% CI)*	P-*value*	*Sham mean change (95% CI)*	P-*value*
All patients	(*n* = 48)		(*n* = 26)		(*n* = 20)	
Δ25(OHD)	6.59 (−1.39 to 14.57)	0.06	4.80 (−6.78 to 16.39)	0.18	8.74 (−2.25 to 19.72)	0.16
ΔOsteocalcin	1.43 (−0.17 to 3.02)	0.09	1.99 (−0.58 to 4.56)	0.19	0.72 (−0.88 to 2.31)	0.26
Δβ-CTx	0.0053 (−0.016 to 0.027)	0.91	0.006 (−0.026 to 0.038)	0.72	0.005 (−0.021 to 0.031)	0.43
ΔP1NP	2.02 (−2.03 to 6.07)	0.54	4.29 (−2.20 to 10.78)	0.25	−0.84 (−4.78 to 3.10)	0.66
CPAP-compliant patients	(*n* = 18)		(*n* = 12)		(*n* = 6)	
Δ25(OHD)	10.02 (−0.54 to 20.59)	0.09	8.13 (−5.50 to 21.76)	0.24	13.80 (−3.65 to 31.25)	0.17
ΔOsteocalcin	**3.27 (1.20 to 5.35)**	**0.01**	2.99 (0.07 to 5.91)	0.07	**3.83 (1.40 to 6.27)**	**0.046**
Δβ-CTx	0.023 (−0.010 to 0.056)	0.31	0.019 (−0.026 to 0.064)	0.88	**0.033 (−0.005 to 0.071)**	**0.04**
ΔP1NP	4.17 (−0.52 to 8.85)	0.16	3.42 (−2.95 to 9.79)	0.41	5.67 (−0.89 to 12.22)	0.25
Patients with severe OSA	(*n* = 28)		(*n* = 16)		(*n* = 12)	
Δ25(OHD)	**9.60 (−1.20 to 20.39)**	**0.045**	5.45 (−10.31 to 21.21)	0.25	15.13 (1.07 to 29.18)	0.07
ΔOsteocalcin	1.89 (−0.32 to 4.10)	0.09	2.53 (−1.05 to 6.10)	0.16	0.97 (−0.67 to 2.61)	0.37
Δβ-CTx	0.0060 (−0.025 to 0.037)	0.94	0.018 (−0.027 to 0.063)	0.86	−0.015 (−0.049 to 0.018)	0.77
ΔP1NP	1.93 (−3.75 to 7.60)	0.82	4.06 (−4.99 to 13.12)	0.38	−1.18 (−5.70 to 3.33)	0.30
Sleepy patients	(*n* = 29)		(*n* = 16)		(*n* = 13)	
Δ25(OHD)	**14.04 (4.86 to 23.23)**	**0.007**	12.69 (0.46 to 24.93)	0.056	15.71 (1.30 to 30.11)	0.055
ΔOsteocalcin	2.08 (−0.14 to 4.31)	0.054	2.47 (−1.29 to 6.23)	0.26	1.61 (−0.37 to 3.58)	0.07
Δβ-CTx	0.0076 (−0.020 to 0.035)	0.74	0.015 (−0.026 to 0.055)	0.61	−0.002 (−0.038 to 0.035)	0.88
ΔP1NP	2.24 (−3.52 to 8.00)	0.76	4.56 (−4.85 to 13.98)	0.62	−0.62 (−6.23 to 5.00)	0.89

The *P*-value denotes the significance of the change as determined by the Wilcoxon rank-sum test.
β-CTx, collagen-type 1 cross-linked C-telopeptide; 25(OHD), 25-hydroxyvitamin D; CPAP, continuous positive airway pressure; OSA, obstructive sleep apnea; P1NP, N-terminal propeptide of type 1 collagen. Bold values indicate statistically significant values.

Correlations with glucose metabolism

When assessing correlations between vitamin D, bone turnover markers and metabolic variables, osteocalcin showed a significant correlation with IGF-1 at 24 weeks ($r = 0.35$, $P = 0.02$) and also with glucose levels at baseline ($r = 0.25$, $P = 0.046$). In addition, change in osteocalcin was significantly correlated with change in insulin at 24 weeks ($r = -0.48$, $P = 0.03$) and change in IGF-1 at 24 weeks ($r = 0.41$, $P = 0.01$). In addition, P1NP was correlated with IGF-1 at 24 weeks ($r = 0.32$, $P = 0.03$) and insulin levels both at baseline ($r = 0.32$, $P = 0.02$) and 24 weeks ($r = 0.32$, $P = 0.047$).

