Sleep-Disordered Breathing and Mandibular Bone Mass as Assessed Through Panoramic Radiographs

By

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Abstract

Sleep-disordered breathing (SDB) and low bone mass (i.e., osteoporosis) are common conditions affecting the adult population with an estimated prevalence of around 15%. Recent studies have assessed a potential association between SDB and reduction in bone mass in adult population. Nevertheless the results of these studies have been inconsistent. Moreover, it is still not known whether this potential association is also manifested in children. This thesis presents a systematic review with meta-analysis to synthesize the existing evidence on the potential association between SDB (including its severe form, i.e., obstructive sleep apnea [OSA]) and low bone mass in adults. In addition, the thesis contains a group of retrospective cross-sectional studies that were conducted in children to test the possible impact of SDB on their bone mass.

We *first* illustrated that the association between SDB (more specifically OSA) and low bone mass in adults is plausible. However, the supporting evidence has potential risk of bias and available data is inconsistent.

We *next* shown, through conducting 2 retrospective *cross-sectional* studies, that the association between SDB and low bone mass may also exist in children. Indeed, results of the *first* cross-sectional study illustrated that risk of SDB, as suggested by the Paediatric Sleep Questionnaire (PSQ), may be associated with thinner mandibular cortical thickness (which has been strongly linked to skeletal bone density) in children. This possible association was also supported by the results of the *second* cross-sectional study, as it was illustrated that in children with a PSG-diagnosed severe form of SDB as induced by OSA

was linked to a negative association with mandibular cortical width. We suggested that PSG-diagnosed OSA children had a thinner mandibular cortical width compared to healthy children with no reported sleep-breathing symptoms.

Taken together, we believe that the identifying trends support the probable existence of an association between SDB and low bone mass in adults and in children. This should justify the implementation of future studies that rely on large-scale, multi-centers, clinical trials for a better assessment of this possible association for early diagnosis and intervention.

Preface

This thesis is an original work of Hazem Eimar. The research project, of which this thesis is a part, received ethics approval from the University of Alberta Research Board, Project name "Obstructive Sleep Apnea and Mandibular Cortical Thickness: A Retrospective Study", Pro00063547, Date: March 17, 2016.

This thesis is composed of 3 manuscripts. Materials presented in this thesis represent original contributions to current knowledge. Authors' contributions to the work are described below for each of these manuscripts:

Chapter 2:

HE performed the literature search and drafted the manuscript. HS performed the meta-analysis. SG and DI helped in study selection process. JEM, DG1 and DG2 revised and edited the manuscript content. CF supervised and designed the review manuscript.

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Chapter 3:

HE performed the radiographic scanning of the dry-skull specimens, analyzed the data and drafted the manuscript. AC helped in imaging machine set-ups and analyzing the

data. AC, DG1 and DG2 revised and edited the manuscript content. CF supervised and designed the manuscript.

This work is prepared for submission as part of study presented at chapter 4: **Eimar H**, Mohammed A. Q. Al-Saleh, Cortes AR, Gozal D, Graf D, Flores-Mir C. Potential Impact of Sleep Disordered Breathing on Mandibular Cortical Thickness in Children.

Chapter 4:

HE designed the project, collected and analyzed the data and drafted the manuscript. MA helped in data collection. AC, DG1 and DG2 revised and edited the manuscript content. CF supervised and designed the manuscript.

This work is prepared for submission as part of study presented at chapter 4: **Eimar H**, Mohammed A. Q. Al-Saleh, Cortes AR, Gozal D, Graf D, Flores-Mir C. Potential Impact of Sleep Disordered Breathing on Mandibular Cortical Thickness in Children.

Dedication

This thesis is dedicated to my parents for their endless love and support throughout the course of this thesis. It is also dedicated to my wife, Hala, and kids, Nadia and Zaid, for all the wonderful things they bring to my life.

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First and Foremost, I would like to express my most sincere appreciation and gratitude to my committee members for their guidance and advice. A special thanks to my supervisor and mentor Dr. Carlos Flores-Mir, for his patience, support, motivation, immense knowledge and for the countless hours of reading and editing my thesis. I would also like to thank Dr. Daniel Graf for the consistent support he provided throughout my MSc studies. I appreciate all his feedback, long discussion and the learning opportunities he provided. In addition, I would like to thank Dr. Gozal for his excellent feedback and support he provided during my studies.

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CHAPTER 1: Introduction and Aim of the Work

Sleep-disordered breathing (SDB) is a common condition, with a wide spectrum of severity ranging from habitual snoring to obstructive sleep apnea (OSA) affecting approximately 10-12% of children and up to 35% of the adult population [1-5]. Recent research suggested possible links between SDB (including its severe form i.e., OSA) and bone remodeling, evidenced as inhibition of growth or reduction in bone mass (osteoporosis or risk of fracture). OSA is characterized by intermittent hypoxia events, which may impose a negative effect on bone cells, and result in loss of bone formation and/or increase in bone resorption, thus accelerating bone mass loss [6]. In addition, OSA could also facilitate bone loss through indirect physiological mechanisms [6]: elevations in the activity of the sympathetic nervous system (SNS) [7], disruption in the secretion of the circadian rhythm hormone melatonin , and reductions in serum levels of 25-OH vitamin D [8, 9]. In order to asses whether this potential association between OSA and reduction in bone mass does exist, several clinical studies were performed in adult population. The results of these studies have been inconsistent; hence, a definitive answer about this possible association cannot be given. Thus, a systematic review (and possibly combined with meta-analysis) covering these clinical studies in adult population is warranted. Moreover, no clinical studies assessed the possible association between SDB and bone mass in children.

1.1. Hypothesis:

SDB (including its severe form i.e., OSA) is associated with low bone mass in adult and children populations.

1.2. Specific Aims:

a. *Assess the existing evidence on the potential association between OSA and low bone mass in adult population through a systematic review with a meta-analysis.*

b. *Assess the impact of SDB, as suggested by Pediatric Sleep Questionnaire (PSQ), on the thickness of mandibular cortical thickness, which has been strongly linked to skeletal bone density, in a sample of children – Retrospective cross-sectional study.*

c. *Assess the mandibular cortical thickness, which has been strongly linked to skeletal bone density, in children with PSG-diagnosed OSA and children with no reported sleep breathing disorders – Retrospective cross-sectional study.*

CHAPTER 2: Systematic Review of the Literature

Association between Sleep Apnea and Low Bone Mass in Adults: A Systematic Review and Meta-Analysis

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2.1. Abstract:

Purpose: To synthesize existing evidence on the potential association between sleep apnea and low bone mass in adults.

Methods: Electronic searches of five databases were performed. The inclusion criteria consisted of studies in humans that assessed potential associations between sleep apnea and bone metabolic diseases in an adult population. For diagnosis of sleep apnea overnight polysomnography, home polygraphy, or validated records from health-care databases were considered. Reduced bone density, osteoporosis, serum/urinary levels for markers of bone formation and resorption, or risk of fractures caused without history of trauma were considered indicators of low bone mass. A random effects model meta-analysis was applied when possible.

Results: Of 963 relevant references, 12 studies met our inclusion criteria and were assessed to be of medium to low bias. Nine out of 12 studies reported an association between sleep apnea and low bone mass (increased bone resorption markers, reduced bone density and higher risk of osteoporosis). Two studies did not report a significant association, whereas one study reported an increase of bone density in sleep apnea patients compared to non-sleep apnea patients. Meta-analysis of 2 studies (n=112,258 patients) showed that sleep apnea was a significant risk factor for osteoporosis (odds ratio (OR), 1.92; 95%CI, 1.24 to 2.97; I^2=66%); females only had an OR of 2.56 (95% CI, 1.96 to 3.34; I^2=0%) while the OR in males was of 2.03 (95% CI, 1.24 to 3.35; I^2 =38%).

Conclusions: An association between sleep apnea and low bone mass in adults is plausible, but supporting evidence has a risk of bias and is inconsistent.

2.2. Introduction:

Low bone mass (i.e., osteoporosis) and sleep disturbances (i.e., sleep apnea) are common conditions affecting the adult population with an estimated prevalence of around 15% [3, 10]. Both conditions are associated with life-threatening complications and significant economic burden [11, 12]. With an increasing elderly population due to longer life expectancy, the costs associated with the treatment and management of both diseases are rising steadily [6].

Osteoporosis is a relatively common bone disease characterized by reduced bone mass and deterioration of bone tissues [10]. Osteoporosis increases the risk of bone fractures, and can cause disfigurement, severe pain, loss of mobility, low self-esteem and an overall reduction in quality of life [13]. Ten million U.S. adults over the age of 50 are reported to suffer from this disease [14]. In 2005, the cost of treating osteoporotic fractures in U.S. was estimated at $19 billion [15].

Sleep apnea is the most common sleep breathing disorder in U.S [3]. It is characterized by reductions or cessation of airflow while sleeping resulting in intermittently low oxygen levels, as well as disrupted sleep. Reduction in oxygen tissue tension may in turn lead to serious morbidities such as hypertension, ischemic heart disease and arrhythmias, cerebrovascular disease, reduced sexual function, excessive daytime sleepiness, work-related injuries, depression and impaired cognitive state [16, 17]. Sleep apnea affects approximately one in seven adults [1]. The costs of treating sleep apnea and

sleep apnea-related motor vehicle accidents in the US are estimated to be $3.4 and $15.9 billion, respectively [18].

The intermittent hypoxia that is linked to sleep apneic events elicits sustained sympathetic activation and has also been implicated in reductions in the serum levels of melatonin (anti-oxidant molecule) [19], two perturbations with inherent implications on bone biology [6]. Several studies have assessed the potential association between sleep apnea and reduction in bone mass (osteoporosis or risk of bone fracture). The results of these studies, however, have been inconsistent and contradictory, most likely as a consequence of including different populations and potential bias in the study designs. Furthermore, the validity constructs of the putative association between low bone mass and sleep apnea has not undergone a formal systematic review. The aim of the current study was to assess the evidence behind the association between sleep apnea (central or obstructive) and bone mass (low bone mass [osteopenia or / and osteoporosis], or increased number of non-traumatic bone fracture) in adult populations. Confirming the association between sleep apnea and bone mass, as well as the direction of this effect, will help to determine whether the presence of sleep apnea should alter screening recommendations for osteoporosis or treatment of this condition in patients with sleep apnea.

2.3. Methods:

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement checklist was used as a template [20] for this systematic review and meta-analysis. The protocol was registered with PROSPERO at http://www.crd.york.ac.uk/Prospero/ (Registration number CRD42015020753).

Study Design and Eligibility Criteria

Studies were included if the participants were adults (age over 18 years) diagnosed with sleep apnea who underwent measurement of bone health. Diagnosis of sleep apnea required either an overnight polysomnography (PSG) conducted at a sleep laboratory, a portable monitor in the home (home sleep apnea testing), or through a validated definition of a health administrative database. Bone health assessment included at least one of the followings: bone mineral density readings (obtained by Dual-energy X-ray Absorptiometry [DXA] with either a Z-score or T-score, quantitative computed tomography, quantitative ultrasound methods and peripheral magnetic resonance imaging), records for osteopenia or osteoporosis through a validated definition of a health administrative database, serum/urinary levels for markers of bone formation and resorption (bone turnover), or number of non-traumatic fractures that is associated with reduced bone density such as proximal femur, pubic rami, ribs, distal radius, proximal humerus and vertebral body. Studies were only considered if bone assessment for sleep apnea patients were compared to control patients without sleep apnea. Included studies have to be either clinical trials, cohorts, cross sectionals, case-controls, or epidemiological studies, without restriction on sample size or geographic locations. Reviews, case reports, case series, letters, and personal opinions were excluded.

Search strategy

Electronic database searches were completed by a senior health science librarian on July 31, 2016. No language restrictions were applied. Detailed individual search strategies were applied to the following databases: MEDLINE, EMBASE, PubMed,

CINHAL Plus and All EMB Reviews. A grey literature search was undertaken through Google Scholar (scholar.google. com) using the same keywords search strategy. This search was limited to the first 100 hits. Investigators and experts in the field were also contacted to identify any missing studies. Lastly, the reference lists of included articles were reviewed to identify additional articles meeting the search criteria that were not captured through the search.

Key words and medical subject headings (MeSH) were properly adapted to each database (**Supplementary Table 2.10.1**). All references were managed by reference manager software EndNote X7 (Thomson Reuters). Two independent reviewers (HE and SG) reviewed all articles independently for inclusion. For first level screening, the title and abstracts were reviewed to identify articles that did not meet inclusion. All articles where there was insufficient information in the title and/or abstract were included for the next level of screening. In the second level of screening, the same reviewers evaluated the full text articles for all articles included at the end of the first level screening and applied the same criteria for inclusion. Any disagreement in inclusion status between the two reviewers was resolved with a third reviewer (DI)

Information collection process

Data were extracted, using a customized abstraction form for the included studies included at the end of second level screening. Data extraction included authors, year of publication, study design, population, demographic features of the sample, age, sex, sample size, method used to determine sleep apnea and bone mass diagnoses, covariates, outcome measures, and conclusions. Two reviewers (HE and SG) independently performed the

information extraction. Extracted data from each reviewer was then combined and compared for accuracy. Discrepancies were resolved with a third reviewer. Reviewers attempted to contact authors if further or missing information about the studies was required.

Assessment of risk of bias within the selected studies

Selected studies were appraised based on the Newcastle-Ottawa Scale (NOS) for assessing risk of bias (RoB) in observational studies [21]. This tool was developed to assess RoB in non-randomized studies. Its content validity and inter-rater reliability have been established. The NOS proposes predefined criteria, some of which have to be further detailed for this specific topic. These criteria were defined in a consensus meeting with all authors (criteria are presented in Supplementary information) before assessing the studies. Briefly, cross-sectional studies were assessed for quality of selection (case definition, representativeness of the cases, selection of controls and definition of controls); comparability (confounding); and exposure (ascertainment of exposure, similarity of the applied methods on cases and controls, and non-response rate). Longitudinal studies were assessed for quality of selection (representativeness, selection of controls, ascertainment of exposure, no bone diseases at start of study); comparability (confounding); and outcome (assessment of outcome, length and adequacy of follow-up). Sex and weight were identified as important confounders. The NOS uses a star system to evaluate the above-mentioned criteria. A black star "★"was assigned for each criterion met by the study; otherwise a white star of black margin "☆" was reported. Studies could be awarded a maximum score of 9 points (black stars). Studies with scores of 5 points (black stars) or more were

considered to be of moderate to good quality. For randomized clinical trials the Cochrane RoB tool[22] use was planned; however, it was considered unlikely that clinical trials about this specific topic would be identified. Two reviewers (HE and SG) assessed RoB of the included studies independently, in duplicate. Discrepancies were resolved with a third reviewer (DI).

Data synthesis

The association between sleep apnea and risk of osteoporosis overall, and separately for males and females were conducted using odd ratios (ORs). Risk of osteoporosis was defined by bone densitometers readings with T-score less than -2.5 or Z-score isles that -2 in a specific bone region. A random effects model was used to pool data [22]. Heterogeneity across the studies was assessed using the T^2 and the I^2 statistic, with guide for interpretation as follows: 0% to 30%, not important; 30% to 50%, moderate heterogeneity; 50% to 100%, considerable heterogeneity [23]. Clinical heterogeneity was examined by assessing the characteristics of the selected studies, including similarity between patients and outcome measures used. We planned to test publication bias using a funnel plot, if at least 10 studies are to be included in a meta-analysis, by visually assessing the degree of funnel plot asymmetry [24, 25]. All results are reported with 95% confidence intervals (CIs) and the pooled effect estimate was considered significant if P was <0.05. Analyses were conducted using the Review Manager 5.2 software (RevMan 2014, The Nordic Cochrane Centre, Copenhagen, Denmark).

