Skeletal Outcomes Following Intensive Care (SOFter)

The effect of critical illness on bone metabolism and bone mineral density

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A thesis submitted for the degree of Doctor of Philosophy at Monash University in 2018

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Word Count 36,568

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Abstract

Over the last two decades research on intensive care outcomes has broadened from a focus on mortality, to include morbidity and quality of life in survivors. Although the presence of long-term disability in survivors or critical illness is established, the ability to attenuate or prevent these outcomes remains elusive. A specific area where critical illness may adversely affect the wellbeing of survivors relates to accelerated bone turnover, resulting in increased risk of fragility fracture and mortality following critical illness. The aims of this thesis were to examine the evidence for accelerated bone turnover following critical illness, observe the change in bone mineral density and fracture risk following critical illness, and if justified propose an interventional trial of anti-fracture therapy.

There is increasing and consistent evidence of abnormal bone metabolism during critical illness. The pattern is consistent with uncoupling of bone formation and resorption, with accelerated bone loss beginning early in critical illness, persisting for weeks to months, and normalising over the following year. In contrast, bone formation remains within normal limits. The magnitude and duration of change, effect of pre-morbid and critical illness related factors, an effect of critical illness on measurement, require further investigation.

A major component of this thesis is the comparison of bone density data from critically ill patients to a well-defined control population, the Geelong Osteoporosis Study (GOS). The GOS cohort is used as a control population for comparison of absolute and annualised change in BMD, for a prospective study of ICU patients ventilated for greater than 24-hours and followed up for 2-years, and a nested cohort study, of GOS participants admitted to ICU. There is evidence of skeletal impact of increased bone turnover associated with critical illness, with accelerated loss of bone mass after critical illness persisting for up to 2-years. In addition, a high proportion of patients are osteopaenic or osteoporotic after ICU, suggesting a disease burden that may contribute to long-term morbidity and mortality. This is particularly evident in older women, a population at most risk of additional bone loss. There is also evidence of increased risk of fragility fracture after critical illness, again particularly in the highest risk group of older women.

There is preliminary evidence that anti-fracture interventions may be effective at attenuating bone loss, and reducing mortality, after critical illness. The protocol for a phase II safety and efficacy randomised controlled trial, of denosumab compared to placebo in post-menopausal women requiring prolonged mechanical ventilation, has been developed as part of this thesis.

Finally, a $3,847,885 AUD NHMRC Project Grant was developed and submitted on behalf of an Australia-wide collaboration by the ANZIC-RC, in 2018. This 1420 patient, multi-centre, three-armed, randomised, controlled, double-blinded, clinical trial is designed to determine if administration of denosumab or zoledronic acid, to mechanically ventilated post-menopausal women, during critical illness improves 2-year outcomes compared to placebo.

In summary this thesis has achieved its aims, and significantly added to the body of literature relating to critical illness associated bone loss that has emerged over the last decade.

Lay Summary

In the years after critical illness, survivors are more likely to experience disability and disease. The recognition, prevention and treatment of this is evolving. Loss of bone mass after critical illness may contribute to disability, because of an increased risk of fracture and death. This thesis investigates the relationship between critical illness, bone loss, and fracture, and proposes a study to prevent this from occurring.

Publications and presentations arising during enrolment in this thesis

Published journal articles

1. Orford N, Cattigan C, Brennan SL, Kotowicz M, Pasco J, Cooper DJ. The association between critical illness and changes in bone turnover in adults: a systematic review. *Osteoporos Int*. 2014;25(10):2335-2346. doi:10.1007/s00198-014-2734-1. Impact Factor 3.6

2. Orford NR, Bailey M, Bellomo R, et al. The association of time and medications with changes in bone mineral density in the 2 years after critical illness. *Crit Care*. 2017;21(1):69. doi:10.1186/s13054-017-1657-6. Impact Factor 5.4

3. Orford NR, Lane SE, Bailey M, et al. Changes in Bone Mineral Density in the Year after Critical Illness. *Am J Respir Crit Care Med*. 2016;193(7):736-744. doi:10.1164/rccm.201508-1514OC. Impact Factor 13.2

4. Orford NR, Saunders K, Merriman E, et al. Skeletal morbidity among survivors of critical illness. *Crit Care Med*. 2011;39(6):1295-1300. doi:10.1097/CCM.0b013e318211ff3d.Impact Factor 7.4

In press journal articles

1. Orford NR, Bailey M, Bellomo R, et al Changes in Bone Mineral Density in Women before Critical Illness: A Matched Controls Nested Cohort Study. *JCEM.* Submitted March 2018

Conference presentations

1. 2016: Phase 2 pilot interventional trial of denosumab to prevent bone loss in older female survivors of critical illness. VICTORI Group, Melbourne.

2. 2015: Effect of critical illness on bone turnover. ANZICS CTG ASM, Noosa.

3. 2014: Vitamin D in critical illness, what's the latest? 4th Nutrition in the Critically Ill Symposium, Melbourne.

Grants, scholarships, related to research reported in this thesis

Grants received

1. 2017: Malcolm Fisher Intensive Care Foundation Grant $14,638. “Effect of denosumab on bone turnover markers in critically ill women - A safety and feasibility, randomised, placebo, controlled trial”

2. 2011: Intensive Care Foundation Grant $28,000. “A prospective observational study of critical illness related changes in bone mineral density, bone turnover and calcium metabolism”.

Grants submitted

1. 2018: NHMRC Project Grant (APP1156428) $ 3,847,855. "DeNosumab or Zoledronic Acid for Fracture Prevention in Postmenopausal Critically Ill Women – A Randomised Controlled Trial" (The No Fracture Trial)

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 4 original papers published in peer reviewed journals and 1 unpublished publications. The core theme of the thesis is critical illness associated bone loss. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the ANZIC-RC and Department of Epidemiology and Preventive Medicine, under the supervision of Professor DJ Cooper.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters 2,3,4,5,6 my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author names and % contribution	Co-author Monash student
2	The association between critical illness and bone turnover in adults: a systematic review	Published	80% Concept, search method and conduct, manuscript prepare	C Cattigan, review 4% SL Brennan, review 4% M Kotowicz, design, review 4% J Pasco, design, review 4%	No for all
				DJ Cooper, review 4%	
3	Skeletal morbidity among survivors of critical illness	Published	65% Concept, design, data linkage,, analysis, manuscript prepare	K Saunders, review 4% E Merriman, data linkage 3% M Henry, analysis, review 5% J Pasco, design, analysis, review 10% P Stow, data linkage 3% M Kotowicz, design, analysis, review 10%	No for all

4	Changes in Bone Mineral Density in the Year after Critical Illness	Published	60% Concept, design, enrolment, data analysis, manuscript prepare	S Lane, data analysis, review 5% M Bailey, data analysis, review 5% J Pasco, design, analysis, review 5% C Cattigan, review 1% T Elderkin, data analysis 2% SL Brennan, review 2% R Bellomo, design, analysis, review 10% DJ Cooper, design, analysis, review 5% M Kotowicz, design, analysis, review 5%	No for all
5	The association of time and medications with changes in BMD in the 2 years after critical illness	Published	60% Concept, design, data analysis, manuscript prepare	M Bailey, design, analysis, review 15% R Bellomo, design, analysis, review 10% J Pasco, design, analysis, review 5% C Cattigan, review 1% T Elderkin, review 1% SL Brennan, review 1% DJ Cooper, review 2% M Kotowicz, design, analysis, review 5%	No for all
6	Changes in BMD in Women before Critical Illness compared to Population Matched Controls	Submitted and under review	60% Concept, design, data analysis, manuscript prepare	M Bailey, design, analysis, review 15% R Bellomo, design, analysis, review 15% J Pasco, design, review 5% DJ Cooper, review 2% M Kotowicz, review 3%	No for all

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: [redacted] **Date:** 22nd April 2018

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: **Date:** 23rd April 2018

Acknowledgements

I wish to record my acknowledgement and gratitude for financial support from;

Intensive Care Foundation for competitive grants awarded in 20111 and 2017

Intensive Care Unit Research Group, Barwon Health for support for the conduct of observational trials in this thesis.

I also wish to record my acknowledgement and gratitude to;

The University Hospital Geelong Intensive Care Unit staff, my colleagues, for their support during the course of this thesis.

The Geelong Osteoporosis Study, in particular Professor's Kotowicz and Pasco for their patient mentorship, collaboration, and advice.

The Australian and New Zealand Intensive Care Research Centre for guiding me through the conduct of a PhD.

Professor Rinaldo Bellomo for his constant, infectious, support and encouragement for the last decade.

Professor Michael Bailey for his patient advice, repeated lessons on statistical analysis, and constant encouragement.

Professor Jamie Cooper for being available when needed, and ensuring I stayed on track.

The critically ill patients and their families from South Western Victoria, who motivated me to learn more about improving survivorship.

Finally, to my wife sharyn, my children Angus, Finn, and Clara, thankyou for your patience, love, support, and constant encouragement.

