Bipolar Disorder, Associated Treatments and Bone Health

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Bipolar Disorder, Associated Treatments and Bone Health

by

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Doctor of Philosophy

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Manuscripts

i. Included in this thesis

1. **Chandrasekaran V**, Brennan-Olsen SL, Stuart AL, Pasco JA, Berk M, Hodge JM and Williams LJ. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review protocol. *BMJ Open.* 2017;7:e013981. doi: 10.1136/bmjopen-2016-013981
2. **Chandrasekaran V**, Brennan-Olsen SL, Stuart AL, Pasco JA, Berk M, Hodge JM and Williams LJ. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review. *J Affect Disord*. 2019 Apr 15;249:262-269. doi: 10.1016/j.jad.2019.02.013. Epub 2019 Feb 6.
3. **Chandrasekaran V**, Pasco JA, Stuart AL, Brennan-Olsen SL, Berk M, Hodge JM, Samarasinghe RM and Williams LJ. Anticonvulsant use and bone health in a population-based study of men and women: cross-sectional data from the Geelong Osteoporosis Study. *BMC Musculoskelet Disord.* 2021 Feb 11;22(1):172. doi: 10.1186/s12891-021-04042-w.
4. **Chandrasekaran V,** Stuart AL, Pasco JA, Brennan-Olsen SL, Berk M, Hodge JM, Samarasinghe RM and Williams LJ. Anticonvulsant use and fracture: a case-control study. *[Accepted at J Musculoskelet Neuronal Interact on 14/02/2021]*
5. **Chandrasekaran V**, Hodge JM, Samarasinghe RM, Pasco JA, Berk M and Williams LJ. Comparison of the effects of anticonvulsants on osteoclast and osteoblast formation and function. *[Submitted to Biological Psychiatry]*

ii. Other publications

1. Williams LJ, Berk M, Hodge JM, Kotowicz MA, Stuart AL, **Chandrasekaran V**, Cleminson JR, Pasco JA. Selective Serotonin Reuptake Inhibitors (SSRIs) and markers of bone turnover in men. *Calcif Tissue Int.* 2018. doi: 10.1007/s00223-018-0398-0
2. Williams LJ, Stuart AL, Berk M, Brennan-Olsen SL, Hodge JM, Cowdery S, **Chandrasekaran V**, Pasco JA. Bone health in bipolar disorder: a study protocol for a case–control study in Australia. *BMJ Open*. 2020;0:e032821. doi:10.1136/bmjopen-2019-032821
3. Aslam H, Marx W, Rocks T, Loughman A, **Chandrasekaran V**, Ruusunen A, Dawson SL, West M, Mullarkey E, Pasco JA, Jacka F. The Effect of dairy and dairy derivatives on gut microbiota: a systematic literature review. *Gut Microbes.* 2020 Nov 9;12(1):1799533

Conference Presentations

i. National

1. **Chandrasekaran V**, Hodge JM, Samarasinghe RM, Stuart AL, Pasco JA, Berk M, Brennan-Olsen SL, Williams LJ. Comparison of anticonvulsants on osteoclast and osteoblast formation and function (SMHR 2019).
2. **Chandrasekaran V**, Brennan-Olsen SL, Stuart AL, et al. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review (ANZBMS 2018).
3. **Chandrasekaran V**, Brennan-Olsen SL, Stuart AL, et al. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review protocol. (SMHR 2017)
4. Williams LJ, Stuart AL, Quirk SE, Pasco JA, Brennan-Olsen SL, Hodge JM, **Chandrasekaran V**, Berk M. Personality disorder and physical health comorbidities: A link with bone health? Submitted to: Conference (SMHR 2016)

ii. International

1. **Chandrasekaran V**, Hodge JM, Samarasinghe RM, Stuart AL, Pasco JA, Berk M, Brennan-Olsen SL, Williams LJ. Comparison of anticonvulsants on osteoclast formation and function. 14th World Congress of Biological Psychiatry (WFSBP), Vancouver, Canada, 2-6 June 2019.
2. **Chandrasekaran V**, Brennan-Olsen SL, Stuart AL, et al. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review. The 21st Annual ISBD Conference, Sydney, Australia, 20-23 March 2019.

3. **Chandrasekaran V**, Stuart AL, Pasco JA, Berk M, Hodge JM, Brennan-Olsen SL, Williams LJ. Quantitative Heel Ultrasound (QUS) and anticonvulsant use in a population-based study. IOF Regional 7th Asia-Pacific Osteoporosis Conference, Sydney, Australia, 30 November-1 December 2018
4. **Chandrasekaran V**, Brennan-Olsen SL, Stuart AL, et al. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review. 2018 ANZBMS Conference, Queenstown, New Zealand, 2-5 September 2018.
5. **Chandrasekaran V**, Brennan-Olsen SL, Stuart AL, et al. Associations between bipolar spectrum disorder and bone health: a systematic review. World Psychiatric Association (WPA) Thematic Congress, Melbourne, Australia, 25-28 February 2018. (e-Poster Discussion Presentation)
6. **Chandrasekaran V**, Brennan-Olsen SL, Stuart AL, et al. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review protocol. Australian Society for Bipolar and Depressive Disorders Ltd (ASBDD), Melbourne, Australia, 15-17 September 2017.
7. Williams LJ, Stuart AL, Quirk SE, Berk M, Brennan-Olsen SL, Hodge JM, **Chandrasekaran V**, Cleminson J, Pasco JA. Personality disorders and bone: Data from the Geelong Osteoporosis Study (GOS). World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (WCO-IOF- ESCEO-2017), Florence, Italy, 23-26 March 2017.
8. Davis J, Stuart AL, Jacka FN, **Chandrasekaran V**, Pasco JA. Feasibility of participants providing stool samples when displaying anxiety or depressive symptoms. International Federation of Psychiatric Epidemiology (IFPE), Melbourne, Australia, 17-20 October 2017.

Abstract

Bipolar disorder is a severe and recurrent mental illness associated with mental and physical health comorbidities such as anxiety and substance use disorders, cardiovascular disease, diabetes, osteoarthritis and metabolic disease. Another potential comorbidity is poor bone health. Given a relationship between unipolar depression and bone health exists, whether or not this extends to bipolar disorder, as well as corresponding mood stabilising treatments such as anticonvulsants, is yet to be thoroughly examined. This mixed-methods, thesis by publication identified evidence at both a clinical and cellular level, regarding the interplay between bipolar disorder, anticonvulsant use and bone health.

This mixed-methods project was divided into three parts incorporating a systematic review (Part A), epidemiological studies (Part B) and bone cell *in vitro* studies (Part C).

Part A: Manuscript I outlines the systematic review methodology utilised and Manuscript II identified, evaluated and discussed the literature investigating the link between bipolar disorder and bone health in adults aged ≥18. An e-search of medical and social science databases identified three cohort studies (n=344,497), which met the predetermined criteria, out of the 1,409 articles screened. Bipolar disorder was associated with a 20-80% higher fracture risk, and increased fracture incidence (21.4 vs 10.8 per 1,000 person years in those with and without bipolar disorder, respectively). Fracture-free survival time in those with bipolar disorder worsened with advancing age and was reduced by 10-30% in women compared to men.

Part B: Manuscript III was a cross-sectional study conducted to investigate the association between anticonvulsant use and bone mineral density (BMD; measured using dual-energy X-ray absorptiometry) and bone quality (measured using quantitative heel ultrasound, QUS), using data from men (n=926; 24-73 years) and women (n=1070; 21-94 years) participating in

the Geelong Osteoporosis Study. Men taking anticonvulsants had lower adjusted mean BMD at the spine and hip; and all QUS parameters (BUA, SOS, SI) were lower compared to non-users. In women, anticonvulsant use was associated with lower adjusted mean BMD at the hip, and the QUS parameter, BUA.

Manuscript IV was a case-control study investigating the relationship between anticonvulsant use and fracture in 1,458 participants (51.8% women) who had sustained a radiologically confirmed fracture compared to 1,796 participants (46.5% women) who had not sustained a fracture. Findings suggested that men and women taking anticonvulsants had an increased fracture risk compared to non-users independent of demographic, lifestyle and medication related factors.

Part C: Manuscript V reported the effects of specific anticonvulsants on osteoclast (OC) and osteoblast (OB) formation and function, using human bone cell models. OC and OB were cultured in the presence or absence of four anticonvulsants [valproic acid (VPA), carbamazepine (CBZ), lamotrigine (LMT) and (GBP)] for 14-21 days. VPA promoted OC fusion at high concentrations (100μM) compared to CBZ and LMT, which inhibited all OC parameters at this concentration, whereas GBP had no impact up to supraphysiological concentrations (1mM). All four drugs dose-dependently inhibited early OB differentiation (ALP), but with order of potency: VPA>CBZ>LMT followed by GBP, which was 100-fold less potent. Only VPA demonstrated capacity to inhibit extracellular matrix mineralisation by OB.

In conclusion, individuals with bipolar disorder have an increased risk of fracture, independent of age, sex, comorbidities and medication use. Furthermore, anticonvulsant use is associated with lower bone quantity and quality, for men and possibly women, and a higher fracture risk, independent of demographic, lifestyle, medical and other medication related factors. In the

laboratory, intra-class differences on bone cell formation and function were evident, with VPA being the most potent, producing larger, more active OC and inhibiting OB formation within the therapeutic range. While further exploration of the mechanisms of action, risk and treatment factors is needed, our findings suggest that monitoring bone health among individuals with bipolar disorder and also users of anticonvulsants is warranted.

List of Figures

Abbreviations

25(OH)D	25-hydroxy vitamin D
5-HTR	Serotonin Receptor
5-HTT	Serotonin Transporter
ABS	Australian Bureau of Statistics
ADOPT	Antiepileptic Drug and Osteoporosis Prevention Trial
ALP	Alkaline Phosphatase
ALZ	Alizarin Red
ANOVA	Analysis of Covariance
ANZBMS	Australian and New Zealand Bone and Mineral Society
ASBDD	Australian Society for Bipolar and Depressive Disorders Ltd
BDRS	Bipolar Depression Rating Scale
Bipolar CHOICE	Clinical Health Outcomes Initiative in Comparative Effectiveness for Bipolar Disorder
BMD	Bone Mineral Density
BMI	Body Mass Index
BMP	Bone Morphogenetic Protein
BRU	Bone Remodelling Unit
BSD	Barwon Statistical Division
BUA	Broadband Ultrasound Attenuation
CA1	Cornu Ammonis 1
CAD	Coronary Artery Disease
cAMP	Adenosine Monophosphate
CBMC	Cord Blood Mononuclear Cells
CBZ	Carbamazepine
cDNA	complementary DNA

CI	Confidence Interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CFU-GM	Colony-Forming Unit-Granulocyte and Monocyte
COL1A1	Collagen type 1 alpha 1 chain
CPC	Cetylperidinium Chloride
CRC	Cooperative Research Centres
CRH	Corticotropin Releasing Hormone
CRP	C-Reactive Protein
CSF1R	Colony-Stimulating Factor 1 Receptor (MCSF Receptor)
CTx	C-terminal telopeptide of type 1 collagen
CTSK	Cathepsin K
D2	Dopamine Receptor 2
DC-STAMP	Dendritic Cell–Specific Transmembrane Protein
DDD	Defined Daily Dosage
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulphoxide
DPX-L	Pencil-beam DXA densitometer
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSM-IV	DSM, Fourth Edition
DSM-5	DSM, Fifth Edition
DXA	Dual-energy X-ray Absorptiometry
ECT	Electroconvulsive Therapy
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Fetal Bovine Serum

FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GBP	Gabapentin
GOS	Geelong Osteoporosis Study
GH	Growth Hormone
GPCRs	G-protein Coupled Receptors
hOSM	human Oncostatin M
HPA axis	Hypothalamic–Pituitary–Adrenal axis
HR	Hazards Ratio
ICD	International Classification of Diseases
IFPE	International Federation of Psychiatric Epidemiology
IGF-1	Insulin-like Growth Factor-1
IL	Interleukin
IL-1β	Interleukin-1 β
IL-1RA	Interleukin-1 Receptor Antagonist
IOF	International Osteoporosis Foundation
IRR	Incidence Rate Ratio
IRSAD	Index of Relative Socioeconomic Advantage and Disadvantage
ISBD	International Society for Bipolar Disorder
LMT	Lamotrigine
LPO	lipid peroxidase
MAO	Monoamine Oxidase
MAOI	Monoamine Oxidase Inhibitors
MBF	Medical Benefits Fund of Australia
M-CSF	Macrophage Colony Stimulating Factor

MDD	Major Depressive Disorder
MDE	Major Depressive Episodes
MEM	Minimum Essential Medium
MeSH®	Medical Subject Headings
mtDNA	Mitochondrial DNA
mRNA	messenger RNA
MSC	Mesenchymal Stem Cells
NHIRD	National Health Insurance Research Database
NHMRC	The National Health and Medical Research Council
NIH	National Institutes of Health
NFATc1	Nuclear Factor of Activated T Cells 1
NF-κB	Nuclear Factor kappa B1
$NO\cdot$	Nitric Oxide
NTD	New Taiwanese Dollar
$O_2\cdot$	Superoxide
OB	Osteoblast
OC	Osteoclast
OC-STAMP	Osteoclast Stimulatory Transmembrane Protein
OD	Optical Density
OPG	Osteoprotegerin
OR	Odds Ratio
OSBARD	Other Specified Bipolar and Related Disorders
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PIP2	Phosphatidylinositol 4,5-bisphosphate
pNP	p-nitrophenyl

pNPP	p-nitrophenylphosphate
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRISMA-P	PRISMA Protocols
PROSPERO	International Prospective Register of Systematic Reviews
PROFRAC	PRedictors and Outcomes of incident FRACtures
PTH	Parathyroid Hormone
py	Person Years
qRT-PCR	Real Time PCR, or quantitative PCR
QUS	Quantitative Heel Ultrasound
QUOROM	Quality of Reporting of Meta-analyses
RANK	Receptor Activator of Nuclear Factor κ B
RANKL	Receptor Activator of Nuclear Factor κ B Ligand
RNA	Ribonucleic acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SCID-I/NP	Structured Clinical Interview for DSM, fourth edition, text revision research version, Non-patient edition
SEIFA	Socio-Economic Index For Areas
SES	Socioeconomic Status
SI	Stiffness Index
sIL-2R	Soluble Interleukin-2 Receptor
SIU	Standard International Units
SMHR	Society for Mental Health Research
SOS	Speed of Sound
SSRI	Selective Serotonin Reuptake Inhibitors
TAFE	Technical and Further Education

TBARS	Thiobarbituric Acid Reactive Substances
TNF-α	Tumour Necrosis Factor-α
TPH1	Tryptophan Hydroxylase 1
TRAP	Tartrate-Resistant Acid Phosphatase
UV	Ultraviolet
VPA	Valproic Acid
WCO-IOF- ESCEO	World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases
WFSBP	World Congress of Biological Psychiatry
WHO	World Health Organisation
WPA	World Psychiatric Association
Wnt	Wingless-related integration site

Introduction

Bipolar disorder is a severe, recurrent mental illness affecting approximately 60 million people worldwide (1), with an estimated annual cost of $7.39 billion in Australia (2). Psychological as well as physical health comorbidities such as cardiovascular disease, diabetes, osteoarthritis and metabolic disease are common in bipolar disorder (3); impacting healthy functioning and often requiring lifelong treatment (3, 4).

One potential comorbidity, previously studied in unipolar depression, is an increased loss of bone. Poor bone health, namely osteoporosis and osteopenia, affects an excess of 7.5 million Australians (5), with the treatment of osteoporosis and associated fractures costing $2.75 billion per year (6). Common risk factors such as lifestyle, certain medication use and biological phenomena such as increases in systemic inflammation possibly link bipolar disorder and osteoporosis, but there is a notably small evidence base studying this association (7).

Current bipolar disorder treatment aims to attain mood stabilisation, which may be achieved through polypharmacy. Complex polypharmacy also varies across different mood states and the symptoms presented by each individual (8). Medications such as antidepressant and antipsychotic medication are sometimes prescribed concurrently and may compound the noxious effects seen on bone health (9, 10). Anticonvulsants, or antiepileptic drugs have been studied extensively in the context of epilepsy, but are steadily gaining utility in the treatment of bipolar disorder due to broad spectrum effects (11), and may also affect the progression of osteoporosis (12-14).

Considering the complexity of bipolar disorder treatment, and the burdens associated with potentially increased fracture risk, further exploration on the exact nature of this association is warranted.

Overview of the thesis

This mixed-methods project seeks to identify evidence at both a clinical and cellular level regarding the interplay between bipolar disorder, anticonvulsants and bone health. A systematic review has been conducted to consolidate and evaluate existing research investigating the association between bipolar disorder and bone health. The epidemiological and laboratory arms of this project went onto investigate the effect of one potential mediating factor, namely, anticonvulsant use on bone health.

Chapter Two describes the nature, prevalence and aetiology of bipolar disorder, associated treatment options and medical comorbidity, and then continues on to describe the biology of bone and nature of osteoporosis. An examination of the available literature investigating mood disorders, psychotropic use and bone is then presented, leading into the specific aims and hypotheses of this thesis.

Chapter Three describes the methodology of this thesis separated into three parts. Part A consists of a published protocol (Manuscript I) describing the methods followed for the systematic review of the literature regarding the association between bipolar disorder and bone health. Part B describes the study designs, participants, data collection, statistical analyses and ethical considerations of the epidemiological studies. Part C describes the methods used in conducting the *in vitro* study to investigate the effects of specific anticonvulsants on the formation and function of OC and OB, and the corresponding analyses.

Chapter Four aims to collate, evaluate, and discuss the literature examining the link between bipolar disorder and bone health (Manuscript II).

Chapter Five explores associations between anticonvulsants and bone health in two parts, utilising data from the GOS in Manuscript III, and PROFRAC study in Manuscript IV. Part C

of Chapter Five also explores the effects of anticonvulsants on human OC and OB formation and function *in vitro* (Manuscript V).

Chapters Six summarises and discusses the key findings of the thesis. Strengths and limitations and future directions are also presented. In a final section, the conclusions drawn from this thesis are presented.

Chapter 1: Literature Review

1.1 Bipolar disorder

Bipolar disorder is a severe mental illness characterised by fluctuating manic or hypomanic and depressive mood episodes (15), with the potential for recurrence, psychosis and suicide (16). Affecting approximately 2.4% of the population (17) and ranked the sixth leading cause of disability in the world amongst individuals aged 15-44 years (18), bipolar disorder is associated with significant emotional, social and financial burdens (19, 20). Although there is some debate as to whether bipolar symptoms appear during childhood, age of symptom onset is generally during adolescence. However, it is not uncommon for bipolar disorder to go undiagnosed until adulthood since the appearance of manic symptoms is an important requirement for the diagnosis of bipolar disorder. Bipolar disorder is often misdiagnosed as unipolar depression up to 47% of the time (21), and the rate of conversion from unipolar depression to bipolar disorder over the individual's lifespan is estimated to be 10-20% (22, 23). Two possible explanations for this discrepancy are the delay in the appearance of manic symptoms and that current methods for diagnosis mainly involve a clinical interview, which, in the absence of biological markers is criticised as lacking objectivity (24).

1.1.1 Definition of bipolar disorder

The most widely acknowledged definition of bipolar disorder is outlined in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) (25). A diagnosis is made based on the nature of the episodes experienced by the individual. Major depressive episodes (MDEs) are more often seen in the course of bipolar disorder than mania (21). The DSM-5 defines an MDE as the presence of five of nine diagnostic symptoms with a minimum duration of 2 weeks, and a change from previous functioning. Symptoms include persistent depressed mood, markedly diminished interest or pleasure towards day-to-day activities, significant, unintentional changes in weight and sleep patterns, psychomotor agitation or retardation and low energy. Other

symptoms include feelings of worthlessness, indecisiveness, inappropriate guilt, a diminished ability to focus, and recurrent thoughts of death and recurrent suicidal ideation. These changes can only be classified as an MDE if they are not linked to grief, a medical condition or substance use (26, 27). A diagnosis of major depressive disorder (MDD) tends to be given (also termed unipolar depression throughout) until the individual experiences a manic episode (25).

Mania is described in the DSM-5 as the presence of at least three of the following diagnostic symptoms to a significant degree, or four, if the mood is only irritable; and a change from previous functioning. Mania constitutes persistently elevated mood and high energy, characterised by impulsivity, euphoria, grandiosity, a flight of ideas, decreased need for sleep, increased verbosity and irritability, as reported or observed. Other symptoms include an increase in goal directed activity or psychomotor agitation. Diagnostic criteria for mania and hypomania have become more specific with the release of the DSM-5, requiring the individual to have increased energy/activity in addition to elated or irritable mood (25). The appearance of hypomania along with an MDE qualifies as a diagnosis of bipolar II disorder (26, 27). Hypomania refers to distinct periods of 4 or more days with mania-like symptoms, as observed or reported (26), but of insufficient severity to meet criteria for full-fledged mania (28, 29).

Cyclothymic disorder is a chronic, fluctuating mood disturbance, consisting mainly of several periods of hypomanic symptoms and numerous periods of depressive symptoms. However, the number, severity, duration or pervasiveness of the symptoms are of insufficient number to be classified as bipolar I disorder or bipolar II disorder (27).

Some substances of abuse and other prescription drugs can give rise to manic behaviour, and this phenomenon can be classified accordingly (30). When symptoms are distinctly bipolar-like and cannot be linked to a medical condition or substance use, but not enough to be classified as bipolar type I, type II, or cyclothymic disorder it is classified as other specified

bipolar and related disorders (OSBARD). Characteristic symptoms of bipolar and related disorders that do not meet full criteria for any of the above disorders are classified as 'unspecified bipolar and related disorder'.

1.1.2 Prevalence of bipolar disorder

Prevalence estimates for bipolar disorder have been reported to be between 0.5 and 5% (31). The World Mental Health Survey Initiative (2011) carried out face-to-face, household surveys, interviewing 61,392 community dwelling adults living in 11 countries across Europe, Asia and the Americas. The lifetime prevalence of bipolar spectrum disorder (incorporating bipolar I, bipolar II and subthreshold bipolar disorder) was reported to be 2.4% (17). The National Comorbidity Survey (2007) found the lifetime prevalence to be 1% for bipolar I disorder, 1.2% for bipolar II disorder and 2-3% for bipolar disorder not otherwise specified (DSM-IV criteria), from surveys administered throughout North America (32). The National Survey of Mental Health and Wellbeing (2007) found that the 12-month prevalence of bipolar disorder in Australia was 1.8% (95% CI 1.2-2.4) in men, 1.7% (95% CI 1.3-2.2) in women and 1.8% (95% CI 1.4-2.2) overall (33).

1.1.3 Aetiology

1.1.3.1 Genetic basis and heritability

Bipolar disorder is thought to result from the interplay of multiple genes versus the effects of one dysfunctional gene (34, 35). Genome wide association studies have implicated 88 different alleles (>40 genes) that could be responsible for the pathophysiology of bipolar disorder, although many individual studies were not replicated (35, 36). Twin and familial studies have established that bipolar disorder is highly heritable, with genetic influences explaining 60-85% of risk (37). Yet, incomplete concordance between monozygotic twins suggests that

environmental and epigenetic factors may also have a role to play in the aetiology of bipolar disorder (34).

1.1.3.2 Inflammation

Inflammation is an acute, localised, protective pathophysiological response to tissue damage or injury, and is one of the most commonly discussed theories to explain bipolar disorder (15, 38-41). Studies suggest that there is the involvement of chronic, mild inflammation in the brain and the periphery in the progression of bipolar disorder. Elevated cytokine levels, or neuroinflammation, has been associated with both mania and depression (42), although different subsets of cytokines have been shown to be activated at different phases (43-47).

Biomarkers of inflammation identified to have significance in bipolar disorder are interleukin-6 (IL-6), interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), soluble interleukin-2 receptor (sIL-2R), and interleukin-1 receptor antagonist (IL-1RA) (48, 49). Goldsmith et al (2016) conducted a meta-analysis of cytokine network alterations in psychiatric patients, and found that IL-6, TNF-α, sIL-2R and IL-1RA were elevated in acute mania; and IL-6, IL-1β and sIL-2R were significantly elevated in euthymic (but not depressed) patients (49). However, inflammation appears to persist in periods of euthymia as well, possibly leading to neuroprogression and the cognitive decline associated with chronic bipolar disorder (43-47).

Chronic neuroinflammation results in increased production of free radicals, decreased mitochondrial function, lipid peroxidation and excitotoxicity. All these stressors may lead to increased glutamatergic transmission and a resulting influx of intracellular calcium, which may then result in a potential neurodegenerative effect due to neurotoxicity (15). This effect could lead to progressive atrophy of the brain mass, and the subsequent ventricular enlargement, explained later in this section (35).

1.1.3.3 Oxidative stress

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to be intrinsically beneficial, due to their vital role in cell signalling and homeostatic processes; and they are products of a healthy metabolism. Often arising from the electron transport chain, which occurs in the inner mitochondrial membrane, energy generation sometimes results in the production of ROS (O_2· or NO·) (50). While being beneficial in small concentrations, the overproduction of ROS/RNS have a systemic degenerative effect, affecting multiple organs. Often implicated in ageing, oxidative stress is thought to begin due to the leakage of ROS, and the subsequent mitochondrial dysfunction, resulting in decreased energy generation (15) and cytotoxic damage (50). Oxidative stress is also closely linked with neuropsychiatric symptoms (15, 50, 51), owing to the high metabolic status of the brain, and its acute sensitivity to redox status (52).

Oxidative stress biomarkers linked with bipolar disorder are thiobarbituric acid reactive substances (TBARS), nitric oxide (NO·) and lipid peroxidase (LPO) (48). Meta-analyses have found that individuals with a bipolar diagnosis have significantly elevated levels of TBARS, NO· and LPO compared to healthy controls (52, 53); in particular, TBARS is elevated in mania (48).

1.1.3.4 Mitochondrial dysfunction

Approximately 16-21% of those with mitochondrial diseases have also been shown to have a bipolar diagnosis (54-56), which is 20 times higher than the general population (57). Mutations in mitochondrial DNA (mtDNA) have been implicated in bipolar disorder due to altered intracellular calcium levels. The basis of this research was a result of another study, conducted in 1992, which suggested that the accumulation of partially deleted mtDNA in the brain, rather than in the muscles causes recurrent MDEs (58). This line of research spawned several other

studies examining the selective expression of mitochondria-related genes in brains, post-mortem. Collectively, these studies suggest that mitochondrial dysfunction may contribute to the neuroprogression of bipolar disorder (59-61).

1.1.3.5 Neural circuitry

Histopathological studies have shown that there are subtle, yet observable changes in the brain of individuals with bipolar disorder, such as a general decrease in neuronal density and size in the anterior cingulate cortex (62), decreased glial density in prefrontal cortical areas (63), and smaller pyramidal cells in the CA1 region of the hippocampus (64). Abnormal myelination is also observed in the prefrontal deep white matter of those with bipolar disorder (65). Interestingly, a more pronounced decrease in the glia/neuron ratio and the glial density in the amygdala was observed amongst the patients who were not treated with lithium or valproate (66). This neuroprotective effect was also observed in neuroimaging studies, where patients treated with lithium had larger amygdalae and hippocampi compared to individuals not treated with lithium and healthy volunteers (67). Furthermore, increased severity, decreased responsiveness to therapy, cognitive impairment and a progressive loss of grey matter; and thereby ventricular enlargement, has been associated with the chronicity of bipolar disorder (15).

1.1.4 Treatment

While a single treatment that can treat all phases and presentations of bipolar disorder would be ideal, and many patients do respond to lithium in this way, combination therapy is often necessary in order to achieve remission (68). In a large prospective cohort study (n=2712), Golden et al (2017) found that bipolar I-depressed patients were most likely to receive the most complex treatment regiments, compared to bipolar-I manic, bipolar-I mixed or bipolar-II patients (8). Prophylaxis, relapse prevention, side-effects and tolerability all become important

considerations in order to improve adherence to treatment (68, 69). Based on the symptoms presented, a gamut of antipsychotics, mood stabilisers, anticonvulsants, antidepressants, and anxiolytics are usually used to treat the individual (22, 70).

1.1.4.1 Lithium

One of the earliest breakthroughs in bipolar disorder treatment was the discovery of the therapeutic potential of lithium, mainly in being a non-sedating replacement to existing therapeutics (70, 71). One implicated mechanism of action is that lithium displaces magnesium, an important cofactor involved in promoting signal transduction in a number of signalling pathways (71). Although lithium has remained the treatment of choice since its discovery, due to its sustained efficacy, particularly in the treatment of depressed and mixed states in bipolar I disorder (8, 72), its protective effects (73-75) and relatively benign side effect profile, other therapies are necessary in order to treat the 20-40% of individuals who are lithium-resistant (70, 76-78).