2.4. Results

Study selection

As described in **Figure 2.8.1**, 963 publications were initially retrieved from 5 electronic databases and Google Scholar, equating to 676 unique publications after removal of the duplicates. At the end of first screening level, 653 publications were excluded (Phase I). Full texts of the remaining 23 publications were then retrieved and assessed for eligibility, after which 11 were excluded (see **Supplementary Table 2.10.2** on reasons for exclusion**)**. No additional articles were identified for inclusion after screening the references lists of the included 12 full-text publications. Therefore, a total of 12 studies [26-37] were finally included in this review.

Studies characteristics

Included studies in this review are summarized in **Table 2.9.1**. Included studies were published between 2012 and 2016; no studies prior 2012 were identified. There was heterogeneity among the selected studies in the study design, selection of the participants, demographic data of the participants, method of diagnosis for sleep apnea, bone mass assessment, methods of statistical analysis, outcomes presented and covariates adjusted in each study. Ten studies were cross-sectional studies [26-35], and 2 were longitudinal population level cohort studies [36, 37]. Three studies were conducted in Taiwan [31, 36, 37], two in Turkey [29, 30, 32, 33], Italy [27, 35], and Japan [26, 34] and one in France [28] and. Sample sizes varied markedly between studies (46 [32] to 134,070 [37]). None of the studies included participants <18 years. Five studies evaluated only male-participants [26, 29, 32, 33, 35], the rest included both male and female participants. Selection criteria

for the participants (Sleep apnea and controls) varied among the studies. Eight studies [26, 29, 31, 33-37] had their controls selected based on a matching process (based on one variable or more such as age, sex, body mass index, energy expenditure and physical activity, medical condition/ medications). In all studies, sleep apnea was diagnosed through nocturnal PSG [26, 27, 29-37] or ambulatory polygraphy [28]. Seven studies assessed serum/urinary levels for specific markers for bone metabolism turnover [26, 29, 30, 32-35]. Five studies assess bone mineral density through direct readings from DXA [27-30, 33] or CT scan [34]. Three studies evaluated the previous clinical records/codes for of DXA or osteoporosis [31, 36, 37]. One study evaluated the incidence of pathological fracture in sleep apnea patients and in patients with no sleep disorders [37]. Included covariates, used for statistical model analysis, were varied among the selected studies. Among these covariates are the age [26, 27, 32, 34], sex [26, 27, 36, 37], body mass index /weight [26-29, 31, 33], energy expenditure and physical activity [28, 31, 35], medical condition and medications [26, 34, 36, 37].

Risk of bias within the included studies

Risk of bias assessment of the individual included studies is detailed in **Table 2.9.2** (for cross sectional studies) and **Table 2.9.3** (for longitudinal studies). All studies were considered to be at medium to low risk of bias potential. The main limitations were: selection criteria for the cases, reporting of non-response rate (for cross sectional studies).

*The association between sleep apnea and bone metabolism marker*s

Seven cross sectional studies investigated the association between sleep apnea and different bone metabolism markers [26, 29, 30, 32-35]. Overall the findings reported by

these studies were not consistent. As seen in **Table 2.9.1**, the results of Tomiyami *et al* study [26] showed that the severity of sleep apnea was correlated positively with the urinary level of the bone resorption marker cross-linked C terminal telopeptide of collagen (CTX), which indicates degradation of mature type I collagen found predominantly in bone, and that the CTX levels decrease significantly following three months of continuous positive air way pressure (CPAP) treatment. The same study reported no significant association between AHI and markers for bone formation (osteocalcin and bone-specific alkaline phosphatase). Terzi *et al* [33] evaluated the following bone turnover markers in patients with and without sleep apnea: calcium, phosphorous, parathyroid hormone, thyroid stimulating hormone, alkaline phosphatase, 25-OH- Vitamin D, osteocalcin and β-CTX bone turnover markers. Among the examined markers, only β-CTX (a specific marker for bone resorption) was significantly higher in sleep apnea patients compared to non sleep apnea patients. However, the levels of β-CTX were not significantly associated with the severity of sleep apnea (R=0.195, P_{value}=0.303), whereas osteocalcin levels showed significant negative association with the AHI (R=-0.521, P_{value}=0.003) Liguori *et al* [35] reported that SA patients had lower vitamin D compared to non-SA patients, whereas parathyroid hormone, fibrinogen and C-reactive protein serum levels in SA were significantly lower than non-SA patients. On the other hand, the findings reported by Uzkeser *et al* [29], Yuceege *et al* [30], Aslan *et al* [32] and Hamada *et al* [34] showed the lack of an association between the severity of sleep apnea with either bone formation markers (serum levels of calcium, phosphate, bone-specific alkaline phosphatase, Para-thyroid hormone and 25-hydroxyl vitamin D3, insulin growth factor 1) or with bone resorption markers (urinary level of deoxypyridinoline [DPD]).

The association between sleep apnea and bone density

Eleven (9 cross-sectional [26-35] and 2 longitudinal [36, 37]) studies investigated the association between sleep apnea and low bone mass (bone density or osteoporosis). For cross-sectional studies, the diagnosis sleep apnea relied on corresponding AHI values, whereas bone density (BMD or T-score) readings were obtained by DXA [27-33, 35-37] or CT scan [34] applied on the following locations: Lumbar spine [27-35], the femoral neck [27-30, 32, 33, 35], total hip [27] and total femur [29, 35] regions. For longitudinal studies [36, 37], the association of sleep apnea and bone density was based on records reported in medical databases.

Of the 11 studies examining the association between sleep apnea and bone density, 8 studies [29-31, 33-37] showed that sleep apnea patients were more likely to suffer from reduced bone density or/and osteoporosis compared to non-sleep apnea patients. By contrast, 2 studies showed no differences between sleep apnea patients and controls [27, 32] and one study showed that sleep apnea patients had higher bone mineral density compared to non-sleep apnea patients [28]. Five [30, 31, 34, 36, 37] showed that females with sleep apnea are at higher risk of developing bone diseases. Eight [29-31, 33-37] studies showed that males with sleep apnea are at higher risk of developing bone diseases (reduced bone density and /or increased risk of osteoporosis).

Only 4 [28, 32, 36, 37] out of 12 studies provided the exact numbers of participants with the diagnosis of osteoporosis. The diagnosis of osteoporosis from these studies was based on a T-score less than -2.5. None of studies reported the risk of osteoporosis using a Z-score. Two studies were cross-sectional observational studies [28,

32] and the other 2 studies were longitudinal population-cohort studies [36, 37]. One of these cross-sectional observational studies, which was conducted by Sforza *et al* [28], has different characteristics compared to the other study as the risk of osteoporosis was evaluated in older age participants (average age in Sforza *et al* study was 68.6 ± 0.03 year compared to 40 – 68 year age range in the other study). Additionally, the risk of osteoporosis in the cross-sectional observational studies was based on direct readings of T-score compared to incidence rate ratio (IRR) of osteoporosis from a the same validated health database in the 2 longitudinal population-cohort studies. It has to be noted that the time frame overlaps of the same database between both studies, even though it was minimal (1998-2001 vs. 2000-2008). The two studies conducted by Chen *et al* [36] and Yen *et al* [37] were finally included in the meta-analysis for assessment of the association between sleep apnea and risk of osteoporosis. However, neither Yen *et al* nor Chen *et al* studies reported the bone area/s where T-scores were collected. The heterogeneity between the studies found in the meta-analysis was high; therefore, a random model was chosen [38]. As noted before their data came from the same database and some minimal time overlap between both studies is noted. The pooled odd ratios (ORs) from 2 studies (n=112,258 patients) showed that sleep apnea was a significant risk factor for osteoporosis in both males and females (OR, 1.92; 95%CI, 1.24 to 2.97; I^2=66%) (**Figure 2.8.2**). When the analysis performed separately, the association between sleep apnea and risk of osteoporosis was also statistically significant in females (OR, 2.56; 95% CI, 1.96 to 3.34; I^2=0%)) and in males (OR, 2.03; 95% CI, 1.24 to 3.35; I^2=38%), respectively (**Figure 2.8.2**). Worthy of note that the OR result was smaller when both males and females were combined in one group in comparison to ORs calculated separately for males and females due to the fact that

the heterogeneity index (I^2) was high in the combined males and females group. The association analysis (based on Pearson correlation [R]) between severity of sleep apnea (based on AHI) and bone density (represented by BMD or T score using lumbar spine and femoral bone) were also evaluated. The association results were not consistent. For lumbar spine, BMD (g/cm^2) readings were found to be negatively associated with AHI (n=21, R=0.4, P_{value}=0.023) [29], positively associated with AHI (n=832, R=0.219, P_{value}=0.010 [28]; n=50, R=0.280, P_{value}=0.134 [33]), and no evidence of a significant association with AHI (n=115, R=0.00, P_{value}=not reported) [27]. T scores of lumbar spine were negatively associated with AHI (n=21, R=0.5, P_{value}=0.012 [29]; n=85, R=0.250, P_{value}=0.021 [30]; n=66, R=0.249, P_{value}=0.044 [31]) or positively associated with AHI (n=832, R=0.159, P_{value}=0.010 [28]) and no evidence of a significant association with AHI (n=46, R=-0.199, P_{value}=0.185 [32]; n=50, R=0.283, P_{value}=0.130 [33]). BMD (gm/cm^2) of femoral sites was negatively associated with AHI (n=50, R=0.558, P_{value}=0.001 [33]), positively associated with AHI (n=832, R=0.200, P_{value}=0.010 [28]) or not significant (n=115, R=-0.11, P_{value}=not reported [27]). T scores of femoral sites were negatively associated with AHI (n=50, R=0.556, P_{value}=0.001 [33]), positively associated with AHI (n=832, R=0.141, P_{value}=0.010 [28]; n=85, R=0.242, P_{value}=0.029 [30]) and no evidence of a significant association with AHI (n=46, R=-0.177, P_{value}=0.240) .

The association between sleep apnea and risk of pathological bone fracture.

Only one study by Yen *et al* [37] evaluated the incidence of osteoporosis with or without pathological bone fracture in sleep apnea patients *vs.* non-sleep disorder patients. In this study, the authors reported that sleep apnea patients were associated with an increased

risk of osteoporosis without fracture compared to patients without sleep disorders (HR = 3.37, 95%CI = 2.69–4.21).

2.5. Discussion

Summary of evidence

The present systematic review used an electronic search strategy to identify articles examining the association between sleep apnea and bone mass. Using this search strategy, 12 studies (10 cross-sectionals studies [26-35] and 2 longitudinal studies [36, 37]) were finally identified. All studies were considered to be at medium or low risk of bias potential. The quality of the information retrieved from the finally selected studies warranted a meaningful statistical combination from only 2 studies**.** In summary, the limited available evidence suggests that the association between sleep apnea and low bone mass in adults is plausible, but supporting evidence limited and inconsistent.

Sleep Apnea and Bone Metabolism Markers

Only 7 cross sectional studies investigated the association between sleep apnea and different bone metabolism markers [26, 29, 30, 32-35]. The findings reported by these studies suggest overall a lack of association between the severity of sleep apnea, based on apnea-hypopnea index (AHI) readings, and bone formation markers. However, of the findings regarding an association between the severity of sleep apnea and bone resorption markers were contradictory. Some of the contradictions between the studies results may be related to the variations in study designs, and selection of the cases and control groups (See **Table 2.9.1**). Moreover, it may be difficult to compare bone markers results among those

studies since it is known that variations in specimen collection and storage, analytical methods used, and differences in diet and fasting condition of the studied patients may affect the overall readings of the analyzed bone markers [39]. In addition, the nature of some of these markers, such as serum calcium, phosphorous and 25-Hydroxyl vitamin 3D levels, is under strict metabolic control and unlikely to show long-term changes indicating altered bone metabolism (unless long-term variations in their levels were measured) [40]. Accordingly, in order to clarify the potential association between sleep apnea and bone metabolic markers, future research should focus in assessing those markers (such as CTX and bone-specific alkaline phosphatase) that indicate long-term changes of bone metabolism. Also, future studies should assess those markers at different time points: before the diagnosis with sleep apnea, changes in their levels during sleep apnea with or without treatment. Indeed only one study conducted by Tomiyami *et al* [26], assessed different bone metabolic markers at 2-time point: before and following 3-month of treatment of sleep apnea with CPAP therapy. Although, 3-month of sleep apnea treatment might not be enough period of time to detect changes in bone density (in relative to those approved medications for osteoporosis [39]), the results of Tomiyami *et al* showed the urinary level of CTX was significantly reduced in those sleep apnea treated patients [26].

Sleep Apnea and Bone Density

In this review, we summarized the results from 11 (9 cross-sectional [26-35] and 2 longitudinal [36, 37]) studies investigated the association between sleep apnea and bone mass (bone density and / or osteoporosis). No consensus was secured when trying to reconcile the findings of these selected studies; however, the majority (8 [29-31, 33-37] out

of these 11 studies) reported that sleep apnea patients are at higher risk of developing bone diseases compared to non-sleep apnea patients. One study [28] showed that sleep apnea patients exhibit higher bone density and lower risk of osteoporosis compared to non-sleep apnea patients, whereas 2 studies [27, 32] reported no significant differences in bone density and risk of osteoporosis in sleep apnea patients and controls. The meta-analysis conducted in an effort to determine the risk of developing osteoporosis in female and male populations included the data from only 2 studies [36, 37], due to the differences in the study design. Results of our meta-analysis showed that both females and males with sleep apnea are at higher risk of developing osteoporosis compared to females and males with no sleep apnea.

Possible links between sleep apnea and bone density

The mechanisms by which sleep apnea affects bone biology are still not well delineated. A recent review by Swanson *et al* described possible links between sleep apnea and bone mass in details [6]. Sleep apnea is characterized by (intermittent hypoxia events), which greatly differs in the transcriptional pathways being activated in comparison with continuous hypoxia [41]. Accordingly, it was hypothesized that the intermittent hypoxia of sleep apnea may impose a negative effect on bone cells, and result in loss of bone formation or bone mass [6]. In addition, sleep apnea could also facilitate bone loss through indirect physiological mechanisms [6]: Elevations in the tonic and reactive activity of the sympathetic nervous system (SNS) [7] could underlie bone loss since hyperactivity of the SNS has now been firmly established as a negative regulator of bone mass [42, 43]. Similarly, intermittent hypoxia may lead to disruption in the secretion of the circadian

rhythm hormone melatonin [1], a known positive regulator of bone mass [44, 45]. Another possible pathway, which may cause low bone mass in sleep apnea patients, is the observed reduction of the serum level of 25-OH vitamin D in these patients [8, 9]. 25-OH vitamin D helps in increasing the serum levels of calcium, and thus its reduction is known to be associated with increase risk of developing osteomalacia (calcium-depleted bone) [46] and increase the risk of fracture [47]. Also, sleep apnea is known to stimulate the inflammation process[48], which can activate osteoclasts and inhibits the deposition of bone mineral [49]**.** Also, the oxidative stress induced by the hypoxia / normoxia cycles in sleep apnea may stimulate bone resorption and thus bone loss [50]. Other pathways may rise from sleep apnea associated endocrine disorders such as hypogonadism, obesity and glucose intolerance [51, 52], which are correlated with low bone mass [53]. Moreover, sleep apnea patients are known to suffer from obesity and diabetes mellitus type 2 [54], medical conditions that disrupt the bone compositions leading to increase the risk of fracture that is unrelated to bone mineral density [55]. Additionally, several previous studies reported that sleep apnea is occurring in genetically related subjects and among certain ethnic groups [56, 57]. It might be that, at least in some patients, the genetic predisposition for developing sleep apnea also results in a genetic predisposition for low bone mass. However, future research will have to be conducted in order to address these hypotheses.