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Abbreviations

ALP	Alkaline phosphatase
APACHE	Acute physiology and chronic health evaluation score
ARDS	Acute respiratory distress syndrome
AP	Anterior posterior
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular unit
BSL	Blood sugar level
BTM	Bone turnover markers
CI	Confidence interval
CRP	C-reactive protein
CRRT	Continuous renal replacement therapy
CT	Computed topography
CTX	Carboxy-terminal cross-linked telopeptide of type 1 collagen
CV	Coefficient of variation
DPD	Deoxypyridinoline
DXA	Dual energy x-ray absorptiometry
EQ VAS	EuroQol visual analog scale
FDA	Food and drug administration
FRAX®	Fracture risk assessment tool
GH	Growth hormone
GHRP	Growth hormone-releasing peptide
GnRH	Gonadotrophin-releasing hormone
GOS	Geelong Osteoporosis Study
HR	Hazard ration
ICU	Intensive Care Unit
IGF	Insulin-like growth factor
IL-1	Interleukin-1
ITAM	Immunomodulatory tyrosine-based activation motif factor
IQR	Interquartile range
LOS	Length of stay
LOWESS	Locally weighted polynomial regression
M-CSF	Macrophage-colony-stimulating factor
MRI	Magnetic resonance imaging
NTX	Collagen type 1 cross-linked N-telopeptide
OC	Osteocalcin
OPG	Osteoprogeretin
PBMC	Peripheral blood mononuclear cells
PBS	Pharmaceutical benefits scheme
P1CP	Procollagen type 1 C peptide
P1NP	Procollagen type 1 N peptide
PTH	Parathyroid hormone
PQCT	Peripheral quantitative CT
PYD	Pyridinoline
RANKL	Receptor activator of nuclear factor-κB ligand
RCT	Randomised controlled trial

RMANOVA	Repeat measure analysis of variance
SAE	Serious adverse event
SALP	Skeletal alkaline phosphatase
SD	Standard deviation
SE	Standard error
TNF	Tumour necrosis factor
TRH	Thyroid releasing hormone
TGF-β	Transforming growth factor-β
UHG	University Hospital Geelong
VEGF	Vascular endothelial growth factor
WHO	World Health Organisations

Chapter 1: Introduction - The need to identify target diseases to reduce the health impact of surviving critical illness

1.1 Introduction

Over the last two decades research on intensive care (ICU) outcomes has broadened from a focus on mortality, to include morbidity and quality of life in survivors[1-10]. As a result the consequences of surviving critical illness compared to pre-illness status and general population controls, are increasingly well described, including impaired physical function[6,11-13], cognitive impairment[14-16], and psychological distress[17-19]. With spending on critical illness in the US consuming 16.9% to 38.4% of hospital care costs, and 5.2% to 11.2% of the total US National Healthcare Budget spent on critical illness in hospital and in the year after discharge from hospital[20], it is not surprising that efforts to improve quality of survival and reduce disability after ICU are increasing.

Although the presence of long-term disability in survivors of critical illness is established, the ability to attenuate or prevent these outcomes remains elusive. The relative contributions to long-term recovery of pre-critical illness co-morbidities and trajectory of disability from critical illness related factors[7,21-25] remain unresolved, and specific diseases that cause impaired recovery have not been identified. As a result interventions to improve recovery after critical illness have had a general functional focus - physical therapy programs[26,27], mental health support[28], and follow-up clinics[29-31] - and have met with limited success[32]. The identification of target diseases - either pre-existing chronic disease exacerbated by critical illness, or new chronic disease caused by critical illness - their relationship to critical illness, risk factors, duration, and magnitude of effect, could provide areas to focus future interventional trials aimed at modifying disease progress and improving long-term outcomes.

1.2 Endocrine dysfunction and outcomes after critical illness

The understanding of short and long-term relevance of endocrine changes associated with critical illness reflect the broader uncertainty about factors that contribute to impaired recovery. The perturbations in endocrine function observed during critical illness may be adaptive or maladaptive, provide a marker of severity of the illness and correlate with outcomes, but are not necessarily be involved in causation. The contribution of changes in acute and chronic endocrine function to critical care outcomes is a significant area of critical

care research[33,34]. Associations between impaired plasma levels of cortisol, vasopressin, growth hormone, glycaemic control, thyroid function, sex hormones, vitamin D, and short-term survival have led to trials investigating the effect of hormone replacement or correction of neuroendocrine perturbations on short-term mortality[35-43]. Overall the progress of these trials have followed a pattern of benefit observed in small single-centre studies, with lack of benefit or evidence of harm when tested in a multi-centre, randomised controlled design [35-40].

Although trials manipulating endocrine abnormalities observed during critical illness have failed to consistently show benefit, there is evidence to support the hypothesis that specific changes in acute and chronic endocrine function result in potentially modifiable changes in long-term outcomes following critical illness. For example, the NICE-SUGAR trial, a large multi-centre randomised controlled trial comparing tight glycaemic control (BSL 4.5-6.0 mmol/L) to standard care (BSL <10.0 mmol/L) in adults expected to remain in ICU for at least 3-days, reported an increase in 90-day mortality in the treatment arm[40]. This increase in mortality related to cardiovascular death, and both short and long-term endocrine responses to critical illness and tight glycaemic control have been hypothesised as causative. A relationship between pre-critical illness glycaemic state and outcome has been described[44], suggesting long-term glycaemic state is an important consideration when manipulating glycaemic control during critical illness. This theory is supported by outpatient research reporting increased risk or lack of benefit associated with aggressive management of hyperglycemia in patients with type 2 diabetes mellitus[45,46]. Finally, a dysfunctional counter-regulatory endothelial response to critical illness related tight glycaemic control has been proposed as the mechanism for increased cardiovascular morbidity[47]. In summary, short and long-term changes in endocrine function may contribute to specific post-ICU morbidity.

Another specific area where critical illness may adversely affect the wellbeing of survivors relates to accelerated bone turnover, resulting in increased risk of fragility fracture and mortality following critical illness. Demonstration of reduced bone mass and increased bone loss following critical illness, and description of the degree, incidence, time course, and risk factors for these changes may provide a basis for interventional trials of anti-resorptive therapy to prevent bone loss and reduce fracture risk in survivors of critical illness. The goal of this thesis is to examine the current evidence for accelerated bone turnover following critical illness, observe the change in bone mineral density and fracture risk following critical illness, and if justified propose an interventional trial of antiresorptive therapy.

1.3 Osteoporosis and intensive care

Bone density normally peaks after puberty and into the third decade, remains relatively constant until the age of 50 years, and thereafter decreases through life. Osteoporosis, a chronic progressive disease and major public health issue[48], is characterized by low bone mass, micro-architectural bone disruption, and skeletal fragility leading to fracture[49]. The lifetime risk of osteoporotic spine, hip, or wrist fracture is 30-40% in developed countries, and the lifetime risk of hip fracture is one in six in white females[50], with significant associated health burden of mortality, morbidity, and cost[51,52]. However, as few as 13-27% of patients with osteoporosis are treated following a fragility fracture, suggesting osteoporosis remains an under diagnosed disease[53,54].

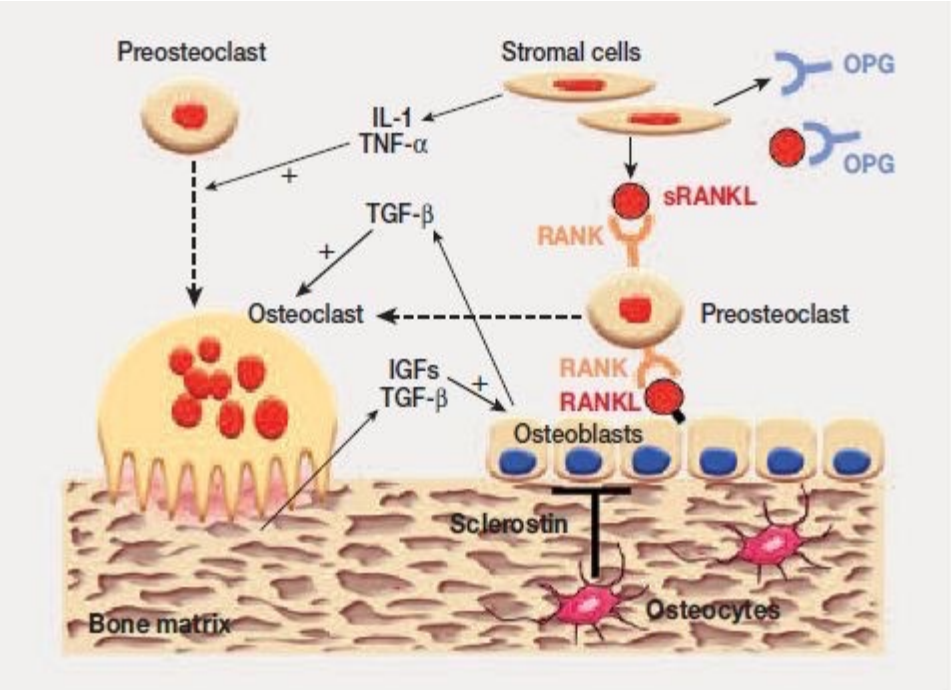
The factors that contribute to loss of bone strength and increased risk of fragility fracture can be broadly divided into material composition and structure. Bone consists of a woven triple helix of cross-linked type I collagen, stiffened by crystals of calcium hydroxyapatite. This results in an approximately 60% mineralized structure that is flexible enough to deform under loading conditions, stiff enough to tolerate load without cracking, and light enough to facilitate movement. Bone is divided into cortical (compact) and trabecular (spongy/ cancellous) bone. Cortical bone is the denser, stronger outer shell, performing the main functions of support and lever. The functional unit of cortical bone is the osteon, which form overlapping parallel “bricks” surrounded by concentric lamellae of mineralized collagen fibres around the central Haversian Canal. This structure not only permits the functions of bone, but limits the propagation of cracking as the entry of cracks is limited by the cement line that separates osteons, largely limiting cracks to the older, more densely interstitial bone between osteons[50,55]. Trabecular bone, is softer, with a higher surface area to mass ratio, and is found at the end of long bones, proximal to joints, and on interior vertebral surfaces. The higher surface and vascularity make it more suitable for metabolic activity and haematopoiesis. In addition the more porous design allows more deforming energy to be absorbed without cracking, ideal for the compressive forces applied to vertebral bone[55].