1.1.4.2 Antidepressants

Since mania only constitutes approximately 12% of the life of an individual with bipolar disorder, there is a higher propensity for clinicians to focus on antidepressant treatments (22). Drugs such as Isoniazid and Iproniazid were serendipitously discovered to have psychostimulatory effects on the brain through the inhibition of the enzyme monoamine oxidase (MAO) (79, 80). However, as with many early psychotropic agents, monoamine oxidase inhibitors (MAOIs) had serious side effect profiles, such as hypertension and intracranial haemorrhages, hepatotoxicity, neurotoxicity and some cholinergic effects (79). Although MAOIs are still used in treatment, they are only used in cases where the patient has shown an intolerance to other drugs or when electroconvulsive therapy (ECT) is contraindicated (79).

Tricyclic agents were similarly introduced, purported to target the same pathway while being relatively less toxic and more efficacious when compared to MAOIs (81). While highly efficacious in treating depressive symptoms (82), tricyclic agents are also known to have several harmful off-target effects, such as adrenergic, histaminergic and cholinergic effects (83) and in the context of bipolar disorder can drive switching into mania, rapid cycling and mixed states.

Selective Serotonin Reuptake Inhibitors (SSRIs) have gained popularity in clinical practice as a first-choice antidepressant, due to their efficacy and targeting the relatively benign serotonergic pathway (80, 82). However, several studies have shown that treatment with antidepressants, in the absence of an anti-manic agent can evoke a switch to (hypo)mania, and induce rapid cycling (22, 35, 70) and have uncertain benefits. Antipsychotics and mood stabilisers, such as anticonvulsants are sometimes prescribed in combination with antidepressants in bipolar disorder treatment (84).

1.1.4.3 Antipsychotics

Antipsychotics target the dopamine and serotonin neurotransmitter systems (85). First generation antipsychotics, called 'typical' antipsychotics act on the dopaminergic system, but the high risk of extrapyramidal symptoms (86) and potential neurotoxicity from combined treatments (85) necessitated the development of modern, 'atypical' antipsychotics. Atypical antipsychotics act on both the serotonergic signalling system and the dopaminergic system (87).

Most studies agree that monotherapy is not consistently successful (11, 77, 88, 89). Cipriani et al. (2011) suggests that treatment with antipsychotics is significantly more effective for the treatment of mania compared to other pharmacological treatments tested, such as mood

stabilisers, while Glue et al. (2015) suggests a combination of antipsychotics and mood stabilisers is most effective (88, 90).

1.1.4.4 Anticonvulsants

Anticonvulsants were fortuitously discovered to have utility in the treatment of non-epileptic diseases (91, 92), particularly manic states; which was supported by early observations that ECT was highly efficacious (80% antimanic response) in the treatment of mania, potentially due to its anticonvulsive effect (11, 78, 93).

Potassium bromide was the first known anticonvulsive agent, which was followed by phenobarbital and phenytoin, between 1857 and 1938 (94). The discovery of first-generation anticonvulsants, such as carbamazepine (CBZ) (95, 96), valpromide and valproic acid (VPA) (91, 92, 97), followed over the next few decades; which were shown to treat disorders such as epilepsy by reversing their neuroexcitatory effects.

Post et al. (1986) hypothesised that the conceptualisation of mood disorder as convulsive equivalents may explain the efficacy of some anticonvulsants in that, they decrease the kindling effects that trigger subsequent episodes (97-100). Since anticonvulsants including CBZ and VPA have been included in the gamut of bipolar disorder treatments, their antimanic properties became apparent following preliminary evidence showing them to better mood stabilisers compared to existing agents (78). The need for improved drugs in terms of both, safety and efficacy then made way for newer compounds, such as lamotrigine (LMT) and gabapentin (GBP).

A number of studies suggest mechanisms vary for the different anticonvulsants, although the mechanism of action in bipolar disorder treatment is yet to be elucidated (101-103). Current overarching theories support that anticonvulsants have agonistic effects on the glutamatergic

and GABAergic neuronal pathways (104), and/or calcium, sodium and potassium voltage-gated channels, thereby reversing their neuroexcitatory effects (78). An earlier review mentioned voltage-clamp experiments carried out in mammalian and amphibian systems, where phenytoin and CBZ were found to have a dampening effect on high frequency repetitive firing of action potential (105). This effect maintained signal duration and amplitude by enhancing sodium channel inactivation (105). Although fewer voltage-clamp experiments have been conducted to study the mechanism of action of VPA, it is thought to have a similar effect, indicating that this could be an important target for bipolar disorder treatment (101).

This differential interaction of anticonvulsants with intracellular signalling pathways, and the resulting effects may interfere with other common pathways, leading to comorbid conditions (101). For example, a disturbed calcium homeostasis can affect a number of systems such as the central nervous system and the musculoskeletal systems, among others (106). Furthermore, newer agents, classified as second-generation anticonvulsants were introduced due to their improved specificity (107, 108).

Prescription patterns of anticonvulsants in the treatment of bipolar disorder vary, with one recent study (n=992) reporting that 66.5% of their participants with bipolar disorder took anticonvulsants (109), and an earlier study (n=450) reporting 86%, very often in combination with other medications (110, 111). While several anticonvulsants have been considered for bipolar disorder treatment (101, 107, 112), treatments such as CBZ (113), LMT and VPA (114, 115) have been reported to have the best mood stabilising effects. In particular, LMT is thought to have the best antidepressant efficacy data.

Wang et al (2003) suggested that VPA is most effective in patients unexposed to antidepressants or stimulants. However, divalproex is better tolerated than valproate (108). They also suggested that CBZ is effective in treating non-classical bipolar disorder, and is more

tolerated in manic patients compared to depressed or euthymic individuals. Due to complexity of use such as drug-drug interactions, it is preferred as an alternative than first-line intervention (108). Numerous studies suggest that newer agents, GBP and LMT are comparatively benign, although limited in efficacy and have utility in maintenance therapy or in bipolar II disorder treatment (13, 108, 116, 117).

1.1.5 Medical comorbidity

A World Health Organisation (WHO) study on the global burden of neuropsychiatric illnesses established that bipolar disorder is one of the foremost global health burdens, responsible for more disability-adjusted life years compared to other major neurological conditions and all forms of cancer due to its recurrent, lifelong nature (17, 118, 119). Although this mortality may also be attributable to prescribed psychotropic medications, substance use, unemployment, suicide, and an unhealthy lifestyle (120), it is estimated that the effects of medical comorbidities lead to ~60% increased likelihood of premature death (121).

The Clinical Health Outcomes Initiative in Comparative Effectiveness for Bipolar Disorder (Bipolar CHOICE) study reported that 96.3% of participants had at least one other comorbid medical illness (122). Bipolar disorder has been associated with a number of comorbid medical conditions, such as gastric ulcers, cardiovascular disease, chronic kidney disease and rheumatoid arthritis (123, 124), with a more severe illness course associated with a higher medical illness burden (3). Other comorbid physiological conditions previously associated include the metabolic syndrome, migraine, diabetes, obesity and hepatitis C virus infections (125). These factors contribute to an increase in the medical illness burden and economic burden of the disease. More recently, another possible comorbidity that has been investigated is poor bone health.

1.2 Bone health

Bone is a multicellular organ (Figure 1a) involved in a constant, dynamic process of renewal, called bone remodelling, mediated by cells specialised in bone formation and resorption. The bone marrow microenvironment is actively involved in the renewal of cells in the immune and circulatory system (126).

1.2.1 Bone cells

1.2.1.1 Osteoclasts (OC)

OC function to resorb bone and are terminally differentiated, multinucleate cells that arise from the mononuclear fraction of the haematopoietic stem cell lineage (127). OC precursors migrate from the bone marrow to sites of bone resorption, where they further differentiate and fuse, to form large, actively resorbing cells that remove the mineralised bone matrix. Excess calcium is deposited and stored on the bone, therefore, resorption also liberates minerals such as calcium, into the blood stream (128, 129).

Cytokines essential for osteoclastogenesis to occur include macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANKL), both of which are secreted by OB, osteocytes and stromal cells (128). Osteoprotegerin (OPG) is a soluble decoy receptor that binds RANKL and the balance between RANKL and OPG regulates OC activity.

1.2.1.2 Osteoblasts (OB)

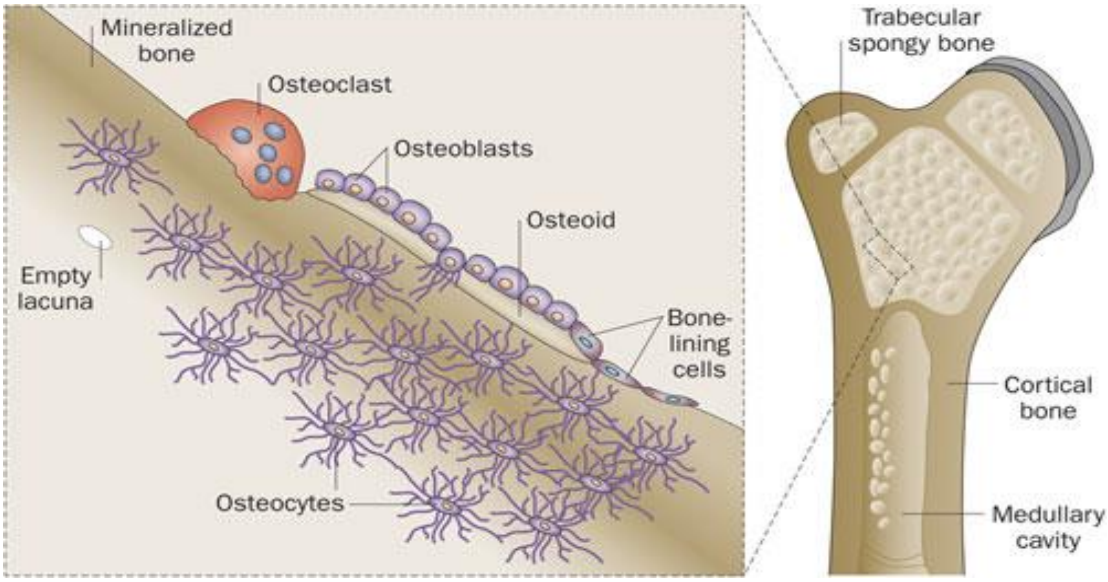
OB make up under 4-6% of the total number of bone surface cells in adult bone and are responsible for bone formation. They are cuboidal, alkaline phosphatase (ALP) positive cells that serve a crucial role in bone matrix mineralisation (128, 130). OB are derived from mesenchymal stem cells (MSC), and their differentiation is controlled by bone morphogenetic

protein (BMP), small doses of parathyroid hormone (PTH) and involves Wingless-related integration site (Wnt) intracellular signalling pathway, which is essential to bone development and maintenance (128, 131).

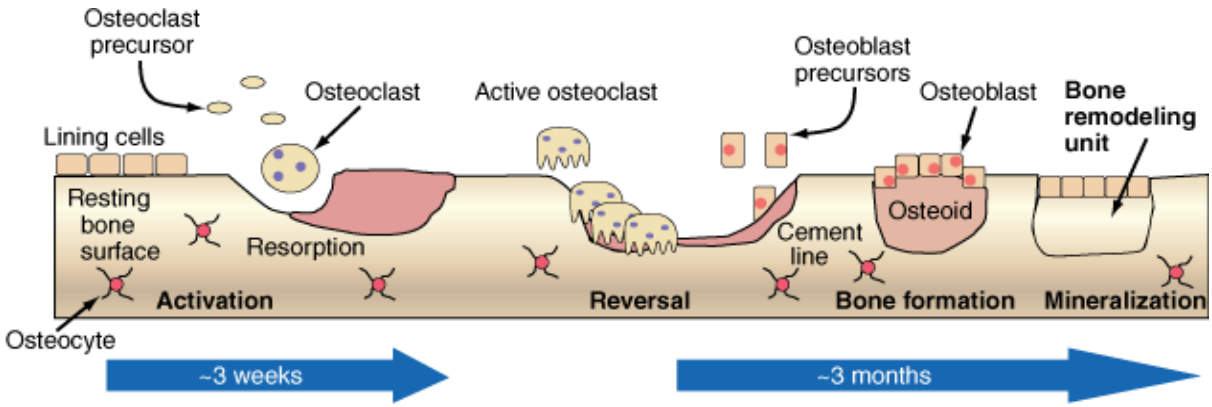
Bone matrix synthesis by OB occurs in two stages – the deposition of the organic matrix, followed by its mineralisation (128). Once mineralisation is complete, OB can either be buried within the matrix as osteocytes, transformed into inactive surface bone lining cells, or undergo apoptosis (128, 132).

1.2.2 Bone remodelling

Bone remodelling refers to the process whereby bone strength and integrity are maintained by constant renewal and consists of four main phases – resorption, reversal, formation and resting (Figure 1b) (129). OC and OB work together to repair injury in response to chemical and mechanical stressors that cause the erosion of bone. The RANKL/RANK/OPG pathway plays a vital role in the formation and survival of OC and OB (133). RANKL plays a key role in the activation, function and survival of OC. RANKL is expressed by OB, and binds to its receptor, RANK, which is expressed on the membrane of OC precursors. RANK signalling promotes cell fusion, culminating in giant, multinucleated OC (Figure 1a,b). OC, OB and lining cells form a temporary anatomical structure at a resorption site called a bone remodelling unit (BRU) (Figure 1b) (134). Individual OC resorb bone by creating a contained, acidic microenvironment, called a ‘sealed zone’ (128).



(a)



(b)

Figure 1 (a) Bone cells involved in remodelling. (b) A schematic view of bone remodelling.

Binding of the RANK ligand to its receptor RANK on OC precursors promotes activation, upon which OC precursors fuse to form giant, multinucleate, bone-resorbing OC that attach to the site of bone damage and secrete enzymes that dissolve bone. This activity leaves behind 'resorption pits'. Macrophage cells clear the cell debris, and osteocytes signal lining cells to turn into active OB and move towards a resorption pit. Activated OB form the osteoid, which mineralises over the next few months, due to calcium deposition. Mature OB then undergo apoptosis or get buried under bone matrix to form osteocytes (135, 136).

The ruffled border is a unique adaptation of the OC, which transports proteolytic enzymes and protons into the resorption compartment in order to dissolve hydroxyapatite that results from the proteolytic cleavage of bone matrix (137). Once resorption is complete, osteal macrophages and lining cells clear all the cell debris (138), OC undergo apoptosis and resorption stops. Simultaneously, OC stimulate the differentiation of the OB precursors, in order to activate bone formation (139). This phase is called 'reversal' or 'coupling' because it connects the resorption phase to the formation phase (131). The next phase involves the deposition and mineralisation of the new bone matrix, called osteoid, which consists of 88% type I collagen, 10% non-collagenous proteins and 1-2% of lipids and glycosaminoglycans (see Figure 1b). Remodelling terminates at the site once the resorbed bone is completely replaced (129).

1.2.3 Bone loss

Bone remodelling is tightly regulated by ALP, an enzyme which assists in bone mineralisation to determine the BMD of an individual (140). Measuring BMD enables the detection of poor bone health. If a mutation occurs in any of the key genes involved in the regulation of this process, diseases/conditions can result. When bone resorption is defective, conditions resulting from elevated BMD occur, such as osteopetrosis or osteosclerosis (129). Alternatively, age-related oestrogen withdrawal in postmenopausal women, and changes in ROS, PTH and IGF-1 levels in men lead to a decrease in BMD (129, 141).

According to the WHO, osteopenia is defined by bone densitometry as a T score -1 to -2.5, a T score of -2.5 and below the young adult mean BMD is indicative of osteoporosis, with severe osteoporosis also having to include a history of fracture (142).

Osteoporosis is a multifactorial and progressive condition; age-related factors, genetic predisposition, muscular degeneration, hormonal and inflammatory conditions are known to accelerate osteodegeneration (129). Further, modifiable factors such as poor nutrition, low

body weight, substance use, smoking and lack of exercise, biological factors such as excess production of bone resorption factors and certain medical conditions and medications, such as asthma or diabetes are known to contribute to accelerated bone loss (6, 141, 143, 144).

1.3 Mood disorders and bone

1.3.1 Unipolar depression and bone

A growing body of research suggests a possible association between unipolar depression and osteoporosis (145-149). Cizza et al (2010) performed a meta-analysis, and found that 76% of eligible articles supported an inverse association between depression and bone health, concluding that individuals with MDD had lower BMD of the femoral neck, total femur and spine (148). Schweiger et al (2016) had similar findings, suggesting that further exploration into potential mediating factors is warranted (149). In a more recent meta-analysis, Qiu et al (2018) added that a diagnosis of MDD is associated with a 1.24 times higher risk of fracture (147).

Aloumanis et al (2013) suggested that a diagnosis of depression may lead to poor bone health due to factors such as dietary and metabolic imbalances, physical inactivity, alcohol use, cigarette smoking, and substance use (146). However, the association between depression and osteoporosis is thought to be bidirectional. For instance, a diagnosis of osteoporosis may also be associated with factors that may lead to depression, such as impaired physical ability, chronic pain, isolation, decreased self-esteem and quality of life (146).

It seems bone mass remains negatively correlated with depressive symptoms, even when known risk factors have been considered (122, 150), however, it is important to observe pathophysiological factors involved, to better understand this association. Numerous endocrine factors have been proposed to explain the relationship between depression and osteoporosis

(146). Of these, most commonly implicated is the hypothalamic-pituitary-adrenal axis (HPA axis), leading to varying degrees of cortisol suppression (151, 152), oestrogen withdrawal (149) and hyperprolactinemia (153, 154). Inflammatory factors are commonly implicated in both depression and osteoporosis, another potential explanatory mechanism linking depression and bone loss. Furthermore, hyperprolactinemia may also be a potential mediator of the link between depression and poor bone health (155-157).

In a meta-analysis of cohort studies, Wu et al (2010) found fracture risk was greater in individuals with depression, which may be mediated by antidepressant use (145). Whether psychotropic medications affect bone loss independent of the effects of the illness has been debated (106), with one meta-analysis (158) and two smaller studies (159, 160) suggesting that antidepressant use was not associated with decreased bone mass, however, evidence supporting a relationship between antidepressant use and increased bone loss is far more abundant (148).

1.3.2 Bipolar disorder and bone health

In contrast to the growing body of research investigating the association between unipolar depression and bone, there are relatively far fewer studies investigating the association between bipolar disorder, per se and bone health.

Mezuk et al. (2010) conducted a large prospective cohort study (n=67,387, <5% female, aged ≥50), whereby they identified that a bipolar disorder diagnosis was linked to a 20% increased fracture risk, independent of the use of anticonvulsants (12). Similarly, Hsu et al. (2016) conducted a nationwide cohort study in Taiwan (n=236,355, >60% female, aged ≥16), and found that bipolar disorder was a risk factor for fractures, irrespective of comorbidities (161). Su et al. (2017) conducted a more recent study (n=40,755, >60% female, aged≥16) utilising the same administrative database, and had similar findings. They found that bipolar disorder was associated with a 5.9% higher risk of fracture when compared to matched controls.

However, they also found that this effect was not compounded by the use of antipsychotics or mood stabilizers (93).

Cui et al (2019) recently conducted Mendelian randomization analysis to explore causal interfaces between bipolar disorder and BMD, and had findings that were at odds with existing works suggesting an association between bipolar disorder and fracture. Their findings did not support a causal relationship between bipolar disorder and BMD status (162). Given no further work is available, exploration is warranted.

1.4 Psychotropic use and bone

The three classes of medications commonly prescribed to treat bipolar disorder, antidepressants, antipsychotics and anticonvulsants have been shown to be individually noxious to bone (155).

1.4.1 Antidepressants and bone

A number of epidemiological studies to date have now shown antidepressants to have a deleterious effect on bone, with an increased risk of both osteoporosis and fracture (145, 146, 163-165). A large Danish case-control study (n=498,617) found that antidepressants dose-dependently increased fracture risk, and that SSRIs may be associated with a higher fracture risk than tricyclic agents (9, 166, 167). Interestingly, this pattern was found to diminish upon discontinuation (9).

Considering recent findings that peripheral serotonergic receptors exist on bone cells and the common use of SSRIs in practice, which target the serotonergic system; the focus of most studies remains on the serotonergic system (9). Serotonergic receptors are now known to also be found in the gastrointestinal and cardiovascular systems (9). OC, OB and osteocytes also express serotonin receptors, with serotonin also shown to affect bone metabolism (168). Since

serotonin does not cross the blood-brain barrier, central (5%) and peripheral serotonin (95%) are functionally distinct (9) and interestingly appear to have opposite effects on bone mass (169). Hodge et al. (2013) analysed bone cells for the expression of serotonergic receptor expression, and studied the effects of 5 SSRIs on human OC and OB *in vitro* (170). They found that the serotonin receptor 2A (5-HTR2A) was expressed only in OB, 5-HTR2B expression increased from precursor to mature OC; and tryptophan hydroxylase 1 (TPH1), transporter (5-HTT), and 5-HTR1B were expressed by both. They also found that four of the five SSRIs tested inhibited OC formation and resorption dose dependently and inhibited ALP and bone mineralisation by the OB, but only at the highest dose (30μM). Citalopram was the least potent (170).

A number of *in vivo* animal studies have also been conducted adding to the evidence base. Battaglino et al. (2006) found fluoxetine treatment leads to a nett gain in trabecular bone mass unless in an oestrogen deficient state, in which case, it does not prevent bone loss (171). Some studies in rodents also suggest potential contenders for fracture healing through increases in endosteal and trabecular bone, such as long-term serotonin administration (172), and phosphodiesterase inhibitors (173). Gold et al. (2015) found that chronic administration antidepressants in male Sprague Dawley rats resulted in impaired bone strength (122).

1.4.2 Antipsychotics and bone

Antipsychotics are a mainstay in neuropsychiatric treatment and act by post-synaptic blockade of primarily dopaminergic, and serotonergic receptors in the brain (174). First generation agents, haloperidol, chlorpromazine and trifluoperazine, were the most commonly issued typical antipsychotics and of the newer, second generation or atypical agents, olanzapine, risperidone, aripiprazole and quetiapine were the most commonly prescribed (175, 176). Antipsychotic use has generally been associated with an increased risk of fracture (177-182);

with a recent meta-analysis reporting a 1.5-fold increase in fracture risk attributable to antipsychotic use (10). Wang et al (2014) treated patients (n=120) with a new schizophrenia diagnosis, and found BMD to be significantly lower in patients treated with clozapine, quetiapine or aripiprazole compared to healthy controls, with this occurring within 12 months of commencing treatment (183). A large Swedish study identified adults with two consecutive prescriptions of risperidone (n=38,211), other atypical antipsychotics (n=60,691), or typical antipsychotics (n=17,445) within three months, aiming to estimate the likelihood of developing osteoporosis-related fractures. Their findings showed that first generation antipsychotics pose a higher risk of fracture in comparison to second generation antipsychotics (184).

Dopamine receptor 2 (D2) receptor binding was thought to elevate prolactin levels (185), initially associated with an increased risk of osteoporosis (178, 186, 187). Although there is some evidence suggesting that prolactin has a direct effect on human OB (188), this antipsychotic-induced bone loss attributed to hyperprolactinemia was later believed to be due to a potential effect of hypogonadism-mediated sex hormone withdrawal (156).

Studies relating to the molecular mechanisms that potentially underlie the association between antipsychotics and bone loss are also limited. Costa et al. (2011) performed rodent studies, studying the effects of clozapine and haloperidol in rats. They found that *in vivo*, clozapine caused a 15% decrease in BMD within 42 days of commencing treatment. Clozapine decreased OB differentiation, OB mitogenesis and OC differentiation dose-dependently i*n vitro*, whereas haloperidol did not seem to affect rats *in vivo*, or rat cells *in vitro* (189). Motyl et al. (2012) studied the effects of risperidone in mice and reported that it significantly increased the number of OC in culture, but OB differentiation remained unchanged (190). In a mammalian study conducted on 9-24-month-old pigtail macaques, quetiapine and risperidone had very little effect on body weight or bone growth rates (191).

1.4.3 Anticonvulsants and bone

Anticonvulsant use, and effects on bone health have been of interest due to their widespread utility in treating chronic illnesses such as epilepsy. In a sample of 71 patients taking anticonvulsants for over 6 months, at least 50% developed clinical or subclinical bone disorders (192, 193). Similarly, in a small study of nineteen pre-menopausal women on long term combination treatment with VPA and atypical antipsychotics revealed that 47% of women had osteopenia or osteoporosis linked with long term psychotropic use (194).

Anticonvulsant use has also been implicated in several off-target effects such as hepatotoxicity, cognitive decline, metabolic disturbances, movement and behavioural disorders and likely bone abnormalities (195). A review conducted by Lee et al (2010) on epidemiological studies investigating the effects of anticonvulsant use on BMD and fracture found that anticonvulsant use increases fracture risk by 1.2-2.4 times, and that Cytochrome P450-inducing anticonvulsants upregulate enzymes involved in vitamin D metabolism, leading to a more pronounced deleterious effect (14). These enzymes convert 25-hydroxy vitamin D (25(OH) vitamin D) into inactive metabolites that lead to calcium resorption with consecutive secondary hyperparathyroidism (195). This unavailability of vitamin D is thought to compound bone loss.

Existing pre-clinical studies explaining the association between anticonvulsants and bone loss are limited. VPA is the best studied anticonvulsant, due to its wide usage and it has been associated with decreased BMD of the lumbar spine and femoral neck and increased serum ALP and PTH levels (196, 197). Humphrey et al. (2013) found that treatment with VPA significantly decreased the concentration of two important bone proteins, osteonectin and type 1 collagen, while leaving over a thousand other proteins unaffected, in an OB-like cell line (102, 198). In an earlier study, Feldkamp et al. (2000) recognised that phenytoin and CBZ had direct effects on OB-like cells, likely leading to the observed decreases in BMD (199). Further,

anticonvulsants are thought to have differential effects due to the drugs having different molecular targets (200).

Other implicated mechanisms that have been proposed to contribute to this association are interference with vitamin K metabolism, inhibition of calcitonin secretion, PTH resistance, and impaired calcium absorption (201, 202). While anticonvulsant use and bone health has been predominantly investigated in paediatric or geriatric populations with epilepsy, anticonvulsant users tend to be of all ages and have a vast array of clinical indications, including off-label use (203, 204).

1.5 Aims and hypotheses

Given that a relationship exists between unipolar depression, antidepressants and poor bone health, it is therefore possible that a similar relationship exists between bipolar disorder, mood stabilising treatments, such as anticonvulsants and bone. Such associations are yet to be thoroughly examined. This mixed-methods project will seek to identify evidence at both a clinical and cellular level, regarding the interplay between bipolar disorder, anticonvulsant use and bone health.

Specifically, the aims of this thesis by publication are to:

- investigate the association between bipolar disorder, anticonvulsant use and bone health in human studies (Manuscripts II, III and IV)
- determine the cellular and molecular effects of specific anticonvulsants on an *in vitro* human bone cell model (Manuscript V)

It is hypothesised that:

- both bipolar disorder and anticonvulsants are associated with increased bone fragility

- anticonvulsants regulate bone cell formation and function.

Chapter 2: Methods

Part A: Systematic review

2.1.1 Manuscript I

Chandrasekaran V, Brennan-Olsen SL, Stuart AL, Pasco JA, Berk M, Hodge JM and Williams LJ. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review protocol. *BMJ Open.* 2017;7:e013981. doi: 10.1136/bmjopen-2016-013981

AUTHORSHIP STATEMENT

1. Details of publication and executive author

Title of Publication		**Publication details**
Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review protocol		*BMJ Open.* 2017 Feb 28;7(2):e013981.
Name of executive author	**School/Institute/Division if based at Deakin; Organisation and address if non-Deakin**	**Email or phone**
Vinoomika Chandrasekaran	IMPACT SRC, School of Medicine	veena.c@deakin.edu.au

2. Inclusion of publication in a thesis

Is it intended to include this publication in a higher degree by research (HDR) thesis?	Yes	If Yes, please complete Section 3 If No, go straight to Section 4.

3. HDR thesis author's declaration

Name of HDR thesis author if different from above. (If the same, write "as above")	**School/Institute/Division if based at Deakin**	**Thesis title**
As above	IMPACT SRC, School of Medicine	Bipolar Disorder, Associated Treatments and Bone Health
If there are multiple authors, give a full description of HDR thesis author's contribution to the publication (for example, how much did you contribute to the conception of the		

project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)		
Under the guidance of my HDR supervisors, I conceptualised the systematic review methodology and developing a search strategy. I drafted the initial manuscript and contributed to edits and approved final version.		
I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.	Signature and date	20/02/2019 Signature Redacted by Library

4. Description of all author contributions

Name and affiliation of author	**Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)**
Sharon L Brennan-Olsen, University of Melbourne	Conceptualised the research question, revised the search strategy and methodology, edited, revised and approved the manuscript.
Amanda L Stuart, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Julie A Pasco, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Michael Berk, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Jason M Hodge, Deakin University	Revised, edited, and approved the methodological process and the manuscript.