Recent translational studies using animal models assessed the associations between sleep apnea and low bone mass. The results of these studies were not consistent. A recent study by Song *et al* showed that after 5 weeks (which is equivalent to 8.3 years of age in humans) of exposure to intermittent hypoxia (mimicking sleep apnea),

oophorectomized female Sprague-Dawley rats had reduced lumbar and femoral bone mineral densities compared to oophorectomized female exposed to normal oxygen levels [58]. It was also shown that the expression of the following osteogenic and osteolysis genes: Runt-related transcription factor 2 Runx2, collagen type 1 (Col I), Alkaline phosphatase (ALP) and Tartrate-resistant acid phosphatase (TRAP) 5b, were lower in oophorectomized rats exposed to intermittent oxygen levels compared to oophorectomized rats exposed to normal oxygen levels. However, there were no differences between gene expression of osteocalcin and Receptor activator of nuclear factor kappa-B ligand (RANKL) between the two rat groups. Another recent study by Torres *et al* showed no variation in trabecular bone mineral density in male, female and orchidectomized 14-week old C57BL/6 mice exposed, for 32 days, to intermittent hypoxia (mimicking sleep apnea in some extent) compared to non-exposed mice [59]. A possible explanation to the preserved bone mass in rats exposed to intermittent hypoxia in Torres *et al* study is that the intermittent hypoxia induced physiological repair mechanisms, including the activation of the adult mesenchyme stem cells from the bone marrow [60] that has the capability to differentiate to bone forming cells (osteoblasts). Another explanation is that 32-day protocol, which is equivalent to ~4.2 years of age in humans, might not be enough since most sleep apnea patients left undiagnosed or untreated for many years. Another recent study by Oishi *et al* [61] showed that a 3-week exposure to intermittent hypoxia decreased the size of mandibular and viscerocranial bones in early adolescent old male Sprague-Dawley rats. However, the bone mineral density in the mandibular condyle and inter-radicular alveolar bone were increased in intermittent exposed rats compared to rats exposed to normal level of oxygen. Overall, in the absence of mechanistic studies that

firmly establish causality in animal models, extrapolation from such published evidence to the clinical settings is only inferential, and does not provide conclusive biological plausibility.

Limitations

Some methodological limitations of this review should be considered. First, limited available evidence consisting of only 10 cross-sectional studies [26-35] and 2 retrospective longitudinal studies [36, 37] were found. No randomized clinical trials were identified; as such studies would be questionable and premature due to ethical concerns. Second, regarding the available evidence, the selected studies applied different methodological designs: cross-sectional and longitudinal studies. The inclusion and exclusion criteria, and the assessment of comorbidities common to both sleep apnea and bone mass were different among the studies. Also, the sample size was different among the studies. Additionally, there was heterogeneity in the statistical method of analysis and the covariates (possible risk factors for developing bone diseases) adjusted in each study. Among these covariates; age, food consumption and physical activity (energy expenditure) are known to have direct effect on bone density. For instance, the range and average age of the included participants varied dramatically among the studies and thus, could affect the outcomes. It is known that people lose bone mass as they age. This age-related bone loss progresses slowly in both sexes after age of 40-50, with more rapid loss in female surrounding the menopausal transition. After age of 70, bone loss progresses at higher rate in both sexes [62]. Accordingly, future studies will have to be performed to assess the possible association between SA and bone density at different age subgroups. Other

important covariates that may affect the reported outcomes in the selected studies are the body weight (body mass index) and daily physical activity. Body weight and physical activity were shown to be the primary determinant of bone mineral density [63, 64]. Only 4 of the selected studies assessed the differences in physical activity between SA and matched controls [28, 34, 35]. The methods used for the assessment of the physical activity varied among these studies (incremental shuttle walk test [ISWT] [31], daily energy expenditure [DEE] [28], physical activity index [PAI] [34] and Epworth Sleepiness Scale [ESS] [35]). In 3 out the 4 studies, the daily physical activity were similar between SA and matched participants, and yet the SA subjects had lower bone density compared to matched controls [31, 34, 35]. The forth study, the daily physical activity was higher in SA participants compared to their matched controls, and it was found that the SA subjects had higher bone density than their matched controls [28]. In a follow up study by the same group, the reduced physical activity stood up as the best predictor for low bone mass (osteoporosis) [65]. Accordingly, future studies will have to control for the energy expenditure and physical activity among the participants for better assessment of the association between SA and bone density.

In addition, the study groups varied among the studies [(sleep apnea vs. non-sleep apnea) [26, 29, 33-37], (mild- vs. moderate- vs. severe-sleep apnea) [27], (moderate- and severe-sleep apnea vs. non- or mild-sleep apnea) [28, 30, 32], (severe-sleep apnea vs. non-, mild- or moderate-sleep apnea) [30], (COPD with sleep apnea vs. COPD without sleep apnea) [31]]. For the longitudinal studies, the diagnoses of sleep apnea and low bone mass were based on previously collected reports from the same health database, the National

Health Insurance Research Database (NIHRD) of Taiwan, which may increase the risk of recall or misclassification bias and risk of surveillance, which refers to "the more you look, the more you find". Additionally, although the Taiwan NIHRD covers 99.9% of the population, it lacks for information on laboratory examination, life style (including daily activity, body weight, tobacco use and drinking) and family history of systemic chronic diseases, which may influence the outcomes reported by these two studies. Moreover, both studies did not report the follow-up frequency, number of visits, or likelihood of testing bone density in sleep apnea and control groups, which may affect the results of meta-analysis. Also, there was a lack of assessment for the association between the severity of sleep apnea and bone mineral density in the included longitudinal studies. In 10 of the included studies [26-35], the sample consisted of patients who sought treatment for sleep disturbances, and thus may not be representative of the general population in-terms of incidence of apnea and incidence of osteoporosis. Additionally, the selected cross-sectional studies assessed the association between the sleep apnea and bone health at only one point in time, thereby restricting the imputed validity of the findings. Moreover, the included studies in this review reported the bone density findings using the t-score. T-score classifies the bone density into three categories (normal, osteopenic and osteoporosis) based on whether the readings above or below the normal variability in a bone mineral density measurement in a young population (30 year old) [66]. Accordingly, applying t-score for assessment of bone density in old adult (older than 30 years old) will not discriminate whether the low bone mass was a result of age related bone deterioration or due to a secondary cause. It would be more informative if the bone density data were reported in z-score, as this score relies in comparison to age-matched mean bone mineral density [66].

Therefore, if the bone density z-score data of a specific patient was less than what is expected to be for the patient's age, this will call for further investigation for the secondary cause of the reduction in bone density that is unrelated to age. Accordingly, further research should focus on applying the z-score for bone density assessment if a secondary cause of bone loss (such as sleep apnea) is expected. Due to all these limitations, methodologically stronger research, including well-designed prospective longitudinal cohort studies and interventional studies operating under optimal constructs for the diagnosis of sleep apnea and bone metabolism are clearly needed.

The results from the meta-analysis should be considered cautiously. Although a meta-analysis can be technically justified and be useful if properly framed, at the same time it may be dangerous if their results are considered without a proper conceptual/philosophical framework. Both included studies considered data from the same database with some time overlap. Some of the known risk factors of using retrospective data from large national databases have been already noted.

2.6. Conclusions

In conclusion, this systematic review provides insights into our state of knowledge on a potential association between sleep apnea and bone diseases. Findings of this systematic review suggest that the possibility that sleep apnea constitutes risk factor for developing bone diseases such as reduced bone density and osteoporosis cannot be formally dispelled nor conclusively proven. The incongruence among the reported findings may be traced back to methodological differences in the reporting of bone mass proxy variables or in the different populations included to date. However, the trends supporting the existence

of an association between sleep apnea and low bone mass appear to justify implementation of future studies that rely on large-scale, multi-centers, clinical trials that possibly address all the above limitations, which might influence the study outcomes. If confirmatory, then implementation of appropriate screening tools for early diagnosis of bone diseases in sleep apnea patients may be warranted such as to decrease the inherent morbidity and mortality of these two co-existing conditions.

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2.8. Figures:

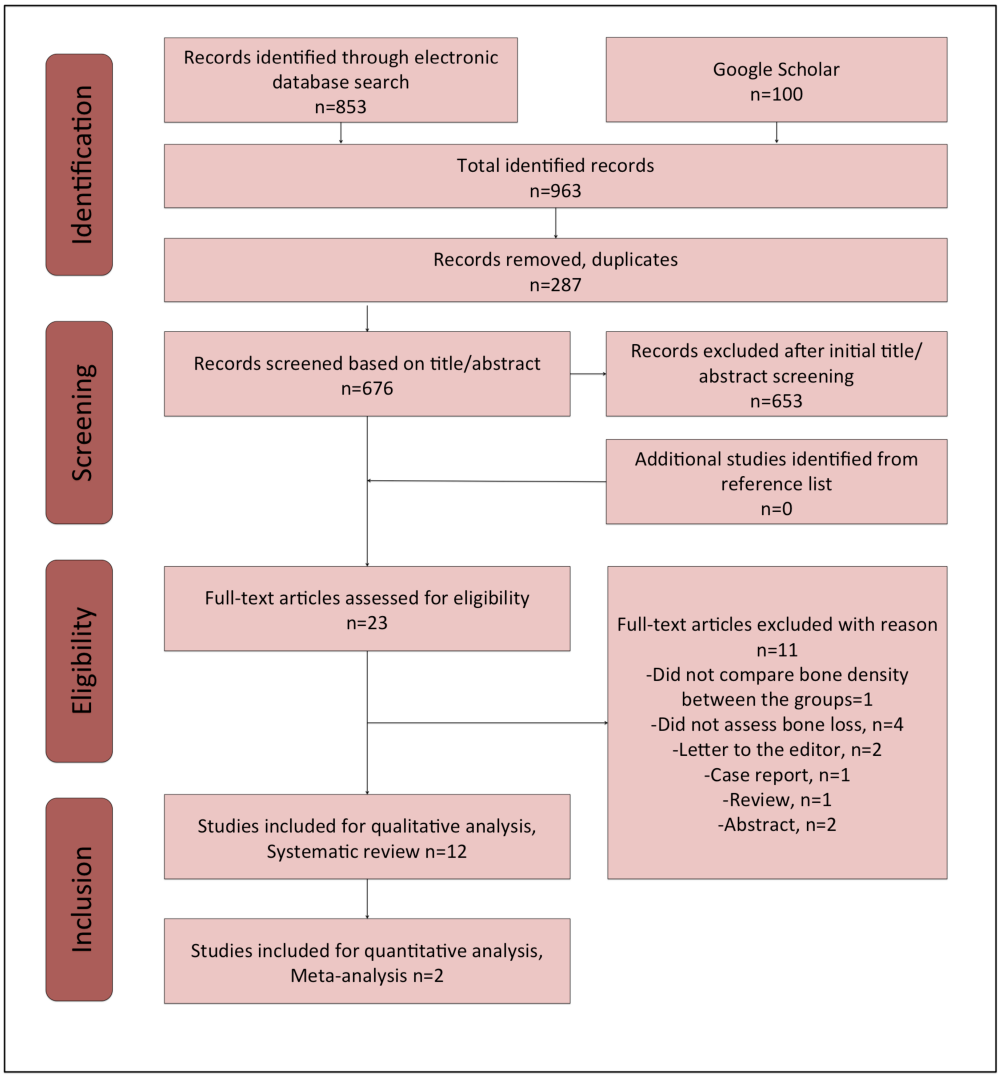

Figure 2.8.1. Flow diagram of the published data search according to the PRISMA statement.

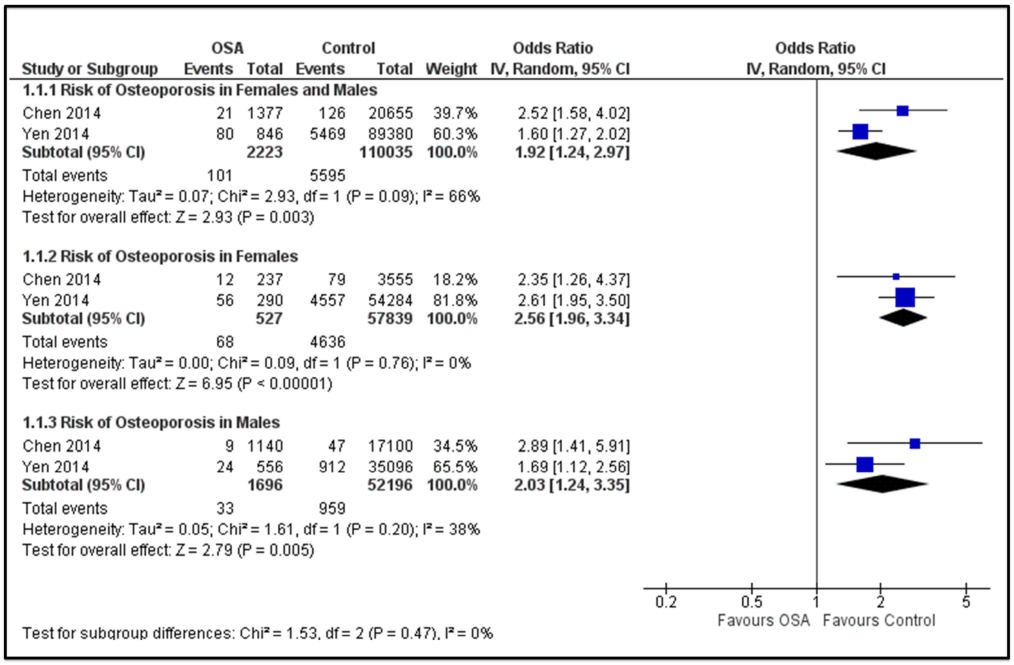

Figure 2.8.2. Meta-analysis of the effect of the sleep apnea diagnosis on the odds ratio of osteoporosis for the longitudinal studies. Forest plots of comparison of risk of osteoporosis in female and male sleep apnea- vs. sex-matched control-patients, female sleep apnea- vs. sex-matched control-patients, and male sleep apnea- vs. sex-matched control-patients.

2.9. Tables:

Table 2.9.1. Overview of the cross-sectional studies (n=10) and longitudinal studies (n=2) on sleep apnea and bone mass.