The process of bone modeling during growth results in changes to the size and contours of bone internally and externally that establish peak bone strength. Bone remodeling thereafter works to maintain bone strength during ageing, a process that requires osteoclast and osteoblast activity to be tightly coupled with equilibrium of mechanical, nutritional, immune and endocrine factors[7,8,9]. Remodelling, resorption, then replacement, occurs asynchronously through the skeleton, and involves 5-10% of the skeleton per year. At a cellular level this process occurs in discrete clusters of osteoclasts and osteoblasts called the basic multicellular unit (BMU).

In adults, the first step in this remodeling process is probably triggered by the death or deformation of osteocytes. Osteocytes are terminally differentiated osteoblasts, embedded in lacunae in the bone matrix (osteoid), with a dense network of communicating cytoplasmic processes that connect them to other osteocytes and lining cells. It is likely that they respond to deformation, or death, by signaling the need for adaptive remodeling, a process that involves canopy cells lining bone, endothelial cells, vascular, and immune cells. The observation that conditions associated with osteocyte apoptosis (oestrogen deficiency, corticosteroid therapy, increasing age), result in decreased bone strength before bone loss occurs support this theory[55-57].

The replication, differentiation, activity, and lifespan of osteoclast and osteoblast progenitors are determined by growth factors from matrix, cytokines, circulating hormones, soluble and membrane products of osteoclasts and their precursors, signals from osteocytes, and immune cells from osteoblast lineage (Figure 1). Osteoclasts are derived from hemopoietic precursors from the capillary blood supply and marrow, and are closely related to macrophages. Differentiation from osteoclast precursor to mature osteoclast requires signals from macrophage-colony-stimulating factor (M-CSF), receptor activator of nuclear factor-κB ligand (RANKL), and vascular endothelial growth factor (VEGF). RANKL is abundantly expressed by osteoblasts, bone marrow stromal cells, and T and B-lymphocytes. RANKL binds to RANK receptor on osteoclasts, stimulating activity. Osteoblasts also release the cytokine receptor osteoprogeretin (OPG), a RANKL decoy/antagonist. Osteoprotegerin, a member of the TNF receptor superfamily, acts as a decoy receptor for receptor activator of nuclear factor kappa B ligand (RANKL), and prevents RANK mediated regulation of inflammation, innate immunity, apoptosis, and blocking maturation and activity of osteoclast precursors. Osteoblasts are stimulated by vitamin D, parathyroid hormone, and growth factors released from bone matrix during resorption and produced by osteoblasts themselves. Many of these local factors also contribute to osteoblast and osteoclast apoptosis. Trabecular bone is resorbed by osteoclasts and cleaned up by macrophages, followed by the differentiation of osteoblasts precursors to repair and replace bone gaps. Cortical bone is resorbed by osteoclasts, followed by maturing osteoblasts, space filled by vessels, nerves, connective tissues.

Figure 1: Osteoblast-osteoclast communication during remodelling



***Abbreviations**: IGF, insulin-like growth factor; IL-1, interleukin-1; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor κB; sRANKL, soluble receptor activator of nuclear factor κB ligand; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α. (P.J.Marie. Bone remodeling: A social network of cells. Medicographia 2012;34:149-154)*

During human growth the BMU has a positive bone balance, that is the amount of bone resorbed is less than that formed, so that each modelling event results in an increase in bone. As the skeleton reaches it programmed dimensions, the need for a positive BMU balance lessens, shifting to a negative balance as bone formation decreases. The two factors that determine the actual rate of bone gain or loss are the remodelling rate, and magnitude of change in each BMU. Rapid remodelling, independent of the balance in each BMU, is associated with fracture as more densely mineralised, older bone is replaced with less dense bone with reduced stiffness [58]. Also, excavated resorption sites remain temporally unfilled, creating areas that predispose to microdamage and fracture. Finally isomerisation and maturation of collagen is impaired.

Bone loss associated with increased bone turnover occurs in numerous conditions, including menopause, myeloma, rheumatoid arthritis, bone metastases, suppression of sex hormones (prostate cancer, breast cancer, tamoxifen), and in the presence of pro-inflammatory cytokines (IL-1, TNF)[59]. Oestrogen deficiency increases the rate of remodelling

and the volume of bone resorption by prolonging the life span of osteoclasts, and decreasing the life span of osteoblasts. This leads to trabecular thinning, loss of connectivity between trabeculae, cortical thinning, and increased cortical porosity. As a result bone fragility is more common in women than men, partly because the production of sex hormones does not decrease rapidly in men, with no subsequent increase in remodelling rate. The bone fragility and fractures observed in osteoporosis vary in pathogenesis, with some related to reduced bone mineral density, others a reduced density of osteocytes, and high, normal, or low rates of remodelling.

1.3 Assessment of osteoporosis

Bone Mineral Density

The measurement of BMD by dual energy x-ray absorptiometry (DXA) at the proximal femur and lumbar spine forms the basis of assessment and treatment of osteoporosis, with change in BMD estimated to account for 60-80% of variance in bone strength[19]. BMD values in individuals are expressed as an absolute value (g/cm^2), and in relation to a reference young adult population in standard deviation (SD) units, the T-score. The T-score is the number of standard deviations above or below the young adult mean, with cut-off values calculated from the Australian reference ranges[60,61]. The WHO operational definition[62] of osteoporosis includes normal (T-score > -1.0), osteopaenic (T-score -2.5 to -1.0), or osteoporotic (T-score <-2.5). Established osteoporosis is defined as a T-score below -2.5 in the presence of one or more fragility fractures [20]. BMD measurement is also used to estimate fracture risk, providing a continuous relationship with no absolute cut-off threshold that discriminates who will and will not fracture. Individuals with a 1SD decrease in BMD compared to their age-matched peers will have an approximate 2-fold increase risk of fractures in their remaining lifetime. This increases to 4-fold increase in fracture risk for a T-score of -2.5 [18]. In addition to categorisation of osteoporosis, BMD is used to assess response to treatment, as a primary outcome measure in antiresorptive trials, and to calculate fracture risk. Change in BMD over one year is the standard for interventional research studies[63-67], as BMD undergoes relatively small changes over time, of a magnitude similar to measurement error (short-term precision in vivo for Lunar DXA (GE Healthcare, Madison, USA) is 1.6% for the femoral neck and 0.6% for the lumbar spine[1]).

Bone Turnover Markers

Biochemical markers of bone turnover also have a role in the assessment of bone loss. Although the diagnosis of osteoporosis is not based on evaluation of biochemical markers,

they are used in predicting the rate of bone loss and subsequent fracture risk[68,69]. Overall BTMs are separated into markers of bone resorption and bone formation [70]. The bone resorption markers include urinary collagen type 1 cross-linked N-telopeptide (NTX), pyridinoline (Pyd) or deoxypyridinoline (Dpd), carboxy-terminal cross-linked telopeptide of type 1 collagen (ICTP/CTX). Bone formation markers include skeletal alkaline phosphatase (SALP), osteocalcin (OC), procollagen type 1 C peptide (P1CP) and procollagen type 1 N peptide (P1NP). Although divided into formation and resorption markers, BTM levels are affected by a number of factors, requiring more complex interpretation. Osteocalcin is a marker of osteoblast function and bone formation, but smaller fragments are derivatives of bone resorption and detected in assay. The bone formation markers P1NP and P1CP are both procollagen terminal extension peptides, but P1NP is more specific for bone formation. Also a number of BTMs are affected by biological factors including age, gender, co-existing disease, and medications. Examples include decreased excretion of CTX in renal failure and sensitivity of OC to glucocorticoid exposure [70].

Markers for bone turnover are generally higher in those with osteoporosis compared to healthy controls, although there is considerable overlap. The combined use of BMD measurement and biochemical markers may be helpful in risk assessment, especially in those women who are not identified as at risk by BMD measurement alone [23]. Levels of bone markers decrease rapidly with antiresorptive therapies, with 30-60% decreases after 3-6 months. The short-term decrease in bone markers predicts the effects of antiresorptive agents on bone mass and fracture risk over the subsequent 2-year, thus providing a useful measure of treatment efficacy [24].

Bone Strength: Microindentation and microarchitecture

Bone strength is a function of bone mass, bone structure (size, geometry and microarchitecture) and the material properties of the matrix [71]. Most fragility fractures occur in individuals with osteopenia or normal range BMD [72,73]. This is because BMD assesses the calcium content of the region of interest providing an indirect measure of bone mass and limited information regarding bone microarchitecture and material properties. Bone microarchitecture and material properties of the matrix play a key role in the resistance to propagation of microdamage that compromise bone strength. The pathogenesis of skeletal fragility arises at the level of the individual BMUs, with a bone remodelling balance that results in incomplete filling of resorption lacunae in the adult skeleton[74]. Remodelling occurs on bone surfaces, with trabecular bone loss greater due to its high surface area. This leads to in loss of trabecular plates, loss of connectivity and trabecular perforation[58].