Lana J Williams, Deakin University	Conceptualised the research question, revised the search strategy and methodology, edited, revised and approved the manuscript.

5. Author Declarations

I agree to be named as one of the authors of this work, and confirm:

i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
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iii. that the description in Section 4 of my contribution(s) to this publication is accurate,
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6. Other contributor declarations

I agree to be named as a non-author contributor to this work.

Name and affiliation of contributor	**Contribution**	**Signature* and date**
N/A		

* If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author

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No data presented, protocol only.			

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Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review protocol

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ABSTRACT

Introduction: Bipolar spectrum disorder is a chronic, episodic illness, associated with significant personal, social and economic burden. It is estimated to affect ~2.4% of the population worldwide and is commonly associated with psychological and/or physiological comorbidities. Osteoporosis is one such comorbidity, a disease of bone that is asymptomatic until a fracture occurs. This systematic review attempts to capture, collate, assess and discuss the literature investigating the association between bipolar spectrum disorder and bone health.

Methods and analysis: We aim to identify articles that investigate the association between bipolar spectrum disorder and bone health in adults by systematically searching the MEDLINE, PubMed, OVID and CINAHL databases. Two independent reviewers will determine eligibility of studies according to predetermined criteria, and methodological quality will be assessed using a previously published scoring system. A meta-analysis will be conducted, and statistical methods will be used to identify and control for heterogeneity, if possible. If numerical syntheses are prevented due to statistical heterogeneity, a best evidence synthesis will be conducted to assess the level of evidence for associations between bipolar spectrum disorder and bone health.

Ethics and dissemination: Ethical permission will not be required for this systematic review since only published data will be used. This protocol will be registered with PROSPERO. Findings of the review will be published in a peer-reviewed scientific journal, and will be presented to clinical and population health audiences at national and international conferences.

Strengths and limitations of this study

- This systematic review will explore a novel and covert clinical area.
- It will comprehensively assess existing literature that investigates associations between bipolar spectrum disorder and bone health.
- Potential confounders and/or mediators of the relationship will be identified.
- Two authors will independently confirm study selection, and undertake data extraction and methodological assessment.
- A potential limitation of this review may be the paucity of data available due to this being a nascent area of enquiry, and that there may be much heterogeneity in available studies.

INTRODUCTION

Bipolar spectrum disorder, a mental disorder characterised by biphasic fluctuations in mood, is a severe, chronic, episodic illness, which generally necessitates pharmacotherapy and/or psychotherapy. It is estimated to affect ~2.4% of the population[1] and has been ranked the sixth leading cause of disability in the world, among individuals aged 15–44 years.[2] The related direct and indirect costs associated with bipolar spectrum disorder are substantial.[3 4] The burden of bipolar spectrum disorder is experienced on many levels—by the sufferer, their immediate family and friends and also by the healthcare system. Symptom burden and disease course is often worsened in the presence of psychological and/or physiological comorbidities.[5 6]

Psychiatric disorders, including bipolar spectrum disorder, have been associated with early mortality, with ~60% of this excess mortality due to chronic physical illness.[7] A particularly common comorbidity of unipolar depression is osteoporosis.[8 9] Yet it is normatively overlooked, due to being asymptomatic until fracture occurs. Osteoporosis is a global public health issue, estimated to affect nearly 49 million individuals in industrialised countries, with this on the rise as a consequence of the ageing population.[10 11] The rising global economic burden related to the direct and indirect costs of medical care and rehabilitation of individuals with osteoporotic fractures is concerning.[12 13] Both clinically diagnosed unipolar depression and depressive symptoms have been shown to be

associated with deficits in bone mineral density (BMD), bone loss over time and increased fracture risk in men and women.[9 14 15] Furthermore, antidepressants, in particular, selective serotonin reuptake inhibitors used in treatment of depression, have also been shown to be noxious to bone.[16] Other psychotropic medication, namely antipsychotics and anticonvulsants, have also been shown to have a deleterious effect on bone.[17–19] A recent research synthesis with meta-analyses concluded that depression should be considered a serious risk factor for osteoporosis, based on aggregated data showing BMD among individuals with depression to be up to 7.3% lower.[15 20] Another meta-analysis reported depression to be associated with up to a 52% increased risk of fracture.[21] Whether this is true for bipolar spectrum disorder per se is yet to be determined.

Considering the previous research discussing the probable association between unipolar depression and bone, this review would essentially provide a starting point for similar investigations in bipolar spectrum disorder. This review will analyse the existing data, and this information may provide a clearer background into bone fragility associated with bipolar spectrum disorder, enabling the details of this association to be further explored.

Objectives

This systematic review will:

1. Identify published studies that investigate the association between bipolar spectrum disorder and bone health, including BMD and fracture;
2. Evaluate the quality of the methodology used in each of the studies eligible for inclusion in this review;
3. Collate the evidence, including identifying any potential confounding and/or mediating factors in the association between bipolar spectrum disorder and bone health;
4. Perform sensitivity analyses to account for differences between (a) self-reported and diagnosed bipolar spectrum disorder, (b) diagnostic criteria between versions of the Diagnostic and Statistical Manual (DSM) and/or International Classification of Diseases (ICD) and (c) bipolar disorders I and II;
5. Provide a comprehensive synthesis of the findings using previously published methodology.

METHODS

Criteria for considering studies for this review

Articles resulting from cross-sectional, case–control and/or longitudinal studies of bone health (defined as BMD, bone quality, osteoporosis and/or fracture), in adult populations (≥18 years) with bipolar spectrum disorder (defined by self-report, medical records or diagnoses based on any version of the Diagnostic and Statistical Manual of Mental Disorders or International Statistical Classification of Diseases and Related Health Problems criteria), inclusive of any sex or nationality, and published in any year, will be considered as eligible for this review.

Grey literature, case studies, theses and conference presentations will be excluded. Baseline data from randomised control trials will be included and treated as cross-sectional analyses.

Search strategy and data extraction

In order to identify the relevant literature, we will undertake an electronic search strategy to investigate research databases from the disciplines of medical, health and the social sciences (PubMed, OVID, CINAHL, MEDLINE). The following medical subject headings will be applied: “bipolar disorder” AND (“bone” OR “osteoporosis” OR “fracture” OR “bone density”), to identify publications that match our eligibility criteria. For our search strategy, we will also include the key word term of ‘bipolar spectrum disorder’.

No limits will be applied with regard to year of publication. For each database, where appropriate, relevant truncation will be applied. One reviewer will apply the search strategy and identify eligible literature for inclusion by cross-checking with the predetermined eligibility criteria. Two further reviewers will confirm the eligibility of those identified articles. Professional assistance would be sought to interpret articles written in languages other than English, in order to confirm their relevance to the eligibility criteria. Finally, the reference lists of eligible studies will be manually searched by two reviewers.[22]

Assessment of methodological quality of included articles

The methodological scoring system of Lievense *et al*[23] will be employed to assess the methodological quality of included articles (tables 1 and 2). Based on those methodological assessment criteria, each eligible study will be scored, with each study given either a positive or negative score for each criterion. This process of scoring methodological quality reflects cohort studies as the most optimal study design, followed by case–control studies and, finally, cross-sectional study designs. Two reviewers will independently score the methodological quality of each study; should these scores differ, the reviewers will attempt to reconcile any differences, after which a third reviewer would provide final judgement, if necessary. Each study will be ranked according to their total score (%), and deemed as having higher methodological quality if scored above the median, as previously published.[24]

For the meta-analyses, we will determine the population with bipolar spectrum disorder to be our proxy ‘treatment’ group and apply Hunter-Schmidt’s approach,[25] whereby a pooled within-group SD will be used. Effect size will be corrected for measurement error by dividing the effect size by the square root of the reliability coefficient of the dependent variable, whereby measurement error correction equals the effect size divided by the square root of r.

Table 1 Criteria list for assessment of study quality, adapted from Lievense *et al*[23]

Item	Criterion	C/CC/CS
Study population		
1	Uniform point (selection before disease was present)	C/CC/CS
2	Cases and controls drawn from the same population	CC
3	Participation rate >80% for cases/ cohort	C/CC/CS
4	Participation rate>80% for controls	CC
Assessment of risk factor		
5	Exposure assessment blinded	C/CC/CS
6	Exposure measured identically for cases and controls	CC
7	Exposure assessed prior to the outcome	C/CC/CS
Assessment of outcome		
8	Bone health assessed identically in patients with bipolar spectrum disorder.	C/CC/CS
9	Presence of osteoporosis assessed reproducibly	C/CC/CS
10	Osteoporosis identification assessed according to BMD measurements	C/CC/CS
Study design		
11	Prospective design used	C/CC
12	Follow-up time >24 months	C
13	Withdrawals <20%	C
Analysis and data presentation		
14	Appropriate analysis techniques used	C/CC/CS
15	Adjusted for at least age and sex	C/CC/CS

BMD, bone mineral density; C, applicable to cohort studies; CC, applicable to case–control studies; CS, applicable to cross-sectional.

Table 2 Method for determining the level of evidence for best evidence synthesis, adapted from Lievense *et al*; replicated from Brennan *et al*[24]

Level of evidence	Criteria for inclusion in best evidence synthesis
Strong evidence	Generally consistent findings in: Multiple high-quality cohort studies
Moderate evidence	Generally consistent findings in: ▶ 1 high-quality cohort study and >2 high-quality case–control studies ▶ >3 high-quality case–control studies
Limited evidence	Generally consistent findings in: ▶ A single cohort study ▶ 1 or 2 case–control studies or ▶ Multiple cross-sectional studies
Conflicting evidence	Inconsistent findings in >25% of the trials
No evidence	No studies could be found

Presenting and reporting results

PRISMA guidelines[26] will be adhered to, with regard to the presentation of findings from this review, and this protocol adheres to the PRISMA-P guidelines.[27] Numbers and reasons pertaining to inclusion versus exclusion of papers in the context of the predetermined eligibility criteria will be presented in a QUOROM diagram.[28] Key information regarding factors involved in the association between bipolar spectrum disorder and bone health will be identified; these factors may include, but will not be limited to, inflammatory markers, lifestyle behaviours, socioeconomic status, medications and substance use. Our findings will be useful to inform and reach a consensus as to the link between bipolar spectrum disorder and bone health.

A meta-analysis is planned; however, if a numerical synthesis is not possible due to methodological heterogeneity, a 'best evidence synthesis' will be undertaken. A 'best evidence synthesis' would evaluate the level of evidence identified, ranging from no evidence to strong evidence (table 2), as previously published in the musculoskeletal field.[24]

We will also perform sensitivity analyses to account for differences between (1) self-reported and diagnosed bipolar spectrum disorder, (2) diagnostic criteria between versions of the DSM and/or ICD and (3) bipolar disorders I and II.

Dissemination

This protocol will be registered with PROSPERO, an international database of health-related systematic review protocols. The findings of our systematic review will be published in a peer-reviewed scientific journal, and results will be shared at national and/or international conferences relevant to the field of bipolar spectrum disorder and/or bone health.

Ethics

Since only published data will be used in this systematic review, we do not require ethical permission. However, ethical and governance standards will be strictly adhered to, in matters of data management and in the presentation and discussion of our results.

Conclusion

To the best of our knowledge, this will be the first systematic review to identify and evaluate the existing evidence base regarding associations between bipolar spectrum disorder and bone health; and determining the nature of this relationship has both public health and clinical implications. The findings of this review will contribute to existing literature investigating other psychiatric disorders and bone health, and will also provide an evidence base on which resource allocation and clinical and public health strategies aimed at reducing burden associated with both osteoporosis and bipolar spectrum disorder can be founded.

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Contributors All authors conceptualised the research question for this protocol and edited and revised the research question. VC, SLB-O and LJW developed the e-search strategy. All authors edited, revised and approved the methodological processes. VC, SLB-O and LJW drafted the manuscript, and all authors edited and contributed to the writing of this paper. All authors read and approved the final version, and guarantee the review.

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Part B: Epidemiological studies

The relationship between anticonvulsant use and bone health was tested in a population drawn from the Barwon Statistical Division, South Eastern Australia. Manuscript III is a cross-sectional study investigating the effect of anticonvulsant use on bone quantity and quality, utilising data from the GOS. Manuscript IV is a case-control study, investigating the effect of anticonvulsant use on fracture risk, utilising data from the PROFRAC study.

2.2.1 Geelong Osteoporosis Study (Manuscript III)

2.2.1.1 Participants

Participants were drawn from the GOS, a population-based study originally designed to study the epidemiology of osteoporosis in Australia (205). Participants were randomly selected from the electoral roll for the Barwon Statistical Division. Between 1993-1997, a total of 1494 women (77% response), aged 20-94 years agreed to participate. A cohort of 1540 men (67% response) aged 20-94 years was similarly recruited between 2001-2006. Participants have returned for follow-up every 2-5 years (205).

2.2.1.2 Data collection

2.2.1.2.1 Measurement of outcome variables

Bone quantity

Bone mineral density (BMD; g/cm^2) at the spine (PA projection, L2-4) and total hip was measured using dual-energy X-ray absorptiometry (DPX-L (women) and Lunar Prodigy (men), GE, Madison, WI, USA). Trained technicians performed daily calibrations of the densitometers with equipment-specific phantom, and took all measurements.

Bone quality

Quantitative Heel Ultrasound (QUS; Achilles Insight Ultrasonometer, GE Lunar, Madison, WI, USA) was used to determine bone quality at the calcaneus. QUS measures consist of the following parameters: speed of sound [SOS (m/s)], reflecting elasticity and bone density, broadband ultrasound attenuation [BUA (dB/MHz)], reflecting microarchitecture and bone density and stiffness index [SI (%)], a calculated clinical index (205).

2.2.1.2.2 Measurement of exposure variables

Medication use

Current medication use was determined. Participants were requested to bring a medication list or containers to their appointment to ensure accurate reporting. Exposure to anticonvulsants, and other medications known to affect bone, such as oral glucocorticoids, bisphosphonates, other psychotropics and thyroid medication were coded based on the Australian Index of Medications guidelines (206).

Anthropometric measurements

Height and weight were measured to the nearest 0.1 cm and 0.1 kg respectively. Body mass index (BMI) was calculated (kg/m^2).

Socio-demographic data

Area-based rankings of relative socioeconomic advantage in terms of access to social and material resources were sourced by cross-checking the residential address for each participant with the corresponding Australian Bureau of Statistics (ABS) census collection district. ABS software used to access the socio-economic index for areas (SEIFA) scores. These scores provide a set of indices that indicate relative socio-economic advantage or disadvantage at the small area level (IRSAD). Scores were categorised into quintiles whereby socioeconomic

status (SES) quintile 1 was considered most disadvantaged and SES quintile 5 was most advantaged.

Lifestyle factors

Daily alcohol use (g/day) and calcium intake (mg/day) was determined from a validated food frequency questionnaire (207). Habitual physical activity level was classified as active if vigorous or light exercise was performed regularly; otherwise participants were classified as sedentary (208). Current cigarette smoking was self-reported.

2.2.1.3 Data management and storage

Data are stored under password protected databases on the mainframe computer of the University Hospital Geelong. These databases are backed up daily from the file server, and updated every day, ensuring that the database is current and up to date. Data are maintained in a relational database management system (Access) that enables the storage of data in tables with each field's properties easily defined. To maintain data integrity and the relationship between tables/fields/data, each is linked by a common key field being a unique identification number. These files are easily convertible for use in statistical packages.

2.2.1.4 Statistical analysis

Differences in characteristics between those using anticonvulsants versus non-users were compared using t-tests for continuous normally distributed variables, Kruskal-Wallis for non-parametric continuous variables and chi-square or Fisher's exact test for discrete variables. Linear regression was used to explore associations between anticonvulsant use and a) BMD (spine and hip) and b) QUS measures (BUA, SOS and SI). Data for men and women were analysed using separate statistical models. Confounders such as age, BMI, smoking status, SES, physical activity and medications known to affect bone were tested sequentially and

retained in the final model when significant ($p<0.05$). Interactions between exposure variables were checked for effect modification in the final models.

2.2.1.5 Ethics

This study has been approved by the Human Research Ethics Committee at Barwon Health (92/01 and 00/56) and Deakin University (2013-320). All participants have provided informed, written consent.

2.2.2 PRedictors and Outcomes of incident FRACtures study (Manuscript IV)

2.2.2.1 Participants

This case-control study utilised data from 1,458 participants (51.8% women) aged ≥20 years, participating in the PROFRAC study and the GOS. Participants had a radiologically confirmed fracture between June 2012 and May 2013 (209), and completed a comprehensive questionnaire sent by mail. Information regarding fracture site and cause was recorded, while individuals were excluded for the following reasons: spontaneous fractures, uncontactable, death, no knowledge of fracture and an inability to provide informed consent. Further details of the recruitment of fracture cases has been published (209). Controls were drawn from the same population living within the Barwon Statistical Division, and matched by age and sex. A total of 1,796 fracture-free participants (46.5% women) were selected from the closest GOS follow-ups: 15-year follow-up for women (2011-2014) and the GOS 5-year follow-up for men (2005-2006). Control participants were fracture-free during June 2012-May 2013.

2.2.2.2 Data collection

2.2.2.2.1 Measurement of outcome variable

Fracture

Fractures were identified using a daily keyword search of all radiological reports from the University Hospital Geelong, based on a previously validated method of fracture ascertainment (210). Only a participant's first fracture during June 2012-May 2013 was recognised for this analysis.

2.2.2.2.2 Measurement of exposure variables

Medication use

Participants self-reported current medication use. Use of anticonvulsants and other medications considered to affect calcium and bone metabolism (adrenal steroid hormones, gonadal hormones, thyroid hormones, anti-fracture agents) were classified for both cases and controls. Cases who started anticonvulsants post-fracture were considered non-users for these analyses.

Demographic data

Highest level of education was self-reported and categorised as primary school education, some secondary school, completed secondary school, Technical and Further Education (TAFE)/Trade other and University.

Lifestyle and medical factors

A five-point scale was utilised to determine current alcohol use. The categories included never, less than once per week, once or twice per week, several times per week or every day. Participants were categorised as active (very active or active) or inactive (limited activity in the home, or chair or bedridden), based on the Metabolic Equivalent of Task values (205, 212).

Current cigarette smoking was self-reported. Date and site of any fractures occurring during adulthood were determined. Participants were classified as fallers if they had fallen to the ground at least once during the past 12 months. History of epilepsy and bipolar disorder diagnoses were self-reported.

2.2.2.3 Data management and storage

As described above, data are stored within password protected databases on the mainframe computer of the University Hospital Geelong, which are easily convertible for use in statistical packages.

2.2.2.4 Statistical analysis

Differences between fracture cases and controls were analysed using t-tests for parametric data, Kruskal Wallis for non-parametric data and Chi Square for categorical data. Binary logistic regression (odds ratio, OR, with 95% confidence intervals, CI) was used to investigate the association between anticonvulsant use and fracture in separate models for men and women. Covariates tested in the models included age, education, physical activity, past adult fracture, falls in the past 12 months, smoking status, alcohol consumption, use of medications known to affect calcium and bone metabolism and self-reported epilepsy and bipolar disorder. Backwards stepwise regression techniques were used to determine the best model and all potential interactions were tested. Analyses were repeated following the removal of minor fractures (finger, thumb and toe).

2.2.2.5 Ethics

This study has also been approved by the Human Research Ethics Committee at Barwon Health (92/01_E9) and Deakin University (2012-060). All participants have provided informed, written consent.

Part C: Bone cell in vitro studies

2.3.1 Effect of anticonvulsants on human OC formation and resorption

As previously described (213), a mononuclear cell fraction containing monocytes and lymphocytes from cord blood, termed Cord Blood Mononuclear Cells (CBMC), were isolated from human umbilical cord blood, and expanded to form colony forming unit-granulocyte macrophage (CFU-GM) derived OC precursors. Similarly, OC precursors were differentiated in 96-well plates containing numbered dentine slices (diameter: 5.5 mm) into mature human OC using cell culture medium (αMEM) containing essential growth factors and cytokines (see Figure 2); with the exception of this experiment being conducted in the presence or absence of five concentrations (10^{-3}-10^{-7}M) each, of four anticonvulsants, namely VPA, GBP, CBZ and LMT for 14 days. Cells were then fixed in 1% paraformaldehyde, tartrate-resistant acid phosphatase activity was quantified and OC formation and function assessed using image analysis software.

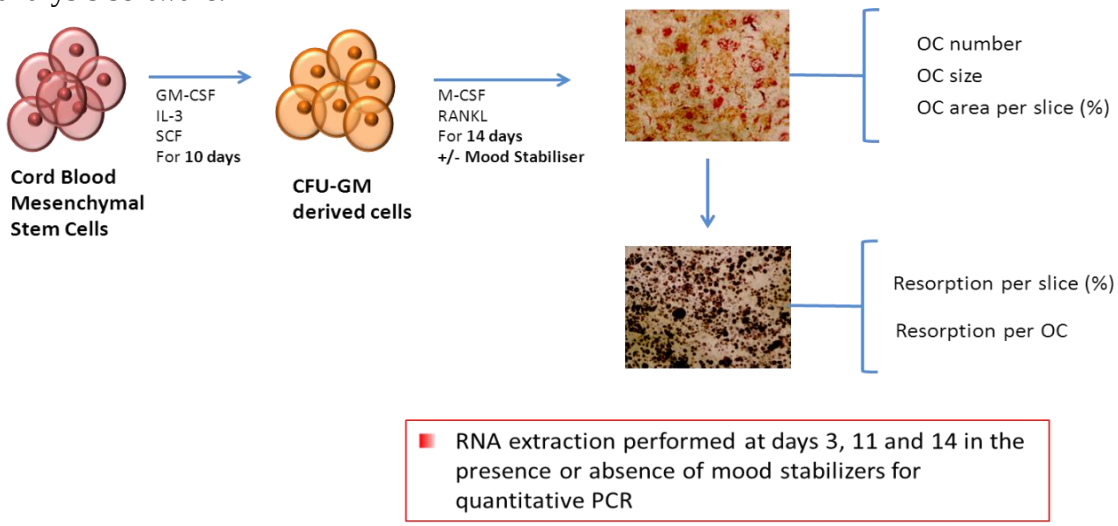

Figure 2: Human OC formation and mineralisation. CBMCs were isolated by Ficoll-Paque density gradient centrifugation and grown in CFU-GM favouring media. Following the expansion of CFU-GM for 10 days, they are seeded on to pre-wetted dentine slices. A fraction of untreated cells is used to test for cell proliferation and viability. Drug treatments are then performed for 14 days and the cells are assessed for the expression of human OC genes such

as Cathepsin K (CTSK), Nuclear Factor kappa B1 (NFκB1), Nuclear Factor of Activated T-cell, cytoplasmic 1 (NFATc1), Colony-stimulating factor 1 receptor (CSF1R), Osteoclast Stimulatory Transmembrane Protein (OC STAMP) and Dendritic Cell–Specific Transmembrane Protein (DC STAMP).

2.3.2 Effect of anticonvulsants on human OB formation and mineralisation

Human adipose tissue sourced from elective abdominoplasty was blunt dissected, collagen-digested, and the released mesenchymal stem cells (MSC) isolated and expanded, as previously described (214). Adherent MSC were seeded in 96-well plates in DMEM containing osteogenic factors vital to OB formation (see Figure 3), at a seeding density of 10^4 cells/well and cultured for 14-21 days in the presence or absence of anticonvulsants, to achieve similar treatment ranges as in OC assays (10^{-3}-10^{-7}M). Human oncostatin M (hOSM), as positive control (214, 215) and osteogenic media (Ost M) as vehicle controls were run alongside these wells. Early OB cultures were assessed for ALP activity on day 7, and mineralization was quantified by staining with alizarin red (ALZ) upon completion of the culture period.

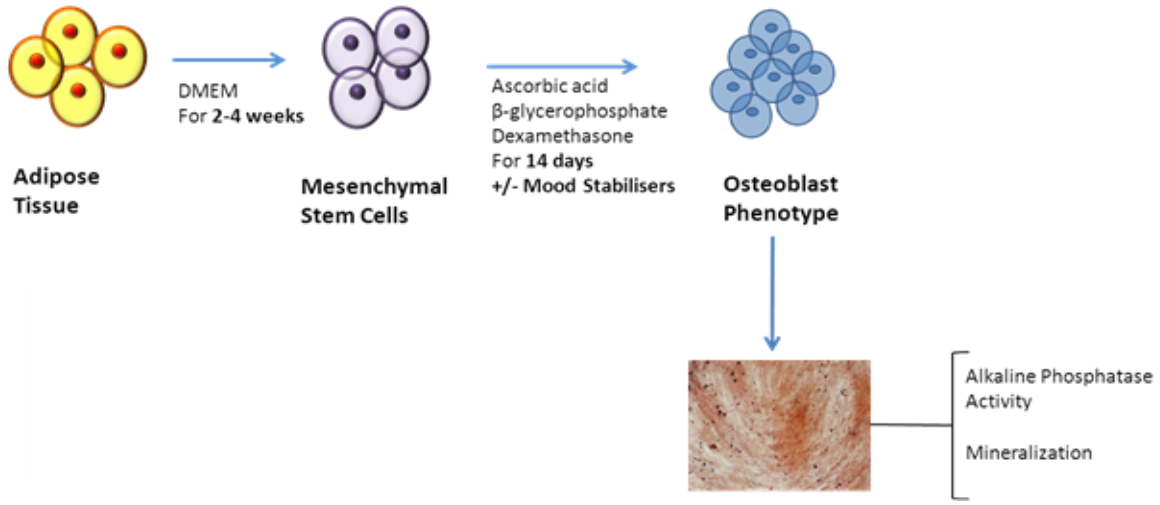

Figure 3: Human OB formation and mineralisation. A primary cell line of adipose-derived MSCs is produced and differentiation into OBs is performed. OB thus derived were treated with anticonvulsants in treatment specific concentrations for 7-21 days. ALP activity was measured at 7 days and mineralisation of extracellular matrix were assessed at 14-21 days.

2.3.3 Effect of anticonvulsants on gene expression by qRT-PCR analysis

Total RNA was isolated from CFU-GM-derived OC precursors that had been cultured in the presence of VPA and GBP for 7-14d at 10^{-4}M or CBZ and LMT for 7-14d at 10^{-5}M. The cell cultures were lysed by the direct addition of Trizol, and reverse transcribed to cDNA using the Superscript® III First Strand Synthesis SuperMix system (Life Technologies) as per manufacturer's instructions. For each treatment mRNA was extracted separately from 3 wells of a 6-well plate and quantitative real-time PCR (qRT-PCR) was performed in duplicate.

To quantify the expression of human OC genes (CTSK, NFκB1, NFATc1, CSF1R, and OC/DC STAMP), we employed qRT-PCR analysis of the cDNA in a 7500 Fast Real-Time PCR System (Applied Biosystems), using TaqMan® Gene Expression Assays (Applied Biosystems Hs00166156 (CTSK), Hs00231653 (NFκB1), Hs00232342 (NFATc1), Hs00234622 (CSF1R), Hs00875776 (OC STAMP) and Hs00984780 (DC STAMP). Relative gene expression units were determined using the formula $2^{-\Delta Ct}$x1000, where ΔCt values represent the difference between the Ct of the gene of interest and GAPDH (amplified using Taqman chemistry with forward primer (5'-gacaggatgcagaaggagattact-3'), reverse primer (5'-tgatccacatctgctggaaggt-3') and the probe (Fam-atcattgctcctcctgagcgcaagtactc-Tamra).

2.3.4 Statistical analysis

Data were expressed as the mean ± SEM, where applicable. Differences between groups were assessed using one-way ANOVA followed by Fisher's multiple comparison test or two-way ANOVA, the general linear model followed by Tukey's post-hoc test (pairwise comparison). Statistical significance was set at $p<0.05$. Groups and drug concentration curves with annotations that do not contain the same letter were considered significantly different.

2.3.5 Ethics

The use of CBMCs (10/154) and adipose tissue (17/90) for this study has also been approved by the Human Research Ethics Committee at Barwon Health and Deakin University.

Chapter 3: Bipolar Disorder and Bone Health

Part A: Systematic review

3.1.1 Manuscript II

Chandrasekaran V, Brennan-Olsen SL, Stuart AL, Pasco JA, Berk M, Hodge JM and Williams LJ. Association between bipolar spectrum disorder and bone health: a meta-analysis and systematic review. *J Affect Disord.* 2019 Apr 15; 249:262-269. doi: 10.1016/j.jad.2019.02.013. Epub 2019 Feb 6.

AUTHORSHIP STATEMENT

1. Details of publication and executive author

Title of Publication		**Publication details**
Bipolar disorder and bone health: A systematic review		*J Affect Disord.* 2019 Apr 15; 249:262-269. doi: 10.1016/j.jad.2019.02.013. Epub 2019 Feb 6.
Name of executive author	**School/Institute/Division if based at Deakin; Organisation and address if non-Deakin**	**Email or phone**
Vinoomika Chandrasekaran	IMPACT SRC, School of Medicine	veena.c@deakin.edu.au

2. Inclusion of publication in a thesis

Is it intended to include this publication in a higher degree by research (HDR) thesis?	Yes	If Yes, please complete Section 3 If No, go straight to Section 4.