DISCUSSION

To our knowledge this is the first sham-controlled RCT assessing the effect of CPAP on vitamin D and bone turnover markers in patients with OSA. CPAP compared with sham did not significantly change either vitamin D levels or concentrations of bone turnover markers after 12 weeks of treatment. At 24 weeks, however, we did find some changes within small subgroups of patients, including patients with severe OSA and patients with excessive sleepiness. Osteocalcin was significantly increased after 24 weeks in the group of patients that complied with CPAP ≥ 4 h night^{-1}. Therefore, it is plausible that CPAP may have late beneficial effects on bone parameters in certain subsamples of OSA patients.

Previous studies have primarily assessed vitamin D levels in patients with OSA (Archontogeorgis *et al*., 2017; Barcelo *et al*., 2013; Erden *et al*., 2014; Kerley *et al*., 2016, 2017; Mete *et al*., 2013; Salepci *et al*., 2017; Toujani *et al*., 2017), whereas markers of bone turnover have not been studied extensively in this patient group (Terzi and Yilmaz, 2016). Barcelo *et al.* (2013) showed, in a group of newly diagnosed patients with OSA, that 55.3% of men and 63.2% of women were vitamin D deficient, and Mete *et al.* (2013) showed significantly lower vitamin D concentrations in OSA compared with controls. Recently, Salepci *et al.* (2017) showed that a large proportion of patients referred for OSA evaluation had vitamin D deficiency, although vitamin D levels did not differ by OSA diagnosis status or severity. In the present study, eight participants (12%) were vitamin D deficient; however, within the group as a whole, the mean baseline levels of vitamin D did not show deficiency, therefore it is possible that we did not include a large enough group of deficient patients to be able to detect any significant change. Furthermore, all three bone turnover markers were within normal reference range for all patients, whereas Terzi and Yilmaz (2016) have shown that patients with OSA compared with controls had higher β-CTx as well as osteocalcin. However, they were all within reference range (Terzi and Yilmaz, 2016), as were the participants in our study. To our knowledge there are no studies on P1NP levels in patients with OSA. Seasonal changes to vitamin D levels are often a confounding factor for studies looking at serum vitamin D levels; however, as data were collected over several years it

is unlikely that our findings were limited by a seasonal effect. In addition, adjusting for season in the multiple regression model with vitamin D did not change the results. Furthermore, when we only included the participants that were vitamin D deficient at baseline, we did observe a significant increase in vitamin D at 24 weeks following CPAP.

In the present study, CPAP did not modulate vitamin D or any of the bone turnover markers in the entire cohort. However, as vitamin D did increase in severe and sleepy patients and as osteocalcin increased in compliant patients after 24 weeks, it is plausible that only some patients with OSA will have beneficial effects from CPAP treatment on bone turnover and vitamin D levels, and it also seems that time/intensity of CPAP use is a factor that impacts the association. Two previous studies have assessed the effect of CPAP on vitamin D (Liguori *et al.*, 2015) and bone resorption (Tomiyama *et al.*, 2008), respectively. Liguori *et al.* (2015) showed in a group of patients with severe OSA compared with controls that CPAP increased vitamin D levels after 7 days of treatment. However, this result was seen only in male patients using CPAP > 4 h night^{-1} and with a residual AHI < 5 per h. The patients with OSA as a group were also vitamin D deficient compared with controls, unlike the majority of our patient group. Furthermore, Liguori *et al.* (2017) have recently shown, in a long-term follow-up study of the same male population, that serum vitamin D levels increased after long-term use of CPAP treatment, although this increase was most pronounced in obese patients. They further showed that compliance played a role, as patients with OSA syndrome with minimal compliance showed no changes in serum vitamin D levels (Liguori *et al.*, 2017). Although this was an uncontrolled study and not a RCT, this nevertheless indicates that effects of CPAP on vitamin D levels may not be present for all patients with OSA syndrome, but rather in subsamples. These data also suggest effects are more pronounced with long-term CPAP use, which is in line also with the results of the present study. Tomiyama *et al.* (2008) showed in a group of pateints with OSA that there was a severity-dependent increase in urinary β-CTx, and that 3 months of CPAP significantly decreased β-CTx levels. One explanation for the discrepancies between their results and ours is that their group of patients had a lower body mass index (BMI) but also that they did not exclude other co-morbidities.