First author, Country, Year, Reference	Study period, Population, Age: range, average Sex	Study groups: SA patients (n) SA Diagnosis	Study groups: Controls	Adjustment for covariates	Bone measurement	Main outcomes
Cross-sectional Studies (n=10)						
Tomiyami, Japan 2008 (20)	*November 2005 – June 2006 *53 patients with clinical suspicious of SA *Age range 27-80yr, average 51yr	*50 patients in total divided into 3 groups: Mild SA: 5≤AHI<15, n=10 Moderate SA: 15≤AHI<30, n=12 Severe SA: AHI≥30, n=28 Moderate- and severe- SA received continuous positive airway pressure (CPAP) therapy *Diagnosis is based on PSG: AHI >=5	15 age- and sex-matched non-SA patients	Age, sex, body mass index, systolic and diastolic blood pressure, serum total cholesterol, serum triglycerides, serum high density lipoprotein cholesterol, fasting plasma glucose, smoking status, medication status for hypertension, diabetes mellitus and dyslipidemia.	*Bone formation markers: plasma concentration of osteocalcin (Oc) and bone-specific alkaline phosphatase (ALP) *Bone resorption markers: urinary concentrations of cross-linked C-terminal telopeptide of type I collagen (CTX)	*Univariate linear regression analysis: -AHI was positively correlated with the bone resorption marker (CTX) (R=0.32, p<0.05). -AHI was not associated with bone formation markers (OC and BAP) *ANOVA analysis: -Bone resorption marker (CTX) was higher in severe SA patients compared with mild or control subjects before start the CPAP therapy *Paired-t-test: -Significant decrease of CTX was observed following 3 months' CPAP (before: 211±107 vs. after: 128±59 μ g/mmol/creatinine; p<0.01).
Mariani, Italy, 2012 (21)	*June 2010 – June 2011*209 patients with clinical suspicious of SA from cohort obese patients *Age range 30-65yrs, average 56yr *Male and female	*115 patients in total divided into 3 groups: Mild SA: 5≤AHI<15, n=50 Moderate SA: 15≤AHI<30, n=33 Severe SA: AHI≥30, n=32 *Diagnosis is based on PSG: AHI >=5	Comparison was done between the three study groups: Mild-, Moderate- and Severe-SA patients	Age, sex, weight, lean mass, metabolic activity, inflammatory indices.	*BMD (gm/cm^2) and t-score was reported using DXA in the lumbar spine (L1–L4), total hip and the femoral neck	*Kruskal-Wallis analysis: -BMD measurements were similar between the study groups (mild, moderate and severe SA) *Spearman's correlation analysis: -Lack of association between AHI and BMD at all bone sites
Sforza, France, 2013 (22)	* Not mentioned *1,011 of cohort volunteered patients (Follow up for 7 yrs) (197 patients were excluded) *Age: Average 68.6yr *Male and female	*459 patients SA patients * Diagnoses is based on ambulatory polygraphy: AHI ≥15	*373 patients who had their AHI <15	Physical activity (Daily energy expenditure [DEE]), body mass index, oxygen desaturation index.	*BMD (gm/cm^2) and t-score was reported using DXA in the lumbar spine (L1–L4) and the femoral neck	*ANOVA analysis: -SA patients had a higher BMD at femoral (0.86 ± 1.46gm/cm^2, -0.78 ± 1.00 T score) compared to non-SA patients (0.82 ± 1.4gm/cm^2; -0.98 ± 1.0 t score) -SA patients had a higher BMD at lumbar spine (0.97 ± 1.6gm/cm^2, -1.0 ± 1.4 T score) compared to non-SA patients (0.93 ± 1gm/cm^2; -0.8 ± 1.4 t score)

Uzkeser, Turkey, 2013 (23)	*Not mentioned *255 patients with clinical suspicious of SA *Age range 37-69yr, median 54yr *Male	*21 patients in total Severity was not assessed *Diagnosis is based on PSG: AHI cut off was not mentioned	26 age- and sex-matched healthy non-SA patients	Body mass index	*Serum calcium (Ca), phosphorus (P), ALP levels, and urinary desoxy-piridinoline (DPD) levels *BMD (gm/cm^2) and t-score was reported using DXA in the lumbar spine (L1–L4), the femoral neck, and total femur region	*Mann-Whitney test: -No significant differences between the serum levels of Ca, P, ALP and urinary DPD among the study groups -Lumbar L1-L4 BMD (gm/cm^2) measurements were lower in SA patients [0.9 (0.6–1.1)] compared to control [0.9 (0.8–1.2)] -Lumbar L1-L4 t score measurements were lower in SA patients [-1.1 (-3.7–0.2)] compared to control [-0.9 (-2.4–1.1)] -Femoral neck BMD (gm/cm^2) measurements were lower in SA patients [0.8 (0.7–1.2)] compared to control [0.9 (0.6–1.9)] -Femoral total BMD (gm/cm2), Femoral neck t score and femoral total t score measurements were similar between the SA- and control-patients *Spearman's correlation analysis: -Significant correlations between AHI with lumbar L1-L4 BMD (R=−0.4) and lumbar L1–L4 t score values (R=−0.5)
Yuceege Turkey, 2015 (24)	*January 2012 - March 2013 *85patients with clinical suspicious of SA *Age range <45yrs, average35.5 ± 5.7 yrs *Male and female	*Two cut-off points: 1) 40 patients, AHI ≥15 2) 55 patients, AHI ≥30 *Diagnosis is based on PGS	1) 45 patients, AHI <15 2) 30 patients, AHI < 30	The adjustment for covariates is not mentioned	*BMD (gm/cm^2) and t-score was reported using DXA in the lumbar spine (L1–L4) and the femoral neck *Serum level of Ca, P, para-thormone (PTH) and 25-OH D3 vitamin.	*Multiple regression analysis: 1) At 1st cut off point: -AHI ≥15 was related with 2965-fold (95% CI 1.12–7.83) increased risk of osteopenia and osteoporosis, -Serum level of Ca, P, PTH and 25-OH D3 were similar among the study groups. 2) At 2nd cut off point: -AHI ≥ 30 was related with 4.26-fold (95% C.I. 1496–12.1) increased risk of osteopenia and osteoporosis. -Serum level of Ca, P, PTH and 25-OH D3 were similar among the study groups *Correlation analysis: -AHI was inversely correlated with total Lumbar T scores (R= −0.250, P = 0.021) and positively correlated with Total Femur T scores (R= 0.242, P = 0.029).

Wang, Taiwan, 2015, [25]	*January 2008 - January 2013 *312 of cohort COPD patients (246 patients were excluded) *Age: average 71.5 yrs *Male and female	*35 COPD patients with SA *Diagnosis is based on PGS: AHI ≥15	*31 age- and sex-matched COPD patients without SA	Body mass index, AHI, oxygen desaturation index, forced, expiratory volume, and physical activity (incremental shuttle walk test [ISWT])	*Records of BMD t-score, which were obtained using DXA of the lumbar spine (L2-4)	*T-Test analysis: -BMD t-score at lumbar spine of COPD patients with SA was significantly lower than in those without SA (−1.99±1.63 versus −1.27±1.14, P=0.045).
Aslan, Turkey, 2015, [26]	*Not mentioned *287 with clinical suspicious of SA (241 patients were excluded) *Age: range 40-68yrs *Male	*24 SA *Diagnosis is based on PSG: AHI ≥ 6	*22 control AHI <5	Age	*BMD (gm/cm^2) and t-score was reported using DXA in the lumbar (L1-L4) and femur neck *Growth hormone, IGF-1, sex hormone-binding globulin, total testosterone and free testosterone levels.	*Fisher's exact test: -T score of the femur and lumbar vertebra was lower in SA compared to controls, however, the difference did not reach the significant level (p value=0.05) *T-Test: -IGF-1, free testosterone, SHBG levels were similar between the 2 groups *Mann-Whiteney test: -Growth hormone and Total testosterone levels were similar between the 2 groups *Pearson correlation analysis: -No significant association between AHI and vertebral-and femoral- T-Scores (r=-0.199, P-value=0.185; r=-0.177, P-value=0.240)
Terzi, Turkey, 2016, [27]	*2012 – 2013 *55 patients with clinical suspicious of SA *Age: average~52yrs for SA and ~51yrs for non-SA *Male	*30 patients with SA Mild SA: 5≤AHI<15, n=0 Moderate SA: 15≤AHI<30, n=11 Severe SA: AHI≥30, n=19 *Diagnosis is based on PSG: AHI >=5	*20 age- and sex-matched non-SA patients	Body mass index	*BMD (gm/cm^2) and t-score was reported using DXA in the lumbar spine (L1–L4) and the femoral neck *Serum level of Ca, P, PTH, thyroid stimulating hormone (TSH), Oc, C-terminal telopeptide of type I collagen (CTX), 25-OH D3 vitamin and ALP	*T-Test analysis: -No significant differences between the levels of Ca, P, PTH, TSH, ALP, 25-OH D3 vitamin and Oc among the study groups -Bone resorption marker (CTX) was higher in SA patients compared with non-SA patients. *Mann-Whitney test: -Lumbar L1-L4 BMD (gm/cm^2) measurements were similar between the groups (SA patients: 1.08 ±0.15, non-SA patients: 1.16 ±0.13), p-value=0.058 -Femoral neck BMD (gm/cm^2) measurements were lower in SA patients (1.04 ±0.15) compared to non-SA patients (1.15 ±0.10), p-value=0.021 *Yates' continuity corrections: -Lumbar L1-L4 t-score measurements were similar between the groups, p-

						*Fisher's exact test: -Femoral neck t-score measurements were lower in SA patients compared to non-SA patients, p-value=0.022 *Univariate analysis: -Significant negative correlations between AHI and Oc, femoral neck T value, and femoral neck BMD value.
Hamada, Japan, 2016, [28]	*2008 – 2011 *718 patients with clinical suspicious *Age: range 20-80yrs *Male and female	*201 patients with SA Mild SA: 5≤AHI<15, n=57 Moderate SA: 15≤AHI<30, n=62 Severe SA: AHI≥30, n=82 *SA diagnosis was based on overnight PSG	*33 age- and sex-matched non-SA patients	Age, hypertension, alveolar-arterial oxygen difference, physical activity (physical activity index ([PAI])	*BMD (mg/ml) was reported using abdominal CT scan for the lumbar spine (L1–L3) *Serum level of Ca, Oc and 25-OH D3 vitamin	* ANOVA analysis: -BMD readings were lower in male patients with severe SA compared to non-SA patients -BMD readings were similar in SA female patients and non-SA female patients -No significant differences in serum level of Ca, Oc and 25-OH D3 vitamin between SA patients and non-SA patients *Spearman's correlation analysis: -AHI was not significantly correlated with BMD values
Liguori, Italy, 2016, [29]	*October 2013 – March 2015 *240 severe SA patients (148 patients were excluded) *Age: average 51.17 ± 11.82yrs *Male	*92 patients with SA *Diagnosis is based on PSG: AHI > 30	*50 age-, BMI- and physical activity-matched non-SA patients	Age, BMI, physical activity (Epworth Sleepiness Scale [ESS])	*BMD (gm/cm^2) and t-score was reported using DXA in the lumbar, the femoral neck and the total femur. *Calcium, vitamin D, parathyroid hormone, fibrinogen, C-reactive protein	*T-Test analysis: -SA patients had significantly lower vitamin D than controls. -SA patients had significantly higher parathyroid hormone, fibrinogen and C-reactive protein serum levels than controls *Chi-square analysis: -SA patients had lower BMD in lumbar and femur neck compared to controls (p-value<0.05). -SA patients and controls did not differ in terms of physical activity. *Pearson Correlation analysis; -Significant correlations between lower BMD in the lumbar spine and femur, lower mean arterial oxygen saturation (SaO2) and SaO2 nadir, and higher time spent with an SaO2 <90%. -Lower BMD in several lumbar spine and femur regions also correlated with higher Epworth Sleepiness Scale (ESS) scores and higher BMI.

Longitudinal-cohort Studies (n=2)

Chen, Taiwan, 2014 (30)	*2000-2008 (Follow up for 6yrs) *1,377 patients *Age: >40yr *Male and female	*1,377 patients *SA diagnosis was based on the following: 1) records of at least two outpatient service claims with the codes of SA and received a PSG test, and 2) single hospitalization with SA among the five claims diagnosis code	*20,655 age- and sex- matched non-SA patients	Age, sex, diabetes, hypertension, hyperlipidemia, chronic kidney disease, stroke, chronic obstructive pulmonary disease, cancer, anxiety, depression, benzodiazepine and zolpidem medications.	*Records for BMD (gm/cm^2) and t-score which were reported using DXA in the lumbar spine (L1–L4) and the femoral neck	*Incidence rate ratio (IRR): -Osteoporosis was higher in SA compared to non SA patients (IRR: 2.52; 95%CI: 1.59 to 3.99) *Cox proportional hazard regressions analysis (HR): -SA patients had 2.7 times more likely to develop osteoporosis during the follow-up period (adjusted HR: 2.739; 95% CI: 1.690 to 4.437)
Yen, Taiwan, 2014, (31)	*1998-2001 (follow up until 2010) *44,690 diagnosed with sleep disorders *Age:>20yrs, average 48.9 ± 14.5yrs *Male and female	*846 out of 44,690 patients had the diagnosis of SA *43,844 out of 44,690 patients had sleep disorders other than SA. *SA diagnosis was based on the medical and family histories, and by PSG	*89,380 age-, sex- and sleep disorder index date- matched non-sleep disturbances patients	Age, sex, diabetes, hypertension, coronary heart disease, obesity, stroke, hyperlipidemia, chronic kidney disease, gout, monthly income, and geographic location.	*Records for BMD (not specified)	*Incidence rate ratio (IRR): -Osteoporosis was higher in SA-sleep disorders compared to non-sleep disorders patients (IRR: 1.47; 95%CI: 1.26 to 1.72) *Cox proportional-hazards regression analysis (HR): -Risk of osteoporosis was higher in SA-sleep disorders patients compared to non-sleep disorders patients (adjusted HR: 2.98, 95%CI: 2.36 to 3.74) -SA-sleep disorders patients were associated with an increased risk of osteoporosis without pathological bone fracture compared to patients without sleep disorders (HR = 3.37, 95%CI = 2.69–4.21).

SA, sleep apnea; PSG, polysomongraphy; AHI, apnea-hypopnea index; BMD, bone mineral density; DXA, dual x-ray absorptiometry; COPD, chronic obstructive pulmonary disorder

Table 2.9.2. Quality assessment criteria used for cross-sectional studies (n=10) through a modified version of Newcastle-Ottawa Scale (NOS) for observational studies.

NOS Criteria	Studies, Year									
	Tomiyami, 2008	Mariani, 2012	Sforza, 2013	Uzkeser, 2013	Yucege, 2015	Wang, 2015	Aslan, 2015	Terzi, 2015	Hamada, 2015	Ligouri, 2016
A. SAMPLE SELECTION CRITERIA (Maximum 4 stars)										
1) Is case definition adequate	★	★	★	☆	★	★	★	★	★	★
2) Representative of cases	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆
3) Selection of controls	★	★	★	★	★	★	★	★	★	★
4) Definition of controls	★	★	★	★	★	★	★	★	★	★
B. COMPARABILITY (Maximum 2 stars)										
Comparability of cases and controls on the basis of the design or analysis	★ ☆	★ ☆	★ ☆	★ ☆	★ ☆	★ ☆	★ ☆	★ ☆	★ ★	★ ★
C. EXPOSURE (Maximum 3 stars)										
1) Ascertainment of exposure	☆	★	★	★	★	★	★	★	★	☆
2) Same method of ascertainment for cases and controls	★	★	★	★	★	★	★	★	★	★
3) Non-response rate	☆	☆	☆	★	☆	☆	★	☆	☆	★
Total (Maximum 9 stars)	**5**	**6**	**6**	**6**	**6**	**6**	**7**	**6**	**7**	**7**

Table 2.9.3. Quality assessment criteria used for longitudinal studies (n=2) through a modified version of Newcastle-Ottawa Scale (NOS) for population based studies.