3. HDR thesis author's declaration

Name of HDR thesis author if different from above. (If the same, write "as above")	**School/Institute/Division if based at Deakin**	**Thesis title**
As above	IMPACT SRC, School of Medicine	Bipolar Disorder, Associated Treatments and Bone Health

If there are multiple authors, give a full description of HDR thesis author's contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)		
Under the guidance of my HDR supervisors, I worked on executing the steps of the systematic review methodology; developing a search strategy, conducted exclusions, data extraction and presenting of the results. I drafted the initial manuscript and contributed to edits and approved final version.		
I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.	Signature and date	20/02/2019 Signature Redacted by Library

4. Description of all author contributions

Name and affiliation of author	**Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)**
Sharon L Brennan-Olsen, University of Melbourne	Conceptualised the research question, revised the search strategy and methodology, performed data extraction, drafted, edited, revised and approved the manuscript.
Amanda L Stuart, Deakin University	Confirmed exclusions, performed methodological scoring, edited, revised and approved the manuscript.
Julie A Pasco, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Michael Berk, Deakin University	Revised, edited, and approved the methodological process and the manuscript.

Jason M Hodge, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Lana J Williams, Deakin University	Conceptualised the research question, search strategy and methodology, performed methodological scoring, drafted, edited the manuscript and approved the final version.

5. Author Declarations

I agree to be named as one of the authors of this work, and confirm:

i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
ii. that there are no other authors according to these criteria,
iii. that the description in Section 4 of my contribution(s) to this publication is accurate,
iv. that the data on which these findings are based are stored as set out in Section 7 below.

If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further

v. consent to the incorporation of the publication into the candidate's HDR thesis submitted to Deakin University and, if the higher degree is awarded, the subsequent publication of the thesis by the university (subject to relevant Copyright provisions).

Name of author	**Signature***	**Date**
Sharon L Brennan-Olsen		21st February 2019
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Julie A Pasco		21st February 2019

Michael Berk		21st February 2019
Jason M Hodge		21st February 2019
Lana J Williams		21st February 2019

6. Other contributor declarations

I agree to be named as a non-author contributor to this work.

Name and affiliation of contributor	**Contribution**	**Signature* and date**
A/Prof Seetal Dodd, Deakin University	Translated the abstract of a manuscript written in French	16th July 2020
Mrs. Sophia Xin Sui	Translated the title of a manuscript written in Chinese	21st February 2019

* If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author

7. Data storage

The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member

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Data format	**Storage Location**	**Date lodged**	**Name of custodian if other than the executive author**
Review, accessed previously published work.			

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Review article

Bipolar disorder and bone health: A systematic review

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ABSTRACT

Background: Bipolar disorder is a chronic, episodic mental illness, affecting around 2.4% of the population worldwide. Psychological and/or physiological comorbidities are a common consequence, and osteoporosis is one such possible comorbidity. Thus, this systematic review aimed to collate, evaluate, and discuss the literature examining the link between bipolar disorder and bone health.

Methods: We conducted an e-search of PubMed/OVID/MEDLINE, PsychINFO and CINAHL to identify studies that investigated associations between bipolar disorder and bone in adults aged ≥18. Two reviewers determined eligibility according to pre-determined criteria, and methodological quality was assessed using a previously published methodological scoring system. Due to heterogeneity, a best-evidence synthesis was performed.

Results: Our search yielded 1409 articles, of which three (all cohorts) met predetermined criteria. The studies from Taiwan and the United States of America analysed administrative data, albeit spanning different years, and comprised a total of 344,497 participants. No studies investigating bone quantity or quality were identified. Bipolar disorder was associated with an increased risk of fracture (range 20–80%); and fracture-free survival time for those with bipolar disorder decreased substantially with advancing age, and for women (10–30% shorter than men). Fracture incidence per 1000 person years (py) was 21.4 and 10.8 in those with and without bipolar disorder, respectively.

Limitations: Limited data and marked methodological heterogeneity prevented the pooling of these data for a numerical synthesis.

Conclusions: Increased fracture risk was observed in individuals with bipolar disorder, independent of older age, sex, comorbidities and medication use. The operative mechanisms, risk and treatment factors warrant further enquiry.

1. Introduction

Bipolar disorder is a leading cause of years lived with disability in the world (World Health Organisation, 2003). Aside from psychological comorbidities, non-communicable physical disorders are common across psychiatric illnesses (Sanna et al., 2013). Poor bone health is no exception, with recent data showing associations between unipolar depression and reductions in bone mineral density (BMD) and increased fracture risk in men and women across the lifespan (Cizza et al., 2010; Fernandes et al., 2016; Williams et al., 2016). A research synthesis with meta-analyses concluded that major depression should be considered a risk factor for osteoporosis onset that is as powerful as recognised risk factors such as smoking, physical inactivity and inadequate calcium nutrition (Cizza et al., 2010). Mezuk, 2008

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0165-0327/

A review from 2008 alluded to the possibility that bipolar disorder, associated lifestyle factors and/or the medications commonly used to treat the disorder, could also be detrimental to bone health (Mezuk, 2008). The lifetime risk for hip, vertebral and wrist fractures alone has been estimated to be approximately 40% (World Health Organisation, 2003). Fractures contribute to loss of independence, limited mobility, chronic pain and an overall increased mortality rate (Murray and Lopez, 1996; Otmar et al., 2013; Williams et al., 2011); the direct and indirect costs of osteoporosis, osteopenia, and the resulting fractures between 2012–2022 is estimated to be $33.6 billion in Australia (Watts et al., 2013), with similar studies showing the growing economic impact of osteoporosis worldwide (Cauley, 2013; Hernlund et al., 2013).

In order to add to the growing evidence base studying this association, this systematic review aims to collate existing data regarding the association between bipolar disorder and bone health.

2. Methods

The published protocol for this systematic review (Chandrasekaran et al., 2017) is registered with the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42017074372).

2.1. Eligibility criteria for considering studies for this review

We considered cohort, cross-sectional and/or case-control study designs that investigated the association between bipolar disorder and bone health in adult populations (≥18 years) to be eligible for inclusion in this review. Bipolar disorder was defined by hospital records or diagnoses based on criteria from any edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association 2000, 2013) or International Statistical Classification of Diseases and Related Health Problems (ICD) (Medicode(Firm), 1996), and bone health was defined as bone quality, bone mineral density (BMD), osteoporosis or fracture. Eligible studies could comprise populations of either sex, be published in any year, conducted in any country, and published in any language.

Publications including case studies, grey literature, theses and conference presentations were excluded. Clinical trials were also excluded; however, baseline data from clinical trials (where available) were included.

2.2. Search strategy and data extraction

We performed an electronic search strategy on the 7th August 2017 and applied the following medical subject headings (MeSH): “bipolar disorder” AND (“bone” OR “osteoporosis” OR “fracture” OR “bone density”) to identify relevant literature in research databases from the medical, health and social sciences disciplines (PubMed/OVID/MEDLINE, PsychINFO and CINAHL). Our search strategy also included the key word term ‘bipolar spectrum disorder’ and no limits were applied in terms of year of publication or age of participants.

One reviewer (VC) cross-checked articles against the pre-determined criteria to determine eligibility for inclusion (Chandrasekaran et al., 2017). Professional assistance was sought to interpret articles written in French and Chinese, to confirm their relevance to the eligibility criteria. Of those articles excluded, 10% were cross-checked by a second reviewer (ALS), and the final selection was confirmed by two reviewers (ALS, LJW). Finally, two reviewers (VC, ALS) screened the reference lists of eligible studies in order to locate any further articles previously unidentified by the e-search strategy.

For the multivariate analyses, the key factors mediating the association between bipolar disorder and bone health were considered, and a summary of their effects reported (Tables 2 and 3).

2.3. Assessment of methodological quality of included articles

A modified version of a previously published methodological scoring system (Lievense et al., 2001) was utilised to assess the methodological quality of eligible articles. Lievense et al.’s scoring system suggests that cohort studies have the most optimal study design, followed by case-control studies, and finally, cross-sectional study designs (Lievense et al., 2001). Each included study was scored based on the Lievense et al. methodological assessment criteria and given an affirmative (1) or negative score (0) for each criterion, or, where a score was unclear a question mark (?) was given. Two reviewers (ALS, LJW) independently scored the methodological quality of each study, and where scores differed reviewers attempted to reconcile any differences, after which a third reviewer (SLB-O) provided final judgement.

2.4. Presenting and reporting results

The findings from this review are presented in accordance with PRISMA guidelines (Moher et al., 2009). Fig. 1 provides a summary of the yield ascertained from the e-search strategy, and exclusions at each stage of eligibility assessment. Overall, the search of databases yielded a total of 1409 articles, of which 157 were duplicates. Of the remaining 1252 potentially relevant studies, and based on the pre-determined eligibility criteria, 1158 were excluded based on the title, whilst a further 54 were excluded based on the abstract. Full texts of the remaining 40 articles were examined and contrasted against the pre-determined eligibility criteria, from which a further 37 articles were excluded, leaving three articles for inclusion in this review. No further articles were identified when the reference lists of the three included articles were screened.

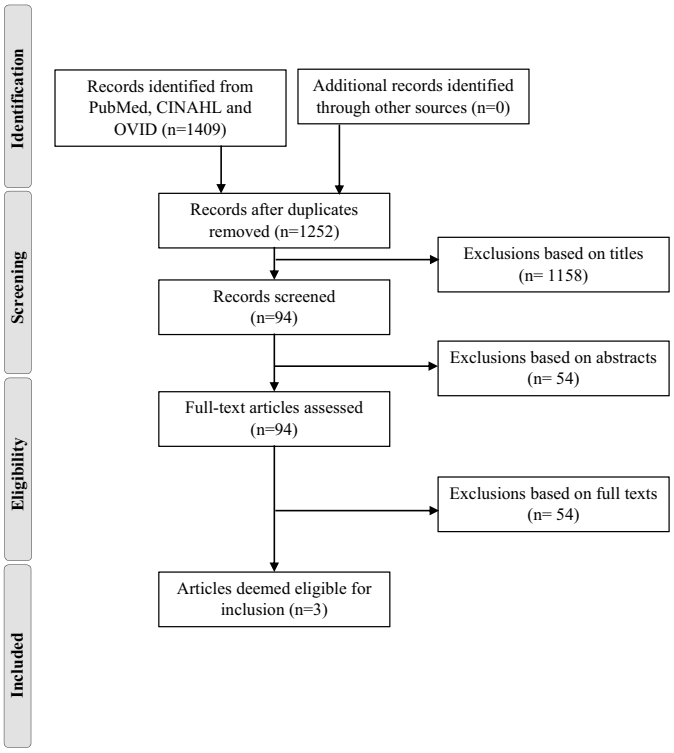

Fig. 1. Flowchart of the identification of eligible studies from the systematic search of PubMed, PsychINFO and CINAHL databases, based on the PRISMA flowchart (Moher et al., 2009).

Table 1
Descriptive characteristics of eligible studies (all of which were cohorts) included in this review, presented in alphabetical order.

First author (ref), country, year	Years of data collection; Follow up period	*N* = (% female)	Population description	Age (years) mean (± SD)	Fracture - method of identification	Bipolar disorder - method of identification
Hsu et al., 2016	2001–2011 Followed up until: fracture, 31/12/2011 or withdrawal from insurance program.	236,355 (62.4)	Patients identified from administrative records (NHIRD) with a new diagnosis of bipolar disorder between 2001and 2008 vs. those with no history of bipolar disorder in the NHIRD, frequency-matched 4:1 by age (per 5 years), sex and index year	Total sample: * Bipolar disorder: 43.7 (± 15.5) No bipolar disorder: 43.8 (± 15.5)	ICD–9–CM codes 800–829	ICD-9-CM code 296
Mezuk et al, USA, 2010	2002–2006 Followed up for 1428 days (~ 4 years).	67,387 (3.5)	Patients identified from the administrative records (VA) with a diagnosis of bipolar disorder in 2002 vs. VA healthcare system users with no history of bipolar disorder between 1999–2001	Total sample: ≥50 Bipolar disorder: 62.0 (± 9.9) No bipolar disorder: 67.5 (± 10.2)	ICD-9-CM codes: 800–829 and V-codes†: 733.1x, V54.1x and V54.2.x	ICD-9-CM codes 296.0–296.1 and 296.4–296.8
Su et al., 2017	1997–2013 Followed up until: fracture, death or 31/12/2013.	40,755 (61.8)	Patients identified from administrative records (NHIRD) with a diagnosis of bipolar disorder after 1998 vs. adults with no history of bipolar disorder in a 10:1 ratio matched by age, sex, and index year	Total sample: ≥16 Bipolar disorder: 43 (± 17.2) No bipolar disorder: 43 (± 17.2)	ICD–9–CM codes 800–829	Inpatient diagnosis with bipolar disorder and/or ≥2 medical records of outpatient care for bipolar disorder within 12 months ICD-9-CM codes 296.0, 296.4–296.8 and 296.89

† *V-Codes (V01-V91) – defined in the ICD-9-CM as 'Supplementary Classification of Factors Influencing Health Status and Contact with Health Services'.*

ICD-9-CM – International Classification of Diseases, Ninth Revision, Clinical Modification; NHIRD - National Health Insurance Research Database; SD – Standard Deviation; VA – Veterans Administration.

* *Data not provided.*

3. Results

3.1. Methodological quality and heterogeneity

During the methodological quality assessment, an initial inter-rater reliability of 91.7% was achieved, and all discrepancies were resolved after one consensus meeting and consultation with the third reviewer (SLB-O). The overall mean score of methodological quality was 77.8% (range 75.0–83.3%). Given the small number of studies included in this review, all studies were included in the best-evidence synthesis.

Heterogeneity tests, determined using RevMan, revealed that whilst methodological heterogeneity was high (I^2 statistic 78%), clinical heterogeneity was very low (I^2 statistic 11%), (Higgins et al., 2002), thus we determined that pooling these data for a meta-analysis would provide misleading findings for clinical applicability, and instead performed a best-evidence synthesis.

3.2. Description of the studies

Table 1 presents an overview of the three included studies, all of which were cohort by design, and published in 2010 (Mezuk et al., 2010), 2016 (Hsu et al., 2016) and 2017 (Su et al., 2017). Two studies were performed in Taiwan and used the same administrative dataset (Hsu et al., 2016; Su et al., 2017) albeit spanning different years, whilst the third study was performed using an administrative dataset from the United States of America (USA) (Mezuk et al., 2010). Sample sizes ranged from 40,755 (Su et al., 2017) to 236,355 (Hsu et al., 2016), and varied in terms of male: female ratio, with one study comprising only 3.5% female participants (Mezuk et al., 2010), whilst the other two studies each comprised approximately 62% female participants (Hsu et al., 2016; Su et al., 2017).

Although all three studies met the eligibility criteria in terms of investigating adults aged 18 years and over, two studies also encompassed individuals aged 16 years or older (Hsu et al., 2016; Su et al., 2017). Each of the three included studies used ICD codes (9th Revision – Clinical Modification [ICD-9-CM]) to identify both bipolar disorder and fracture (Hsu et al., 2016; Mezuk et al., 2010; Su et al., 2017). All three studies performed multivariable modelling using Cox Proportional Hazards (although adjustments varied between studies (Hsu et al., 2016; Mezuk et al., 2010; Su et al., 2017), whilst incident fracture rates per 1000 py for individuals with and without bipolar disorder was available in one study (Hsu et al., 2016) (Table 2).

3.3. Incident fracture rates

Fracture incidence per 1000 py was 10.8 in those without bipolar disorder compared to 21.4 in those with bipolar disorder (Hsu et al., 2016) (Table 2).

3.4. Multivariable results

Table 3 presents results from multivariable analyses, all of which found adults with bipolar disorder to have an independent, increased risk of fracture compared to those without bipolar disorder.

3.4.1. Overall time to fracture

In the USA study (Mezuk et al., 2010) reported those with bipolar disorder to have a 21% increased risk of fracture compared to those without bipolar disorder. This was similar to one of the Taiwanese studies (Su et al., 2017), in which those with bipolar disorder, compared to those without, had a 32% increased risk of fracture; however, in the other study from Taiwan (Hsu et al., 2016) reported a 79% increased risk of fracture for those with, compared to without bipolar disorder.

3.4.2. Sex

The larger of the two Taiwanese studies stratified by sex, and reported that, independent of age and comorbidities, men with bipolar disorder had 63% higher fracture risk than those without, whilst women with bipolar disorder had 91% higher fracture risk (Hsu et al., 2016). A comparison between the sexes by the other Taiwanese study, reported a 10% increase in fracture risk in women compared to men (Su et al., 2017), independent of advancing age, two or more comorbidities, prevalent osteoporosis, substance abuse, and medication use (Su et al., 2017).

3.4.3. Effects of age

Differences between the two Taiwanese studies were also observed in terms of age-group specific associations between bipolar disorder and fracture. One study (Hsu et al., 2016) reported that with advancing age, the adjusted hazards ratios of fracture reduced in those with bipolar disorder, whilst the other Taiwanese study (Su et al., 2017) reported the opposite, whereby the fracture risk in those with bipolar disorder increased with advancing age. However, when excluding the younger age group (which contained individuals aged from 16–35 years, and thus incorporating adolescents), the findings of Hsu et al. showed that fracture risk in adults with bipolar disorder was between 1.4–1.8-fold higher than in those without bipolar disorder (Hsu et al., 2016). Due to methodological heterogeneity, comparisons with the study by Su et al. are difficult, however, compared to the younger age group of 16–34 years that were held as referent, the smaller of the two Taiwanese studies (Su et al., 2017) showed that the fracture risk was 3-fold higher in those aged 80 years or older with bipolar disorder.

3.4.4. Comorbidities

Hsu et al. reported that, among individuals with comorbidities, those with bipolar disorder had an 88% increased fracture risk compared to those without bipolar disorder (Hsu et al., 2016). Su et al. reported that having two or more comorbidities increased the fracture risk by 18%, whilst osteoporosis alcohol-related disorders increased fracture risk by 74% and 32%, respectively. Substance abuse increased fracture risk 2.65-fold (Su et al., 2017).

3.4.5. Pharmacological treatments

In those with bipolar disorder, Su et al. reported that the use of benzodiazepines or hypnotics at or above 28DDD (Defined Daily Dosage) increased fracture risk by 22% and 21%, respectively (Su et al., 2017).

3.4.6. Income and urbanisation

Higher income and urbanisation decreased the risk of fracture

Table. 2
Incident fracture rates per 1000 py and incident rate ratios (IRR) in adults with and without bipolar disorder.

First author (ref), country, year	Incidence per 1000 py (95%CI)	IRR (95% CI)	Summary of associations
Hsu et al., 2016	No bipolar disorder: 10.8 (*) Bipolar disorder: 21.4 (*)	No bipolar disorder: (referent) Bipolar disorder: 1.98 (1.93–2.02)	Increased fracture incidence in adults with bipolar disorder

IRR – Incident Rate Ratio, py - Person Years.
* Data not provided/available.

Table. 3
Multivariable results pertaining to the association between bipolar disorder and fracture.

First author (ref), country, year	Adjusted results, presented as HR (95%CI)	Adjusted for confounders	Summary of associations
Hsu et al., 2016	*Total sample:* No bipolar disorder: (referent) Bipolar disorder: 1.79 (95%CI 1.73–1.84) *Sexes:* Men: No bipolar disorder: (referent) Bipolar disorder: 1.63 (95%CI 1.55–1.72) Women: No bipolar disorder; (referent) Bipolar disorder: 1.91 (95%CI 1.84–1.99) *Age groups:* < 35 years: No bipolar disorder: (referent) Bipolar disorder: 2.10 (95%CI 1.96–2.25) 35–64 years: No bipolar disorder: (referent) Bipolar disorder: 1.76 (95%CI 1.69–1.83) ≥ 65 years: No bipolar disorder: (referent) Bipolar disorder: 1.42 (95%CI 1.33–1.53) *Comorbidity:* No comorbidity: No bipolar disorder: (referent) Bipolar disorder: 1.92 (95%CI 1.83–2.01) With comorbidity: No bipolar disorder: (referent) Bipolar disorder: 1.88 (95%CI 1.81–1.96)	Age, sex, comorbidities (diabetes, hypertension, hyperlipidemia, CAD, osteoporosis, stroke, epilepsy, alcohol-related illness) As above As above As above	*Total sample:* Increased fracture risk was shorter in adults with bipolar disorder *Sexes:* For both sexes, increased fracture risk was shorter in those with bipolar disorder: women had the highest fracture risk in this cohort. *Age groups:* Increased fracture risk in adults with bipolar disorder reduced with advancing age *Comorbidity:* Fracture risk was highest in adults with bipolar disorder, regardless of prevalent comorbidity
Mezuk et al, USA, 2010	*Total sample:* No bipolar disorder: (referent) Bipolar disorder: 1.21 (95%CI 1.10–1.33)	Age, sex, medication use (anticonvulsants, benzodiazepines, antipsychotics, SSRIs, TCA, antiresorptives), Charlson comorbidity index, race, marital status, depression status, alcohol/substance use diagnosis, ability to pay for service, service-connected disability	Fracture risk was higher in adults with bipolar disorder
Su et al., 2017	*Total sample:* No bipolar disorder: (referent) Bipolar disorder: 1.32 (95%CI 1.20–1.45)	Age, sex, comorbidities (osteoporosis, diabetes mellitus, hypertension, rheumatoid arthritis, senile dementia, alcohol related disorder, substance abuse), medication use (benzodiazepines, hypnotics, prednisolone), Charlson comorbidity index, urbanization, income, extrapyramidal symptoms	Fracture risk was higher in adults with bipolar disorder
	Sexes: Men: (referent) Women: 1.12 (95%CI 1.06–1.19)	As above	Fracture risk in adults with bipolar disorder was higher in women than men
	Age groups: 16–34 years: (referent) 35–49 years:1.28 (95%CI 1.17–1.39) 50–64 years: 2.25 (95%CI 2.06–2.45) 65–79: 3.78 (95%CI 3.42–4.18) ≥ 80 years: 4.51 (95%CI 3.83–5.31)	As above	Fracture risk in adults with bipolar disorder increased with advancing age
	Comorbidity: No osteoporosis: (referent) Osteoporosis: 1.74 (95%CI 1.50–2.03) No diabetes mellitus: (referent) Diabetes mellitus: 1.09 (95%CI 0.97–1.22) No hypertension: (referent) Hypertension: 0.98 (95%CI 0.90–1.06)	As above	Fracture risk in adults with bipolar disorder was higher in those with osteoporosis and substance abuse

(*continued on next page*)

Table. 3 (*continued*)

First author (ref), country, year	Adjusted results, presented as HR (95%CI)	Adjusted for confounders	Summary of associations
	No rheumatoid arthritis: (referent) Rheumatoid arthritis: 0.91 (95%CI 0.61–1.37) No senile dementia: (referent) Senile dementia: 1.15 (95%CI 0.66–1.98) No alcohol-related disorder: (referent) Alcohol-related disorder: 1.32 (95%CI 0.84–2.10) No substance abuse: (referent) Substance abuse: 2.65 (95%CI 1.37–5.15)		
	Extrapyramidal symptoms: 1.08 (95%CI 0.99–1.99)	As above	No association
	Charlson Comorbidity Index: 1: 1.08 (95%CI 0.99–1.17) 2: 1.18 (95%CI 1.06–1.33) ≥3: 1.16 (95%CI 1.02–1.31)	As above	Fracture risk in adults with bipolar disorder was higher in those with 2 or more comorbidities
	Medications: Use < 28 DDD: (referent) Benzodiazepine (≥28DDD): 1.22 (95%CI 1.10–1.34) Use < 28 DDD: (referent) Hypnotics (≥28DDD): 1.12 (95%CI 1.06–1.37) Use < 28 DDD: (referent) Prednisolone (≥28DDD): 0.65 (95%CI 0.44–0.98)	As above	Fracture risk in adults with bipolar disorder was higher in those who took benzodiazepine and hypnotics at or above the DDD of 28.
	Income No income: (referent) NTD1-15,840: 0.93 (95%CI 0.85–1.01) NTD15,841–25,000: 0.94 (95%CI 0.87–1.01) ≥ NTD25,001: 0.91 (95%CI 0.84–1.02)	As above	No association
	Urbanisation Low: (referent) Moderate: 0.91 (95%CI 0.81–1.02) High: 0.86 (95%CI 0.78–0.96) Very High: 0.79 (95%CI 0.71–0.89)	As above	No association

CAD – Coronary Artery Disease, CI – Confidence Interval, DDD- Defined Daily Dosage, HR – Hazards Ratio, NTD – New *Taiwanese Dollar; SSRIs – selective serotonin reuptake inhibitors, TCA – tricyclic antidepressants.*

amongst individuals with bipolar disorder (Su et al., 2017).

4. Discussion

This systematic review identified and evaluated the largely understudied area of research investigating the association between bipolar disorder and bone health. Fracture risk was reported to be higher for those with bipolar disorder compared to those without, independent of age, sex, comorbidities and medication use.

These findings contribute to the previous literature, suggesting unipolar depression is associated with increases in fracture risk (Williams et al., 2016). Unipolar depression has also been associated with reductions in BMD and bone quality, associations that are yet to be explored in those with bipolar disorder (Cizza et al., 2010; Fernandes et al., 2016; Williams et al., 2011). There are several mechanisms that could be used to explain these reported findings. Biological factors including inflammation, mitochondrial dysfunction, oxidative stress and endocrine factors have common pathways that can affect neuroprogression in bipolar disorder while also promoting bone loss (Grande et al., 2016; Vieta et al., 2018). Elevated cytokine levels, or neuroinflammation is associated with both mania and depression (Wadee et al., 2002). Chronic neuroinflammation results in increased free radicals, decreased mitochondrial function, lipid peroxidation and excitotoxicity. All these stressors may lead to increased intracellular and glutamate levels, which may then result in a neuroprogressive effect due to neurotoxicity (Berk et al., 2011). Studies have also shown inflammation to be a key mediator in the development of osteodegenerative diseases such as osteoporosis (Ginaldi et al., 2005; Lacativa and Farias, 2010; Mundy, 2007; Pasco et al, 2006). Furthermore, there are several commonly implicated lifestyle factors that might also affect this association including cigarette smoking, physical inactivity, dietary calcium imbalances, vitamin D and alcohol and substance use (Grande et al., 2016). Two of the three studies included in this review highlighted psychotropic use as an essential factor to consider in the relationship between bipolar disorder and bone health (Mezuk et al., 2010; Su et al., 2017). Three of the major agents used in the treatment of bipolar disorder, antidepressants, antipsychotics and anticonvulsants, have been shown to be independently detrimental to bone.

Selective serotonin reuptake inhibitors (SSRIs) and tricyclics are associated with increased fracture risk (Rizzoli et al., 2012), and a preclinical study has shown that SSRIs affect osteoblast formation and function (Hodge et al., 2013). Similarly, recent meta-analyses suggest that antipsychotic use is associated with a 1.5-fold increase in fracture risk, where higher bone loss was associated with age, female sex and prolonged prolactin-elevating antipsychotic use (Chen et al., 2016; Lee et al., 2017; Wu et al., 2013). While hyperprolactinemia is often used to explain the deleterious effects of antipsychotics on bone, a conclusive explanation of the effect of hyperprolactinemia on BMD is yet to be determined (Chen et al., 2016; Lee et al., 2017; Wu et al., 2013). A review conducted by Lee et al (2010) showed anticonvulsants to be associated with increased fracture risk. While the harmful effects of anticonvulsants on bone health has been established in the treatment of epilepsy, the influence of anticonvulsants as a psychotropic drug in populations with mental presentations is yet to be ascertained.

Due to the nature and severity of the symptoms associated with bipolar disorder, hospitalisation is often required. Notably, Su et al. studied the effect of severity of bipolar disorder and history of psychiatric hospitalisation on fracture risk, and observed that fracture risk increased significantly with increased severity and psychiatric hospitalisation (Su et al., 2017). A similar pattern was seen in the data presented by Hsu et al., where fracture risk increased with an increased frequency of hospital care, however this study did not specify whether psychiatric treatment was provided (Hsu et al., 2016).