Trabecularisation of endocortical surfaces maintains the surface area for bone remodelling and contributes to cortical thinning and net bone loss[75,76]. Ageing is also associated with periosteal bone apposition that can compensate to some extent for endocortical bone loss and maintain cortical thickness[77,78]. The net effect of these processes is an increase in bone size and medullary width, cortical thinning and loss of structural elements in the trabecular compartment, resulting in increased bone fragility.

Microindentation

Bone material strength at the tissue level can be assessed by testing bone microindentation, a method that allows assessment of susceptibility to fracture through measurement of the thick cortex of the mid-tibia using a new device known as the OsteoProbe[79]. This device uses bone microindentation to measure tissue mechanical properties *in vivo* by quantifying indentation distance in relation to a reference value and expressing the ratio in bone material strength index (BMSi) units. A greater BMSi indicates more resistance to crack propagation; hence, the BMSi is a direct measure of fracture resistance.

Bone remodelling at the basic multicellular units influences the material properties of bone replacing old mineralised bone with new matrix, increasing the heterogeneity of the skeleton and increasing its resistance of the propagation of microdamage that ultimately leads to mechanical failure of bone, that is, fracture. Moreover, other elements like microporosity, collagen and non-collagen proteins properties, degree of mineralisation, water content or tissue homogeneity, among others, contribute to the mechanical properties of the bone tissue. The more susceptible the bone to fracture, the more fragile the bone and the further the test probe will indent the bone[80]. By measuring indentation distances in relation to a reference material, we will be able to assess the ability of bone to resist crack generation and propagation – the anatomical basis for fracture. Bone microindentation technology has been used successfully to evaluate alterations in bone material properties in patients with type 2 diabetes[80], atypical femur fractures[81] and in response to therapy in patients with glucocorticoid-induced osteoporosis[82].

Microarchitecture

The tomographic nature of peripheral quantitative CT (pQCT) affords the distinctive ability to differentiate cortical and trabecular bone and provides imaging that is superior dual energy x-ray absorptiometry (DXA) as it differentiates cortical and trabecular bone. The effective radiation exposure with pQCT is very low (1.2 µSv per set of scans); due to the low radiation dose, no radiological protection measures need to be taken. It provides measures of volumetric trabecular and cortical BMD, cortical thickness, cortical density and periosteal

circumference and estimated bone strength at the tibial and radial mid-shaft sites.

In a matched case-control study of postmenopausal French women, 101 cases with fragility fracture over 13 years of follow-up were matched with fracture-free controls[83]. Vertebral and nonvertebral fractures were associated with low volumetric BMD and architectural deterioration of trabecular and cortical bone as assessed by high-resolution pQCT at the distal radius and tibia, independent of areal BMD. Cases had decreased trabecular volume, cortical thickness, trabecular number and trabecular thickness. Similarly, in another study using high resolution pQCT, osteoporotic women had lower density, cortical thickness and increased trabecular separation than osteopaenic women and, among osteopaenic women, those with fracture had lower trabecular density and more heterogeneous trabecular distribution [84].

With ageing, women undergo loss of trabeculae with an increase in trabecular separation, whereas men start with thicker trabeculae and experience less age-related microstructural damage. Because decreases in trabecular number substantially affect bone strength, this finding may explain, at least in part, the protection men have against age-related increases in distal forearm fractures. More recent findings suggest that development of intracortical porosity may play an important role in compromising bone strength [85,86] and that this could explain the high proportion of non-vertebral fractures that occur with ageing at predominantly cortical sites [85]. In an Australian study of 185 female twin pairs aged 40-61 years, postmenopausal women had higher levels of remodelling markers that were associated with larger intracortical surface area rather than with the progressively diminishing trabecular surface area[86]. Identification of intracortical, endocortical and trabecular bone surface area are beyond the resolution of contemporary DXA analysis and are, therefore, not accounted for using BMD from DXA.

1.4 Consequences of osteoporosis

Fracture, fracture risk, and associated morbidity

In the US osteoporosis results in 1.5 million fractures per year, the vast majority occurring in postmenopausal women[87]. The consequences of fragility fractures is devastating in terms of mortality, morbidity, and cost [51,52]. A review of fractures in the Geelong region revealed almost all hip fracture and 27% of non-hip fracture were hospitalised. Homes were modified in 14% of cases, and 32% of the women purchased or hired equipment to assist with activities of daily living. Three-quarters of women with hip, pelvis, or lower limb fractures

were confined to the home, had to walk with a walking aid, or could walk only short distances for several weeks. After a year, nearly one-half had not regained prefracture mobility. One-seventh of women with upper-limb fractures did not venture outside the home for at least 6 weeks, and half of all cases needed help with personal care and housework during the first 6 weeks. After 6 months, 3.4% of all patients, 19.6% of hip, 12.8% of humeral, and 4.7% of spine fracture patients required assistance with bathing and showering. After a year, more than half of the hip fracture cases remained restricted regarding housework, gardening, and transport. In summary, a fracture, regardless of site, has a major impact on a woman's lifestyle and well-being for at least a year [51]. Despite the known consequences, as few as 13-27% of patients with osteoporosis are treated following a fragility fracture, suggesting osteoporosis remains an under diagnosed disease [53,54].

It is also important to note that although the individual risk of fracture is highest in women with osteoporosis, almost three-quarters of fractures occur in women with osteopaenia or normal bone mass, due to the increase proportion of the population in these categories (Table 1)[72,73,88]. This means effective reduction of fragility fractures requires attention to women with osteopaenia in particular. This limitation of BMD for fracture prediction has led to the development of fracture risk prevention models that incorporate clinical factors and BMD, including the FRAX® fracture risk assessment tool [89]. These tools are widely used to estimate 10-year probability of hip and major osteoporotic fracture.

Table 1: Distribution and fracture risk of women by BMD category

Category	T-score	Population	Any fracture	Adj RR fracture
Normal	> -1.0	37.6%	16.6%	1.0
Osteopaenia	-1.0 to -2.5	48.0 %	56.5%	2.67 (1.55-4.61)
Osteoporosis	< - 2.50	14.5%	26.9%	3.72 (2.00-6.90)

Pasco J, et al. Osteoporosis Int. 2006;17:1404-1409

Mortality

The consequences of osteoporosis extend beyond the morbidity associated with fragility fractures, to mortality. Between 10 to 20% of people who sustain a hip fracture die within one year [50], the risk highest in the first six-months and decreasing over time. The Dubbo Osteoporosis Epidemiology Study reported an increased mortality in participants who sustained a fracture compared to the general population. For both sexes, mortality rates were increased in the first 5-years following fracture, declining thereafter to towards general

population mortality rates. The exception to this was hip fracture, where mortality rates remained elevated to 10-years [90].

The cause of this long-term fracture-mortality association remains unresolved, with debate about the relative contributions of underlying comorbidities, frailty, and low bone density. Vertebral fractures are associated with an increased pulmonary and cardiovascular mortality that extends beyond the first year and increases with the number of vertebral fractures. However few of these deaths are directly attributable to hip fracture; most result from chronic illnesses that lead to both fracture and early death [50]. The Study of Osteoporotic Fractures reported that hip and pelvic fractures were associated with a RR of death of 2.4, but only 14% of deaths were attributable to the fracture[91]. Similarly, analysis of the Swedish patient register concluded that 17-32% of deaths after hip fracture, and 28% of deaths after vertebral fracture were attributable to the fracture [92,93]. The Dubbo Osteoporosis Epidemiology Study reported cardiac, respiratory, cerebrovascular disease, and malignancy as the major cause of death following fracture. A direct association of mortality with fracture was suggested by the increased risk observed in the years immediately following fracture followed by a gradual return to population levels, as well as the observation that mortality risk increased again after subsequent fracture[90].

The evidence for association between osteoporosis and increased mortality is further strengthened by the relationship between osteoporosis treatments and reduced mortality. A large prospective RCT comparing annual intravenous zoledronic acid administered within 3-months of hip fracture to placebo in 1065 adults reported a reduced incidence of second hip fracture and non-vertebral fracture, and a reduction in all-cause mortality by 28% [94]. A meta-analysis of RCTs investigating approved doses of medication with proven efficacy in preventing vertebral and non-vertebral fractures, with a duration of at least 12 months and reporting mortality, identified eight studies of four agents (risedronate, strontium ranelate, zoledronic acid, and denosumab), providing data of over 1400 deaths in approximately 40,000 subjects. Overall osteoporosis treatment was associated with an 11% reduction in mortality (RR 0.89, 95%CI 0.80-0.99, p=0.036)[95]. Meta-regression analyses revealed mortality reduction was not related to mean age, incidence of hip or non-vertebral fracture in the placebo group, or non-vertebral fracture risk reduction, but was associated with the baseline mortality rate of the placebo group (P=0.03). In the four studies where the placebo mortality rate was greater than 10 per 1000 patient years (range 13.9-70.2 deaths per 1000 patient-years), there was a significant reduction in mortality (RR 0.83; 95% CI 0.72-0.94, p=0.0052), compared to no reduction in mortality in studies where placebo mortality rate was

less than 10 per 1000 years (RR 1.01, 95% CI 0.87-1.19, p=0.86)[95]. The mortality effect appeared to be similar across the different classes of agents in the study. It is important to note that 9 of 10 trials were performed in postmenopausal women with low bone mineral density, and that only 1 trial reported a significant mortality benefit. The authors conclude that to better understand this mortality should be included as pre-specified endpoint in future studies of osteoporosis therapies.