Barcelo *et al.* (2013) have shown an inverse relationship between 25(OHD) levels and diabetes and also metabolic syndrome in patients with OSA, showing 25(OHD) as a potential link between the two. In addition, Kerley *et al.* (2017) showed in a group of patients with OSA that supplementation with vitamin D improved several metabolic variables, such as decreases in both low-density lipoprotein and lipoprotein-associated phospholipase A2, as well as trends toward decreased fasting glucose and increased high-density lipoprotein. However, as we only observed a modification of 25(OHD) and bone turnover markers in certain subsamples of patients and with increased CPAP treatment time frames, this might indicate that OSA impacts energy metabolism only in certain patients. Further research is needed to clarify these relationships. In a review by Swanson *et al.* (2015), it is suggested that OSA may affect bone turnover marker through several mechanisms such as hypoxia but also sleep loss and fragmentation. In the present study, there was no indication of any difference in sleep variables between the groups and adjusting for sleep variables did not alter these results. Furthermore, it is interesting that we could show a significant association between osteocalcin and glucose and insulin levels. Brennan-Speranza and Conigrave (2015) reported in a review that there are positive correlations between serum osteocalcin levels and measures of metabolic health, opening up the possibility that osteocalcin could provide a link between OSA and glucose metabolism, insulin resistance and metabolic syndrome. It should be noted that the uncarboxylated form of osteocalcin is often purported to associate with glucose metabolism, and this was not assessed in the current study (Brennan-Speranza and Conigrave, 2015). As OSA is a risk factor for myocardial infarction (MI), it is likely that this is a possible link, although future studies are required to explore this relationship and mechanism further. In the present study, we showed that 24 weeks of CPAP had beneficial effects on osteocalcin in some subgroups. It is likely that these are also the groups with the highest risk of MI, although this would need to be clarified. There is a lack of studies investigating P1NP in OSA.

This study used an extensive RCT protocol in a well-controlled group of patients with OSA. Furthermore, the current study followed patients for a longer period of time than any previous studies. The patient group as a whole was a good representation of patients with OSA in terms of BMI and severity. Despite this, there are some limitations to consider. Both the full sample study group and subgroups were relatively small and were all male, which are both important limitations that may have an impact on the generalizability of the study results. It is also plausible that the current patients, while all being diagnosed with OSA, may nevertheless be considered too generally healthy to represent patients with severe OSA, which could potentially have influenced our results. In addition, as the study did not select excessively sleepy patients, it is thus possible that the sleepy patients in the cohort, in whom effects were observed, may represent a different phenotype of OSA compared with the non-sleepy group, although they may also represent a group of people with reduced sunlight exposure. It should further be noted that overall compliance was moderate (although on par with compliance found in trials and 'real-world'; McEvoy *et al.*, 2016) and, although changes in bone turnover markers were seen in the CPAP-compliant group, this could have influenced the results. The obvious effects of seasonality and sunlight exposure on vitamin D are well defined in the literature. Thus, the relationship between 25(OHD) and any disease and/or intervention, including OSA and CPAP use, as in the

current study, can be obscured if the seasonal variation is not properly adjusted for. The regression adjustment model we used in the current study is well accepted (Demidenko, 2004; Draper and Smith, 1998), although we note that there is still a risk of not fully eliminating the variable effects that may influence the data, particularly given the periodic, rather than linear nature of seasonality. The regression adjustment we used in the current study, however, is more likely to underestimate than overestimate a relationship between CPAP use and vitamin D levels (Zhang *et al.*, 2011). Future RCTs should be performed in specific subgroups of patients, for example patients with severe OSA or sleepiness, and also further assess the effect of CPAP compliance. In addition, studies in women are warranted. Furthermore, as this was a secondary analysis study it was not power calculated for these outcomes. Nonetheless, as this is the first sham-controlled RCT in this field it adds valuable information to the field of OSA treatment and effects on bone metabolism and potentially glucose metabolism.

In conclusion, 12 weeks of CPAP did not modulate vitamin D or any of the bone turnover markers compared with sham. However, it is plausible that CPAP may have beneficial effects on vitamin D levels and bone turnover markers in selected groups of patients with OSA.