4.1. Strengths and limitations of the selected articles

The selected studies are each cohort by design, and are therefore considered optimal. Samples have been drawn from large national health insurance and administrative databases and are therefore sufficiently powered. All three studies (Hsu et al., 2016; Mezuk et al., 2010; Su et al., 2017) investigated the risk of fracture in individuals with bipolar disorder, and used the ICD-9-CM as their instrument of bipolar disorder identification. The limitations of the selected studies primarily lie in the heterogeneity. Mezuk et al. investigate fracture risk in an older cohort ($\geq$50 years) in the USA (Mezuk et al., 2010), consisting of a small percentage of women ($<$10%), while the Taiwanese studies include a much larger age range (16–80+) consisting of more women ($\sim$60%) than men in their analyses (Hsu et al., 2016; Su et al., 2017). Only two of the three selected studies adjusted for medications (Mezuk et al., 2010; Su et al., 2017), which is a key factor in studying the association between bipolar disorder and bone health (Yatham et al., 2018). Further, only one study adjusted for the use of antiresorptives (Mezuk et al., 2010). Another notable factor is that two of the studies have used the same Taiwanese national database (NHIRD), and yet show inconsistent results. Su et al. showed an increasing risk of fracture with age, while Hsu et al. showed a decrease in fracture risk with age. This may be explained in part by the fact that Hsu et al. also included the ICD-9-CM code 296, which includes two codes that diagnose major depressive disorder (296.2, 296.3). In addition, the bipolar disorder diagnosis was not confirmed with a structured clinical interview. A final limitation of the identified studies was the lack of information on additional confounding factors such as sociodemographic factors, lifestyle behaviours such as smoking status, diet, alcohol consumption and physical activity, disease course and exact severity. This may be of particular importance with individuals with more severe symptoms of bipolar disorder due to behavioural manifestations typically observed during manic episodes such as violence, impulsivity and risk-taking behaviours, which may expose them to high-risk situations (Hsu et al., 2016). Only Su et al. considered the effects of urbanisation and socioeconomic status on the association between bipolar disorder and fractures, and found that increased fracture risk was associated with lower income and low urbanisation (Su et al., 2017).

4.2. Strengths and limitations of this review

During each step of the selection of included articles, as with our scoring process, our method involved a second author confirming a percentage of the exclusions and the scoring; and where necessary, a third author providing final judgement. This ensures the fidelity of the systematic process of article selection and in producing this synthesis. While this review provides a comprehensive synthesis of the association between bipolar disorder and fracture, discussing potential mediators of this nascent area of enquiry was limited by the paucity of available studies, and a meta-analysis was prevented by clinical heterogeneity and data for the two Taiwanese studies being sourced from the same database.

5. Conclusion

According to the objectives stated in our published protocol (Chandrasekaran et al, 2017), we systematically identified published literature investigating the association between bipolar disorder and bone health, and evaluated the methodological quality of the identified studies. We also discussed the known potential confounding and/or mediating factors in our review. However, owing to the heterogeneity between the identified studies, the data could not be pooled for a numerical synthesis. Therefore, a comprehensive synthesis of the extracted data has been provided.

To our knowledge, this is the first review of its kind to systematically investigate the association between bipolar disorder and bone health. It remains to be determined whether associations exist in different populations and after key confounding factors are taken into consideration. Moreover, underlying mechanisms need to be explored. This systematic review provides a comprehensive synthesis of existing literature to inform further enquiry in this burgeoning field of research; which will in turn, provide a basis for important clinical decisions in the treatment of bipolar disorder.

Author statement

VC, LW and SLB-O developed the e-search strategy. VC conducted the exclusions, which were confirmed by ALS. LJW and ALS conducted the methodological scoring. SLB-O and VC performed data extraction. All authors edited, revised and approved the methodological processes. VC, SLB-O and LJW drafted the manuscript, and all authors edited and contributed to the writing of this paper. All authors read and approved the final version, and guarantee the review.

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Conflict of interest

None of the authors have any relevant conflicts of interest related to the work under consideration for publication. S.L.B-O has received Honorarium fees from Amgen Australia and Pfizer Australia, and

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Chapter 4: Anticonvulsants and Bone Health

Part B: Epidemiological studies

4.1.1 Manuscript III

Chandrasekaran V, Pasco JA, Stuart AL, Brennan-Olsen SL, Berk M, Hodge JM, Samarasinghe RM and Williams LJ. Anticonvulsant use and bone health in a population-based study of men and women: cross-sectional data from the Geelong Osteoporosis Study. *BMC Musculoskelet Disord*. 2021 Feb 11; 22(1):172.

AUTHORSHIP STATEMENT

1. Details of publication and executive author

Title of Publication		**Publication details**
Anticonvulsant use and bone health in a population-based study of men and women: cross-sectional data from the Geelong Osteoporosis Study.		*BMC Musculoskelet Disord*. 2021 Feb 11;22(1):172.
Name of executive author	**School/Institute/Division if based at Deakin; Organisation and address if non-Deakin**	**Email or phone**
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2. Inclusion of publication in a thesis

Is it intended to include this publication in a higher degree by research (HDR) thesis?	Yes	If Yes, please complete Section 3 If No, go straight to Section 4.

3. HDR thesis author's declaration

Name of HDR thesis author if different from above. (If the same, write "as above")	**School/Institute/Division if based at Deakin**	**Thesis title**
As above	IMPACT SRC, School of Medicine	Bipolar Disorder, Associated Treatments and Bone Health

If there are multiple authors, give a full description of HDR thesis author's contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)		
Under the guidance of my HDR supervisors, I conceptualised the methodology and drafted the initial manuscript, contributed to edits and approved final version.		
I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.	**Signature and date**	28/05/2020 Signature Redacted by Library

4. Description of all author contributions

Name and affiliation of author	**Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)**
Julie A Pasco, Deakin University	Designed the methodology, revised, edited, and approved the methodological process and the manuscript.
Amanda L Stuart, Deakin University	Conceptualised the research question, revised the methodology, performed data analyses, edited, revised and approved the manuscript.
Sharon L Brennan-Olsen, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Michael Berk, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Jason M Hodge, Deakin University	Revised, edited, and approved the methodological process and the manuscript.

Rasika M Samarasinghe, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Lana J Williams, Deakin University	Conceptualised the research question, revised the methodology, edited, revised and approved the manuscript.

5. Author Declarations

I agree to be named as one of the authors of this work, and confirm:

i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
ii. that there are no other authors according to these criteria,
iii. that the description in Section 4 of my contribution(s) to this publication is accurate,
iv. that the data on which these findings are based are stored as set out in Section 7 below.

If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further

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Name of author	**Signature***	**Date**
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Sharon L Brennan-Olsen		18th July 2020

Michael Berk		13th July 2020
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Rasika M Samarasinghe		15th July 2020
Lana J Williams		8th July 2020

6. Other contributor declarations

I agree to be named as a non-author contributor to this work.

Name and affiliation of contributor	**Contribution**	**Signature* and date**
N/A		

* If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author

7. Data storage

The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

Data format	Storage Location	Date lodged	Name of custodian if other than the executive author
Microsoft Access Database	University Hospital, Geelong		Prof Julie Pasco

BMC Musculoskeletal Disorders

RESEARCH ARTICLE Open Access

Anticonvulsant use and bone health in a population-based study of men and women: cross-sectional data from the Geelong Osteoporosis Study

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Abstract

Background: Anticonvulsant use has been linked to bone deficits in specific patient populations. We studied the association between anticonvulsant use and bone health in a population-based sample of men and women.

Methods: Data from 926 men (24-73 yr) and 1070 women (21-94 yr) participating in the Geelong Osteoporosis Study were included. Bone mineral density (BMD, g/cm^2) of the PA-spine and total hip was measured using dual-energy X-ray absorptiometry (Lunar). Bone quality was determined using quantitative heel ultrasound (QUS). Anthropometry was conducted and socioeconomic status was determined. Medication and lifestyle information was obtained via questionnaire. Linear regression was used to test associations between anticonvulsant use and bone health before and after adjustment for potential confounders.

Results: Seventeen (1.8%) men and 20 (1.9%) women reported anticonvulsant use. In men, anticonvulsant users had 9.1% lower adjusted mean BMD at the spine and hip compared to non-users. Body mass index was an effect modifier at the spine. Anticonvulsant users also had 1.8% lower speed of sound (SOS), 10.6% lower broadband ultrasound attenuation (BUA) and 13.7% lower stiffness index (SI) compared to non-users. In women, BMD tended to be lower at the hip compared to non-users as with the bone quality measure, BUA. No significant associations were observed at the spine or the other bone quality measures, SOS and SI.

Conclusion: Our data suggest that bone quantity and quality, assessed using BMD and QUS, are lower for men and possibly women who use anticonvulsants. While further exploration into potential mechanisms is needed, our findings suggest that monitoring bone health among users of anticonvulsants is warranted.

Keywords: Bone mineral density, Quantitative heel ultrasound, Anticonvulsants, Osteoporosis, psychiatry, neuroscience, medical comorbidity

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Background

Osteoporosis is an osteodegenerative disease of multifactorial aetiology, often undiagnosed until a fracture occurs [1]. As a growing public health concern, osteoporosis also affects independence, quality of life and increases risk of mortality [2]. The lifetime risk of developing an osteoporotic fracture in industrialised nations is 40–56% for women and 20–30% for men above the age of 50 years [3]. Among those aged 50 years and over, the prevalence of osteoporosis or low bone mass is estimated to increase by 31% between 2012 and 2022 [4].

Dual energy X-ray absorptiometry (DXA) measures bone mineral density (BMD), and is currently the key assessment tool for diagnosing osteoporosis [5]. However, being a 2-dimensional measure, BMD does not sufficiently explain variance in fracture outcomes amongst individuals with similar BMD measures [6], thus additional bone measures may be useful. Quantitative heel ultrasound (QUS) is a radiation-free, portable, non-invasive, inexpensive screening method to assess bone quality, measuring 3-dimensional parameters such as bone structure and elasticity to explain variance in bone strength [5, 7]. Trabecular bone in sites such as the vertebral body and the calcaneus is more susceptible to the effects of altered metabolism [8].

The risk factors for osteoporosis are multifactorial and include age, poor lifestyle, inadequate nutrition, smoking, substance abuse and certain medications and illnesses [1, 9]. Several medications, including anticonvulsants such as valproate, have been associated with increased fracture risk [10]. Anticonvulsants are a mainstay in the treatment of psychiatric and neurological illnesses and have been theorised to have agonistic effects on the glutamatergic and GABAergic neuronal pathways [11] and/or calcium, sodium and potassium voltage gated channels, thereby reversing their neuroexcitatory effects [12]. Other possible mechanisms associated with an anticonvulsant-related decline in bone health are impaired calcium absorption or decreased calcium availability due to calcitonin deficiency, altered sex hormones, liver enzyme induction, inhibition of response to parathyroid hormone or hyperparathyroidism [13, 14]. Further, any potential anticonvulsant-induced alterations in body composition may additionally influence fracture risk [15].

Anticonvulsants have been associated with increased fracture risk in adults [10, 16] and decreased BMD in children [17]. Few studies have investigated anticonvulsant use on QUS parameters [18, 19]. A preclinical analysis of levetiracetam use in rats decreased bone quality, but not BMD, suggesting that DXA measurements may not sufficiently detect all anticonvulsant-related bone deficits [20]. Large studies simultaneously assessing how anticonvulsant use affects bone quality and quantity, after considering potential confounding factors are also sparse. Thus, we aimed to assess the relationship between anticonvulsant use and bone quantity and quality in a large population-based sample of men and women.

Methods

Participants

This study utilised data from men and women participating in the Geelong Osteoporosis Study (GOS), a large, ongoing, population-based study conducted in the Barwon Statistical Division in south-eastern Australia [21]. Initially, 1494 women (aged 20–94 years, participation 77.1%) and 1540 men (aged 20–93 years, participation 67%) were randomly selected from the Commonwealth of Australia electoral rolls (where voting is compulsory), between 1994 and 1997, and 2001 and 2006, respectively. Participants have been invited for two to five yearly assessments. An additional sample of 246 women aged 20–29 years was recruited (participation 70.9%) between 2004 and 2008, allowing for continuing investigation of the full adult age range.

For this cross-sectional analysis, data collected at the 10-year follow-up for women and 5-year follow-up for men was utilised. Of the1127 women who participated in the 10-year follow-up, participants for whom bone data were not available were excluded, resulting in a final sample of 1070, aged 20–94 years. Of the 978 men who participated in their 5-year follow-up, similarly, participants for whom bone data were not available were excluded, resulting in a final sample of 926, aged 24–98 years.

Measurement of the outcome variables

BMD (g/cm^2) was measured at the spine (PA projection, L2–4) and total hip for men (Lunar Prodigy, GE, Madison, WI, USA) and women (DPX-L, GE, Madison, WI) [21]. Trained technicians carried out all examinations and performed daily calibrations of the densitometers with an equipment-specific phantom.

At the same time as BMD was measured, bone quality was determined by calcaneus QUS (Achilles Insight Ultrasonometer, GE Lunar, Madison, WI, USA) of the left heel, yielding the following parameters: broadband ultrasound attenuation [BUA (dB/MHz)], reflecting microarchitecture and bone density, speed of sound [SOS (m/s)], reflecting elasticity and bone density and stiffness index [SI (%)], a calculated clinical index [22]. SOS and BUA measure the speed and frequency-dependent ultrasound attenuation of ultrasound signals passing through soft tissue and trabecular bone [23]. SI is a combined parameter, calculated from these primary measures [24].

Measurement of exposure variables

The following data were collected concurrently with the bone health assessments:

Medication use

Current medication use was determined via self-report. Participants were requested to bring a medication list or containers to their appointment to ensure accurate reporting. Exposure to anticonvulsants, and other medications known to affect bone, such as oral glucocorticoids, bisphosphonates, and thyroid medication were coded based on the Australian index of medications guidelines.

Questionnaire data

Information on daily alcohol use (g/day) and calcium intake (mg/day) was determined using a validated food frequency questionnaire [25]. Participants were classified as smokers if they reported current use at the time of assessment and habitual physical activity level was classified as active if vigorous or light exercise was performed regularly; otherwise participants were classified as sedentary.

Other markers of bone health

Osteoporosis status was identified as T-score < − 2.5 at either the spine or total hip [26, 27]. Information regarding previous adult fracture was ascertained via radiological reports from medical imaging centres servicing the region. This method of fracture ascertainment has been previously validated [28].

Anthropometric measures

Body mass index (BMI) was calculated (kg/m^2) from height, measured to the nearest 0.1 cm, and body weight measured to the nearest 0.1 kg.

Socioeconomic status

Area-based socio-economic status (SES) was established by matching the x-y coordinates of participants' residential addresses to the Australian Bureau of Statistics' Index of Relative Socioeconomic Advantage and Disadvantage (IRSAD) data to determine a score for each participant. The IRSAD consists of information regarding income and skill level. IRSAD scores for this study were determined according to cut points of the Barwon Statistical Division and categorised into quintiles whereby SES quintile 1 was considered most disadvantaged and SES quintile 5 was most advantaged.

Statistical analyses

Minitab (Version 18; Minitab, State College Pa) was used to perform statistical analyses. Differences between anticonvulsant users and non-users were detected using t-tests for continuous normally distributed variables, Kruskal-Wallis for non-parametric continuous variables and chi-square or Fisher's exact test for discrete variables. Multiple regression was used to explore associations between anticonvulsant use and a) BMD and b) QUS measures (BUA, SOS and SI). Data for men and women were analysed using separate statistical models. Confounders including age, BMI, smoking status, SES, physical activity and medications known to affect bone were tested sequentially and retained in the final model when significant ($p < 0.05$). Interactions between exposure variables were checked for effect modification in the final models.

Results

Male sample

Seventeen men (1.8%) were anticonvulsant users at the time of assessment; phenytoin ($n = 7$), carbamazepine ($n = 5$), sodium valproate (n = 5), pregabalin ($n = 1$) and clonazepam (n = 1). Anticonvulsant users were older, less active, consumed less alcohol and were more likely to have a previous adult fracture than non-users; otherwise the groups were similar in regard to BMI, smoking status, calcium intake, osteoporosis status and SES (Table 1). Unadjusted BMD and QUS parameters are shown in Table 1.

BMI was an effect modifier in the relationship between anticonvulsant use and BMD at the spine, with the relationship differing for those above and below a BMI of 28.5 kg/m^2. For example, for users vs non-users, age- and BMI- adjusted mean BMD at the spine for men with a BMI of 25 kg/m^2 was 1.154 (95% CI 1.039–1.269) vs 1.275 (1.260–1.290) g/cm^2, a BMI of 27 kg/m^2 was 1.231 (95% CI 1.138–1.324) vs 1.291 (1.278–1.304) g/cm^2, a BMI of 29 kg/m^2 was 1.308 (95% CI 1.204–1.412) vs 1.307 (1.293–1.321) g/cm^2 and a BMI of 31 kg/m^2 was 1.385 (95% CI 1.244–1.527) vs 1.323 (1.306–1.340) g/cm^2 (all $p = 0.033$). At the hip, age- and BMI- adjusted mean BMD was lower among anticonvulsant users compared to non-users [0.887 (95% CI 0.830–0.944) vs 0.976 (0.968–0.984) g/cm^2, $p = 0.002$]. Smoking, physical activity, alcohol and calcium intake, SES and medications known to affect bone did not contribute to the final models.

After adjustment for age, BMI, smoking, alcohol and calcium intake, anticonvulsant use was associated with lower adjusted mean BUA [107.5 (95% CI 99.4–115.7) vs 120.3 (119.2–121.4) dB/MHz, p = 0.002], SOS [1543.1 (95% CI 1521.8–1564.4) vs 1572.0 (1569.1–1574.9) m/sec, $p = 0.008$] and SI [86.2 (95% CI 76.1–96.2) vs 99.9 (98.6–101.3) %, p = 0.008] compared to non-use. Physical activity, SES and medications known to affect bone did not contribute to the final models.

Female sample

Twenty (1.9%) women were anticonvulsant users at the time of assessment; carbamazepine ($n = 6$), sodium valproate ($n = 4$), clonazepam ($n = 5$), gabapentin ($n = 3$),

Table 1 Characteristics of participants, expressed as median (interquartile range), mean (± standard deviation) or n (%)

	Men				Women			
	All	**Users**	**Non-users**	**p**	**All**	**Users**	**Non-users**	**p**
	***n* = 926**	***n* = 17**	***n* = 909**		***n* = 1070**	***n* = 20**	***n* = 1050**	
Age (yr)	60.0 (46.5–73.2)	66.3 (54.0–84.8)	59.9 (46.2–73.1)	0.05	51.0 (34.7–65.7)	52.3 (34.0–65.1)	51.0 (34.7–65.9)	0.90
BMI (kg/m^2)	27.2 (24.7–29.6)	27.8 (24.6–34.9)	27.1 (24.7–49.9)	0.93	26.3 (23.4–30.9)	27.5 (22.3–40.3)	26.2 (23.4–55.0)	0.98
Smoking (current)	104 (11.3%)	3 (17.7%)	101 (11.2%)	0.40	153 (14.3%)	3 (15.0%)	150 (14.3%)	0.93
Physical activity (active)	659 (71.5%)	7 (41.2%)	652 (72.0%)	0.005	835 (78.1%)	11 (55.0%)	824 (78.6%)	0.01
Calcium intake (mg/day)	882 (689.0–1136.2)	899 (596.0–1132.5)	880 (689.3–1136.3)	1.000	864 (659.4–1091.0)	976 (785.9–1206.5)	864 (659.0–1089.2)	0.16
Alcohol use (g/day)	12.0 (2.1–28.8)	0.4 (0.0–7.9)	12.6 (2.3–29.4)	< 0.001	2.7 (0.3–12.0)	0.7 (0.0–7.5)	2.8 (0.3–12.2)	0.07
Medication (current)								
Glucocorticoids	10 (1.1%)	0 (0%)	10 (1.1%)	–	16 (1.5%)	1 (5.0%)	15 (1.4%)	0.26
Thyroid-affecting medications	10 (1.1%)	0 (0%)	10 (1.1%)	–	47 (4.4%)	1 (5.0%)	46 (4.4%)	0.60
Agents affecting calcium	15 (1.6%)	0 (0%)	15 (1.7%)	–	40 (3.7%)	3 (15.0%)	37 (3.5%)	0.04
Socioeconomic status				0.67				0.36
Quintile 1 (lowest)	150 (16.2%)	1 (5.9%)	149 (16.4%)		170 (16.0%)	2 (10.0%)	168 (16.1%)	
Quintile 2	186 (20.1%)	4 (23.5%)	182 (20.0%)		224 (21.0%)	2 (10.0%)	222 (21.2%)	
Quintile 3	178 (19.2%)	5 (29.4%)	173 (19.0%)		243 (22.8%)	7 (35.0%)	236 (22.6%)	
Quintile 4	203 (21.9%)	4 (23.5%)	199 (21.9%)		211 (19.8%)	6 (30.0%)	205 (19.6%)	
Quintile 5	209 (22.6%)	3 (17.7%)	206 (22.7%)		218 (20.5%)	3 (15.0%)	215 (20.6%)	
Prior adult fracture	269 (29.1%)	9 (52.9%)	260 (28.6%)	0.03	82 (7.7%)	5 (25.0%)	77 (7.3%)	0.003
BMD (g/cm^2)								
Spine	1.293 ± 0.198	1.258 ± 0.277	1.294 ± 0.197	0.47	1.209 ± 0.188	1.172 ± 0.223	1.210 ± 0.187	0.39
Hip	0.980 ± 0.137	0.928 ± 0.143	1.066 ± 0.142	< 0.001	0.941 ± 0.166	0.803 ± 0.142	0.853 ± 0.161	0.12
Osteoporosis (current)	29 (8.2%)	3 (25.0%)	26 (7.6%)	0.07	74 (7.0%)	3 (15.0%)	71 (6.8%)	0.16
QUS								
SOS (m/sec)	1572.1 ± 41.4	1540.0 ± 57.0	1572.7 ± 40.9	0.003	1571.1 ± 39.0	1562.9 ± 38.8	1571.2 ± 39.0	0.42
BUA (dB/MHz)	120.4 ± 16.0	106.5 ± 17.6	120.7 ± 15.9	0.001	111.0 ± 16.4	103.9 ± 21.0	111.1 ± 16.3	0.09
SI (%)	99.8 ± 20.5	84.4 ± 26.9	100.1 ± 20.2	0.003	94.7 ± 20.7	91.8 ± 28.5	94.7 ± 20.5	0.57

lamotrigine ($n = 1$) and pregabalin (n = 1). Anticonvulsant users were less active, more likely to have a previous fracture and were more likely to take agents affecting calcium than non-users; otherwise the groups did not differ in regards to age, BMI, smoking status, calcium intake, alcohol use, SES, osteoporosis status or use of thyroid-affecting hormones and adrenal steroid hormones (Table 1). Unadjusted BMD and QUS parameters for users and non-users are shown in Table 1.

After adjustment for age and BMI, there were no differences detected in BMD at the spine between users and non-users of anticonvulsants [1.164 (95% CI 1.090–1.239) vs 1.200 (1.190–1.211) g/cm^2, $p = 0.345$], however, anticonvulsant users tended to have lower BMD at the hip compared to non-users [0.876 (0.821–0.931) vs 0.930 (0.922–0.938) units, $p = 0.058$].

After adjustment for age and BMI, anticonvulsant use was not associated with mean SOS [1565.0 (95% CI 1547.8–1582.1) vs 1571.0 (1568.7–1573.3) m/sec, $p = 0.493$] or SI [90.8 (95% CI 82.8–98.9) vs 93.7 (92.6–94.8) %, $p = 0.484$] but anticonvulsant users tended to have lower BUA than non-users [104.5 (95% CI 97.8–111.3) vs 110.7 (109.8–111.6) dB/MHz, $p = 0.075$].

Discussion

This cross-sectional study investigated associations between anticonvulsant use and bone health in a large population-based sample of men and women. After adjustment for confounders, BMD and QUS parameters were generally lower in men using anticonvulsants compared to non-users. In women, this was not clearly the case, with the difference in BMD and QUS parameters between anticonvulsant users and non-users not reaching statistical significance.

Only two studies have looked at how anticonvulsant use affects QUS measures albeit in children and young adults. A small Italian study ($n = 164$, aged 2–21 years) investigating how anticonvulsants affect DXA and QUS measures in girls with or without Rett Syndrome found that anticonvulsant therapy was associated with lower bone measures, although fracture risk was not elevated [18]. Similarly, a cross-sectional study conducted in Spain found that valproate, but not carbamazepine, phenobarbital, lamotrigine, topiramate, vigabatrine or phenytoin was associated with lower QUS measures in children ($n = 65$, aged 6.5 ± 3.1 years) compared to non-users; despite having 27.3% of their treated group taking two or more anticonvulsants [19]. On the other hand, several studies have looked at associations between anticonvulsant use and BMD [29–32], although most studies have been conducted in patients with epilepsy and in paediatric populations [10]. It has been estimated that over 50% of anticonvulsant users develop bone anomalies [29], and studies conducted over time have shown that long-term use is associated with increased fracture risk and associated bone disease [30, 32]. In a large, multiethnic, postmenopausal cohort ($n = 138,667$, aged 50–79 years), anticonvulsant use was associated with an increased fracture risk, which was further increased with polypharmacy and enzyme-inducing anticonvulsants rather than the non-enzyme inducing type [30]. Interestingly, anticonvulsant use was not associated with BMD at the spine, hip and total body, but was associated with falls risk. While our results reflect the association between anticonvulsants and bone health in adults, it appears that our findings are concordant with the existing literature, that while anticonvulsant use may be broadly associated with bone deficits, associations in girls and women are less clear.

Chronic anticonvulsant use has been associated with a 1.2–2.4-fold increase in fracture risk [33]; explained in part by hepatic enzyme-inducing anticonvulsants known to increase conversion of 25-hydroxyvitamin D into inactive metabolites [34]. Mezuk et al. [35] observed that although enzyme-inducing anticonvulsants may pose a greater fracture risk (HR 2.19, 95% CI: 1.97–2.43), non-enzyme-inducing anticonvulsants are also associated with an increased fracture risk (HR 1.66, 95% CI: 1.54–1.79). Additionally, Lee et al. [33] observed that first generation anticonvulsants such as valproic acid, carbamazepine, phenytoin and phenobarbital are associated with an increased rate of fragility fractures, when compared to newer agents.

Confounding by indication is another factor possibly playing a role. Anticonvulsants are used in the treatment of epilepsy and bipolar disorder, with both having been shown to be independently associated with increased fracture risk. In a recent systematic review, bipolar disorder was associated with a 20–80% increased risk of fracture, independent of age, sex, medication and comorbidities [9]. Decreased physical activity and other modifiable risk factors, such as diet, substance use, smoking, SES, sun exposure, medical comorbidities, polypharmacy and drug-induced metabolic imbalances may also contribute to the overall decline in bone health [36–38]. Falling, associated with seizures in epilepsy, has been proposed to be another mediating factor [39].

The results of this study, when taken in context of research showing that anticonvulsant use independently increases fracture risk, add to existing clinical findings, and may assist treatment decisions in managing an already vulnerable population. Adequate calcium supplementation could counteract the deleterious effects of anticonvulsants [40], while clinical trial evidence suggests that the association between calcium supplementation and improved bone health is weak [41]. The antiepileptic drug and osteoporosis prevention trial (ADOPT) reported similar findings, where over 69% of their cohort

($n = 80$ men, aged ≥58 years) had improved BMD measures with adequate calcium supplementation [42].

Both strengths and limitations need to be taken into consideration. Strengths of this study include the representative nature of the sample spanning the full adult age range, and the number of confounding variables tested. A limitation of this study is its cross-sectional nature, preventing conclusions on how BMD and QUS measures varied over time. Second, vitamin D status has not been considered. Other factors for consideration are a likely healthy participant bias and the small number of anticonvulsant users is an additional consideration, which also limited subgroup analyses of specific agents. Lastly, any unidentified confounding may affect our findings.

Conclusion

Both DXA and QUS measures in our study suggest an anticonvulsant-associated deficit in bone health, at least for men. Further exploration into mechanisms are warranted, as this information may inform clinical decisions and enable necessary preventative measures to be taken when prescribing anticonvulsants.

Abbreviations

BMD: Bone mineral density; DXA: Dual-energy X-ray absorptiometry; QUS: Quantitative heel ultrasound; SOS: Speed of sound; BUA: Broadband ultrasound attenuation; SI: Stiffness index; GOS: Geelong Osteoporosis Study; IRSAD: Index of Relative Socioeconomic Advantage and Disadvantage; BMI: Body mass index; HR: Hazard ratio; CI: Confidence interval; SES: Socioeconomic status; ADOPT: Antiepileptic drug and osteoporosis prevention trial

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Authors' contributions

VC, ALS and LW designed the study and prepared the first draft of this manuscript. ALS performed statistical analyses. All authors reviewed and contributed to the intellectual content in this manuscript, approved the final version and guarantee this work.

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Availability of data and materials

Participants' data available on request from Professor Julie Pasco, due to privacy/ethical restrictions.

Ethics approval and consent to participate

Ethics approval was obtained from the Human Research Ethics Committee at Barwon Health. All participants provided informed, written consent.

Consent for publication

Not applicable.