There is emerging evidence to explain the non-fracture relationship between high bone turnover, cardiovascular and immune-modulating mechanisms, and mortality[96]. Anti-resorptive agents, such as bisphosphonates, may be taken up by calcified blood vessels and inhibit the mevalonate pathway leading to alterations of nitric oxide generation and atherogenesis, including monocyte adhesion to the endothelial surface, platelet aggregation vascular smooth muscle cell proliferation, and vasoconstriction. The HORIZON trial reported a similar rate of occurrence of pneumonia, cancer, and cardiovascular disease in patients treated with placebo or zoledronic acid, but a reduced mortality rate associated with the conditions in the zoledronic acid group[97].

In addition an association between elevated levels of BTMs and increased mortality has been described in cancer and non-cancer populations. In an elderly residential care cohort, increased serum CTX and P1NP were associated with increased mortality, with CTX associated with increased risk of death from cardiovascular causes[96]. Decreased levels of osteocalcin and increased levels of CTX were associated with increased cardiovascular and all-cause mortality in male and female patients referred for coronary angiogram [98,99]. In older ambulatory females serum OPG levels were independently associated with all-cause mortality, and cardiovascular mortality[91], while in systemic amyloidosis without bone lytic lesion, OPG was associated with all-cause mortality [100].

1.5 Rationale for further investigation of critical illness related bone loss

If critical illness were associated with increased bone loss, increased risk of fracture, and associated morbidity and mortality, this would contribute significantly to survivor health burden; with the average cost of hip fracture in Australia estimated at $16,000, and average length of hospital stay of thirteen days[10]. Furthermore fragility fractures are associated with excess mortality, pain, immobility, and reduced functional capacity resulting in significant quality of life issues[12,16,17,11]. Finally, the availability of target interventions to prevent or attenuate acute bone loss following critical illness provides the incentive to further explore this area of clinical research.

The following chapter reviews the evidence for an association between critical illness and altered bone turnover, which provides the rationale for further investigation in this area. A number of studies have identified a relationship between critical illness requiring mechanical ventilatory support and increased bone turnover, with increased osteoclastic bone resorption, increased immature osteoblast number and activity, and reduced activity of mature osteoblasts of the magnitude described in postmenopausal females, or metabolic bone disease [69,101-103].

The evidence describing the effect of known osteoporosis risk factors and critical illness related factors on BTMs in critical illness, the longitudinal changes in BMD following critical illness, and comparison to non-critically ill controls is limited. The subsequent chapters in this thesis describe the results of a longitudinal observational study of changes in BTMs and BMD in survivors of critical illness, factors associated with these changes, and comparison to a large prospective community based osteoporosis study, the Geelong Osteoporosis Study. With no studies to date describing changes in BMD following critical illness, this represents an important contribution to the literature.

1.6 The population controls used in this thesis - The Geelong Osteoporosis Study

A major component of this thesis is the comparison of BMD data from critically ill patients to a well-defined control population, the Geelong Osteoporosis Study (GOS). The GOS was a recruited random population-based sample of women (ages 20-94) and men (ages 20-94) from the Commonwealth Electoral Rolls for an area surrounding Geelong in Southern Australia called the Barwon Statistical Division. As voting is compulsory in Australia the electoral roll provides a comprehensive listing of adults (age ≥ 18 years). For both male and female cohorts the sample was age-stratified with a minimum of 100 in each 5-yr age stratum between ages 20 and 69, and a minimum of 200 in the age 70-79 yr group, and the over 80 yr group. The cohort includes 1494 females and 1467 men. As part of the study BMD's are performed second yearly in the female cohort and five yearly in the male cohort. The annual decline in lumbar spine in the GOS population is normally distributed, with a standard deviation of 0.06% at the lumbar spine in males, and 0.25% at the lumbar spine in females.

In this thesis the GOS cohort is used as a control population for comparison of absolute and annualised change in BMD. The first use of a GOS cohort is a prospective study of ICU patients ventilated for greater than 24-hours and followed up for 2-years. Participants who

completed BMD measurements at ICU discharge and one-year will be matched to GOS controls by age, sex, and BMI, in a one-to-four fashion using Mahalanobis weights, without replacement, to compare annualised change in BMD. The average treatment effect for the ICU participants will be estimated via linear regression including the covariates used for matching, an indicator variable for whether the participant was admitted to the ICU or was a (GOS) population control, and a random effect to account for correlation induced by the matching. The second use of the GOS population is a nested cohort study, where participants in GOS admitted to Intensive Care will be identified through database linkage. The ICU cohort will be matched to non-ICU GOS participants by age and gender, and absolute BMD and change in BMD before and after ICU compared where possible. This will provide unique evidence of pre-ICU trajectory of disease compared to a matched control population.

The final component of this thesis is a protocol for a phase II interventional study aimed at reducing bone resorption after critical illness, based on the proposed observational studies. The testing of antiresorptive therapies, with a proven role in osteoporosis and other models of accelerated bone loss, in a critically ill population, would be a unique and valuable addition to the literature.

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Chapter 2: The association between critical illness and changes in bone turnover in adults: A systematic review

This chapter is a systematic review and synthesis of the literature regarding the association between critical illness and changes to bone turnover in the early period of this thesis. Studies were rated upon their methodological quality, and a best-evidence synthesis was used to summarise the results. Overall 11 studies were identified and assessed, and moderate evidence of a positive association between critical illness requiring intensive care admission and bone turnover was reported. It was recognised that data was limited, and risk factors and the nature of the relationship were not yet understood. The study was published in *Osteoporosis International* in 2014.

Osteoporos Int
DOI 10.1007/s00198-014-2734-1

REVIEW

The association between critical illness and changes in bone turnover in adults: a systematic review

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Received: 18 December 2013 / Accepted: 22 April 2014

Abstract
Summary Critical illness may lead to altered bone turnover and associated adverse health outcomes. This systematic review found moderate evidence for a positive association between critical illness and increased bone turnover. Prospective cohort studies that identify the extent and risk factors for critical illness related bone loss are required.
Introduction Intensive care patients face health issues that extend beyond their critical illness and result in significant morbidity and mortality. Critical illness may result in altered bone turnover due to associated immobilisation, inflammation, exposure to medications that effect bone and calcium metabolism, and endocrine dysfunction. The aim of this study was to synthesise the existing evidence for altered bone turnover in adults admitted to intensive care.
Methods A literature search using MEDLINE and EMBASE was performed from 1965 to March 2013. Reviewed studies investigated the relationship between critical illness and evidence of altered bone turnover (bone turnover markers, bone mineral density, or fracture). Studies were rated upon their methodological quality, and a best-evidence synthesis was used to summarise the results.
Results Four cohort and seven case–control studies were identified for inclusion, of which five studies were rated as being of higher methodological quality. Ten of the studies measured bone turnover markers, and one study fracture rate. Findings were consistent across studies, and best-evidence analysis resulted in a conclusion that moderate evidence exists for an association between critical illness requiring admission to intensive care and altered bone turnover.
Conclusion A positive association between critical illness requiring intensive care admission and bone turnover exists, although data are limited, and the risk factors and the nature of the relationship are not yet understood. Prospective cohort studies that identify risk factors and extent of critical illness related bone turnover changes are required.

Keywords Bone turnover · Critical care · Intensive care · Mechanical ventilation · Osteoporosis

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Published online: 07 May 2014

Introduction

Intensive care patients face health issues after their critical illness including increased mortality, reduced quality of life, reduced return to work, and ongoing economic and social costs to families and caregivers when compared to pre-illness and general population controls [1–7]. Despite an increasing awareness of long-term sequelae of critical illness, the identification of specific pathophysiologies amenable to intervention remains elusive. Osteoporosis is a major public health problem, and is widely recognised as a chronic progressive disease with multifactorial aetiology [8]. The epidemiology and risk factors for primary and secondary osteoporosis are well described, including increasing age, female gender, low body mass index (BMI), smoking, excessive alcohol intake, positive family history, medications such as glucocorticoids, and predisposing disease or medical condition such as hyperthyroidism [8, 9]. However, as few as 13–27 % of patients with osteoporosis are treated following a fragility fracture, suggesting it remains an under-diagnosed disease [10, 11]. Critical illness, with its associated immobilisation, inflammation, and endocrine dysfunction, may lead to accelerated bone turnover. When combined with an ageing population with undiagnosed osteoporosis, this accelerated bone turnover may contribute to the burden of morbidity and mortality observed in survivors of intensive care [12, 13]. In a review of metabolic bone disease in the intensive care unit (ICU), Hollander et al. concluded an interventional study of bisphosphonates in survivors of ICU is needed [14]. However, there is no systematic review of the evidence for accelerated bone turnover following critical illness, or of the nature and risk factors for this relationship.