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AUTHOR CONTRIBUTIONS

Drs Hoyos, Phillips, Yee, Herrmann, Grunstein and Liu were all involved in the study design and data collection; Drs Theorell-Haglöw, Hoyos, Phillips, Brennan-Speranza, Grunstein and Liu contributed to the data analysis; Dr Theorell-Haglöw drafted the manuscript, and all authors contributed in the review and completion of the manuscript.

DISCLOSURE STATEMENT

Drs Theorell-Haglöw, Hoyos, Phillips, Yee, Herrmann, Brennan-Speranza, Grunstein and Liu have nothing to disclose.

REFERENCES

Archontogeorgis, K., Nena, E., Papanas, N. *et al.* Vitamin D levels in middle-aged patients with obstructive sleep apnoea syndrome. *Curr. Vasc. Pharmacol.*, 2017 doi: 10.2174/1570161115666170529085708. [Epub ahead of print].

Arnardottir, E. S., Bjornsdottir, E., Olafsdottir, K. A., Benediktsdottir, B. and Gislason, T. Obstructive sleep apnoea in the general population: highly prevalent but minimal symptoms. *Eur. Respir. J.*, 2016, 47: 194–202.

Barcelo, A., Esquinas, C., Pierola, J. *et al.* Vitamin D status and parathyroid hormone levels in patients with obstructive sleep apnea. *Respiration*, 2013, 86: 295–301.

Biver, E. Use of bone turnover markers in clinical practice. *Curr. Opin. Endocrinol. Diabetes Obes.*, 2012, 19: 468–473.

Bozkurt, N. C., Cakal, E., Sahin, M., Ozkaya, E. C., Firat, H. and Delibasi, T. The relation of serum 25-hydroxyvitamin-D levels with severity of obstructive sleep apnea and glucose metabolism abnormalities. *Endocrine*, 2012, 41: 518–525.

Brennan-Speranza, T. C. and Conigrave, A. D. Osteocalcin: an osteoblast-derived polypeptide hormone that modulates whole body energy metabolism. *Calcif. Tissue Int.*, 2015, 96: 1–10.

Chakhtoura, M., Nasrallah, M. and Chami, H. Bone loss in obesity and obstructive sleep apnea: a review of literature. *J. Clin. Sleep Med.*, 2015, 11: 575–580.

Demidenko, E. *Mixed Models: Theory and Applications*. Wiley, Hoboken, NJ, 2004.

Draper, N. and Smith, H. *Applied Regression Analysis*, 3rd edn. Wiley, New York, 1998.

Erden, E. S., Genc, S., Motor, S. *et al.* Investigation of serum bisphenol A, vitamin D, and parathyroid hormone levels in patients with obstructive sleep apnea syndrome. *Endocrine*, 2014, 45: 311–318.

Franklin, K. A. and Lindberg, E. Obstructive sleep apnea is a common disorder in the population – a review on the epidemiology of sleep apnea. *J. Thorac. Dis.*, 2015, 7: 1311–1322.

Herrmann, M., Sullivan, D. R., Veillard, A. S. *et al.* Serum 25-hydroxyvitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes. *Diabetes Care*, 2015, 38: 521–528.

Holick, M. F. Vitamin D deficiency. *N. Engl. J. Med.*, 2007, 357: 266–281.

Hoyos, C. M., Killick, R., Yee, B. J., Phillips, C. L., Grunstein, R. R. and Liu, P. Y. Cardiometabolic changes after continuous positive airway pressure for obstructive sleep apnoea: a randomised sham-controlled study. *Thorax*, 2012, 67: 1081–1089.

Ip, M. S., Lam, B., Ng, M. M., Lam, W. K., Tsang, K. W. and Lam, K. S. Obstructive sleep apnea is independently associated with insulin resistance. *Am. J. Respir. Crit. Care Med.*, 2002, 165: 670–676.

Johns, M. W. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*, 1991, 14: 540–545.

Kerley, C. P., Hutchinson, K., Bolger, K., Mcgowan, A., Faul, J. and Cormican, L. Serum vitamin D is significantly inversely associated with disease severity in Caucasian adults with obstructive sleep apnea syndrome. *Sleep*, 2016, 39: 293–300.

Kerley, C. P., Hutchinson, K., Bramham, J., McGowan, A., Faul, J. and Cormican, L. Vitamin D improves selected metabolic parameters but not neuropsychological or quality of life indices in OSA: a pilot study. *J. Clin. Sleep Med.*, 2017, 13: 19–26.