NOS Criteria	Studies, Year	
	Chen, 2014	Yen, 2014
A. SAMPLE SELECTION CRITERIA (Maximum 4 stars)		
1) Representativeness of the exposed cohort	★	★
2) Selection of the non-exposed cohort	★	★
2) Ascertainment of exposure	★	★
4) Demonstration that outcome of interest was not present at start of study (no bone disease at start of study)	★	★
B. COMPARABILITY (Maximum 2 stars)		
Comparability of cohort on the basis of the design or analysis	★★	★★
C. Outcome (Maximum 3 stars)		
1) Assessment of outcome	★	☆
2) Was follow-up long enough for outcomes to occur	★	★
3) Adequacy of follow-up of cohorts	★	★
Total (Maximum 9 stars)	**9**	**8**

2.10. Supplementary information:

Supplementary Table 2.10.1. The electronic databases searched and the search strategy used in the systematic review as July 31, 2016.

Databases of published studies	Search strategy Used	Hits
MEDLINE Searched via OVID (1946 –July 31, 2016) ovidsp.tx.ovid.com	(sleep apnea.mp. OR exp Sleep Apnea Syndromes/) AND (exp Osteoporosis, Postmenopausal/ OR exp Osteoporosis/ OR osteoporosis.mp. OR bone mineral density.mp. OR exp Bone Density/ OR exp Fractures, Bone/) AND limit to humans	87
PubMed Searched at 1946 –July 31, 2016 http://www.ncbi.nlm.nih.gov/pubmed/	("sleep apnoea"[All Fields] OR "sleep apnea syndromes"[MeSH Terms] OR ("sleep"[All Fields] AND "apnea"[All Fields] AND "syndromes"[All Fields]) OR "sleep apnea syndromes"[All Fields] OR ("sleep"[All Fields] AND "apnea"[All Fields]) OR "sleep apnea"[All Fields]) AND (("bone density"[MeSH Terms] OR ("bone"[All Fields] AND "density"[All Fields]) OR "bone density"[All Fields]) OR ("osteoporosis, postmenopausal" [MeSH Terms] OR ("osteoporosis"[All Fields] AND "postmenopausal"[All Fields]) OR "postmenopausal osteoporosis"[All Fields] OR "osteoporosis"[All Fields] OR "osteoporosis"[MeSH Terms]) OR ("fractures, bone"[MeSH Terms] OR ("fractures"[All Fields] AND "bone"[All Fields]) OR "bone fractures"[All Fields] OR "fracture"[All Fields]))	130
ALL EMB Reviews Searched via OVID (1946 –July 31, 2016)	(sleep apnea.mp. OR exp Sleep Apnea Syndromes/) AND (exp Osteoporosis, Postmenopausal/ OR exp Osteoporosis/ OR osteoporosis.mp. OR bone mineral	7

	density.mp. OR exp Bone Density/ OR exp Fractures, Bone/) AND limit to humans	
CINAHL Plus Searched at 1937 –July 31, 2016 web.b.ebscohost.com	(sleep apnoea OR sleep apnea syndromes) OR (sleep AND apnea AND syndromes) OR Sleep apnea syndromes OR (sleep AND apnea) OR sleep apnea) AND ((bone density OR (bone AND density) OR bone density) OR (osteoporosis, postmenopausal OR (osteoporosis AND postmenopausal) OR postmenopausal osteoporosis OR osteoporosis OR osteoporosis) OR (fractures, bone OR (fractures AND bone) OR bone fractures OR fracture))	86
EMBASE Searched via ScienceDirect (1974 – July 31, 2016) www.embase.com	(sleep apnea.mp. or exp sleep disordered breathing/) AND (exp osteoporosis/ OR osteoporosis.mp. OR Bone fracture.mp OR exp Fracture OR bone density.mp or ex Bone density/) AND limit to human	543

Supplementary Table 2.10.2. Articles excluded with reasons

Author, Reference	**Year of Publication**	**Reason for exclusion**
Chakhtoura M[67]	2015	Case report and review paper
Swanson CM[68]	2015	Review
Huang HL[69]	2014	Abstract
Mehra R[70]	2007	Did not assess bone loss as an outcome.
Mehra R[71]	2008	Did not assess bone loss as an outcome.
Khan K[72]	2013	Did not assess bone loss as an outcome.
Sanguankeo A[73]	2015	Abstract
Polesel DN[74]	2013	Letter to the editor
Schiza SE[75]	2013	Letter to the editor
Zhang JG[76]	2011	Did not assess bone loss as an outcome.
Sforza E [65]	2016	Did not compare bone density between sleep apnea and non-sleep patients

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

Observational STUDIES

Review: Sleep apnea and bone loss

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition (SA) adequate?
   a) Yes, with independent validation (eg. self reported doctor's diagnosis, reference to primary record source, or diagnosis by PSG).
   b) Yes, based on self-reports.
   c) No description.
2) Representativeness of the cases
   a) Consecutive or obviously representative series of cases (random sample of cases).
   b) Potential for selection biases or not stated.
3) Selection of Controls
   a) Community controls (same community as cases).
   b) Hospital controls.
   c) No description.
4) Definition of Controls
   a) No history of disease (endpoint).
   b) No description of source.

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) Study controls for gender.
   b) Study controls for weight (eg. BMI, overweight, obesity).

Exposure

1) Ascertainment of exposure
   a) Doctor's diagnosis (not self-reported doctor's diagnosis) OR Use of objective measurements (eg.,DXA).
   b) Parent/self reported doctor's diagnosis OR Serum /urinary level of bone formation and resorption markers OR use of anti-osteoporosis medication.
   d) Written self report.
   e) No description.
2) Same method of ascertainment for cases and controls
   a) Yes.
   b) No.
3) Non-Response rate
   a) Same rate for both groups.
   b) Non-respondents described.
   c) Rate different and no designation.

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

CASE CONTROL STUDIES

Review: Obstructive sleep apnea and bone loss

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition (OSA) adequate?
   a) Yes, with independent validation (eg. self reported doctor's diagnosis, reference to primary record source, or diagnosis by PSG).
   b) Yes, based on self-reports.
   c) No description.
2) Representativeness of the cases
   a) Consecutive or obviously representative series of cases (random sample of cases).
   b) Potential for selection biases or not stated.
3) Selection of Controls
   a) Community controls (same community as cases).
   b) Hospital controls.
   c) No description.
4) Definition of Controls
   a) No history of disease (endpoint).
   b) No description of source.

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) Study controls for gender.
   b) Study controls for weight (eg. BMI, overweight, obesity).

Exposure

1) Ascertainment of exposure
   a) Doctor's diagnosis (not self-reported doctor's diagnosis) OR Use of objective measurements (eg.,DXA).
   b) Parent/self reported doctor's diagnosis OR Serum /urinary level of bone formation and resorption markers OR use of anti-osteoporosis medication.
   d) Written self-report.
   e) No description.
2) Same method of ascertainment for cases and controls
   a) Yes.
   b) No.
3) Non-Response rate
   a) Same rate for both groups.
   b) Non-respondents described.
   c) Rate different and no designation.

Preface for chapter 3 and 4:

In chapter 2, we systemically reviewed the clinical studies that assessed the association between SDB (including OSA) and bone mass in adults. Findings of this systematic review suggest that an association between OSA and low bone mass (such as reduced bone density and osteoporosis) in adults is possible. SDB is also common in children [5]. However, it is still not known whether SDB impacts the bone density in children.

Bone mineral density can be appraised using indices measured from dental panoramic radiographs. Among all the radiographic indices, the mandibular cortical width (MCW) exhibits the highest sensitivity and specificity for detecting reductions in bone mineral density [77].

Measurements of the MCW have only been validated on standard dental panoramic radiographs [77]; however, the radiographic data of the participants in chapter 4 included panoramic images reconstructed from cone beam computed tomography (CBCT). Thus, we, *first,* assessed the reliability in establishing these indices from reconstructed CBCT images relying on an *ex vivo* experiment on 10 well-preserved dry human-skull specimens (chapter 3). *Then*, we presented the results of 2 retrospective *cross-sectional* studies conducted in order to investigate the association between sleep disturbance and bone mass in children (chapter 4).

CHAPTER 3. Mandibular Cortical Width

Comparison of the Mandibular Cortical Width on Standard Panoramic Images and Panoramic Images Generated from Cone Beam Computed Tomography

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3.1. Abstract:

Purpose: Establish whether panoramic images reconstructed from cone beam computed tomography (CBCT) could be utilized to assess the mandibular cortical width (MCW), which have only been validated on standard dental panoramic radiographic images.

Methods: Ten well-preserved, dry human skull specimens were exposed to standard dental panoramic- and CBCT-imaging using a standardized protocol. MCW measurements were calculated on the panoramic images (standard dental panoramic images and those reconstructed from CBCT) twice by the same observer and once by a second observer using ImageJ 1.47v software. Intraclass correlation (ICC) statistical tests were used for assessments of the intra-observer reliability and inter-observer agreement. A paired sample t-test was used to determine whether the mean differences of MCW calculated from

panoramic images reconstructed from CBCT are different from those calculated using the standard dental panoramic radiographs.

Results: MCW measurements had excellent intra-observer reliability and inter-observer agreement (ICC values for both tests were more than 0.80) when performed either on standard panoramic images or panoramic images constructed from CBCT scanning. MCW readings performed on panoramic images reconstructed from CBCT scanning (4.1 ± 0.7mm) were similar to those readings performed on standard panoramic images (4.2 ± 0.7mm; p-value >0.05).

Conclusion: MCW performed on panoramic radiographic images reconstructed from CBCT may be used as a screening tool to evaluate the cortical thickness of the mandible.

3.2. Introduction:

Reduction in bone density is a relatively common disease characterized by changes in the micro-architectural structure of bone that leads to fragile bones that are more likely to fracture. Approximately over 200 million people suffer from this disease, which can cause a loss of mobility and an overall reduction of the quality of life [78]. The gold standard for diagnosis of osteoporosis is the Dual-energy X-ray Absorptiometry (DXA). This noninvasive diagnostic tool estimates the quantity of bone by measuring its radiopacity (bone mineral density). Other techniques that are used to diagnose the reduction of bone density are peripheral quantitative tomography, quantitative ultrasound methods and peripheral magnetic resonance imaging [79]. Recently, it has been reported that bone density can also be evaluated using quantitative and/or qualitative indices measured from dental panoramic radiographs [80]. Since panoramic radiographs are frequent diagnostics

procedures during routine dental checkups [81], it was hypothesized that these indices could be used as screening tools to identify patients at a high risk of bone density diseases such osteopenia and osteoporosis.

The most commonly used indices performed on dental radiographs are mandibular cortical width (quantitative index), mandibular cortical width (quantitative index) and the Klemetti index (qualitative index). Based on a recent systematic review with meta-analysis, the mandibular cortical width (MCW) was reported to have the highest sensitivity (0.602, 95% confidence interval [CI], 0.398-0.775) and specificity (0.708, 95%CI, 0.568-0.817) for detecting the reduction in bone density [77]. MCW is the mandibular cortical width at the mental foramen region.

Cone-beam computed tomography (CBCT) is relatively a new diagnostic tool that has offered the dentists several advantages in diagnosis and treatment planning. Accordingly, more dentists are utilizing the CBCT in their dental practices [82]. However, the use of CBCT to assess mandibular bone density by applying MCW performed on standard panoramic radiographs has not been verified yet.

The present study aimed to compare the values of MCW measured from standard panoramic radiographs and panoramic radiographs reconstructed from CBCT images. The study hypothesis was that values of MCW measured from standard panoramic radiographs are similar to those measured from panoramic radiographic images reconstructed from CBCT images.

3.3. Methods:

An *ex-vivo* experiment was conducted to address the study hypothesis. In this

study, 10 well-preserved dry human skull specimens were used. The skull specimens were exposed to standard panoramic and CBCT imaging. For panoramic imaging, each skull was mounted onto pedestal inside a panoramic imaging machine present at University of Alberta Dental Clinics. Ideal position of each skull, which simulates the desired position of the patient's head, within the panoramic imaging machine was achieved with the help of 2 light guides. The light guides consisted from a horizontal light guide to align the skull with the Frankfort horizontal line (line passing from the infraorbital ridge to the anatomic *Porion*); and a vertical light guide to align the skull with midsagital plane. A tripod assembly and notched bite block between the maxillary and mandibular incisors were applied to secure the ideal position of the skull within the panoramic imaging. The panoramic imaging machine was adjusted to the optimum image density and contrast using the following exposure settings: 57 KVP, 2.0mA and 17.6 seconds. A metallic ruler to correct for potential magnification was used. Upon completion of each imaging process, the generated panoramic radiograph was saved for further analyses in a TIFF format, with 3188 * 1709 pixel and 256 gray levels.

For CBCT imaging, each skull was mounted in a Plexiglas box (26 X 24.6 X 22 cm) placed onto a pedestal inside a CBCT scanner (ICAT, Imaging Science International, Hatfield, PA, USA) present at the University of Alberta Dental Clinics. The iCAT machine was adjusted following the standardized protocol: large field of view 9inx12", voxel size 0.30mm, 120kVp, 23.87mAS, 8.9 seconds. From those scans, panoramic reconstructions images were assembled using Dolphin 3D software (Dolphin Imaging & Management Solutions, Chatsworth, CA, USA). The generated panoramic images from CBCT scanning

were saved as TIFF images.

Obtained panoramic images (either from the standard panoramic imaging machine or those reconstructed from CBCT scanning machine) were analyzed twice by the same observer and once by second observer using ImageJ 1.47v software (National Institutes of Health, Bethesda, MD, USA). Both observers are dentists with expertise in oral radiology.

Mandibular Cortical Width (MCW) was measured at the side of better visualization as previously described [83, 84]. Initially, the mental foramen was identified. A line was drawn on the image, which passed perpendicular to the tangent to the lower border of the mandible and through the center of the mental foramen. The distance, in mm, between the lower border of the mandible to the superior margin of the mandible cortex represents the MCW, was recorded (**Figure 3.8.1**).

Statistical Analyses

The Statistical Package for the Social Sciences (SPSS) 23.0v for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. In all experiments, a value of $p<0.05$ was considered significant.

Reliability assessment for intra-observer and inter-observer:

The measured MCW values performed on the standard panoramic radiographs and those reconstructed from CBCT were assessed for consistency and reproducibility. Consistency and reproducibility are indices of agreement between repeated measures made by the same observer (intra-rater reliability) and between 2 observers (inter-rater reliability), respectively. The intra-rater and inter-rater reliability were investigated by

using Intraclass Correlation Coefficient (ICC) statistical tests. A single measure ANOVA with consistency and absolute agreement under two-way mixed model was chosen to ensure consistency in one rater's individual measurements and absolute agreement between both raters, respectively, while the subjects were chosen randomly. ICC values were interpreted by the general guidelines presented by Portney and Watkins [85].

Comparison between MCW performed on standard panoramic images and those generated from CBCT scanning:

The normality and the presence of outliers in the mean difference between MCW readings performed on standard panoramic images and those generated from CBCT scanning were assessed using Kolmogorov-Smirnov test and Shapiro-Wilk test. After checking the presence of outliers and normal distribution of the mean difference data of MCW readings among the two study groups, parametric analysis (a paired sample t-test) was used to determine whether the mean differences of MCW calculated from panoramic images reconstructed from CBCT are different from those calculated using the standard dental panoramic.

3.4. Results:

Intra-rater and inter-rater reliability

Intra-rater and Inter-rater reliability for the MCW measurements performed on standard panoramic images and those generated from CBCT images were excellent with low measurement errors. In all measurements, the ICC values for ICC values were more than 0.80 (**Table 3.9.1**). Worthy of note that the inter-rater reliability assessment for the

MCW from the panoramic images generated from CBCT was relatively lower (based on the lower ICC value and the wider confidence interval) than the MCW readings from the panoramic images

Linear comparison in MCW performed on standard panoramic images and those generated from CBCT scanning

MCW readings performed on standard panoramic images and those reconstructed from CBCT scanning had means of 4.2 ± 0.7mm and 4.1 ± 0.7mm, respectively (**Figure 3.8.2)**. Result of the paired t test indicates that there was no statistically significant difference in the mean of the MCW performed on standard panoramic images and those images reconstructed from CBCT (p-values for both were >0.05).