In this study, we sought to systematically review and synthesise the current literature regarding the association between critical illness and changes to bone turnover. In addition, we describe ongoing and planned research in this area.

Methods

Search strategy and study selection

To identify studies that examined whether admission to an ICU was associated with changes in bone turnover in adults, we searched MEDLINE (1965 until 31 March 2013) and EMBASE (1974 until 31 March 2013) for citations of relevant articles. Our computerised search strategy employed the following medical subject headings (MeSH/EMTREE) ("mechanical ventilation" or "critical illness" or "chronic critical illness" or "ventilator" or "critical care" or "intensive care") and ("bone turnover" or "bone change" or "fracture" or "bone mineral density" or "bone density" or "bone loss" or "CTX" or "P1NP" or "bone biomarkers" or "bone markers").

Studies were deemed eligible for inclusion in this review if they met the following criteria: full-text original articles; comprised either a cohort, case–control, or a cross-sectional study design; examined, in adults aged ≥18 years, associations between receiving mechanical ventilation in an ICU (with a length of stay 24 h or greater), and de novo change in bone turnover (defined as loss of BMD, increase in bone turnover markers (BTMs), or incident fracture of at least one of the major osteoporotic sites of hip, wrist, humerus, or spine). Patients that were identified as osteoporotic at the time of ICU admission were included.

Studies were excluded if published in languages other than English; utilised animal models; investigated patients with existing neurological illness that results in impaired weight-bearing (including stroke with loss of weight-bearing, spinal cord injury, progressive neurological disease, e.g. multiple sclerosis); were admitted to ICU for reasons of trauma-related fracture, or with existing metabolic bone disease; employed qualitative methodology; or were review articles, editorials, commentaries, dissertations, or were randomised control trials. Where interventional studies reported baseline data that fulfilled inclusion and exclusion criteria prior to intervention, the baseline data were included in the analysis.

We electronically restricted our search to identify articles that were related to human subjects, published in English, and available in full-text. Reference lists of relevant studies deemed eligible for inclusion were manually searched, and citations were tracked for those publishing in the field of interest.

Two reviewers confirmed the search strategy (NRO and SLB) and one reviewer performed the computerised search and initial manual search (NRO). Complete details of the search strategy can be obtained from the corresponding author. For each eligible study, two reviewers (NRO and CEC) confirmed the selection of articles based on readings of the full-text article. Where the eligibility of studies was ambiguous, two reviewers (NRO and CEC) held discussions to reach consensus. Where consensus could not be achieved, a third reviewer was consulted (SLB).

Methodological quality assessment

To assess the methodological quality (internal validity) of the included studies, two reviewers (NRO and CEC) undertook independent scoring using an adapted version of the scoring system published by Lievense et al. [15] (Table 1); this methodological approach has previously been employed for reviews of observational studies in the field of musculoskeletal disorders [15, 16]. Both reviewers independently scored each of the criteria as positive (1), negative (0), or not applicable (NA), with a maximum possible score for each study design of 100 %. Where the score afforded to certain criteria differed between the reviewers, discussion was held to achieve

Table 1 Criteria list for assessment of methodological quality for each eligible study, adapted from Lievense et al. [15]

Item criterion	
Study population	
1. Selection at uniform point	C/CC
2. Cases and controls drawn from same population	CC
3. Participation rate ≥80 % for cases/cohort	C/CC
4. Participation rate ≥80 % for controls	CC
Assessment of risk factor	
5. Exposure assessment was blinded	C/CC
6. Exposure measured identically for cases and controls	CC
7. Exposure assessed prior to outcome	C/CC
Assessment of bone turnover	
8. Bone turnover assessed identically in studied population	C/CC
9. Bone turnover reproducibly (coefficient of variation reported)	C/CC
Study design	
10. Prospective design used	C/CC
11. Follow-up time >24 months	C
12. Withdrawals <20 %	C
Analysis and data presentation	
13. Appropriate analysis techniques used	C/CC
14. Adjusted for at least age and gender	C/CC

C applicable to cohort studies, *CC* applicable to case–control studies

consensus; if disagreements were not resolved, a third reviewer (SLB) was consulted to achieve a final judgment. Positive scores were summed to give an overall internal validity score.

Data analysis

As there were limited data and studies were heterogeneous, a "best-evidence" synthesis was preferred rather than a meta-analysis. The studies were divided into subgroups according to the type of study design. A cohort study was judged the most valid design, followed by case–control study. Studies were then ranked according to their methodological quality score (Table 2). A study was considered to be of higher quality if the methodological quality score was greater than the mean quality score of all studies [15–17].

Results

Search results and study characteristics

The results of our search are presented in Fig. 1. Our electronic search strategy identified 13,185 studies, including 2,218 duplicates that were subsequently excluded. Of the remaining 10,967 studies, a total of 21 underwent full-text review to determine eligibility. A further six studies were identified for full-text review from a manual search of citation lists. Of the 27 full-text studies reviewed, 16 were excluded for not meeting the predetermined eligibility criteria, resulting in 11 studies included in the final analysis [18–28]. In three of the case–control studies, ICU patients were randomised to an intervention after comparison of baseline data from the ICU cohort was compared to a control cohort [22, 23, 26]. The baseline data from these studies was included in the analysis, whereas the data resulting from the randomised intervention was excluded. One study performed in vivo analysis of osteoclast number and activity in ICU patients compared to controls, with further in vitro analysis of osteoclast cells, osteoblastic cells, and serum activation factors [25]. As the in vitro tests were not recognised tests of bone turnover, they were excluded from the analysis.

Table 2 Criteria list for determining the level of evidence for best-evidence synthesis, adapted from Lievense et al. [15, 16]

Level of evidence	Criteria for inclusion in best-evidence synthesis
Strong evidence	Generally consistent findings:
	Multiple high-quality cohort studies
Moderate evidence	Generally consistent findings:
	One high-quality cohort study and >2 high-quality case–control studies
	>Three high-quality case–control studies
Limited evidence	Generally consistent findings in:
	Single cohort study
	One or two case–control studies or
	Multiple cross-sectional studies
Conflicting evidence	Inconsistent findings in <75 % of the trials
No evidence	No studies could be found

Description of the studies

An overview of the included articles (n=11) is presented in Table 3. Four of the studies were cohort study design [18–21] and the remaining seven were case–control study design [22–28]. Sample sizes ranged from 9 [28] to 739 cases [18]. A group of seven patients were shared by two studies, in which a total of 15 cases [23] and 33 cases [22] were enrolled. In addition, these studies shared the same group of 50 controls.

The criteria for enrolment in the studies included requirement for mechanical ventilation in an ICU [20, 27, 28], duration of mechanical ventilation greater than 48 h [18] or 2 weeks [22–24], chronic critical illness [25], admission to a respiratory care unit for prolonged ventilatory support [19, 21], and ICU length of stay greater than 10 days [26]. Patient populations included mixed adults in nine of the studies [18–21, 24–28], and males only in two studies [22, 23]. A control population was present in the seven case–control

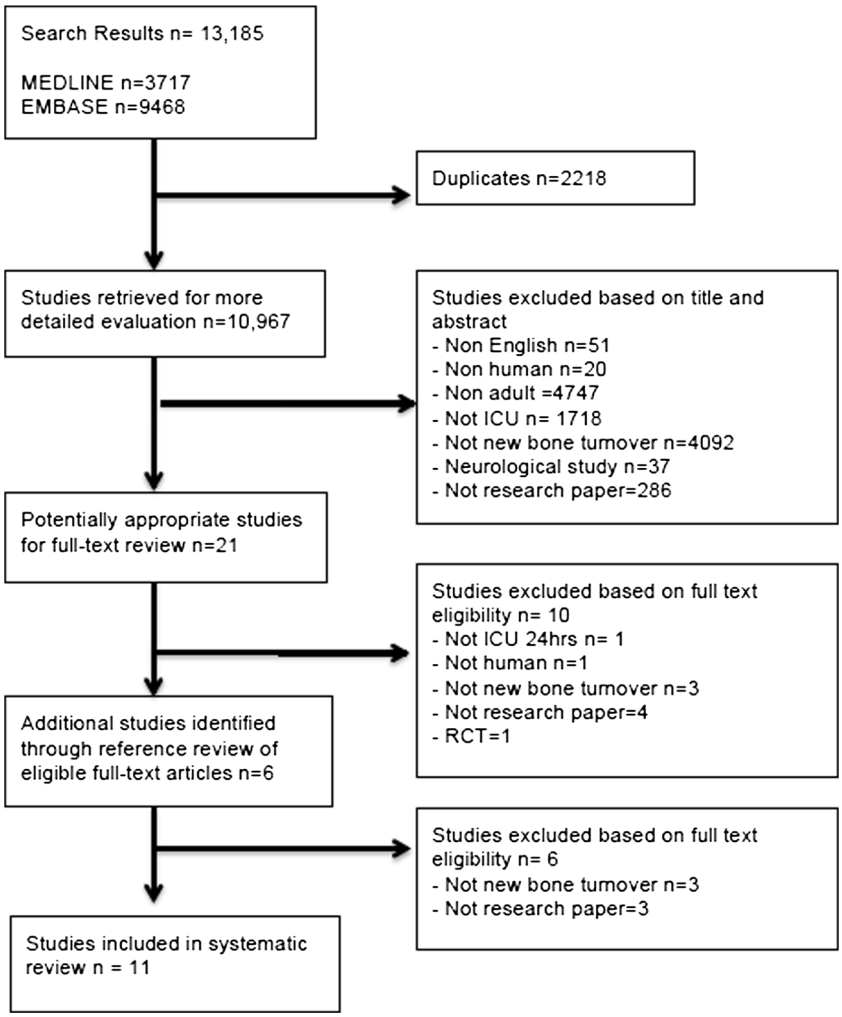

Fig. 1 Summary of systematic search (QUOROM diagram)

studies, and in one of the cohort studies [18]. The controls were age- and gender-matched healthy population-based participants in six studies [18, 22–26], age but not gender-matched healthy controls in one study [28], and non-gender or age-matched participants with a history of rheumatism or mild osteoarthritis in one study [27]. Exclusion criteria included renal, metabolic, and liver disease in five studies [22–24, 26, 28], metabolic and neurological disease in four studies [22–24], and prior medications in four studies [22–24, 26]. The follow-up time for patients ranged from 1 day [24, 25] to 10 years [18].