Competing interests

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Publisher's Note

4.1.2 Manuscript IV

Chandrasekaran V, Stuart AL, Pasco JA, Brennan-Olsen SL, Berk M, Hodge JM, Samarasinghe RM and Williams LJ. Anticonvulsant use and fracture: a case-control study. *[Accepted for publication at J Musculoskelet Neuronal Interact on 14/02/2021]*

AUTHORSHIP STATEMENT

1. Details of publication and executive author

Title of Publication		**Publication details**
Anticonvulsant use and fracture: a case-control study		Accepted for publication at *J Musculoskelet Neuronal Interact* on 14/02/2021.
Name of executive author	**School/Institute/Division if based at Deakin; Organisation and address if non-Deakin**	**Email or phone**
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2. Inclusion of publication in a thesis

Is it intended to include this publication in a higher degree by research (HDR) thesis?	Yes	If Yes, please complete Section 3 If No, go straight to Section 4.

3. HDR thesis author's declaration

Name of HDR thesis author if different from above. (If the same, write "as above")	**School/Institute/Division if based at Deakin**	**Thesis title**
As above	IMPACT SRC, School of Medicine	Bipolar Disorder, Associated Treatments and Bone Health
If there are multiple authors, give a full description of HDR thesis author's contribution to the publication (for example, how much did you contribute to the conception of the		

project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)		
Under the guidance of my HDR supervisors, I conceptualised the methodology and drafted the initial manuscript, contributed to edits and approved final version		
I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.	**Signature and date**	28/05/2020 Signature Redacted by Library

4. Description of all author contributions

Name and affiliation of author	**Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)**
Amanda L Stuart, Deakin University	Conceptualised the research question, revised the methodology, performed data analyses, edited, revised and approved the manuscript.
Julie A Pasco, Deakin University	Designed the methodology, revised, edited, and approved the methodological process and the manuscript.
Sharon L Brennan-Olsen, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Michael Berk, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Jason M Hodge, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Rasika M Samarasinghe	Revised, edited, and approved the methodological process and the manuscript.

Lana J Williams, Deakin University	Conceptualised the research question, revised the search strategy and methodology, edited, revised and approved the manuscript.

5. Author Declarations

I agree to be named as one of the authors of this work, and confirm:

i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
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iii. that the description in Section 4 of my contribution(s) to this publication is accurate,
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Name and affiliation of contributor	**Contribution**	**Signature* and date**
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7. Data storage

The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

Data format	Storage Location	Date lodged	Name of custodian if other than the executive author
Microsoft Access Database	University Hospital, Geelong		Prof Julie Pasco

Accepted Article

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Original Article

Anticonvulsant use and fracture: a case-control study

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Abstract

Objectives: We aimed to investigate fracture risk associated with anticonvulsant use in a population-based sample of men and women. **Methods**: Data from 1,458 participants (51.8% women) with a radiologically confirmed incident fracture (cases) were compared to 1,796 participants (46.5% women) without fracture (controls). Lifestyle factors, medication use and medical history were self-reported. Associations between anticonvulsant use and fracture were explored using binary logistic regression following adjustment for confounders. **Results**: In men, fracture cases and controls differed in age, smoking history, education, alcohol use, and gonadal hormone supplementation. In women, fracture cases and controls differed by previous fracture history, alcohol use, physical activity levels and use of anti-fracture agents. After adjustment for age, pooled anticonvulsant use was associated with a 3.4-fold higher risk of fracture in men and a 1.8-fold higher risk in women. Following further adjustments for confounders these patterns persisted; a 2.8-fold higher fracture risk in men and a 1.8-fold higher fracture risk in women. **Conclusions**: Anticonvulsant use was associated with increased fracture risk, independent of demographic, lifestyle, medical and medication related factors. While further studies exploring potential underlying mechanisms are warranted, regular monitoring of bone health in anticonvulsant users with risk factors may be useful.

Keywords: Anticonvulsant, Fracture, Osteoporosis, Case-Control Study

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Introduction

Anticonvulsants are a mainstay in the treatment of several neurological and psychiatric illnesses, including epilepsy, migraines and bipolar disorder[1,2] due to their efficacy in dampening neuronal excitability[3] and their mood stabilising effects. Anticonvulsants are primarily, United States Food and Drug Administration-approved for the treatment of epilepsy[4], however off-label use of anticonvulsants makes up over 70% of patients taking one or more of the agents. Off-label use appears to be most common for newer agents, and in treating conditions such as bipolar disorder and chronic non-specific pain[4,5]. Prescription patterns in industrialised nations suggest that anticonvulsant use is common in children, but increases again in older adults, including those living in residential care (~10%)[6] and in those living in the community (~1%)[7].

Anticonvulsants are typically prescribed for several years, and in some cases, lifelong[8]. While effective, long-term anticonvulsant use cannot be uncoupled from their resulting adverse effects and potential metabolic imbalances. Long term use may lead to effects such as cardiovascular disease, osteoporosis and increased fracture risk, particularly among older generation anticonvulsants[9,10].

Osteoporosis is a chronic disease with complex and multifactorial pathogenesis, resulting in increased bone fragility[11]. In Australia, 12.4% of patients over the age of 50 years accessing general practice, predominantly women, had a diagnosis of osteoporosis[12]. Decreased bone mass often goes undetected until a fracture occurs[13]. A fracture thereafter increases the likelihood of subsequent fractures, and is associated with decreased quality of life, an augmented mortality risk, increased disability and related hospital admissions[14,15]. In Australia, hip fracture numbers increased by 53% for men and 4.4% in women from the mid-1990s to the mid-2000s[16]. Similarly, in a 2017 estimate, 1 in 4 men and 2 in 5 women in Australia aged 50 years and older were likely to experience an osteoporotic fracture during their lifetime, with an expected cost of approximately $33.6 million dollars over 10 years[17], thus highlighting the importance of fracture prevention.

Some existing studies have found that children and older adults taking anticonvulsants to treat neuropsychiatric illnesses such as epilepsy and bipolar disorder may have an increased risk of fracture; however, studies investigating the effect of anticonvulsant use in the general population and its effect on bone across the adult age range are limited[18]. Thus, we aimed to investigate whether anticonvulsant use is associated with fracture risk in a large population-based sample of men and women aged ≥20 years.

Materials and methods

Participants

This case-control study set within the Barwon Statistical Division (BSD), south-east Australia, utilised data from men and women aged ≥20 years participating in the PRedictors and Outcomes of incident FRACtures study (PROFRAC) and the Geelong Osteoporosis Study (GOS).

PROFRAC consists of 1,458 participants (51.8% women) who had a radiologically confirmed incident fracture between June 2012 and May 2013[19], and completed a comprehensive questionnaire sent by mail. Details of the recruitment of fracture cases has been published[19].

Control participants (fracture-free during June 2012-May 2013) were selected from the GOS, an ongoing, population-based study[20]. A sample consisting of 1,494 women (aged 20-94 years, 77% participation) was randomly-selected from electoral rolls within the BSD[20] between 1994 -1997, and 1,540 men (aged 20-93 years, 67% participation) were recruited between 2001-2006. Control data for this study were drawn from the 15-year follow-up for women (2011-2014) and the 5-year follow-up for men (2005-2006). A total of 1,796 participants (46.5% women) were identified as fracture-free controls.

All participants provided informed, written consent. Ethics approval was obtained from the Barwon Health Human Research Ethics Committee.

Measurement of outcome variable

Fractures were identified using a previously validated method of fracture ascertainment[21] and based on a daily keyword search of all radiological reports at the University Hospital Geelong. Only a participant's first fracture during the study period was recognised for this analysis; however, those with multiple fractures from a single event were classified separately, under *multiple fractures*.

Measurement of exposure variables

Participants self-reported current medication use and date of commencement. Use of anticonvulsants and other medication considered to affect calcium and bone metabolism (adrenal steroid hormones, gonadal hormones, thyroid hormones, anti-fracture agents) were classified for both cases and controls. Cases who started anticonvulsants post-fracture were considered non-users for these analyses.

Highest level of education was self-reported and categorised as primary school education, some secondary school, completed secondary school, Technical and Further Education (TAFE)/Trade/other and University. A five-point scale was utilised to determine current alcohol use. The categories included never, less than once per week, once or twice per week, several times per week or every day. Participants were categorised as active (very active or active) or inactive (limited activity in the home, or chair or bedridden), based on the Metabolic Equivalent of Task values[22,20]. Current cigarette smoking was self-reported. Date and site of any fractures occurring during adulthood was determined. Participants were classified as fallers if they had fallen to the ground at least once during the past 12

Table 1. Characteristics of fracture cases and controls for men and women, results displayed as median (interquartile range) or n (%).

	Men (n=1,664)			Women (n=1,590)		
	Cases n=703	Controls n=961	p	Cases n=755	Controls n=835	p
Age (years)	61.1 (46.3-78.4)	54.7 (39.1-69.5)	**0.012**	64.0 (50.6-74.6)	60.0 (44.4-72.5)	0.228
Education						
Primary school	18 (2.7%)	35 (3.7%)		49 (6.8%)	36 (4.4%)	
Some secondary school	184 (27.5%)	414 (43.1%)	**<0.001**	275 (38.3%)	312 (38.0%)	0.110
Completed secondary school	118 (17.7%)	157 (16.4%)		126 (17.5%)	133 (16.2%)	
TAFE/Trade/Other	235 (35.2%)	229 (23.9%)		133 (18.5%)	185 (22.5%)	
University	113 (16.9%)	125 (13.0%)		136 (18.9%)	156 (19.0%)	
Alcohol (current)						
Never	97 (14.1%)	109 (11.8%)		233 (31.7%)	214 (26.0%)	
Less than once per week Once	145 (21.1%) 223	223 (24.1%)	**<0.001**	184 (25.0%)	245 (29.8%)	**0.032**
or twice per week	(32.5%) 127	201 (21.7%)		139 (18.9%)	178 (21.6%)	
Several times per week	(18.5%)	201 (21.7%)		102 (13.9%)	115 (14.0%)	
Every day	95 (13.8%)	193 (20.8%)		78 (10.6%)	71 (8.6%)	
Prior Adult fracture	246 (36.5%)	318 (34.2%)	0.340	276 (37.7%)	155 (18.7%)	**<0.001**
Faller (12 months)	111 (16.1%)	172 (18.1%)	0.274	177 (23.7%)	224 (27.1%)	0.127
Smoker (current)	152 (21.8%)	108 (11.3%)	**<0.001**	95 (12.8%)	91 (11.0%)	0.291
Physically active (current)	522 (75.3%)	677 (70.9%)	0.050	449 (60.1%)	579 (70.9%)	**<0.001**
Physical conditions (current)						
Epilepsy	13 (1.9%)	14 (1.5%)	0.531	12 (1.6%)	6 (0.7%)	0.101
Bipolar Disorder	5 (0.7%)	4 (0.4%)	0.418	10 (1.3%)	5 (0.6%)	0.135
Medication Use						
Anticonvulsants	32 (4.6%)	18 (1.9%)	**0.001**	40 (5.3%)	24 (2.9%)	**0.014**
Adrenal steroid hormones	15 (2.1%)	10 (1.0%)	0.072	31 (4.1%)	23 (2.8%)	0.137
Gonadal hormones	9 (1.3%)	3 (0.3%)	**0.021**	36 (4.8%)	32 (3.8%)	0.357
Thyroid hormones	8 (1.1%)	10 (1.0%)	0.850	59 (7.8%)	61 (7.3%)	0.701
Anti-fracture agents	17 (2.4%)	17 (1.8%)	0.355	57 (7.6%)	28 (3.4%)	**<0.001**

months. History of epilepsy and bipolar disorder diagnoses were self-reported.

Statistical analyses

Minitab (Version 18; Minitab, State College Pa) was used to perform statistical analyses. Differences between fracture cases and controls were analysed using T-Tests for parametric data, Kruskal Wallis for non-parametric data and Chi Square for categorical data. Binary logistic regression (odds ratio, OR, with 95% confidence intervals, CI) was used to investigate the association between anticonvulsant use and fracture in separate models for men and women. Covariates tested in the models included age, education, physical activity, past adult fracture, falls in the past 12 months, smoking status, alcohol consumption, use of medications known to affect calcium and bone metabolism, duration of anticonvulsant use and self-reported epilepsy and bipolar disorder. Backwards stepwise regression techniques were used to determine the best model and all interactions were tested. Analyses were repeated following the removal of minor fractures (finger, thumb and toe) which did not change the results (data not shown).

Results

Men

There were 703 fractures cases (31 face/skull, 48 clinical vertebra, 42 rib, 10 pelvis, 38 clavicle/scapula, 65 forearm/humerus, 75 wrist, 132 hand/finger/thumb, 24 hip, 52 femur/patella/tibia/fibula, 62 ankle, 72 foot/toes and 52 multiple fracture sites) and 961 controls. Fracture cases and controls differed by age, education level, smoking, alcohol consumption and the use of gonadal hormones (Table 1). There were 50 men (3.0%) exposed to anticonvulsants; carbamazepine (n=13), gabapentin (n=11), phenytoin (n=11), sodium valproate (n=10), clonazepam (n=5), pregabalin (n=5), lamotrigine (n=5), levetiracetam (n=4), quetiapine (n=3), topiramate (n=2) and primidone (n=1). Median duration of use was 79.5 months (IQR 23.5 - 147.8). Exposure to anticonvulsants was documented for 32 of 703 (4.6%) cases and 18 of 961 (1.9%) controls (p=0.001).

Following adjustment for age, anticonvulsant use was associated with a 3.4-fold increased risk of fracture (OR 3.37, 95% CI 1.83-6.20, p<0.001). This relationship persisted after adjustment for past fracture, education,

smoking, alcohol consumption and use of adrenal steroid and gonadal hormones (OR 2.82, 95% CI 1.46-5.42, p=0.002). Further adjustment for smoking status, alcohol consumption, duration of use and diagnoses of epilepsy and bipolar disorder did not affect the findings.

Women

There were 755 fracture cases (18 face/skull, 56 clinical vertebra, 18 rib, 16 pelvis, 11 clavicle/scapula, 91 forearm/humerus, 154 wrist, 41 hand/finger/thumb, 61 hip, 44 femur/patella/tibia/fibula, 90 ankle, 105 foot/toes and 50 multiple fracture sites) and 835 controls. Fracture cases and controls differed by physical activity, alcohol consumption, a history of adult fracture and the use of anti-fracture agents (Table 1). There were 64 women (4.0%) exposed to anticonvulsants; sodium valproate (n=18), clonazepam (n=16), carbamazepine (n=10), pregabalin (n=9), quetiapine (n=9), gabapentin (n=9), lamotrigine (n=6), topiramate (n=4), levetiracetam (n=2), oxcarbazepine (n=1), primidone (n=1), phenytoin (n=1) and vigabatrin (n=1). Median duration of use was 56.5 months (IQR 11.3-152). Exposure to anticonvulsants was documented for 40 of 755 (5.3 %) cases and 24 of 835 (2.9%) controls (p=0.014).

Following adjustment for age, anticonvulsant use was associated with a 1.8-fold increased risk of fracture (OR 1.84, 95% CI 1.09-3.09, p<0.001). This relationship persisted after adjustment for past fracture, falls and activity level (OR 1.81, 95% CI 1.05-3.12, p=0.030). Further adjustment for duration of anticonvulsant use, education, smoking status, alcohol consumption, epilepsy, bipolar disorder and the use of anti-fracture agents, thyroid, gonadal or adrenal steroid hormones did not affect the findings.

Discussion

In this case-control study, current anticonvulsant use was associated with increased fracture risk in both men and women. Specifically, anticonvulsant use was associated with a 2.8-fold higher risk of fracture in men and a 1.9-fold higher fracture risk in women, with these findings persisting after demographic, lifestyle, medical and medication factors were taken into consideration.

While the association between anticonvulsant use and bone health has been noted since the 1970s[23], studies investigating anticonvulsant use and fracture in a population-based sample without indication are variable and limited. Numerous studies investigating bone health among anticonvulsant users included patient groups with epilepsy[24] and other conditions such as bipolar disorder[25] or Rett syndrome[26]. Vestergaard et al (2004) conducted a case-control study studying the association between anticonvulsant use and any fracture in the National Hospital Discharge Register in Denmark (n=124,655, 51.8% women); and found a modest increase in fracture risk in those using anticonvulsants compared to controls[27]. Similarly, Tsiropoulos et al (2008) conducted a case-control study investigating the effect of anticonvulsant use on hip fracture in those admitted for the treatment of hip fracture in Funen County hospitals in Denmark (n=7,557, >70% women), finding that anticonvulsant users were 1.3 times as likely to fracture compared to randomly selected, age- and sex- matched county residents (n=27,575)[10]. Cheng et al (2019) conducted a population study in National Health Insurance Research Dataset in Taiwan (aged ≥50, 63.6% women) to investigate fracture risk in those taking newer generation anticonvulsants[28]. They found that those taking some anticonvulsants such as carbamazepine, gabapentin and oxcarbazepine had a significantly higher fracture risk compared to age-, sex-, and comorbidity-matched controls, while other agents (ie phenytoin, phenobarbital, levetiracetam, valproic acid, topiramate and lamotrigine) did not. Similar to our findings, Jetté et al (2011) found that anticonvulsant use was associated with increased fracture risk in an older population-based sample (aged ≥50, 70.3% women)[29].

Confounding by indication is an important consideration in population-based studies investigating effects of medications[30]. Previous research has shown that epilepsy, independent of anticonvulsant use was associated with an increased fracture risk[31-33]. Recent research findings suggest that the observed increased risk of fracture in those with epilepsy is likely multifactorial, and possibly due to increased falls due to seizure activity, and potentially, anticonvulsant use[34]. Interestingly, there was no difference between the cases and controls in regards to 12 month falls history in this study. Bipolar disorder has also been independently associated with increased fracture risk[9,35]. Recently, we conducted a systematic review to evaluate the current evidence base investigating the association between bipolar disorder and bone health. Our results indicated bipolar disorder was associated with increased fracture risk (range 20-80%), independent of age, sex, comorbidities and medication use[25]. Although, in the current study, consideration of self-report epilepsy and bipolar disorder did not significantly affect our findings.

Research investigating the association between anticonvulsant use and fracture risk has largely been in women-only samples, or those with a higher percentage of women (>60%); potentially due to the current consensus that fracture risk is higher in women than men[36]. Scane et al (1999) conducted a case-control study in men referred to the Bone Clinic in Newcastle in the United Kingdom, and found that patients taking anticonvulsants (n=182, aged 27-79) had a 6.1-fold increased chance of developing a vertebral fracture compared to non-users[37]. Further exploration into potential mechanisms that might explain these sex differences is warranted.

Although not tested in the current study due to power constraints, previous research suggests that some newer anticonvulsants are potentially less detrimental to bone[9]. Anticonvulsants are structurally heterogeneous and differ in mode of action. Therefore, between group differences in major adverse events are an important factor in

prescription decisions. A meta-analysis investigating the association between anticonvulsant use and fracture risk, found that individual anticonvulsants affected bone differently; with the specific agents, topiramate, phenytoin and phenobarbiturate having a 39%, 70% and 78% increase in fracture risk, respectively[24]. All three agents are cytochrome P450-inducing, which comprise a class of anticonvulsants known to upregulate enzymes involved in vitamin D metabolism[38]. These enzymes convert 25-hydroxy vitamin D (25(OH)D) into inactive metabolites that lead to calcium resorption with consecutive secondary hyperparathyroidism[39]. This unavailability of vitamin D is thought to lead to reduced bone mineralisation, which, when coupled with compensatory increases in parathyroid hormone (PTH) production, could lead to increased bone resorption and a net low bone turnover state[40,41]. A study investigating whether enzyme-induction sufficiently explained anticonvulsant-related bone loss at the hip in a community-dwelling sample of older men (n=4,222, aged>70 years) found, however, that non-enzyme-inducing anticonvulsants were independently associated with increased rates of hip bone loss compared to non-use; gabapentin in particular had a 1.4- to 1.8-fold higher adjusted rate of loss compared to non-users[42].

Pre-clinical studies investigating the association between anticonvulsants and bone loss are supportive of the clinical findings but are limited in number. In an osteoblast-like cell line, Humphrey et al. (2013) found that treatment with valproic acid significantly decreased the concentration of two important bone proteins, osteonectin and collagen I, while leaving over a thousand other proteins unaffected[43]. Other studies on osteoblast[44] and fibroblast[45] cell lines suggest that valproic acid may impact cytoskeleton arrangement. Similarly, in a microarray analysis of mouse embryos, valproic acid was shown to alter the microtubule cytoskeleton and actin filaments, and is implicated in teratogenic skeletal phenotypes[46].

Both strengths and limitations are present. The strengths of this study include the wide age range, sample size and ability to test a number of potentially confounding variables. In addition, this study investigated the association between fracture and anticonvulsant use irrespective of indication, which is significant, given its broad utility. Furthermore, both the cases and controls were drawn from the same region and the fracture ascertainment is considered the gold standard. Limitations of this study include the inability to perform subgroup analysis of specific anticonvulsant agents and fracture subtypes due to power constraints and explore potential underlying biological mechanisms such as Vitamin D status. As vertebral fractures can be asymptomatic and remain undiagnosed radiologically, we note that only clinical vertebral fractures were identified. Last, any potential unidentified confounding factors may affect our findings.

Conclusion

In conclusion, anticonvulsant use was found to be associated with an increased fracture risk in both men and women independent of a range of demographic, lifestyle, medical and medication-related factors. Potential mechanisms are yet to be explored; however, this work supports others in suggesting a cautious approach and regular monitoring of bone health in those prescribed anticonvulsants.

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Authors' contributions

VC, ALS and LJW conceptualised the study and collaboratively wrote the first draft of this manuscript. ALS performed statistical analyses. All authors reviewed and contributed to the intellectual content in this manuscript, approved the final version and guarantee this work.

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Part C: Bone cell in vitro studies

4.1.3 Manuscript V

Chandrasekaran V, Hodge JM, Samarasinghe RM, Pasco JA, Berk M, and Williams LJ. Comparison of the effects of anticonvulsants on osteoclast and osteoblast formation and function. *[Submitted to: Biol Psychiatry.]*

AUTHORSHIP STATEMENT

1. Details of publication and executive author

Title of Publication		**Publication details**
Comparison of the effects of anticonvulsants on osteoclast and osteoblast formation and function.		Submitted to *Biol Psychiatry*.
Name of executive author	**School/Institute/Division if based at Deakin; Organisation and address if non-Deakin**	**Email or phone**
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2. Inclusion of publication in a thesis

Is it intended to include this publication in a higher degree by research (HDR) thesis?	Yes	If Yes, please complete Section 3 If No, go straight to Section 4.

3. HDR thesis author's declaration

Name of HDR thesis author if different from above. (If the same, write "as above")	**School/Institute/Division if based at Deakin**	**Thesis title**
As above	IMPACT SRC, School of Medicine	Bipolar Disorder, Associated Treatments and Bone Health
If there are multiple authors, give a full description of HDR thesis author's contribution to the publication (for example, how much did you contribute to the conception of the		

project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)		
Under the guidance of my HDR supervisors, I conceptualised the research question, and used previously published methodology to conduct experiments. I drafted the initial manuscript, contributed to edits and approved final version.		
I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.	**Signature and date**	13/07/2020 Signature Redacted by Library

4. Description of all author contributions

Name and affiliation of author	**Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)**
Jason M Hodge, Deakin University	Designed the methodology, revised, refined, and approved the methodological process and the manuscript.
Rasika M Samarasinghe, Deakin University	Conceptualised the research question, revised and refined the methodology, edited, revised and approved the manuscript.
Julie A Pasco, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
Michael Berk, Deakin University	Revised, edited, and approved the methodological process and the manuscript.
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5. Author Declarations

I agree to be named as one of the authors of this work, and confirm:

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I agree to be named as a non-author contributor to this work.

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Comparison of the effects of anticonvulsants on osteoclast and osteoblast formation and function.

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Abstract

Background: Chronic anticonvulsant use can lead to secondary osteoporosis, marked by microarchitectural deterioration of bone and decreased bone mineral density. Mechanisms of action of this broad class of drugs on bone remain unclear. The actions of four commonly prescribed anticonvulsants, valproic acid (VPA), carbamazepine (CBZ), lamotrigine (LMT) and gabapentin (GBP) were assessed on human osteoclast (OC) and osteoblast (OB) differentiation.

Methods: OC precursors were cultured on dentine slices in the presence of anticonvulsants for 7-14 days and parameters of OC formation and resorption were quantified in addition to expression of key osteoclastogenic genes (CTSK, NFκB1, NFATc1, CSF1R and OC/DC STAMP). Likewise, OB precursors cultured for 7-21 days were assessed for alkaline phosphatase (ALP) and bone mineralisation, respectively.

Results: VPA promoted OC fusion at high concentrations (100μM), compared to CBZ and LMT, which inhibited all OC parameters at this concentration, whereas GBP had no impact up to supraphysiological concentrations (1mM). All four drugs dose-dependently inhibited early OB differentiation (ALP), but with order of potency: VPA>CBZ>LMT followed by GBP, which was 100-fold less potent. Only VPA demonstrated capacity to inhibit extracellular matrix mineralisation by OB.

Conclusions: Comparing classical and newer generation anticonvulsant drugs utilising *in vitro* models of human osteoclast- and osteoblasto- genesis demonstrated intra-class differences. Data suggest that VPA may uncouple bone remodelling, CBZ and LMT may decrease net bone turnover, while GBP appears to have little to no effect on bone cells *in vitro*.

INTRODUCTION

Anticonvulsants comprise a broad class of drugs that have a long history of use in treating neurological and psychiatric illnesses, such as epilepsy, migraine, anxiety and pain as well as bipolar disorder (1, 2). Overarching findings support that anticonvulsants act to dampen excessive firing of neurons in the brain; for instance, VPA acts as an agonist on the glutaminergic and GABAergic neuronal pathways (3, 4), and/or directly block calcium, sodium and potassium voltage-gated channels, thereby reversing their neuroexcitatory effects (5).

Differential interaction of anticonvulsants with off-target intracellular signalling pathways and/or co-prescribed medications (6) lead to unintended side effects commonly seen in first-generation anticonvulsants such as VPA and CBZ. These agents contribute to a noxious side-effect profile (7) including hepatotoxicity, cognitive decline, metabolic disturbances and movement and behavioural disorders. For example, a dysfunctional calcium metabolism can affect a number of systems such as the central nervous and the musculoskeletal systems (8). This observed toxicity led to their replacement with relatively benign alternatives, such as GBP and LMT (9-12), which are considered safer and are associated with fewer drug-drug interactions and a predictable dose response, potentially due to more specific molecular targets (4).

Chronic anticonvulsant use can lead to secondary osteoporosis, marked by progressive microarchitectural deterioration of bone and decreased bone mineral density (BMD). Cytochrome P450-inducing anticonvulsants (often first-generation; such as VPA and CBZ) are associated with pronounced osteodegenerative effects, owing to the upregulation of enzymes involved in vitamin D metabolism, which convert 25-hydroxy vitamin D (25(OH) vitamin D) into inactive metabolites that lead to calcium resorption with consecutive secondary hyperparathyroidism (13). A key determinant of BMD homeostasis, this unavailability of

vitamin D is thought to lead to bone loss. It has been previously estimated that 50% of patients taking anticonvulsants develop clinical or subclinical bone disorders, and therefore, the impact of long-term anticonvulsants use on bone health is a concern (12, 14, 15).

Bone is a multicellular organ involved in a constant, dynamic process of renewal called bone remodelling, mediated by cells specialised in bone resorption (osteoclasts; OC) and formation (osteoblasts; OB). Bone balance is driven by OB-derived osteocytes, which are embedded in bone (16) and intricately regulate molecular mechanisms within the bone microenvironment that determines overall strength and health. Animal studies, conducted primarily in rodents, demonstrate that anticonvulsants exert differential effects on bone health, and warrant further exploration. Rats treated with a low dose of levetiracetam, a second-generation anticonvulsant, had lower bone strength at the femoral neck, while bone mineral content and bone mass remained unaffected (17). When levetiracetam was administered to 16 orchidectomized Wistar rats, reductions of BMD and bone mineral content were seen at the femur (18). Further *in vivo* (19-22) and *in vitro* (23-26) studies have contributed to the controversy surrounding actions on bone, suggesting that individual anticonvulsants within the broader class may have differential effects that warrant further investigation.

At present, only one other study has investigated direct actions of anticonvulsants in human bone cell models (27). In addition, while earlier findings suggested that first-generation anticonvulsants had more deleterious actions on bone than second-generation anticonvulsants (28), more recent studies showed that this distinction is not as clear as initially stated (27, 29, 30). The aim of the present work was to compare the direct effects of two commonly used first-generation anticonvulsants, VPA and CBZ with two second-generation anticonvulsants, GBP and LMT, on human OC and OB formation and function using primary human *in vitro* cell models.