Kushida, C. A., Nichols, D. A., Quan, S. F. *et al.* The Apnea Positive Pressure Long-term Efficacy Study (APPLES): rationale, design, methods, and procedures. *J. Clin. Sleep Med.*, 2006, 2: 288–300.

Liguori, C., Romigi, A., Izzi, F. *et al.* Continuous positive airway pressure treatment increases serum vitamin D levels in male patients with obstructive sleep apnea. *J. Clin. Sleep Med.*, 2015, 11: 603–607.

Liguori, C., Izzi, F., Mercuri, N. B. *et al.* Vitamin D status of male OSAS patients improved after long-term CPAP treatment mainly in obese subjects. *Sleep Med.*, 2017, 29: 81–85.

McCarty, D. E., Chesson, A. L. Jr, Jain, S. K. and Marino, A. A. The link between vitamin D metabolism and sleep medicine. *Sleep Med. Rev.*, 2014, 18: 311–319.

McEvoy, R. D., Antic, N. A., Heeley, E. *et al.* CPAP for prevention of cardiovascular events in obstructive sleep apnea. *N. Engl. J. Med.*, 2016, 375: 919–931.

Mete, T., Yalcin, Y., Berker, D. *et al.* Obstructive sleep apnea syndrome and its association with vitamin D deficiency. *J. Endocrinol. Invest.*, 2013, 36: 681–685.

Parish, J. M. Metabolic syndrome, obstructive sleep apnea, and risk of cardiovascular disease. *Sleep Breath.*, 2012, 16: 595–597.

Peppard, P. E., Young, T., Palta, M. and Skatrud, J. Prospective study of the association between sleep-disordered breathing and hypertension. *N. Engl. J. Med.*, 2000, 342: 1378–1384.

Phillips, C. L., Yee, B. J., Marshall, N. S., Liu, P. Y., Sullivan, D. R. and Grunstein, R. R. Continuous positive airway pressure reduces postprandial lipidemia in obstructive sleep apnea: a randomized, placebo-controlled crossover trial. *Am. J. Respir. Crit. Care Med.*, 2011, 184: 355–361.

Salepci, B., Caglayan, B., Nahid, P. *et al.* Vitamin D deficiency in patients referred for evaluation of obstructive sleep apnea. *J. Clin. Sleep Med.*, 2017, 13: 607–612.

Swanson, C. M., Shea, S. A., Stone, K. L. *et al.* Obstructive sleep apnea and metabolic bone disease: insights into the relationship between bone and sleep. *J. Bone Miner. Res.*, 2015, 30: 199–211.

Takiishi, T., Gysemans, C., Bouillon, R. and Mathieu, C. Vitamin D and diabetes. *Endocrinol. Metab. Clin. North Am.*, 2010, 39: 419–446.

Terzi, R. and Yilmaz, Z. Bone mineral density and changes in bone metabolism in patients with obstructive sleep apnea syndrome. *J. Bone Miner. Metab.*, 2016, 34: 475–481.

Tomiyama, H., Okazaki, R., Inoue, D. *et al.* Link between obstructive sleep apnea and increased bone resorption in men. *Osteoporos. Int.*, 2008, 19: 1185–1192.

Toujani, S., Kaabachi, W., Mjid, M., Hamzaoui, K., Cherif, J. and Beji, M. Vitamin D deficiency and interleukin-17 relationship in severe obstructive sleep apnea-hypopnea syndrome. *Ann. Thorac. Med.*, 2017, 12: 107–113.

Upala, S., Sanguankeo, A. and Congrete, S. Association between obstructive sleep apnea and osteoporosis: a systematic review and meta-analysis. *Int. J. Endocrinol. Metab.*, 2016, 14: e36317.

Zhang, H., Ahn, J. and Yu, K. Comparing statistical methods for removing seasonal variation from vitamin D measurements in case-control studies. *Stat. Interface*, 2011, 4: 85–93.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 (a) Results from regression model analysis using vitamin D at 12 weeks as the outcome variable and variables at baseline as independent variables in the whole group

(b) Results from regression model analysis using osteocalcin at 12 weeks as the outcome variable and variables at baseline as independent variables in the whole group

(c) Results from regression model analysis using CTX at 12 weeks as the outcome variable and variables at baseline as independent variables in the whole group

(d) Results from regression model analysis using P1NP at 12 weeks as the outcome variable and variables at baseline as independent variables in the whole group