Methodological quality assessment

The two reviewers scored 129 items and agreed on 117 items (90.70 % agreement, κ=0.80, standard error (SE) 0.05, 95 % CI 0.70–0.91, strength of agreement considered "very good"). The 12 disagreements were resolved in a single consensus meeting. The range of methodological scores was 55 to 67 % (Table 4), with the mean of quality scores 61 %. Using the mean score as the cut-off point, 5 of 11 were considered to be of higher methodological quality [18, 19, 22–24].

Results of all included studies

Assessment of bone turnover

Table 4 presents the findings of the reviewed studies. The BTM used in the studies included bone resorption and bone formation markers [29]. The bone resorption markers measured included urinary collagen type 1 cross-linked N-telopeptide (NTX) in two studies [19, 21], urinary pyridinoline (Pyd) or deoxypyridinoline (Dpd) in seven studies [20, 22, 23, 26–28], and urine or serum carboxy-terminal cross-linked telopeptide of type 1 collagen (ICTP/BCTX) in two studies [20, 26]. One study reported serum osteoclast precursors (double-positive CD14+/CD11b+) and serum mature osteoclasts (triple-positive CD14+/CD11b+/VNR+) [25]. Bone formation markers were reported in four studies [22–24, 26] and included serum skeletal alkaline phosphatase (SALP), serum osteocalcin (OC), serum procollagen type 1 C peptide

Table 3 Characteristics of the included studies (data are presented as no. (%), mean±SD, median [Inter quartile range [IQR]])

First author, country, year	Design	Subjects no. (% female)	Age	Patient description	Exclusion	Follow-up
Cohort						
Orford et al., Australia, 2011 [18]	Retrospective matched cohort	Cases 739 (35 %)	68 [57–75]	Adult ICU patients, duration of mechanical ventilation >48 h, survived to ICU discharge	Nil	Up to 10 years
		Controls 1,494 (100 %)	54 [37–71]	Age-matched female controls from GOS		Up to 10 years
Nierman et al., USA, 1998 [19]	Prospective cohort	Cases 49 (43 %)	73 [35–96]	Adult patients admitted to RCU with chronic critical illness (median ICU length of stay 20 days [IQR 9,177] prior to RCU admission)	Nil	First 48 h
Lind et al., Sweden, 2000 [20]	Prospective cohort	Cases 26 Sepsis 13 (62 %) Surgery 13 (38 %)	 64±19 62±15	Adult patients admitted to ICU with inclusion criteria (sepsis or major surgery) on Mon–Wed during a 1-year period.	Nil	1–3 days
Nierman et al., USA, 2000 [21]	Retrospective cohort	Cases 55 (45 %)	75 [33–90]	Adult patients admitted to RCU with chronic critical illness (median ICU length of stay 16 days [IQR 1,177] prior to RCU admission) and met eligibility criteria of elevated urine NTX and received treatment for bone hyperresorption between measurements (calcitriol +/−parenteral pamidronate for three consecutive days)	Nil	Average 26 +/−12 days
Case–control						
Van den Berghe et al., Belgium, 2002 [22]	Prospective case–control	Cases 33 (0 %)	67±11	Male adult patients with duration mechanical ventilation >2 weeks, expected ICU LOS at least 2 weeks further; seven patients in this study are shared with previous study [23]	Metabolic, neurological, endocrine disease, liver and renal failure, medications	6 days
		Controls 50 (0 %)	67±8	Age-, BMI-, and gender-matched healthy controls. These are shared with previous study [23]		
Van den Berghe et al., Belgium, 2001 [23]	Prospective case–control	Cases 15 (0 %)	67±12	Male adult patients with duration mechanical ventilation >2 weeks, expected ICU LOS at least further 2 weeks	Metabolic, neurological, endocrine disease, liver and renal failure, medications	6 days
		Controls 50 (0 %)	67±8	Age-, BMI-, and gender-matched healthy controls		
Van den Berghe et al., Belgium, 1999 [24]	Prospective case–control	Cases 14 (29 %)	68±12	Adult patients with duration mechanical ventilation >2 weeks, expected ICU LOS at least 2 further weeks	Metabolic, neurological, endocrine disease, liver and renal failure, medications	10 days
		Controls 65 (23 %)	NA			1 day

Table 3 (continued)

First author, country, year	Design	Subjects no. (% female)	Age	Patient description	Exclusion	Follow-up
				Age- and gender-matched healthy controls		
Owen et al., Belgium, 2012 [25]	Prospective case–control	Cases 12 (29 %)	57 [26–80]	Adult patients with prolonged critical illness (ICU LOS=7–134 days)	Nil	1 day
		Controls 12 (29 %)	57 [23–81]	Age-, gender-, BMI-matched healthy controls		
Van den Berghe et al., Belgium, 2003 [26]	Prospective case–control	Cases 22 (46 %)	62 [36–73]	Adult ICU patients with ICU LOS >10 days	Chronic bone or kidney disease, prior glucocorticoids	10 days
		Controls 22/64 (46 %)	62 [41–73]	Age, gender, BMI matched controls. Analysis of blood samples (n=22) and urine samples (n=64)		
Smith et al. UK, 2002 [27]	Prospective case–control	Cases 23 (56 %)		Adult ICU patients admitted consecutively to ICU (sepsis n=20, trauma n=3)	Nil	1–6 days
		Sepsis 20 (60 %)	65±2.9			
		Trauma 3 (33 %)	20±2.7			
		Controls 29 (55 %)	40±1.8	Adults with soft-tissue rheumatism or mild osteoarthritis (not gender- and age-matched)		
Shapses et al., USA, 1997 [28]	Prospective case–control	Cases 9 (33 %)	73±7	Adult ICU patients requiring postoperative mechanical ventilation after gastrointestinal surgery	Diabetes, liver disease, renal disease	Up to 20 days
		Controls 17 (47 %)	60±2	Age-matched healthy controls		

BMI body mass index, *GOS* Geelong Osteoporosis Study a recruited random population-based sample, *ICU* Intensive Care Unit, *LOS* length of stay, *NA* not available, *NTX* collagen type 1 cross-linked N-telopeptide, *RCU* respiratory care unit

Table 4 Results of eligible studies included in review, presented by study design according to methodological quality assessment score

Study first author	Bone turnover measure	Outcome	Result (presented as OR or HR with 95 % CI ± *p* value, mean ± SD), or median [IQR]					Summary of significant findings	Quality score
Cohort									
Orford et al. [18]	Fracture	Age-adjusted comparison of incident fragility fracture rate between female patients post-ICU discharge and community controls	Adjusted HR 1.65 (95 % CI 1.08–2.52, p=0.02)					Increase in fragility fracture risk in older female ICU survivors compared with age- and gender-matched population control subjects.	64 % (7/11)
Nierman et al. [19]	Biomarker—uNTX	1. Comparison to reference range	Normal (n=4) NTX 43±23 Primary hyperresorption (n=4) NTX 176±88					Increased bone resorption in chronic critically ill patients	64 % (7/11)
		2. Metabolic categorisation into different states	Secondary hyperresorption (n=19) NTX 257±202 Mixed (n=22) NTX 200±122						
Lind et al. [20]	Biomarker—sICTP, uDpd	Comparison between sepsis and major surgery patients	Marker	Sepsis D1	D3	Surgical D1	D3	Increased bone resorption in both septic and surgical patients in ICU	55 % (6/11)
			uDpd	11±7.2	6.9±2.3	9.4±5.5	8.4±5.2		
			sICTP	25±16	25±15	12±13	16±12		
Nierman et al. [21]	Biomarker—uNTX	Comparison to reference range	uNTX 215+175, PTH 81+123					Increased bone resorption in chronic critical illness, with positive association with ICU and hospital LOS	55 % (6/11)
Case–control									
Van den Berghe et al. [22]	Biomarker—sOC, sPICP, sSALP uPyd, uDpd	Comparison between cases and control		Subjects	Controls	p value		Increased bone resorption and synthesis. Positive association between inflammatory cytokines and bone formation markers	67 % (8/12)
			sSALP	22.8±21.2	9.1±5.4	0.0008			
			sPICP	296±175	51±20	< 0.0001			
			sOC	29±17	34±32	0.03			
			uPyd	393±319	63±29	<0.0001			
			uDpd	55±48	12±5	<0.0001			
Van den Berghe et al. [23]	Biomarker—sOC, sPICP, sSALP uPyd, uDpd	Comparison between cases and controls		Subjects	Controls	p value		Increased bone resorption and synthesis. Positive association between inflammatory cytokines and bone formation markers.	67 % (8/12)
			sSALP	19.6±14.8	9.1±5.4	0.02			
			sPICP	325±219	51±20	0.0003			
			sOC	22±10	34±32	0.004			
			uPyd	443±331	63±29	<0.0001			
			uDpd	57±33	12±5	<0.0001			
Study first author	Bone turnover measure	Outcome	Result					Summary of significant findings	Score
Van den Berghe et al. [24]	Biomarker—sOC, sPICP, sSALP uPyd, uDpd	Comparison between cases and controls		Subjects	Controls	p value		Increased bone resorption and synthesis in prolonged critically ill adults. Inverse association between bone resorption markers and thyroid hormones, and positive association between bone formation markers and inflammatory markers.	67 % (8/12)
			sSALP	13.5±11.4	9.1±5.4	NS			
			sPICP	256±108	51.4±20.4	<0.0001			
			sOC	29.5±14.3	34.5±29	0.04			
			uPyd	836±776	61±27	<0.0001			
			uDpd	106±80	12±5	<0.0001			
Owen et al. [25]	Biomarkers Osteoclast precursor	Comparison subjects and controls		Subjects	Controls	p value		Increased circulating osteoclast precursors in critical illness	58 % (7/12)
			Osteoclast precursor	99.1 %	83.9 %	<0.05			