MATERIALS AND METHODS

Materials

Dulbecco's Modified Eagle Medium (DMEM), paraformaldehyde, fast Garnett GBC, penicillin/streptomycin, p-nitrophenyl, p-nitrophenyl phosphate, naphthol AS-BI-phosphate, diethanolamine, alizarin red (ALZ), cetylperidinium chloride (CPC), trypsin, Triton X-100, VPA, GBP, CBZ and LMT were purchased from Sigma-Aldrich (Sydney, Australia). Gibco Minimum Essential Medium (MEM), fetal bovine serum (FBS) and nonessential amino acids (100X) were purchased from Thermo-Fisher Scientific (Melbourne, Australia). Ficoll-Paque was obtained from Pharmacia Biotech (Uppsala, Sweden), and MethoCult GF H4534 (Iscove's modified Dulbecco's medium containing 30% FBS, 1% bovine serum albumin, 1% methylcellulose, 10^{-4} mol/L 2-mercaptoethanol, 2 mmol/L L-glutamine, 50 ng/mL stem cell factor, 10 ng/mL interleukin-3 and 10 ng/mL recombinant human granulocyte/macrophage-colony stimulating factor) was obtained from Stem-Cell Technologies (Melbourne, Australia). Soluble RANKL158–316-GST fusion protein (RANKL) was produced in-house from a construct kindly supplied by Dr. F. Patrick Ross (Hospital for Special Surgery, NY) as previously described (31). All other reagents were analytical grade.

Ethics

Healthy participants undergoing elective abdominoplasty provided informed, written consent for the use of human adipose tissue sections for this work. Similarly, human umbilical cord blood was collected from healthy donors. Ethics approvals for both protocols were granted by the Barwon Health Human Research Ethics Committee and the Deakin University Human Research Ethics Committee.

Quantification of *in vitro* osteoclastogenesis and resorption

Human cord blood collection, isolation of a mononuclear cell fraction, expansion of colony forming unit- granulocyte/macrophage (CFU-GM)-derived OC precursors, differentiation into mature human OC and quantification of bone resorption were conducted as previously described (32). Cell culture medium (MEM) containing 10% FBS, 50 U/mL penicillin, 50 µg/mL streptomycin, nonessential amino acids, 3 mmol/L L-glutamine, 25 ng/mL macrophage-colony stimulating factor and 125 ng/RANKL was used to culture cells in the presence or absence of five concentrations (10^{-3}-10^{-7}M) each, of four anticonvulsants, namely VPA, CBZ, LMT and GBP for 14 days. Drugs were reconstituted in water (VPA and CBZ), or DMSO (LMT and GBP), respectively and appropriate vehicle controls were included in treatment protocols. Cells were fixed in 1% paraformaldehyde and tartrate-resistant acid phosphatase activity was measured. OC formation and capacity to resorb bone (the defining quality of a differentiated OC) were assessed by transmission light microscopy and quantified using microcomputer image analysis software (MCID, Imaging Research Inc., Ontario, Canada). The output provided by the software included OC number, total plan area and resorption. Mean OC size was determined by dividing total plan area per dentine slice by OC number, and resorption per OC was similarly determined by dividing total resorbed area/dentine slice by OC number. Percentage increase or decrease provided in text was calculated relative to control values.

Primary human OB assays

Collagen digestion of blunt-dissected human adipose tissue released mesenchymal stem cells (MSC), which were isolated and expanded, as previously described (33). Adherent MSC were seeded in 6 mm diameter culture wells in DMEM, at 10^4 cells/well, and differentiated towards an OB cell lineage, by the replacement of DMEM with osteogenic medium containing 100µM

ascorbate-2-phosphate, 10mM β-glycerophosphate and 10nM dexamethasone. Cells were cultured for 14-21 days in the presence or absence of anticonvulsants, to obtain the same treatment-specific concentration ranges as described for the OC assays (10^{-3}-10^{-7}M). Drugs were reconstituted in water (VPA and CBZ), or DMSO (LMT and GBP), respectively and appropriate vehicle controls were included in treatment protocols, in addition to hOSM, which served as a positive control (33, 34). Media was changed every 7 days and drug treatments were replaced twice weekly as 20 µL additions of 100x stock, after which ALP (an early OB marker) activity was measured on day 7 and mineralisation was quantified through alizarin red (ALZ) staining upon completion of the culture period.

Quantification of ALP activity

Cellular ALP activity was measured at day 7 of treatment when cells were lysed in 0.1% Triton X-100 for 30 minutes at room temperature. 20 µL of lysates were added to 180 µL of a pre-warmed solution containing 10 mg/mL p-nitrophenylphosphate (pNPP) in 10% v/v diethanolamine buffer (0.5mM $MgCl_2$; pH 9.8). Optical densities (OD) of samples were measured at 410 nm at 37˚C for 30 minutes, at 2.5-minute intervals, using a Tecan Genios Pro photo-spectrometer. Results were converted to standard international units (SIU), corresponding to the conversion by ALP, of 1mM of pNPP to p-nitrophenyl (pNP) per minute. A standard curve was generated by the serial dilution of 1mM pNP in diethanolamine buffer, and data presented as relative SIU.

Quantification of *in vitro* mineralisation by Alizarin Red (ALZ) staining

Mineralising OB (day 21 of culture) were fixed in 1% paraformaldehyde in phosphate-buffered saline for 30 minutes at room temperature and stained with 40mM ALZ (pH 4.2) for 15 minutes at room temperature. Wells were washed 4-6 times to remove background staining, and then solubilised in 3% CPC in 20mM sodium phosphate buffer for 45 minutes. Solubilised solutions

were then transferred to new 6 mm diameter wells and OD measured at 570 nm, and results converted to $CaCl_2$ mg/well. A standard curve was generated by the serial dilution of 1mM ALZ in 3% CPC and similar OD measurements taken. $CaCl_2$ mg/well corresponds to the molar equivalent of ALZ to calcium (1:1); 1mM (mmol/L) represents 22.196ng/L $CaCl_2$.

Quantitative real-time polymerase chain reaction analysis

Since the highest effects on OC formation and activity was observed at higher concentrations, total RNA was isolated from CFU-GM-derived OC precursors that had been cultured in the presence of VPA and GBP for 14 days at 10^{-4}M or CBZ and LMT for 14 days at 10^{-5}M. Treated cells were lysed by the direct addition of Trizol, and reverse transcribed to cDNA using the Superscript® III First Strand Synthesis SuperMix system (Life Technologies) as per manufacturer's instructions. For each treatment RNA was extracted separately from 3 wells of a 6-well plate and quantitative Real-time PCR was performed in duplicate.

To quantify the expression of human OC genes (CTSK, NFκB1, NFATc1, CSF1R and OC STAMP/DC STAMP), we employed Real-time PCR analysis of the cDNA in a 7500 Fast Real-Time PCR System (Applied Biosystems), using TaqMan® Gene Expression Assays (Applied Biosystems Hs00166156 (CTSK), Hs00231653 (NFκB1), Hs00232342 (NFATc1), Hs00234622 (CSF1R), Hs00875776 (OC STAMP) and Hs00984780 (DC STAMP). Relative gene expression units were determined using the formula 2-ΔCtx1000, where ΔCt values represent the difference between the Ct of the gene of interest and GAPDH (amplified using Taqman chemistry with forward primer (5'-gacaggatgcagaaggagattact-3'), reverse primer (5'-tgatccacatctgctggaaggt-3') and the probe (Fam-atcattgctcctcctgagcgcaagtactc-Tamra).

Statistical analyses

Data are expressed as the mean ± SEM where applicable. Differences between groups were determined using either one-way ANOVA followed by Fisher's multiple comparison test or

two-way ANOVA general linear model followed by Fisher's pairwise comparisons. Statistical significance was set at $p \leq 0.05$. Groups and drug concentration curves with annotations that do not contain the same letter were considered significantly different.

RESULTS

Anticonvulsants differentially inhibit OC formation and bone resorption

To determine the effect on OC formation and bone resorption induced by anticonvulsants, OC cells treated with different concentration of drugs were assessed. It was observed that VPA showed no effect on OC formation or resorption over the concentration range (0.1-10μM; Figure 1.1), however, at 100μM, OC number was decreased by 25.9% ($p=0.001$), but with a corresponding increase in OC size and OC total plan area [118.6% ($p<0.001$) and 62.9% ($p=0.013$)], respectively. These large, TRAP+ve, multinucleated OCs were clearly evident on dentine slices, with diameters up to 200 μm and plan areas up to 30,000 μm^2, compared to OCs formed under control conditions of 75 μm and 4,000 μm^2, respectively (Figure 1.1A-C;F). Total resorption was not affected at day 14 of culture, but resorption per OC, reflecting the activation state of mature OCs, was increased by 43.5% ($p=0.011$) at this concentration, with large resorption pits that corresponded with larger OCs evident (Figure 1.1D-F). Cell toxicity was observed at the highest concentration investigated (1mM; data not shown). In comparison, both CBZ and LMT had no effect on any parameter at doses ≤10μM (except for a reduction in total resorption of 33.8% caused by LMT at 10μM), whereas both OC formation and resorption were reduced by ~90% ($p<0.001$) at 100μM for both drugs (Figure 1.2 & 1.3). Both CBZ and LMT were cytotoxic at 1mM, due to the concentration of carrier (DMSO) exceeding 2% v/v of the culture media. GBP, which was water-soluble, had no effect on OC formation and resorption up to a concentration of 1mM (Figure 1.4).

Effects of anticonvulsants on gene expression in OC

To assess whether the anticonvulsants affected OC-associated gene expression, OC precursors were treated with VPA and GBP at 100µM, or CBZ and LMT for 10µM for 7-14 days. Real-time PCR analysis demonstrated that M-CSF receptor expression was unaffected by any drug at 7 days (Figure 2.1A). Expression of NFκB (subunit 1) remained unchanged across the 14-day culture period, both by RANKL alone (Ctrl), and in the presence of anticonvulsants (Figure 2.1B). Treatment with RANKL alone resulted in a sustained increase in NFATc1 expression by 156.9% (p=0.055) and 53.3% (p=0.055) at day 7 and day 14, respectively, but this expression profile with time was not altered by co-treatment with anticonvulsants (Figure 2.1C). Similarly, CTSK expression was dramatically upregulated by 995.6% (p=0.002) at day 7 and further increased by 2805% (p=0.002) at day 14 by RANKL alone, but again, remained unaffected by co-treatment with anticonvulsants (Figure 2.2A).

Neither OC STAMP nor DC STAMP expression levels were altered by co-treatment with VPA at 100µM, a concentration previously demonstrated to increase OC size, although there was a suggestion of a ~100% increase in DC STAMP at day 7 of treatment compared to RANKL alone (non-significant. Figure 2.2B&C).

Anticonvulsants differentially and dose-dependently inhibit OB ALP and mineralisation

All four anticonvulsants demonstrated the capacity to dose-dependently inhibit the production of ALP in osteoblastogenic cells (Figure 3A). Culture media containing osteogenic factors significantly increased the production/activity of ALP compared to basal media (DMEM) alone after 7 days of culture. Co-treatment with hOSM (0.5 ng/mL; stimulator of osteoblastogenesis) increased ALP above osteogenic media control levels by ~60% (data not shown). VPA, CBZ and LMT reduced ALP by 66.8%, 53.4% and 43.8% (p<0.001), respectively, at 10µM, whereas a maximal reduction of 51% by GBP required a 100-fold higher concentration (1mM; Figure

3 A). Mineralisation of extracellular matrix, determined at day 21, was not affected by CBZ, LMT or GBP, whereas VPA reduced Ca^{2+} deposition by ~30% ($p<0.001$) at all concentrations tested (Figure 3B).

DISCUSSION

Anticonvulsants are a popular choice in treating neurological and psychiatric illnesses, including epilepsy, migraine, bipolar disorder and pain. While effective due to a broad-spectrum of action, anticonvulsants also contribute to several unintended off target effects, particularly concerning bone homeostasis. Research investigating the direct effects and mechanism of action of anticonvulsants on OC and OB is limited, but essential, owing to their use in treating diseases such as bipolar disorder, among several others, which may independently affect bone (35, 36).

This study investigated the actions of four anticonvulsants on the processes of human OC and OB formation and activity from primary, precursor cells. First-generation drugs VPA and CBZ, and second-generation GBP and LMT displayed varying actions and potencies. VPA promoted OC fusion at high concentrations (100μM), compared to CBZ and LMT that inhibited all OC parameters at this concentration, whereas GBP had no impact up to supraphysiological concentrations (1mM). All four drugs dose-dependently inhibited early OB differentiation (ALP), but with order of potency: VPA>CBZ>LMT followed by GBP, which was 100-fold less potent. Only VPA demonstrated capacity to inhibit extracellular matrix mineralisation by OB.

Epidemiological, biochemical and preclinical studies suggest a link between anticonvulsant use and bone health. Prolonged use, drug-drug interactions, and interactions with endogenous metabolic pathways such as hepatic enzyme induction and associated suppression of serum vitamin D levels have been implicated in poor bone health (4, 10, 11, 12, 37-38). Mezuk et al (2010) investigated anticonvulsant use and fracture risk in a population of older adults

(n=67,387). They found that anticonvulsant users were ~2.4-fold times more likely to develop a fracture and that anticonvulsant use (vs. never use), current use, and longer duration of use was associated with a higher risk of fracture (39). A review of articles investigating the effect of anticonvulsants on BMD and/or fracture in adults, between 1990-2009 reported similar findings (11). In addition, radiologic and biochemical evidence from the 1960s that investigated anticonvulsant-linked bone loss in epileptic populations identified abnormal vitamin D metabolism, increased bone turnover and decreased BMD in anticonvulsant users compared to nonusers (29, 38, 39-41). Further, a large cohort study (n=138,667) recommended that postmenopausal women using anticonvulsants should be considered a higher risk to fall and/or develop a fracture (38).

Many factors contribute to determining dosage of anticonvulsants including side-effect profile, concurrent medical treatments, spectrum of clinical activity, comorbidities, age and body weight, therefore dose may vary widely for different individuals (4). Bioavailability is further affected by genetic polymorphisms, which modulate drug metabolism and extensive plasma protein binding (primarily albumin) may also affect the overall plasma concentration achieved *in vivo* (4). Second-generation anticonvulsants were designed to overcome this highly variable bioavailability and clearance of first-generation anticonvulsants. For instance, LMT exhibits a first-order linear relationship between daily dose and steady-state concentration (43), while VPA, CBZ (44) and GBP (45) exhibit non-linear pharmacokinetics, and follow a less predictable pattern of absorption and elimination. Although actions of anticonvulsants on OC and OB in this present study were observed at concentrations higher than that typically observed in circulating plasma, actual concentrations achieved in the bone microenvironment and bone marrow plasma may be much higher. For example, fluorinated selective serotonin reuptake inhibitors, fluvoxamine and fluoxetine sequester in the bone microenvironment at

concentrations approaching 100µM, an order of magnitude higher than circulating plasma levels (46).

In the present study VPA stimulated OC fusion at high concentrations, but inhibited OB differentiation in a more sensitive, dose-responsive manner. The larger, more active OC did not increase total resorption when measured at two weeks but indicated that the potential for excessive resorption may exist after longer periods. OC typically contain 2-10 nuclei but can dramatically increase in size at sites of high OC activity in diseases associated with increased resorption, such as rheumatoid arthritis (47), periodontal disease (48, 49) and end-stage renal disease (50). OC containing up to 100 nuclei can be seen in diseases associated with excessive resorption (51, 52). VPA did not impact RANKL-activated expression of the M-CSF receptor and transcription factors NFκB1 and NFATc1 (early markers of osteoclastogenesis), or CTSK, a marker of mature OC resorptive function (53). Although VPA (100µM) increased OC size (in conjunction with a reduction in OC number), neither OC STAMP nor DC STAMP, genes required for pre-OC fusion (54) were altered at day 7. This finding, however, does not exclude these genes from being regulated by VPA at earlier time points in OC differentiation than those investigated in the current study.

Previous studies on OB (25) and fibroblast (26) cell lines have demonstrated that VPA can impact cytoskeleton arrangement. Similarly, a microarray analysis of mouse embryos showed that VPA alters the microtubule cytoskeleton and actin filaments and may be implicated in teratogenic skeletal phenotypes (23). There has been only one similar study investigating anticonvulsants in human bone cells. Rocha et al (2019) reported biphasic effects of VPA on OC and OB formation. At low concentrations (0.1µM and 1µM, respectively), both OC and OB formation was stimulated, whereas concentrations above 10µM inhibited both cell types (27). Although this study also employed human primary cells, OC precursors were derived from adult peripheral blood compared to umbilical cord blood and OB precursors from bone

marrow stem cells compared to adipose tissue derived stem cells in the present study, which possibly accounted for differences in responses observed with VPA. Regardless, the over-arching observations reported here demonstrate the capacity for VPA to increase bone resorption with a corresponding decrease in bone formation. In earlier studies, VPA was shown to stimulate the proliferation and differentiation of the mouse OB cell line MC3T3-E1 (25), however it decreased the proliferation of a mouse mesenchymal stem cell line C3H10T1/2 (56) and decreased the concentration of two important bone proteins, osteonectin and type 1 collagen in human OB like cell line hFOB1.19, while leaving over a thousand other proteins unaffected (56, 57). Taken together, these previous studies and our current findings support a potential role for VPA in negatively modulating bone homeostasis. Recently, a meta-analysis of 18 studies investigating the effects of VPA on BMD and bone metabolism found that VPA markedly decreased BMD at the lumbar spine and femoral neck, although these changes were accompanied with an increased bone-specific ALP (58), demonstrating the complex interactions that are likely occurring between VPA and OC and OB in the bone microenvironment.

Both CBZ and LMT exhibited similar actions in the current study: inhibiting both osteoclastogenesis and OB ALP activity. Consistent with our findings, CBZ decreased BMD via a modest inhibition of OB differentiation of OB-like cells sourced from bone specimens collected during orthopaedic surgery (24). Preclinical studies in rats remain equally contentious, where one study found CBZ to be benign (59), while another study reported that CBZ altered serum levels of Wnt inhibitors in ovariectomised female rats, suggesting that diminished oestrogen levels and altered Wnt expression may partly explain the observed bone loss (20). Further, a meta-analysis of 22 epidemiological studies suggest that CBZ significantly decreased vitamin D and calcium levels, elevated ALP levels and had no significant effects on BMD (60).

Studies investigating LMT in laboratory and clinical settings are limited, and existing evidence is conflicting. Rocha et al (2019) demonstrated that LMT inhibited OC and OB formation (27), while other studies suggest that LMT is benign to bone (10, 61, 62). In this present study, LMT modestly affected OC activity at 10µM, but dramatically decreased all OC parameters (except size) at 100µM. Further, LMT significantly decreased OB formation across all concentrations, compared to controls. Kanda et al (22) found that LMT did not affect bone metabolism in rats, and a recent study in a small epileptic population had similar findings (63).

Observed reductions in OC and OB formation and function seen at higher concentrations of CBZ and LMT in the current study may potentially lead to a low bone turnover state *in vivo*; a condition associated with numerous metabolic disturbances that could result in bone loss and nontraumatic fractures. For instance, chronic kidney disease that contributes to a dynamic bone disease resulting in diminished cancellous bone volume (64) and idiopathic osteoporosis in younger men (65). Consequently, long-term exposure to CBZ and LMT may culminate in bone loss due to a net low bone turnover state.

GBP was the least toxic of the four drugs tested in this study; it did not significantly affect any of the OC parameters but appeared to inhibit OB formation dose-dependently, albeit at much higher concentrations. GBP is considered to have fewer off-target effects and is approved for use as an analgesic or in the adjunctive treatment of partial seizures with or without secondary generalisation (12). Simko et al (19), found that GBP did not affect BMD or mechanical bone strength in rats, however, Kanda et al (22) showed that GBP induced the rarefaction of cancellous bone in rats, which was linked with enhanced bone resorption and decreased bone formation, which in turn suggested a potential decline in bone strength and BMD with chronic use (21). Similarly, two population studies conducted in older adults reported that GBP use was associated with a ~1.7-fold increased risk of fracture (66, 67), in particular, loss of bone at the hip and lumbar spine was observed in adult epileptic populations (61).

In summary, we compared the effects of anticonvulsants on bone cells, and found that the tested anticonvulsants displayed differential effects. VPA stimulated OC fusion while inhibiting OB ALP and mineralisation, actions that may promote an increase in bone turnover *in vivo*. CBZ and LMT inhibited both OC and OB, suggestive of a potential net low bone turnover state *in vivo*, whereas GBP was essentially benign, except at supra-physiological concentrations. Given their wide use for FDA-approved and off-label purposes (68, 69), drug-drug interactions (70), drug-induced comorbidities, potential for polytherapy and chronic use, it is essential that anticonvulsants are prescribed with caution. Considering that epidemiological and basic evidence indicate a link between anticonvulsant use and bone loss, patients taking anticonvulsants may benefit from risk assessment, regular monitoring via bone density scans and/or other treatments to prevent bone fragility.

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DISCLOSURES

Conflict of interest

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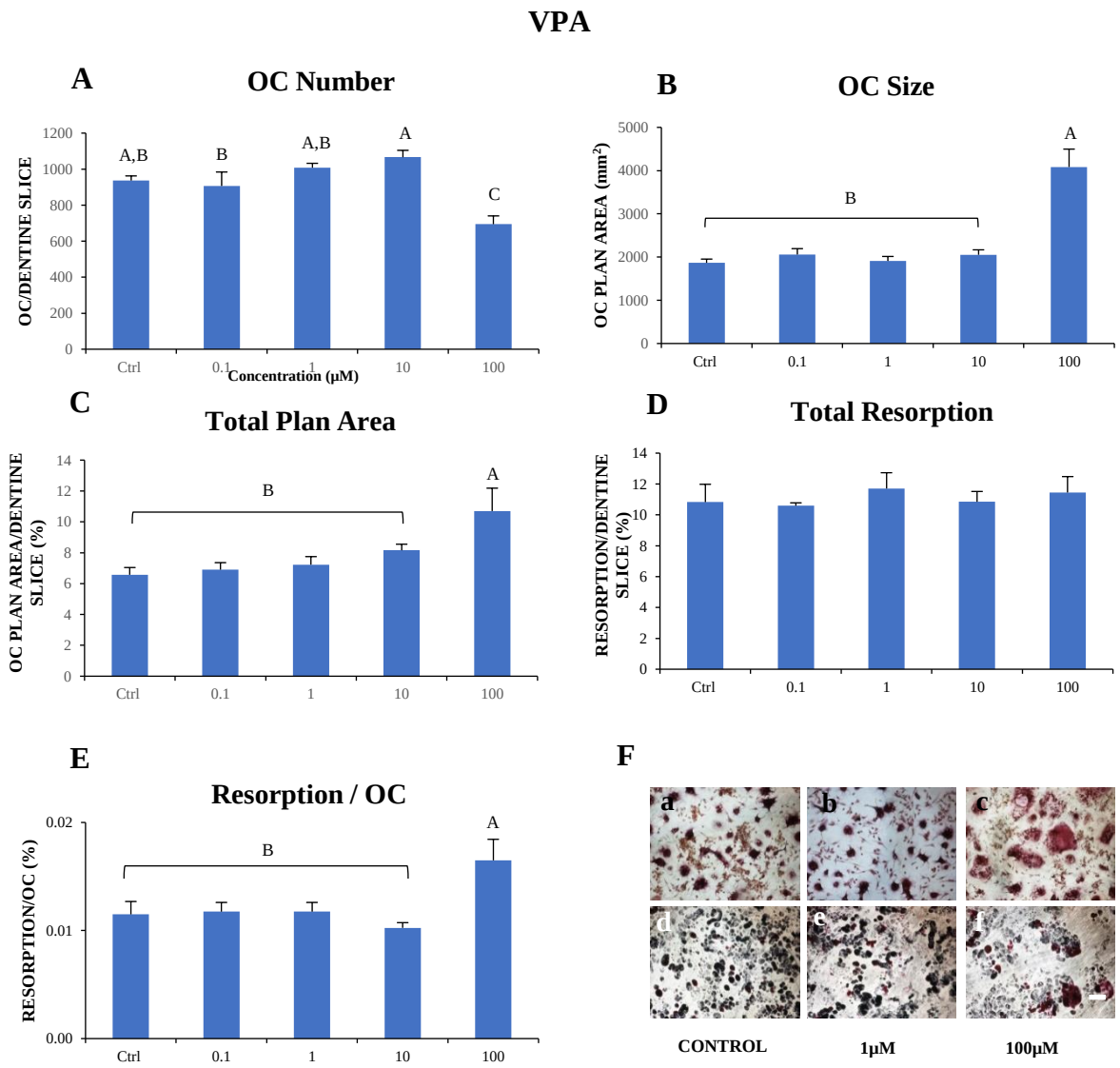

Figure 1.1. Effect of VPA on OC formation and resorption. CFU-GM-derived cells were cultured in RANKL (125 ng/mL) and M-CSF (25 ng/mL) and co-treated with various concentrations (0.1-100µM) of VPA for 14 days. The effects on **(A)** OC number, **(B)** OC size, **(C)** total plan area, **(D)** resorption capacity, and **(E)** individual OC activity were assessed. Data are presented as mean ± SEM (n=4 dentine slices/concentration). Groups with different superscripts are significantly different; $p \leq 0.01$; one-way ANOVA with Fisher's Multiple Comparison test. **(F)** Representative photomicrographs demonstrating the effects of VPA on OC formation (a–c) and resorption (d-f). Scale bar = 100 µm. Representative data from two independent experiments.

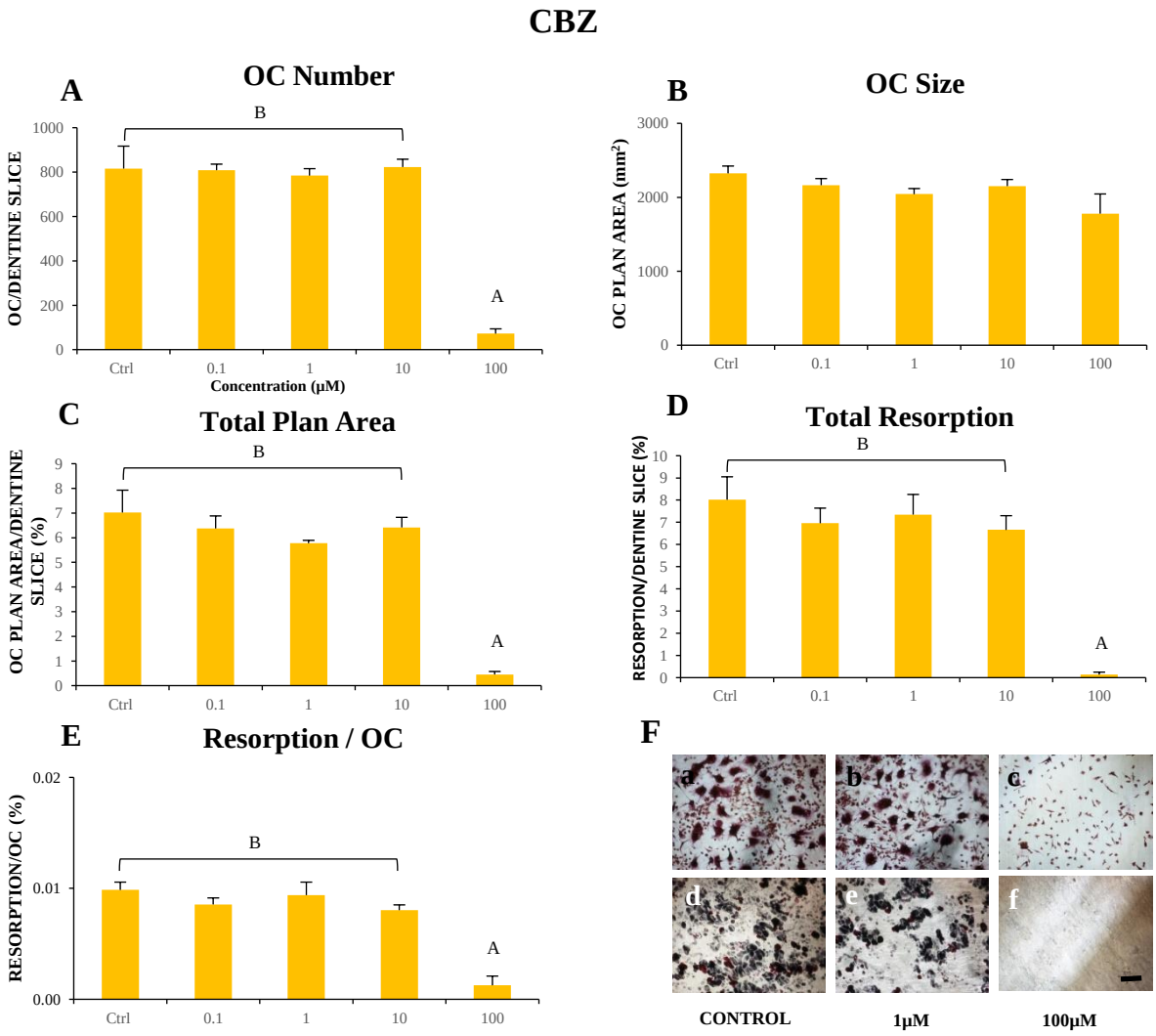

Figure 1.2. Effect of CBZ on OC formation and resorption. CFU-GM-derived cells were cultured in RANKL (125 ng/mL) and M-CSF (25 ng/mL) and co-treated with various concentrations (0.1-100µM) of CBZ for 14 days. The effects on **(A)** OC number, **(B)** OC size, **(C)** total plan area, **(D)** resorption capacity, and **(E)** individual OC activity were assessed. Data are presented as mean ± SEM (n=4 dentine slices/concentration). Groups marked with different superscripts are significantly different; $p<0.001$; one-way ANOVA with Fisher's Multiple Comparison test. **(F)** Representative photomicrographs demonstrating the effects of CBZ on OC formation (a–c) and resorption (d-f). Scale bar = 100 µm. Representative data from two independent experiments.