3.5. Discussion:

MCW measurements on dental panoramic radiographs are a previously validated method for bone density screening [77]. MCW is being commonly used based on the fact that the inferior border of the mandible is clearly visible on the dental panoramic radiographs and the distance between the lower border of the mandible to the mental foramen is relatively constant throughout the adult life [86]. In this study, we evaluated whether the MCW can be utilized panoramic images reconstructed from CBCT for similar purposes. Overall, all the measurements in our study had a high intra-observer and inter-observer reliability. This indicates that the MCW from standard panoramic images or those reconstructed from CBCT scanning can be indistinctly measured from either panoramic image. Of note that the lower ICC value and the wider range of confidence interval in

MCW readings from the panoramic images generated from CBCT relative to standard panoramic images could be related to the fact that the panoramic images generated from CBCT have lower resolutions than the standard dental panoramic radiographic images.

After showing that the measured MCW distances were reliable, the MCW from 10 skulls were calculated. Our findings indicated that mean MCW readings performed from panoramic images are not different from those readings from panoramic images generated from CBCT. This indicates that MCW measured from panoramic images reconstructed from CBCT could be used as screening tool to assess bone density in future research.

Limitation:

There are some factors that should be considered when applying the results of this investigation to clinical situations. In this study, the exact bone density readings from DXA were not collected. These data would be important to verify the exact relationship between MCW performed on panoramic images reconstructed from CBCT scanning. Also true diagnostic values of the process could be reported. Accordingly, future research is required to assess the exact association between MCW readings performed on CBCT images and bone density readings obtained from DXA.

Additional limitation is that the accuracy of measuring the MCW using either the panoramic scanning machine or panoramic images reconstructed from CBCT scanner could not be compared to the actual readings of MCW of these skulls. The only way to calculate the accuracy is to do sagittal sections of the mandibular jaw at the mental foramen, then, measure the MCW and compared to those obtained from the two mentioned imaging

techniques. It was not feasible to destroy the utilized dry-skull specimens, as they are part of a university collection.

This study was performed on dry skull specimens. The landmarks used for measuring the MCW distance may be affected by image quality. However, MCW distance measurements, performed on standard panoramic images were previously validated as a tool to assess bone density successfully. Here, we showed the MCW performed on panoramic images had similar results in comparison to those performed on standard panoramic images.

This study assessed MCW on 10 skulls. Larger sample size would increase the power of the statistical analysis and reduce the cumulative effect of measurement error. Moreover, the 10 skulls were not randomly chosen. Thus, findings from this study should not be inferred to the population as whole.

Although the reliability for measuring MCW showed high level of agreement, there were some minor measurement errors that should be considered in interpreting the numerical data. This could be due to the identification of the landmarks used for measuring the distances. With time and experience in using the ImageJ software, the measurement errors may be reduced.

3.6. Conclusion:

For purpose of our research, the MCW measurements for the mandibular cortical width at the mental foramen area had excellent intra-observer reliability and inter-observer agreement performed either on standard panoramic images or panoramic images generated

from CBCT scanning. Moreover, MCW readings performed on panoramic images reconstructed from CBCT scanning are similar to those readings performed on standard panoramic images. Accordingly, MCW performed on panoramic radiographs reconstructed from CBCT can be used as screening tool to evaluate bone density in future research.

3.7. Acknowledgment

This work was supported by the operating grant from the Alberta Innovates - Health Solutions (AIHS, Grant RES0027174), and a studentship to Hazem Eimar from the Alberta Innovates-Health Solutions (AIHS). We also thank Dr. Lagravere for providing the skull-specimens. We would further acknowledge Dr. Kristopher Currie for his excellent technical support.

3.8. Figures:

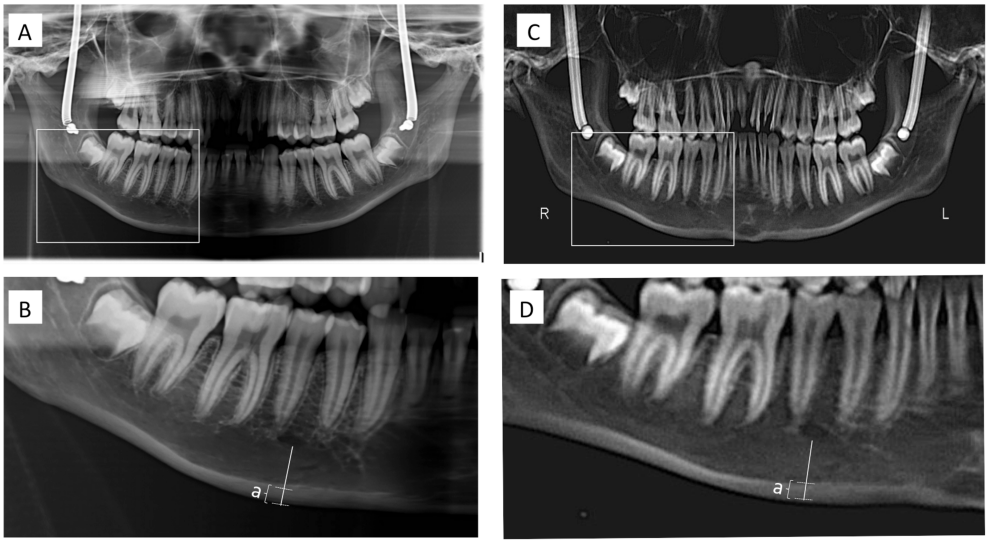

Figure 3.8.1. (A, B) panoramic images of the same skull generated from standard panoramic machine and CBCT scanning machine, respectively. **(C, D)** Higher magnification of the regions shown in panels (A, B), respectively, illustrating the measurement of mandibular cortical width (MCW) at the mental foramen area.

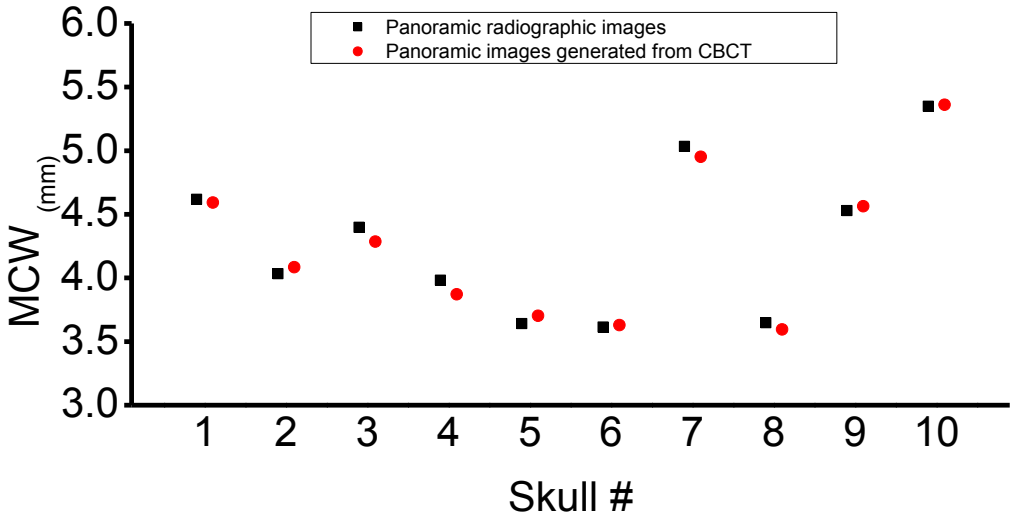

Figure 3.8.2. Scatterplot for the measurements of mandibular cortical width (MCW) performed on panoramic images and those images reconstructed from CBCT scanning.

3.9. Tables:

Table 3.9.1. ICC of Intra-rater and Inter-rater reliability of MCW distances obtained from standard dental panoramic images and panoramic images reconstructed from CBCT scanning.

Measured Distances	ICC	95% Confidence Interval	
		Lower bound	Upper bound
Intra-rater reliability			
Standard panoramic – MCW	0.996	0.983	0.999
CBCT reconstructed panoramic – MCW	0.964	0.863	0.991
Inter-rater reliability			
Standard panoramic – MCW	0.950	0.823	0.987
CBCT reconstructed panoramic – MCW	0.846	0.606	0.959

ICC: Intraclass Correlation Coefficient; MCW: Mandibular cortical width

CHAPTER 4: Sleep-Disordered Breathing and Bone Quality in Children

Potential Impact of Sleep Disordered Breathing on Mandibular Cortical Thickness in Children

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4.1. Abstract:

Background: Mandibular cortical thickness has been strongly linked to skeletal bone density. Preliminary evidence suggests that sleep-disordered breathing (SDB) (i.e., obstructive sleep apnea - OSA) negatively affects bone growth and mineralization, and hence bone density. We hypothesized that children at higher risk for SDB or suffering from OSA may exhibit thinner mandibular cortical thickness.

Methods: Two retrospective cross sectional studies were performed. The *first* study included a cohort of children in which SDB was suggested by the Pediatric Sleep Questionnaire (PSQ) (n=101). Mandibular cortical width (MCW) was measured from panoramic radiographs. The *second* study included comparison of MCW between 24

children with polysomnographically (PSG)-diagnosed OSA and 72 age- and sex-matched control children.

Results: Multiple-predictors regression analysis from the first study indicated that PSQ total scores were negatively associated with MCW (β = -0.398, p <0.001). These findings were further supported by the *second* study illustrating that in children with a severe form of SDB, as induced by OSA severity, there was a negative association with MCW (β = -0.290, p = 0.049). Moreover, PSG-diagnosed OSA children had thinner mandibular cortical thickness (2.9. ± 0.6mm) compared to healthy children (3.5±0.6mm; p =0.002).

Conclusions: Findings suggest that children at risk for SDB exhibit alterations in mandibular bone measures that purportedly may reflect alterations in bone homeostasis.

Key Words: Sleep-disordered breathing; Obstructive Sleep Apnea; Bone Density; Dental radiography; Airway Volume, CBCT radiography; Bone diseases; Mandible; Child.

4.2. Introduction:

Sleep-disordered breathing (SDB) is a common condition, with a wide spectrum of severity ranging from habitual snoring to obstructive sleep apnea (OSA) affecting approximately 10-12% of children and up to 35% of the adult population [1-5]. The more severe form of SDB, i.e., OSA, is characterized by repetitive episodes of upper airway narrowing or collapse during sleep leading to either reductions or cessation of airflow (hypopnea and apnea, respectively), resulting in intermittently low oxygen levels (hypoxia), episodic hypercapnia, as well as disrupted sleep due to recurrent arousals. SDB has been

identified as contributing to a large number of morbidities involving the cardiovascular, endocrine, immune, metabolic and autonomic and central nervous systems [11, 12, 16, 17]. In addition, pediatric SDB has been associated with increased healthcare utilization and costs with the frequency of recurrent respiratory problems being particularly prominent [87].

Several studies conducted in adult populations have suggested that patients with poor sleep quality, such as in SDB patients, may be at higher risk for reduced bone mineral density (such as osteopenia and osteoporosis) as well as increased frequency of bone fractures [26, 29, 31, 33, 34, 36, 37, 88, 89]. Similarly, poor sleep quality also affects children, especially those with enlarged tonsils or/and adenoids [90]. However, it remains unclear whether children with SDB exhibit higher risk for reduced bone density.

Bone mineral density can be appraised using indices measured from dental panoramic radiographs. Among all the radiographic indices, the mandibular cortical width (MCW) demonstrates the highest sensitivity and specificity for detection of reduced bone mineral density [77], with MCW being designated as the width of the inferior border of the mandible below the mental foramen.

Based on aforementioned considerations, we hypothesized that the bone mineral status of the mandible in children, assessed by MCW, could be associated with SDB risk, i.e. evaluated using the Pediatric Sleep Questionnaire (PSQ) [91]. The PSQ is a validated screening tool used to identify patients who are at higher risk of suffering from SDB, and consists of a 22-item scale that evaluates snoring, excessive sleepiness and behavior. PSQ

scores ≥ 8 have been suggested as indicative of high SDB risk, and therefore prompt referral to ear, nose and throat (ENT) specialists for further investigation [92]. We also hypothesized that children who are polysomnographically (PSG)-diagnosed with OSA may exhibit a thinner MCW when compared to matched children with no reported sleep-breathing disturbances.

4.3. Methods

Participants

Approval to conduct the 2 retrospective cross-sectional assessments was obtained from the ethics board of the University of Alberta (Pro00063547). Medical records were retrieved from an existing database of 202 patients diagnosed with SDB who were orthodontically treated at the University of Alberta Orthodontics Clinics before April 30, 2016.

First Study

The *first* study included a convenience sample of children who were evaluated using the Pediatric Sleep Questionnaire (PSQ), and who met the study defined inclusion and exclusion criteria (see below). Subjects were included in the study only if the complete set of records was available: demographic data (age < 18 years, sex, weight, height, body mass index [BMI]), no known genetic or syndromic conditions; craniofacial imaging (including a full view Cone Beam Computed Tomography [CBCT]) as part of the orthodontics diagnostic record. CBCT imaging would have been prescribed to provide a 3-dimenionsal (3D) visualization of the craniofacial skeleton (such as jaw asymmetry), a 3D

evaluation of impacted tooth position, or assessment of the temporomandibular joint complex, among other reasons.

Children diagnosed with conditions known to substantially affect bone metabolism, as well as patients who used medications known to affect bone metabolism, were also excluded. In addition, patients with no craniofacial radiographic images or radiographic images of sub-standard quality were excluded.

Second Study

The second convenience sample consisted of all children (out of the 202 patients existing in the database) who underwent a clinical examination by ear, nose, and throat (ENT) specialists and underwent a full overnight polysomnographic evaluation (PSG) in a sleep laboratory. In addition, these children had a complete set of demographic records and craniofacial imaging as described for the first study. The following sleep-related measures were retrieved from the PSG: total sleep time (TST), stages of sleep time (rapid eye movement [REM]-stage and non–REM (NREM) stages 1-3; reported as percentage of TST), arousal index (number of arousals per hour), and apnea-hypopnea index (AHI) for assessment of the severity of apnea.

Children with symptoms compatible with the presence of sleep disorders but without PSG confirmation were excluded. Moreover, any patient who did not fulfill the inclusion criteria of the first study was also excluded.

For each child with PSG-diagnosed OSA, 3 children with no history or physical findings compatible with SDB (controls) were randomly selected from the same patient

database of the University of Alberta Orthodontics Clinics, and matched for age and sex. Moreover, CBCT evaluation was a pre-requisite, and the same exclusion criteria that were applied to patients with OSA were also applied to controls.

Data collection from the craniofacial radiographic images for both studies

CBCT scans (ICAT, Imaging Science International, Hatfield, PA, USA) were obtained following a standardized protocol consisting of: 0.3-mm voxel, 120kVp, 18.54mA, exposure time of 8.5 seconds and field of view of 16-cm in diameter and 6-cm in height. The aforementioned field of view included only one full dental arch in standard resolution, allowing for a low effective radiation dose (approximately 35 microsieverts), as reported by a study using the same CBCT device [93]. From the aforementioned field of view CBCT scans 3D airway volume, cephalometric and panoramic radiographic analyses images were performed. Cephalometric and panoramic radiographic images were reconstructed and stored using Dolphin 3D software (Dolphin Imaging & Management Solutions, Chatsworth, CA, USA). Cephalometric images were obtained from the right side of the patients. Prior to the reconstruction process, the skull was lined up, with the orbits parallel to the horizontal plane and with a corrected head's rotation. Panoramic images were reconstructed by selecting a custom focal trough that passed through the lingual cusps of the maxillary teeth and extended posterior to the condyles. Focal trough width was adjusted to include the entire length and height of the maxillary and mandibular jaws.