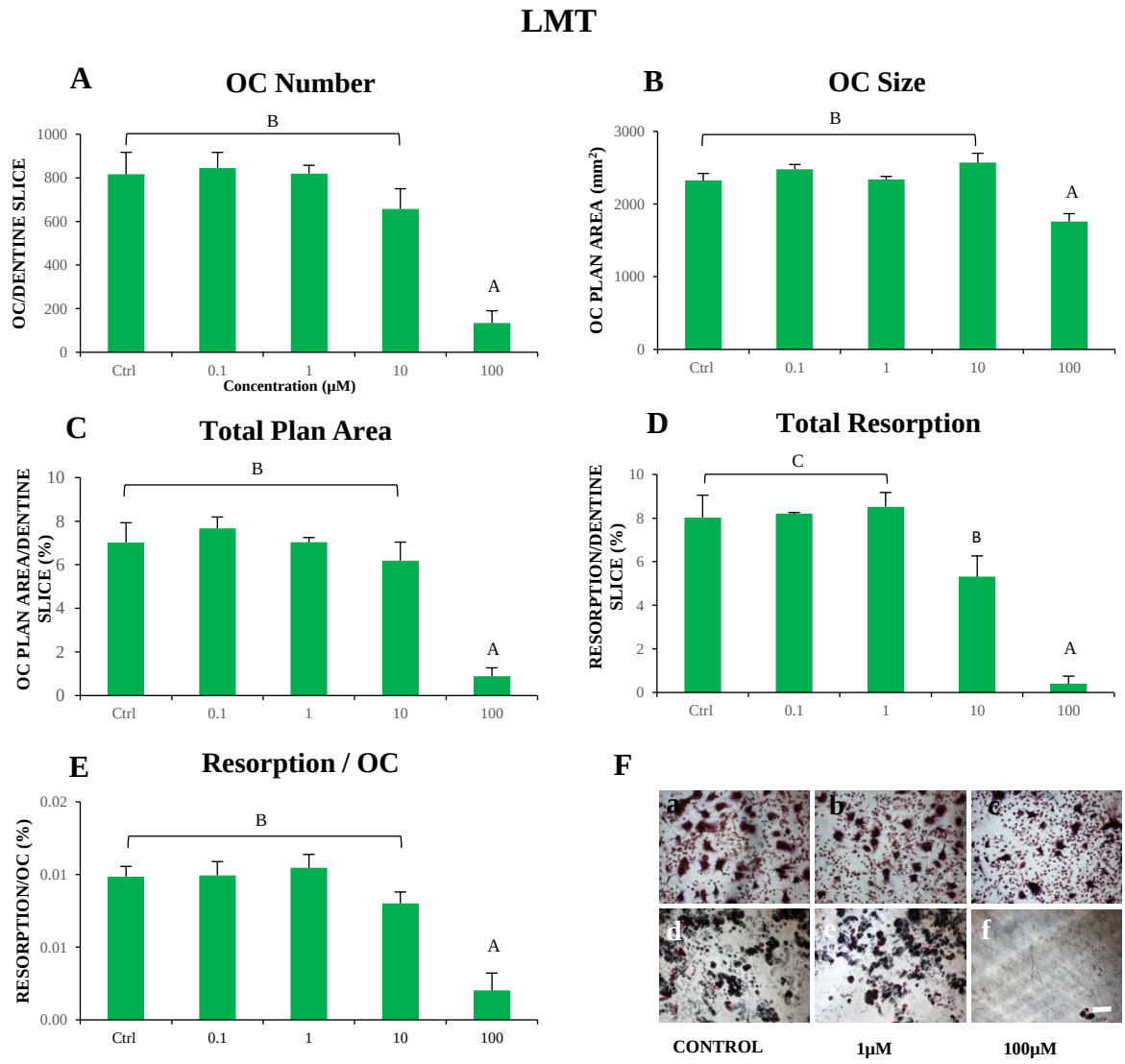

Figure 1.3. Effect of LMT on OC formation and resorption. CFU-GM-derived cells were cultured in RANKL (125 ng/mL) and M-CSF (25 ng/mL) and co-treated with various concentrations (0.1-100µM) of LMT for 14 days. The effects on **(A)** OC number, **(B)** OC size, **(C)** total plan area, **(D)** resorption capacity, and **(E)** individual OC activity were assessed. Data are presented as mean ± SEM (n=4 dentine slices/concentration). Groups marked with different superscripts are significantly different; $p<0.001$; one-way ANOVA with Fisher's Multiple Comparison test. **(F)** Representative photomicrographs demonstrating the effects of VPA on OC formation (a–c) and resorption (d-f). Scale bar = 100 µm. Representative data from two independent experiments.

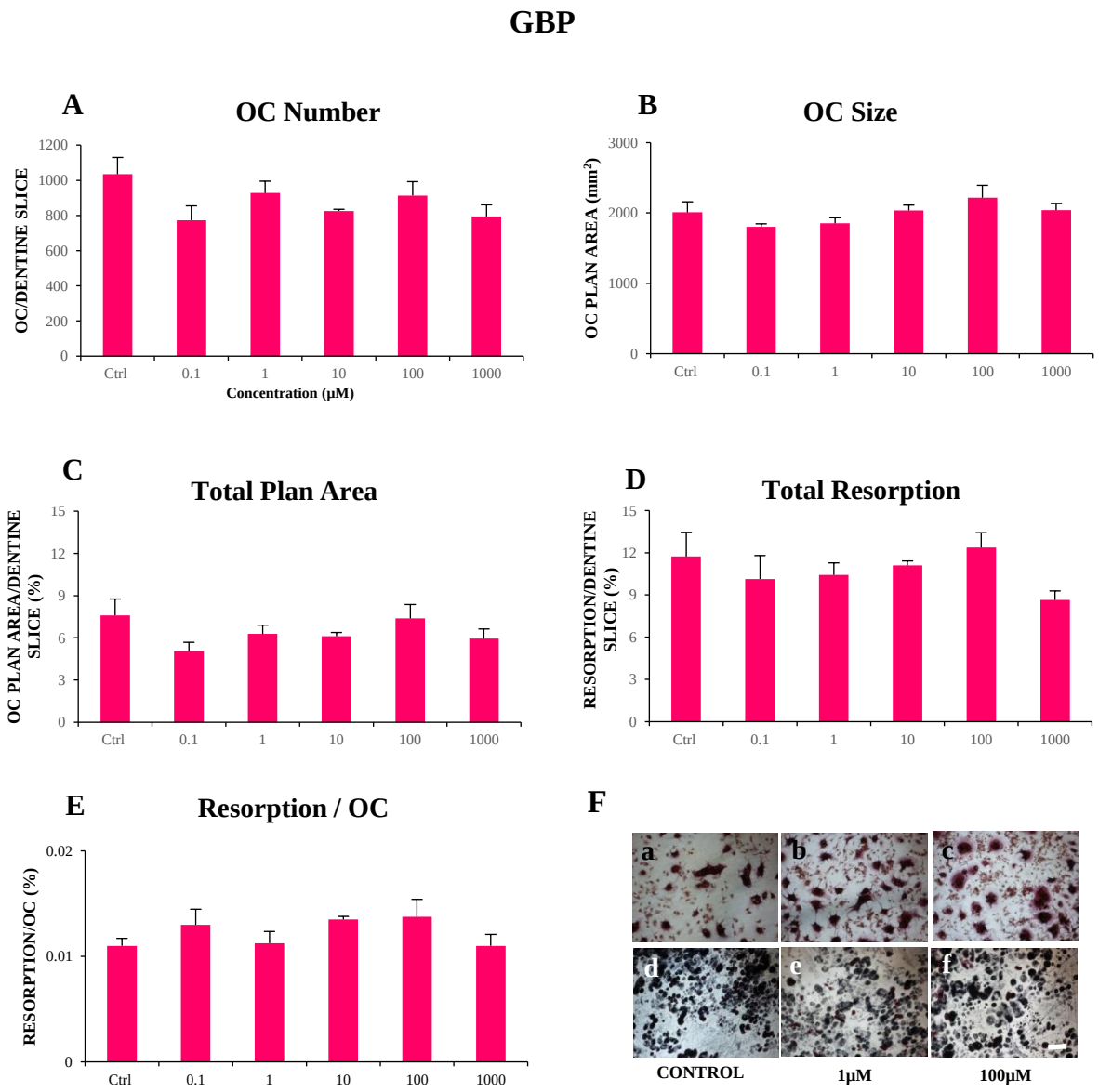

Figure 1.4. Effect of GBP on OC formation and resorption. CFU-GM-derived cells were cultured in RANKL (125 ng/mL) and M-CSF (25 ng/mL) and co-treated with various concentrations (0.1-1000µM) of GBP for 14 days. The effects on **(A)** OC number, **(B)** OC size, **(C)** total plan area, **(D)** resorption capacity, and **(E)** individual OC activity were assessed. Data are presented as mean ± SEM (n=4 dentine slices/concentration). Groups were not significantly different; p>0.01; one-way ANOVA with Fisher's Multiple Comparison test. **(F)** Representative photomicrographs demonstrating the effects of VPA on OC formation (a–c) and resorption (d-f). Scale bar = 100 µm. Representative data from two independent experiments.

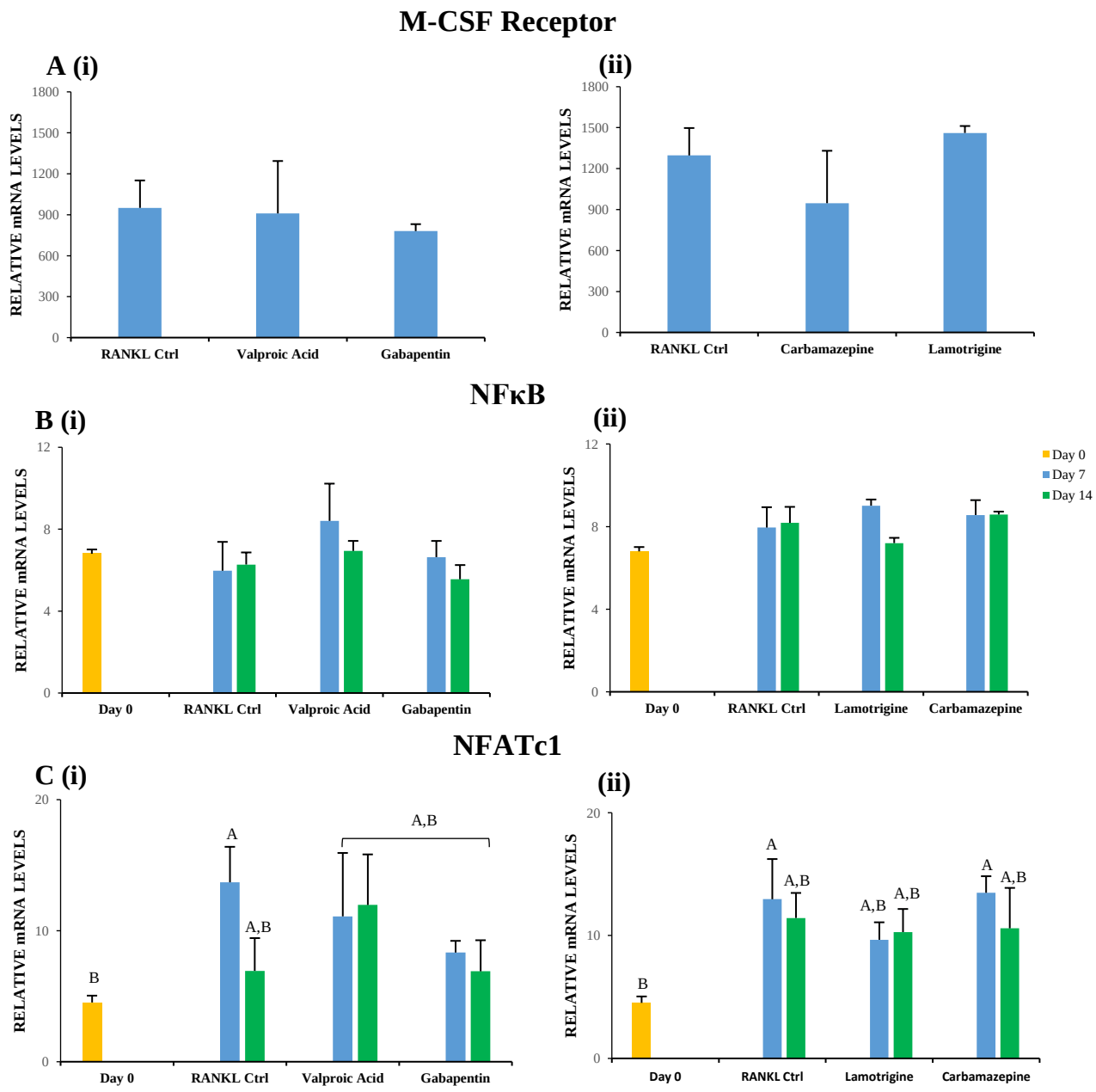

Figure 2.1. Effect of anticonvulsants on OC-related gene expression. CFU-GM-derived cells were cultured in RANKL (125 ng/mL) and M-CSF (25 ng/mL) and co-treated with anticonvulsants (100μM-VPA and GBP; 10μM-CBZ and LMT) for 14 days. Relative gene expression for **(A)** M-CSF receptor at day 7 of treatment, **(B)** NFκB, and **(C)** NFATc1. Vehicle control for VPA and GBP: distilled water; CBZ and LMT: dimethyl sulfoxide. Results are expressed as mean ± SEM (n=4 dentine slices/concentration). Groups with different superscripts are significantly different; $p<0.001$; two-way analysis variance general linear model; Fisher's pairwise comparisons.

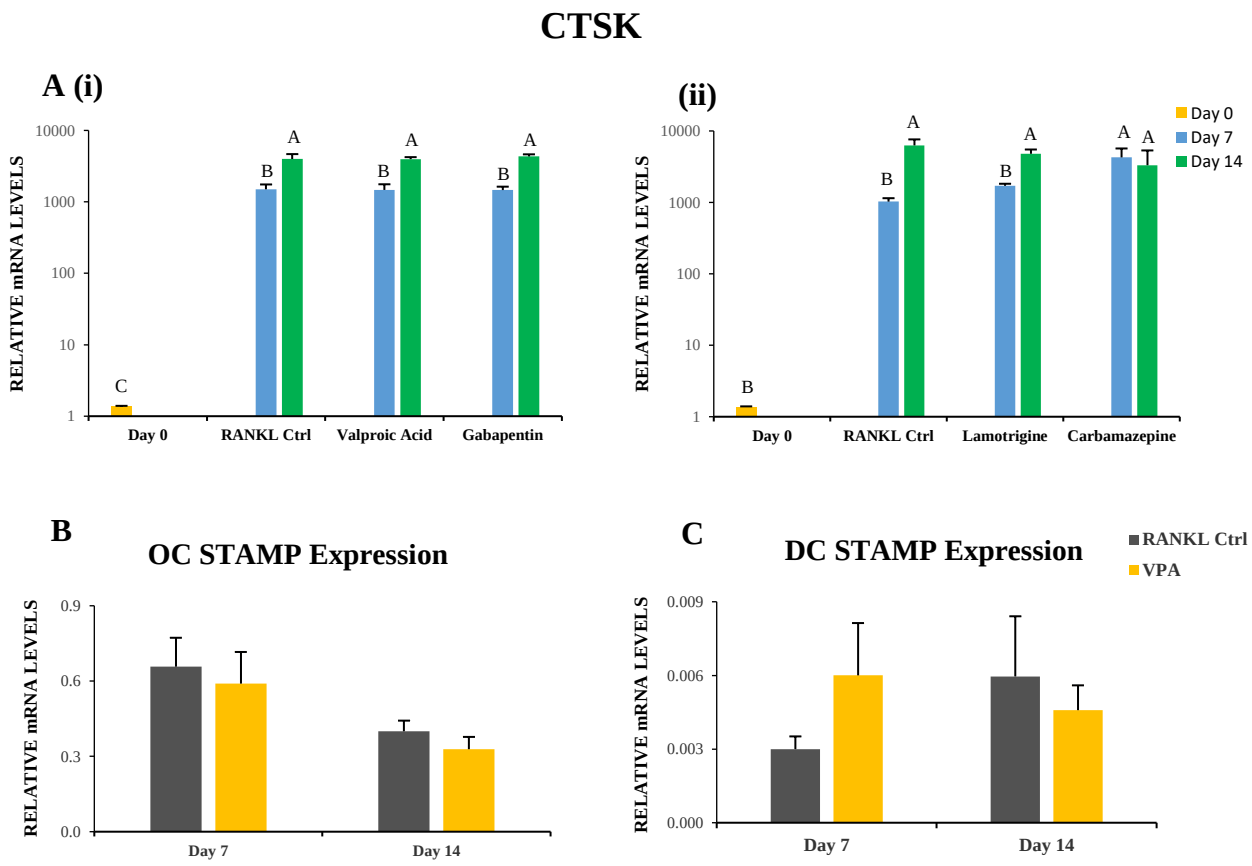

Figure 2.2. Effect of anticonvulsants on expression of CTSK, OC and DC STAMP in OCs. CFU-GM-derived cells were cultured in RANKL (125 ng/mL) and M-CSF (25 ng/mL) and co-treated with anticonvulsants (100µM-VPA and GBP; 10µM-CBZ and LMT) for 14 days. **(A)** Relative gene expression for CTSK. Vehicle control for VPA and GBP: distilled water; CBZ and LMT: dimethyl sulfoxide. **(B)** and **(C)** Effect of VPA on expression of OC and DC STAMP respectively. Results are expressed as mean ± SEM (n=3). Groups with different superscripts are significantly different; $p<0.001$; two-way analysis variance general linear model; Fisher's pairwise comparisons.

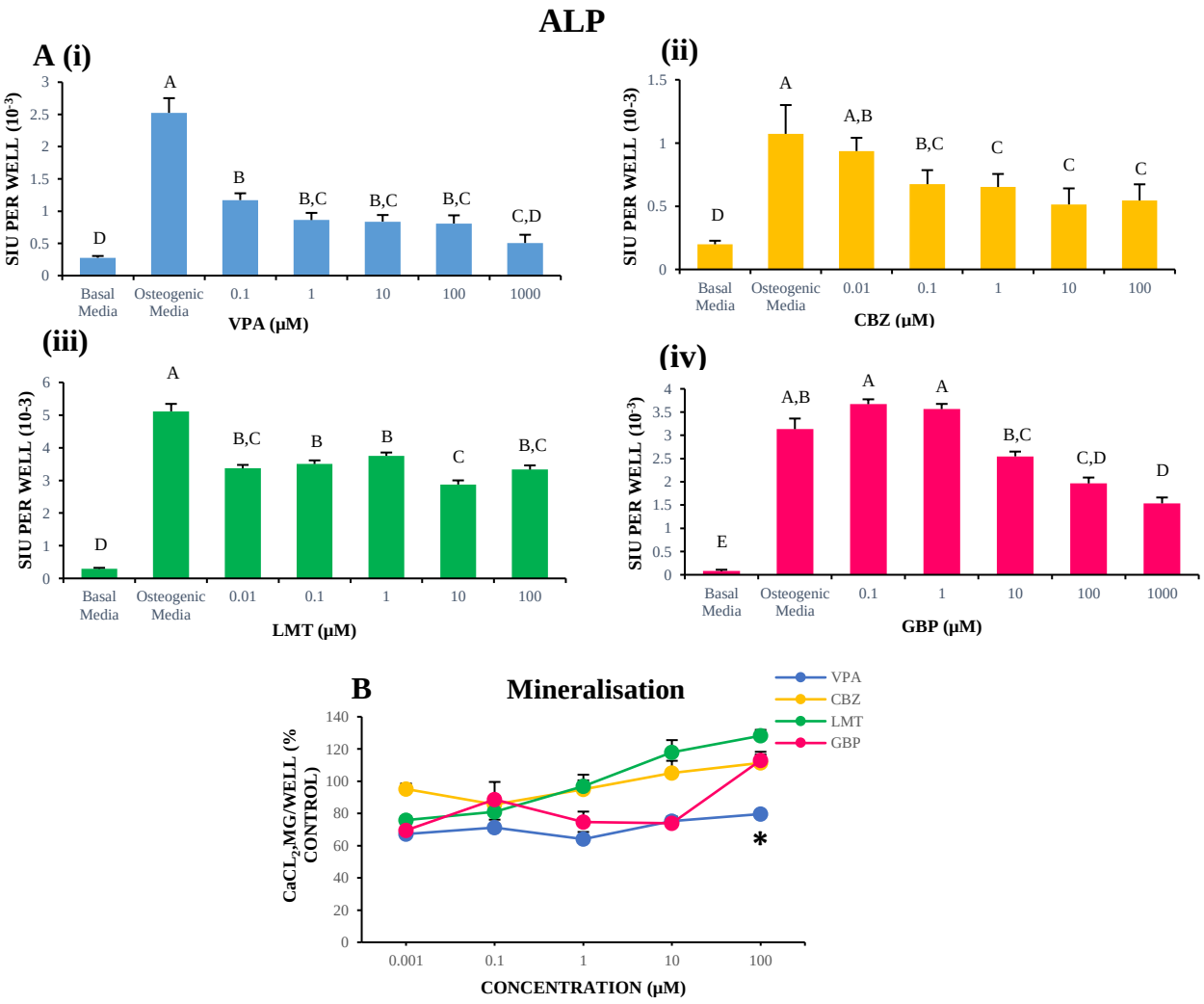

Figure 3. Concentration-dependent effects of anticonvulsants on OB differentiation and matrix mineralisation. MSC were cultured in ascorbate-2-phosphate (100µM), dexamethasone (10nM) and β-glycerophosphate (10mM) and co-treated with various concentrations of the four anticonvulsants for 7 days (ALP activity) or 21 days (mineralisation). Cultures were then assessed for **(A)** ALP activity as a marker of osteoblastogenesis and **(B)** calcium deposition as a marker of matrix mineralisation. Results are expressed as mean ± SEM (n=3 wells/concentration). (A) Groups marked with different superscripts are significantly different; $p<0.001$; one-way ANOVA with Fisher's Multiple Comparison test. (B) *VPA concentration curve significantly different from other drugs; $p<0.001$; two-way analysis variance general linear model; Fisher's pairwise comparisons. Representative data from four independent experiments.

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Chapter 5: Discussion and Conclusion

5.1 Discussion

Given the robust evidence base exploring the relationship between unipolar depression and bone health, this mixed-methods, thesis by publication aimed to identify evidence at both a clinical and cellular level, regarding the interplay between bipolar disorder, anticonvulsant use and bone health. This project evaluated the existing epidemiological evidence investigating the association between bipolar disorder and bone health via a systematic review (Manuscript I and II), which was followed by two epidemiological studies (Manuscripts III and IV) investigating the association between anticonvulsant use and bone health (bone density, quality and fracture) in a population based sample of men and women drawn from the Barwon Statistical Division located in south eastern Australia.

In order to further shed light on the underlying mechanisms behind anticonvulsant-related bone loss reported in clinical and epidemiological studies, human *in vitro* models of OC and OB were employed to assess the direct actions of anticonvulsants on bone cell formation and function (Manuscript V). Since these research findings are discussed in detail in the individual manuscripts (Manuscript I-V), this final discussion aims to summarise key findings to explain how, together, they extend current research and provide a basis for further research.

Via a systematic review, literature investigating the association between bipolar disorder and bone health was identified, collated, evaluated and discussed. Three large, cohort studies met the predetermined criteria. Bipolar disorder was associated with a 20-80% higher fracture risk compared to those without, independent of age, sex, comorbidities and medication use. No previous studies investigating bone quantity or quality were identified. Two of the three identified studies included highlighted psychotropic use as an essential factor in this association.

Given treatment is implicated in the psychiatric disorder-bone relationship and their use in the treatment of bipolar disorder, a cross-sectional study (Manuscript III) was conducted to investigate associations between anticonvulsant use and bone health (quantity and quality) in a large population-based sample of men and women, followed by a case-control study (Manuscript IV) to determine associations between anticonvulsant use and fracture.

As presented in Manuscripts III and IV, after adjustment for key confounders, all three bone endpoints were lower in men using anticonvulsants compared to non-users. In women, anticonvulsant users had lower hip BMD, BUA and increased fracture risk compared to non-users. Previous work has predominately investigated the effects of anticonvulsant use on bone health in children and older adults being treated for epilepsy (216). However, this work has covered the full adult age-range, suggesting that an association with poor bone health may exist across the lifespan of an individual taking anticonvulsants.

Further, although both population-based studies had small numbers of anticonvulsant users, these numbers closely reflected prescription patterns in the region (217).

Including both classical and newer generation anticonvulsant drugs utilising *in vitro* models of human osteoclast- and osteoblasto- genesis demonstrated intra-class differences. Data suggest that VPA may uncouple bone remodelling, CBZ and LMT may decrease net bone turnover, while GBP appears to have little to no effect on bone cells *in vitro*. Such findings have the capacity to influence treatment choices and in turn are of great clinical significance.

5.1.1 Strengths and Limitations

An overall strength of this thesis by publication lies in the mixed-method design, which has provided a comprehensive understanding of this topic of interest. More specifically, the systematic review identified and evaluated three methodologically sound studies which investigated bipolar disorder and bone health in large administrative databases. Three authors

contributed to an exhaustive screening, selection and scoring process, ensuring the fidelity of the synthesis of the extracted data. However, a paucity of published information in this research area limited the investigation of potential mediators of this association and clinical heterogeneity prevented a numerical synthesis.

The epidemiological studies included large, population-based samples of both men and women spanning the full adult range and a large number of confounding variables were able to be tested in statistical models. Limitations of these epidemiological studies include the inability to perform subgroup analysis of specific anticonvulsant agents to determine any potential differential effects (as seen in the *in vitro* studies) due to power limitations, the contribution of clinically diagnosed bipolar disorder to the relationships of interest and the ability to explore other potential underlying biological mechanisms such as inflammation and vitamin D status. The effect of behavioural manifestations during manic episodes were also not explored in this study since the required information was not collected from the participants. Furthermore, treatment compliance was not monitored, the cross-sectional study designs prevented temporal associations from being explored and unidentified confounding may have affected the findings.

The strengths of the laboratory component included investigating the actions of anticonvulsants utilising a well-established human bone cell model (170) and contributing novel findings in an area of research previously conducted predominantly in animal models. In addition, tissue samples were sourced from the same region as the population-based studies and a wide concentration range (10^{-3}M to 10^{-8}M) was employed, ensuring that no drug effects were missed. Research findings would be further strengthened by investigating drug actions in an increased number of independent donors to account for possible genetic variability.

5.1.2 Future directions

Given the epidemiological findings suggesting anticonvulsant use as a class are associated with detrimental effects on bone, at least for men, and that individual anticonvulsants within this broader class may have differential effects on bone cell formation and function, further exploration in larger population based studies are warranted to determine whether these differential effects translate to altered bone outcomes. Such findings have the capacity to influence treatment choice. Another future direction is exploring temporal associations between specific anticonvulsant agents and bone health across sex and age groups as well as the exploration of dose and duration of anticonvulsant use. Lastly, an exploration of disease versus treatment effects in the association with bone health is needed as are studies investigating bipolar disorder and its association with bone quantity and quality. In addition, potential mechanisms to explain the observed fusion and potentially increased resorptive of activity of OC, in conjunction with reduced OB differentiation caused by VPA warrant further exploration. Finally, observing the effects of anticonvulsants on bone health in an *in vivo* model may shed some light on additional factors (e.g. endocrine) that may be contributing to this association.

5.2 Conclusions

In conclusion, this thesis by publication has provided evidence to suggest both bipolar disorder and anticonvulsant use are associated with poor bone health, with anticonvulsants showing potential intra-class differences on bone cell formation and function *in vitro*. Such findings have the capacity to influence treatment choices and in turn are of great clinical significance. While additional exploration into the effects of anticonvulsants over time is warranted, understanding the operative pathways to bone loss has the potential to suggest therapeutic interventions to mitigate this adverse event. Finally, taking all into consideration, it may be

time for the regular monitoring of bone health in individuals diagnosed with bipolar disorder and/or taking anticonvulsants.

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