3D airway volume measurements:

The airway of nasopharynx, oropharynx and total airway (nasopharynx and oropharynx) volume were reported for each subject using the Dolphin 3D software. The boundaries of the nasopharynx and oropharynx airway spaces were defined as previously described [94]. For nasopharynx, the airway space was reconstructed from the intersection of the following lines extending from: *Sella Turcica* point to tip of the odontoid process, the posterior nasal spine to tip of the odontoid process, and *Sella Turcica* point to posterior nasal spine. For oropharynx, the airway space was reconstructed from the intersection of the following lines extending: from the posterior nasal spine to tip of the odontoid process, posterior nasal spine to base of epiglottis, superior border of the 4th cervical vertebra to base of epiglottis to symphysis of the mandible, and line extending from the tip of the odontoid process to posterior superior border of the 4th cervical vertebra.

Cephalometric radiographic analysis:

The following data were obtained from analysis of the cephalometric radiographic images: mandible growth direction and maxilla-mandibular anterior-posterior relationship. Mandible growth direction was recorded using the angular measurement between the Frankfort plane (from *Porion* to *Orbitale* anatomic landmark points) and the mandibular plane (from *Menton* to *Gonion* anatomic landmark points) [95]. The anterior-posterior jaw relationship was determined using an angular measurement from Steiner analysis (A – *Nasion* –B anatomic landmark points).

Panoramic radiographic analysis

TIFF-format panoramic images from all individuals were retrieved from the medical records, analyzed twice by investigators blinded to the PSQ and PSG status using the ImageJ software v1.47 (National Institutes of Health, Bethesda, MD, USA). MCW was determined at the side of better visualization as previously described [83, 84, 96, 97]. Briefly, the mental foramen was identified. A line passing perpendicular to the tangent to the lower border of the mandible and through the center of the mental foramen was drawn. The distance, in mm, between the lower border of the mandible to the superior margin of the mandible cortex, represents the MCW (**Figure 4.8.1**).

Measurements of the MCW have only been validated on standard dental panoramic radiographs [77], but not on panoramic images reconstructed from CBCT. To establish reliability in the determination of MCW from reconstructed CBCT images, an *ex vivo* experiment on 10 well-preserved, dry human-skull specimens was conducted (Chapter 3). Briefly, skulls were exposed to standard dental panoramic- and CBCT imaging using a standardized protocol. MCW measurements were conducted twice on the panoramic images (either from the standard dental panoramic imaging machine or those constructed from CBCT scanning machine) by the same observer and once by a second observer (i.e., dentists with expertise in oral radiology) using ImageJ 1.47v software (**Figure 3.8.1**). Findings from these experiments showed that MCW measurements from CBCT scanning had an excellent intra-observer reliability and inter-observer agreement when compared to standard panoramic images (**Table 3.9.1**). Furthermore, MCW readings performed on panoramic images constructed from CBCT scanning were similar to those readings performed on standard panoramic images (**Figure 3.8.2**). Accordingly, MCW performed on

panoramic radiographic images constructed from CBCT were deemed valid as a screening tool to evaluate the cortical thickness of the mandible.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) 23.0v for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. In all experiments, a value of $p<0.05$ was considered as statistically significant. Continuous variables were reported as mean and standard deviation [SD], whereas categorical variables were summarized as percentages. The MCW distance from the panoramic radiographs reconstructed from CBCT were assessed for consistency (intra-rater reliability) and agreement (inter-rater reliability) using Intraclass Correlation Coefficient (ICC) statistical tests. ICC values were interpreted by the general guidelines presented by Portney and Watkins [85].

For the *first* *cross-sectional* study, single-predictor regression analysis was performed using a general linear model to determine which factors were statistically associated with MCW. Unstandardized coefficients "B", standard of error "SE" and standardized coefficients "β" were reported for each variable. All Factors with a *p* less than 0.25 on the single-predictor analysis were included into the multiple-predictor model. Multiple-predictor regression analysis using a general linear model was performed to determine the factors, which were independently associated with MCW. All included variables were checked for colinearity. Furthermore, participants were divided into 2 groups based on PSQ score (children with PSQ<8 vs. children with PSQ ≥ 8). Differences in participants' characteristics between the two groups were assessed using multivariate

analysis of variance (MANOVA) with Turkey's Post hoc pair-wise comparisons. After checking the normality and homoscedasticity, analysis of covariance (ANCOVA) was performed to assess the differences in MCW between the 2 groups, with age, weight, height, BMI, mandibular growth direction, relationship between jaws, nasopharynx- and oropharynx-airway volumes as covariates. For the *second study*, the same statistical approaches were used as described above.

4.4. Results:

First Study:

A total of 202 patients with potential symptoms suggestive of SDB were identified in the Orthodontic clinic database at the University of Alberta, and 101 patients fulfilled all of the inclusion and exclusion criteria. Participants' general characteristics, PSQ scale scores, and mandibular cortical thickness, as indicated by the MCW, are presented in **Table 4.9.1**.

Intra-rater reliabilities for the MCW performed on panoramic images were excellent in both studies. In all measurements, the ICC values and lower bound of 95%CI for ICC values was more than 0.90.

Results of single- and multiple-predictor regression analyses for all the variables that could be associated with MCW are shown in **Table 4.9.2**. Results from single-predictor analyses showed for all the variables, age, weight, height and BMI, and nasopharynx- and oropharynx-airway volumes, and total PSQ scores were significantly (p <0.05) associated with MCW.

The aforementioned statistically significant variables (p <0.05), plus BMI and antero-posterior jaw relationship (p-values for both were <0.25) were included in the final multiple-predictor linear model. **Table 4.9.2** (right section) shows the results from the multiple-predictor analysis. The regression model predicted MCW, F= 7.218, p < 0.001, R^2= 0.463. However, only the global PSQ score was independently significantly associated with MCW (β= -0.398, p <0.001), whereas age, weight, height, BMI, antero-posterior jaw relationship, nasopharynx-, oropharynx-airway volumes were not.

When the participants were divided into 2 groups based on PSQ total score cut-off value of 8, a significant difference between children with a PSQ < 8 and children with a PSQ ≥ 8 emerged in the MCW measurements (F[1,101]=9.54, p =0.003), even after adjusting for age, weight, height, BMI, mandibular growth direction, relationship between jaws, nasopharynx- and oropharynx-airway volumes. MCW values were significantly lower when PSQ scores were ≥ 8 (2.8 ± 0.4mm) versus PSQ values <8 (3.2 ±0.5mm; p =0.003; **Table 4.9.3)**. However, no significant differences emerged between the 2 PSQ score groups in age, height, weight, BMI, mandible growth direction, antero-posterior jaw relationship, nasopharynx- and oropharynx-airway volumes (**Table 4.9.3)**.

Second Study:

24 children with PSG-diagnosed OSA were identified, and matched with 72 children with no reported symptoms or physical findings suggestive of SDB. The general characteristics for both study groups are reported in **Table 4.9.4**. As MCW values did not

differ between different sexes, (F=0.18, *p*=0.673) they were not considered separately for subsequent analyses.

As reported in **Table 4.9.4**, PSG-diagnosed OSA children had more vertical direction of mandibular growth (28.4 ±8.2 degrees) relative to controls (24.1 ± 5.4 degrees, *p* =0.004). In addition, OSA children had significantly smaller nasopharynx airway volume (3,325 ± 1,233mm^3) in comparison to controls (4,658 ± 1,676 mm^3; *p* =0.004). The oropharynx-airway space was also smaller in OSA children (8,952 ± 2,077mm^3) compared to controls (12,961 ± 6,209mm^3), but did not reach statistical significance (*p*=0.162). Of note, PSQ score of 101 children, from the first study presented above did not reveal significant associations with nasopharynx- and oropharynx- airway volumes as obtained from CBCT imaging (β= -0.135, p=0.177; β= -0.044, p=0.665, respectively). Moreover, the severity of OSA in 24 children, represented by AHI, did not correlate with the nasopharynx- and oropharynx- airway volumes as derived from CBCT scans (β= -0.262, p=0.309; β= -0.068, p=0.796, respectively).

Significant differences between OSA children and controls emerged in the MCW measurements (F[1,108]=11.74, *p* <0.001) after adjusting for age, mandibular growth direction, relationship between jaws, nasopharynx- and oropharynx-airway volumes. MCW values were significantly lower in OSA children (MCW=2.9±0.6mm) compared to control children (MCW=3.5±0.6mm; *p* =0.002; **Table 4.9.4**).

Table 4.9.5. shows the findings for single-predictor and multiple-predictors regression analyses among all the variables that could be associated with MCW in OSA

children. Results from single-predictor analyses showed that MCW was positively associated with age of the OSA children (β=0.74, p <0.01) and negatively with the severity of OSA, represented by the AHI (β= -0.475, p =0.026). Weight, height, BMI, antero-posterior jaw relationship, nasopharynx-, oropharynx-airway volumes were not significantly associated with MCW (p >0.25), thus, were excluded from the final regression model. When multiple-predictors analysis was performed, including age and AHI only, the regression linear model predicted MCW, F= 17.410, p < 0.001, R2= 0.463. Both age (β= -0.674, p <0.001) and AHI (β= -0.290, p =0.049) were significantly associated with MCW.

4.5. Discussion:

The major findings of the present study are that in children with SBD risk, as suggested from the PSQ, and in children with PSG-diagnosed OSA a decreased cortical thickness of the mandible was identified. We also found that the severity of OSA, as indicated by the AHI, was inversely correlated with MCW. Thus, SDB in children may negatively impact on bone health as suggested by the cortical width thickness of the mandible. These findings expand on previous studies in adult cohorts indicating that OSA was associated with bone metabolic diseases such osteopenia and osteoporosis [26, 29-31, 33, 34, 36, 37, 89, 98].

For assessments of bone status in our *cross-sectional* convenience cohorts, we used the cortical width of the mandible, as measured from the panoramic radiographic images reconstructed from the CBCT scanning. We should point out that the most reliable and accepted method for assessment of altered bone mineral density (such as osteoporosis) is derived from Dual-energy X-ray Absorptiometry (DXA), a non-invasive imaging method

that estimates bone density from the radio-opacity of mineralized structures. However, the reduction of bone mineral density can also be detected by other methods, including quantitative tomography, quantitative ultrasound and magnetic resonance imaging [79]. Recently, determination of mandibular cortex thickness indices from dental panoramic radiographs was shown to allow for reliable bone mineral density estimates [77, 80]. As panoramic radiographs are frequently obtained during routine dental check-ups [81]] it has been suggested that such radiographic studies could be used as an initial screening tool for the identification of patients at risk for altered bone density including osteopenia and osteoporosis [77]. In this context, the most commonly used index is the MCW, as the inferior border of the mandible is clearly visible on the dental panoramic radiograph and cortical thickness can be reliably assessed [77]. The usefulness of the MCW was recently confirmed in a systematic review and meta-analysis [77], demonstrating robust sensitivity (0.723, 95% confidence interval [CI] of 0.352-0.926; 0.602) and specificity (0.733, 95%CI of 0.587-0.841) characteristics when detecting reductions in bone density among adults. Moreover, the MCW has been successfully applied in pediatric studies for correlation assessments with different systematic diseases [96, 97, 99]. Although the MCW appears to be both consistent and reliable, some factors should be controlled to minimize its variability, namely appropriate set-up parameters of the imaging technique, high resolution of the images, and proper observer training in identifying the landmarks of the measured distance.

CBCT is a relatively newer diagnostic imaging tool, with a low effective radiation dose, that is being increasingly used by dentists in their clinical practices [82]. CBCT

imaging provides options for 3-D visualization of the craniofacial skeleton, ectopic/impacted teeth, periodontics, bone level evaluation accurate implant placements, 3D evaluation of the temporo-mandibular joints, and upper airway volumetric and contour assessments, from which computational fluid dynamics for airflow estimates can be derived [100, 101]. As the suitability of using CBCT images rather than panoramic radiographs to assess mandibular bone density using the MCW has not been critically ascertained, we validated the approach by comparing images obtained by either way from dry-skull specimens. Measurements from the mandibular cortical width at the mental foramen area had excellent intra-observer reliability and inter-observer agreement performed on panoramic images generated from CBCT scanning. Moreover, MCW readings performed on panoramic images reconstructed from CBCT scanning were similar to those readings performed on standard panoramic images. As a note of caution, to ensure a consistent and reliable MCW, several factors should be considered, namely appropriate set-up parameters of the CBCT imaging machine, high resolution of the images, proper observer training in identifying the landmarks of the measured distance, among others.

In the 2 *cross-sectional* studies reported herein, MCW readings were similar in boys and girls, as previously reported [97], thereby obviating the need for sex-based adjustments in data analyses. In contrast, sex-related differences in bone mineral density among adults are possibly related to discrepant aging trajectories as mediated by hormonal variations (e.g., growth hormone and estrogen), body size, bone size and geometry [102-105].

In both studies, MCW readings increased with age, a finding that was anticipated based on previous reports [97], suggesting a developmental increase in bone mineral density in growing children [106, 107]. Several factors such as growth hormone, steroid hormones, calcium consumption, weight and physical activity exert positive influence on bone mineral density accrual in children [108-111]; however, in the absence of any information on these contributing factors, we cannot comment as to their potential contribution to the differences that were identified in MCW between children with SDB and those without. Indeed, significantly thinner MCW measurements were detected in children with PSQ scores ≥ 8 compared to children with PSQ scores < 8. This cut-off value for the PSQ was used based on previous studies in which the cut-off value was determined as worthy of recommendation for referral to ENT specialists, and indicative of higher pre-PSG risk of OSA [92]. Thus, children at higher risk for SDB appear to have a higher risk for reduced bone growth. The latter findings were further corroborated by the *second* cross-sectional study, as illustrated by the differences in MCW among PSG-diagnosed OSA children and control children. OSA is associated with bone loss (osteopenia and osteoporosis) in adult populations [26, 29-31, 33, 34, 36, 37]. The current study suggests that OSA promotes altered bone biological processes even in children. This might imply that children suffering from OSA have a higher risk for reduced bone formation, and also increased risk for developing future bone disease such as osteoporosis [109, 112]. It might also be that, OSA children suffer from growth retardation or deficiencies in the formation of the craniofacial bones [61], or fail to reach peak skeletal mass [68]. Our study is not suitable to distinguish among these possibilities.

Interestingly, only the overall PSQ score was associated with predictive ability of the MCW, based on our multi-predictors regression analysis. This indicates that SDB risk as assessed by PSQ in children may reflect alterations in sleep that are of relevance to bone morphology, e.g., growth hormone-related pathways, since pulsatile growth hormone release and activity are tightly related to sleep homeostasis and potentially adversely affected by hypoxia [113-117]. Our findings are remarkably similar to recently published studies in adult populations, whereby sleep quality as indicated by the Pittsburgh Sleep Quality Index (PSQI) was associated with a reduced bone stiffness index of the calcaneus bone, assessed by ultrasound bone densitometer [88]. In addition, Kuriyama *et al* also reported that cortical bone thickness of radius, measured by ultrasonic bone densitometer, was thinner in patients who had poor sleep quality (i.e., scored high in PSQI) than those who had better sleep quality [98]. However, results from the published literature on the associations between sleep quantity and bone mass have been somewhat inconsistent. Some studies have shown that low bone mass is associated with short sleep duration [98, 118, 119], long sleep duration [120, 121] or exhibit a U-shape relationship [122]. Since the PSQ does not directly evaluate sleep quality, we cannot specifically infer as to the degree of sleep disruption or overall magnitude of insufficient sleep in our study. However, it is likely that children at high risk for SDB will also suffer from poor sleep quality driven by increased frequency of respiratory-related arousals during sleep [123].

When appraising the association between the severity of OSA (represented by AHI) and bone density, (represented by bone mineral density [g/cm^2] or T-score) in adults, inconsistent findings have been reported. On the one hand, the severity of OSA was

negatively associated with bone density [29-31, 33], whereas on the other hand a positive association between the severity of OSA and bone density was reported [28]. Also, other investigators have reported no significant associations between the severity of OSA and bone density [27, 32]. A recent review by Swanson *et al* described in great detail possible links between sleep breathing disturbances and bone mass [6]. In addition to sleep disruption, intermittent hypoxia events, a major constitutive characteristic of OSA, may also directly impose negative effects on bone cells, and result in loss of bone formation and/or increase in bone resorption, thus accelerating bone mass loss [6]. In addition, OSA could also facilitate bone loss through indirect physiological mechanisms [6], namely elevations in sympathetic nervous system activity [7], disruption in the secretion of the circadian rhythm hormone melatonin [1], and reductions in serum levels of 25-OH vitamin D [8, 9, 124]. However, it might also be hypothesized that alterations to bone biology that may result in altered craniofacial growth (e.g., midfacial hypoplasia) that may result in sleep disordered breathing that could directly affect development of bone mass.

Several limitations of the present study deserve mention. First, our study was retrospective, such that bone density readings from DXA could not be collected. However, the close correlation between MCW and bone mineral density, as measured by DXA from lumbar spine and total body ($r=0.64$, p value <0.05) [97] provides support for the validity of our current results. Although the reliability of MCW readings from the panoramic images generated from the CBCT were assessed; the reliability of the method itself to generate the panoramic images was not evaluated. Additionally, the current study lacked reliability assessment of nasopharynx- and oropharynx-airway space measurements from

the CBCT or those measurements from the cephalometric radiographic images. Moreover, our measurements of the jaw relationship and growth direction were conducted based in lateral view form the cephalometric images created from the CBCT. No measurements were conducted based on coronal and transverse views, as well as a true 3D assessment.

An interesting and as of yet scarcely explored issue is the fact that OSA children exhibited significantly smaller nasopharynx-airway space volumes, as measured from CBCT imaging, compared to controls. Even if regression analysis did not reveal a significant association with the severity of OSA, the potential usefulness of CBCT imaging for SDB evaluation, is currently being explored [125], and as such our findings will need to be prospectively corroborated by other studies. We should also point out that the control children did not undergo PSG evaluation to ascertain the absence of OSA or of any other sleep disturbances; however, we can only infer that confirmatory studies on the absence of OSA in controls would only further enhance the observed differences in MCW reported herein. A third and final limitation in this study is that the duration of OSA is nearly impossible to determine, since symptoms may go unrecognized for several months to years prior to PSG diagnosis. Thus, prospective larger scale studies should be contemplated to corroborate current results.

4.6. Conclusion:

In summary, we present initial evidence suggesting that children at risk for SDB exhibit alterations in mandibular bone measures that purportedly reflect alterations in bone homeostasis.

4.7. Acknowledgment

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4.8. Figures:

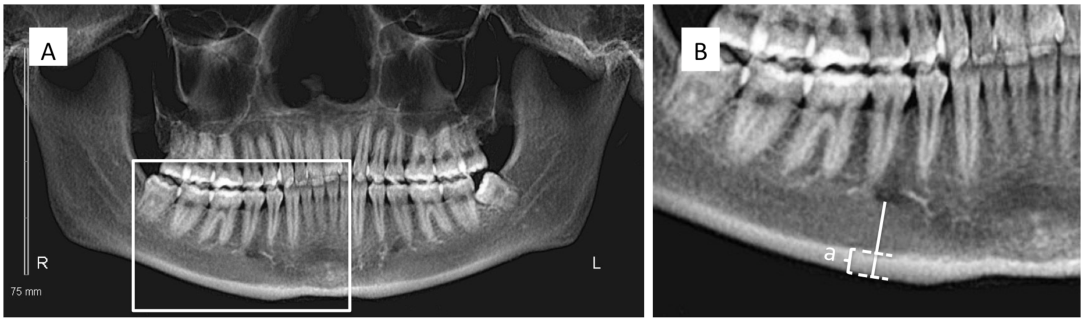

Figure 4.8.1. Cortical bone assessment in children (A**)** a panoramic image and (B) a higher magnification of the region shown in panel (A), illustrating the following measurements: the distance between the lower border of the mandible to the superior margin of the mandibular cortex “a”. The “a” distance, in millimeters, represents the MCW.

4.9. Tables:

Table. 4.9.1. Clinical characteristics of the participants of the first *cross-sectional* cohort

Characteristics	Unit	Mean and SD
Age	Years	10.9 ± 2.6
Girls	n (%)	43 (42.6%)
Height	m	1.47 ± 0.20
Weight	kg	47.4 ± 23.5
BMI	Kg/m^2	20.8 ± 6.1
Mandible growth direction	Degrees	27.7 ± 6.6
Antero-posterior jaw relationship	Degrees	3.3 ± 2.9
Nasopharynx volume	mm^3	2923 ± 1963
Oropharynx volume	mm^3	9037 ± 4437
Total upper airway volume	mm^3	11961 ± 5291
PSQ Score		7.8 ± 4.5

SD represents standard deviation

Abbreviations: MCW: mandibular cortical width; PSQ; pediatric sleep questionnaire; BMI: body mass index

Table 4.9.2. Factors associated with MCW- single-predictor and multiple-predictors analyses

Variable	Single-predictor analysis			Multiple-predictors analysis	
	β	**P value**	**R^2**	**β**	**P value**
Age	0.457	<0.001	0.201	0.056	0.729
Weight	0.379	0.001	0.132	-0.010	0.986
Height	0.524	<0.001	0.265	0.316	0.246
BMI	0.177	0.126	0.018	0.101	0.806
Mandible growth direction	-0.014	0.890*	0.010		
Antero-posterior jaw relationship	-0.171	0.087	0.020	-0.223	0.824
Nasopharynx	0.231	0.020	0.044	0.165	0.103
Oropharynx	0.230	0.021	0.043	-0.012	0.907
PSQ	-0.455	<0.001	0.199	-0.398	<0.001

Adjusted R^2 for the multiple linear model= 0.463, *p*-value <0.001

* Excluded from multiple-predictors analysis as the *p*-value >0.25

Abbreviations: MCW: mandibular cortical width; PSQ; pediatric sleep questionnaire; BMI: body mass index; β: Standardized regression coefficient; R^2: Adjusted R^2 (percent of the variance in the dependent variable explained by the independents)

Table 4.9.3. Clinical characteristics of participants with PSQ < 8 and patients with PSQ ≥ 8.

Characteristics	Unit	PSQ < 8 n= 46 girls/boys 20/46	PSQ ≥ 8 n=55 girls/boys 23/55	P value
Age	years	10.7 ± 3.2	10.2 ± 2.5	0.309
Height	m	1.46 ± 0.19	1.47 ± 0.20	0.891
Weight	kg	43.9 ± 21.5	50.5 ± 25.1	0.236
BMI	Kg/m^2	19.4 ± 5.4	22.1 ± 6.7	0.061
Mandible growth direction	Degrees	27.8 ± 6.2	27.6 ± 6.9	0.905
Antero-posterior jaw relationship	Degrees	3.0 ± 2.9	3.5 ± 2.9	0.389
Nasopharynx volume	mm^3	3045 ± 2394	2823 ± 1540	0.576
Oropharynx volume	mm^3	9353 ± 4141	8779 ± 4688	0.523
MCW	mm	3.2 ± 0.5	2.8 ± 0.4	0.003

Data presented as mean ± standard deviation (SD)

P value was calculated using multivariate analysis of variance (MANOVA) with Turkey's Post hoc pair-wise comparisons.

Abbreviations: MCW: mandibular cortical width; PSQ; pediatric sleep questionnaire; BMI: body mass index

Table 4.9.4. Clinical characteristics of children with OSA and matched controls

Characteristics	Units	Controls n=72 girls/boys 39/ 33	OSA n=24 girls/boys 13/11	P value [a]
Age	years	11.4 ± 2.8	11.4 ± 2.9	0.938
Mandible growth direction	degrees	24.1 ± 5.4	28.4 ±8.2	0.004
Antero-posterior jaw relationship	degrees	2.3 ± 3.2	1.5 ± 3.3	0.189
Nasopharynx airway volume	mm^3	4658 ± 1676	3325 ± 1233	0.004
Oropharynx airway volume	mm^3	12961 ± 6209	8952 ± 2077	0.162
Total sleep time (TST)	min		384.1 ± 116.9	
REM-sleep stage	% of TST		15.5 ± 6.7	
N1-sleep stage	% of TST		3.7 ± 0.5	
N2-sleep stage	% of TST		50.5 ±10.5	
N3-sleep stage	% of TST		30.5 ±12.2	
Arousal index	/hour		12.2 ± 7.9	
AHI	/hour		6.9 ± 12.4	
MCW	mm	3.5 ± 0.6	2.9 ± 0.6	0.002

Data presented as Mean ± Standard deviation (SD)

[a] P value calculated using multivariate analysis of variance (MANOVA) with Turkey's Post hoc pair-wise comparisons.

Abbreviations: TST: Total sleep time; REM-Stage: rapid eye movement-stage; N1-3-Stage: none REM-stages-1-3; AHI: apnea-hypopnea index; MCW: mandibular cortical width

Table 4.9.5. Factors associated with MCW in OSA children- single-predictor and multiple-predictors analyses

Variable	Single-predictor analysis			Multiple-predictors analysis	
	β	**P value**	**R^2**	**β**	**P value**
Age	0.742	<0.001	0.529	0.674	<0.001
Weight	0.215	0.326*	0.046		
Height	0.276	0.364*	0.043		
BMI	0.290	0.626*	0.026		
Mandible growth direction	0.168	0.443*	0.028		
Antero-posterior jaw relationship	-0.217	0.319*	0.047		
Nasopharynx	0.294	0.298*	0.050		
Oropharynx	0.216	0.382*	0.043		
AHI	-0.475	0.026	0.225	-0.290	0.049

Adjusted R^2 for the multiple linear model= 0.647, *p*-value <0.001

* Excluded from multiple-predictors analysis as the *p*-value >0.25

Abbreviations: MCW: mandibular cortical width; PSQ; pediatric sleep questionnaire; BMI: body mass index; β: Standardized regression coefficient; R^2: Adjusted R^2 (percent of the variance in the dependent variable explained by the independents)

CHAPTER 5: Final Discussion and Future Recommendations

The objectives of this thesis were to assess the potential association between SDB and low bone mass in adults and children. From the present set of studies, we concluded that SDB, including sleep apnea, might be associated with bone loss in adult and children populations.

Our systemic review of the literature suggests that the association between SDB (including sleep apnea) and low bone mass (osteopenia and/or osteoporosis, or increased number of non-traumatic bone fracture) in adults is possible. Results from the meta-analysis showed that sleep apnea was a significant risk factor for osteoporosis. Indeed, adult people with sleep apnea have ~2 (in males) to ~2.5 (in females) times the risk of osteoporosis that people who did not have sleep-disordered breathing. However, some limitations of this review should be considered. The quality of the available evidence; consisting only from cross-sectional and retrospective studies with no randomized clinical trials were identified. Moreover, there were differences in the methodological designs, characteristics of the included participants, used diagnostic methods for sleep apnea and bone density, and measured covariates among the selected studies.

The second objective of this thesis was to assess the potential association between SDB and low bone mass in children. From the present set of cross-sectional studies we showed that in children with SDB risk, as suggested from the PSQ, and in children with PSG-diagnosed OSA, a decreased cortical thickness of the mandible was identified. We also found that the severity of OSA, as indicated by the AHI, was inversely correlated with MCW. Thus, SDB in children may negatively impact on bone health as determined by the

cortical width thickness of the mandible. However, some limitations of the present study should be considered. The designs of our studies were retrospective, thus, increasing the risk of recall bias or missing information. Another limitation includes the small sample size and lack of statistical power. Also, bone density readings from DXA could not be collected and the durations of the SDB or sleep apnea were not determined.

For all the above-mentioned limitations, findings form this thesis should be considered cautiously. However, the trends supporting the existence of an association between SDB and low bone mass appear to justify implementation of future studies that rely on large-scale, multi-centers, longitudinal-type prospective cohort clinical trials that possibly address all the above limitations, which might influence the study outcomes. These studies should rely on an adequate sample size of participants based on statistical power calculations and effect size among cohorts analysis, which measures the expected difference between the study groups. Moreover, future studies should adopt safe, reliable and accurate methods for diagnosis of the SDB and reduction in bone density in children and adult populations. For instance, bone density can be reliably measured without exposing the participants to additional radiations using the quantitative ultrasound scanner [126]. This would be very important especially in clinical trials to be conducted on children due to ethical considerations. Other method to estimate bone density in children is the method we applied in our study; the assessment of MCW. However, future studies will have to be conducted in order to determine the validity of using this method in growing children and how it is affected during their pre-adolescence, adolescence and post-adolescence growth periods. If confirmatory, then it would be beneficial to develop a normative MCW reference values across the age, similar to growth curves for children in

height, weight and BMI. These normative data for MCW would be potentially useful to track the child's bone density overtime and determine whether the child is abnormally low or their temporal trajectories in catch up mandibular growth after the treatment. Moreover, future studies should evaluate the levels of specific serum and urine biological markers in order to understand the pathophysiology of both disorders, and how these markers respond to treatment. Also, future studies should apply very specific inclusion and exclusion criteria to minimize any potential effects caused by the differences among the participants in life-style (obesity, smoking and alcohol drinking and exercise), or presence of systematic diseases (such as the cardiovascular diseases, metabolic diseases [i.e., diabetes] and chronic inflammatory diseases [such as periodontal diseases]) on the outcomes. If the findings from these future studies were confirmatory, then implementation of appropriate screening tools for early diagnosis of bone diseases in sleep apnea patients may be warranted such as to decrease the inherent morbidity and mortality of these two co-existing conditions.

Moreover, future research should also be conducted to address all the possible links between SDB and bone biology. It is still not known whether either SDB negatively affects bone health or it's the retardation in growth of the craniofacial bones that facilitates SDB. In one side, SDB is associated with disruption of several physiological events that could possibly negatively bone biology such as: the elevations in the activity of the sympathetic nervous system (SNS) [7], reduction in the secretion of the circadian rhythm hormone melatonin [1], stimulation of the inflammation process [48] and reduction of the serum level of 25-OH vitamin D [8, 9]. Additionally, the genetic predisposition for developing sleep apnea also results in a genetic predisposition for low bone mass. On the other side, growth retardation or deficiencies in the formation of the craniofacial bones [61], or fail to

reach peak skeletal mass [68] may result in smaller nasal, nasopharynx and oropharynx airway chambers. The smaller airway chambers may result in SDB including sleep apnea. This chicken-and-egg dilemma is far from being solved. Therefore, future research will have to be conducted in order to address these hypotheses.

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