Table 4 (continued)

Study first author	Bone turnover measure	Outcome	Result (presented as OR or HR with 95 % CI ± *p* value, mean ± SD), or median [IQR]					Summary of significant findings	Quality score
	Mature osteoclast		Mature osteoclast	0.54 %	0.26 %	0.09			
Van den Berghe et al. [26]	Biomarker—sOC, sPICP, sSALP, sBCTX, SPINP, uPyd, uDpd	Comparison between cases and control		Subjects	Controls	*p* value		Increased bone turnover, characterised by active immature osteoblasts, low activity of mature osteoblasts, and increased osteoclast activity.	58 % (7/12)
			sSALP	9.4±3.9	9.1±5.1	0.7			
			sPICP	388±360	51±23	0.0004			
			sPINP	65 [39,123]	38 [30,46]	0.002			
			sOC	21.8±10.8	34.5±29.0	<0.0001			
			BCTX	0.71 [0.46,1.03]	0.12 [0.08,0.18]	<0.0001			
			uPyd	328 [215,503]	55 [43,71]	<0.0001			
			uDpd	55 [36,76]	11 [8,14]	<0.0001			
Smith et al. [27]	Biomarker—uPyd, uDpd	Comparison between cases and control		ICU sepsis	Controls	ICU trauma	*p* value	Increased bone resorption in sepsis and trauma compared to controls.	58 % (7/12)
			Age	65±2.9	40±1.8	20±2.7	0.001		
		Multiple regression analysis	uPyd	417.0±155	44.7±2.6	207.7±53	0.017	Nitric oxide (NO) not correlated with inhibition of bone resorption.	
			uDpd	67.4±17.2	10.3±0.7	44.8±3.4	0.001		
Shapses et al. [28]	Biomarker—uPyd, uDpd	Comparison between subjects and controls	Increased Pyd and Dpd ($p<0.001$)					Increased bone resorption markers in subjects compared to controls, and increased bone resorption markers in longer stay ICU patients compared to shorter stay	58 % (7/12)

uDpd urinary deoxypyridinoline (ref range 1.3–8.4 nmol/L or 4.3–27.5 nmol/mmol Creat), *sICTP/BCTX* serum carboxy-terminal cross-linked telopeptide of type 1 collagen (ref range 1.9–6.6 μg/L), *IGFBP-3* Insulin-like growth factor-binding protein 3, *M-CSF* macrophage colony stimulating factor, *uNOx* urinary nitric oxide breakdown products nitrate and nitrite (uNOX/creatinine, mmol/mmol), *uNTX* urine collagen type 1 cross-linked N-telopeptide (ref range 12–80 BCE/mmol Cr), *sOC* serum osteocalcin (ref range 13.2–254 μg/L), *PBMC* Peripheral blood mononuclear cell, *sP1CP* serum carboxyl-terminal extension peptide of type 1 procollagen (ref range 75–254 μg/L), *PTH* parathyroid hormone (ref range 12–55 pg/ml), *uPyd* urinary pyridinoline (ref range 27–182 nmol/mmol Creat), *RANKL* receptor activator of NF-kB ligand, *sSALP* serum skeletal alkaline phosphatase (ref range 9.0–15.0 μg/L)

(P1CP), and serum procollagen type 1 N peptide (P1NP). Incident fracture rate post-ICU discharge was reported as the outcome in one study [18].

Results of bone turnover measurement

All studies that measured markers of bone resorption reported an increase in markers compared to controls or reference range, suggesting increased osteoclastic activity. The two studies observing urinary NTX levels in patients admitted to a respiratory care unit (prolonged ventilation unit) reported elevated NTX in 83 % of patients [21], with baseline levels 4- to 6-fold greater than reference range [19, 21] while urinary collagen cross links (Pyd, Dpd) were increased 4- to 14-fold compared to controls [20, 22–24, 26–28], and serum carboxy-terminal cross-linked telopeptide of type 1 collagen was increased 3- to 6-fold compared to controls or reference values [20, 26]. The one study that measured osteoclast precursors and mature osteoclasts in serum described a significant increase in osteoclast precursors in critical illness compared to controls [25].

The studies that reported markers of bone formation described a varied increase in SALP compared to controls, a significant increase in P1CP and P1NP compared to controls, and a significant decrease in osteocalcin compared to controls [22–24, 26]. These results suggest an increase in number and activity of immature osteoblasts, with low activity of mature osteoblasts.

The single study that reported the incidence of new fractures following ICU described an increased incident fragility fracture risk in older female ICU survivors (rate 4.33/100 patient years, 95 % CI 2.72–5.93) compared with age- and gender-matched population controls (rate 2.81/100 patient years, 95 % CI 2.33–3.28) [18].

Exposure variables and increased bone turnover

Three studies reported a relationship between bone resorptive markers and either ICU or hospital length of stay, with a positive correlation between urinary NTX and ICU length of stay (r=0.42, p<0.01) [19], urinary NTX and both hospital (r=0.49, p<0.01) and ICU length of stay (r=0.42, p<0.01) [21], and increased urinary Pyd and Dpd in patients with an ICU length of stay of 5 days or greater compared to less than 5 days [28]. The relationship observed between vitamin D, parathyroid hormone, or calcium status was variable [19, 20]. An association between bone formation markers and inflammatory markers was observed in three studies [22–24], an inverse correlation between bone resorptive markers and thyroid hormones in one study [24], and no correlation between nitric oxide breakdown products and bone resorption markers in one study [27]. The two studies that compared ICU patients with sepsis to other ICU cohort reported an increase in bone resorption markers in sepsis compared to trauma [27] and surgery [20].

Best-evidence analysis

As the reviewed studies employed different methodology had recruited diverse populations (for example, differences in ages, gender and population sizes, among other factors), and examined varying follow-up times, we performed a best-evidence analysis to cater for the high-level heterogeneity. Our best-evidence synthesis included studies that scored above the mean (>61 %) for their methodological quality. Of the eligible studies, two cohort [18, 19] and three case–control studies [22–24] were considered to be of higher methodological quality.

The higher-quality cohort studies described an increase in bone resorption markers, with a positive correlation between markers and duration of ICU stay [19], and an increase in fragility fracture in older women following ICU admission compared to age- and gender-matched healthy controls [18]. The higher-quality case–control studies described an increase in bone resorption, a pattern of increased bone formation consistent with an increased number and activity of immature osteoblasts and decreased activity of mature osteoblasts, and a correlation between inflammatory cytokines and bone resorptive activity [22–24]. These results are consistent with findings of the lower-quality studies.

In summary, the result of our best-evidence analysis is 5 of 11 studies (two cohorts and three case–control) that were considered of higher methodological quality, with consistent results. This is consistent with a conclusion that moderate evidence exists for an association between critical illness requiring intensive care admission and changes in bone turnover.

Discussion

Overall, we identified limited but consistent data that examine the relationship between critical illness requiring ICU admission and bone turnover. A best-evidence analysis of available literature provides moderate evidence for a positive association between critical illness requiring ICU admission and increased bone turnover, a finding in all the studies identified by this analysis. There are insufficient high-quality data available about the factors contributing to the relationship between ICU admission and bone turnover to allow interpretation of the nature of this association.

This review included studies with patients admitted to ICU for mechanical ventilatory support for greater than 24 h, and assessed measures of bone turnover following ICU admission. We chose a relatively inclusive definition of critical illness for this review; as although chronic or prolonged critically ill are

more likely to be at risk of increased bone turnover, the definition for chronic critical illness remains ambiguous [30], and the relationship between critical illness and bone metabolism is relatively unexplored.

The criteria for new bone turnover used in this review included BMD assessment, BTMs, and incident fracture. Measurement of BMD remains the primary tool for fracture risk and osteoporosis treatment assessment, and is the central component of internationally agreed definitions of osteoporosis [31]. An important limitation of the studies identified by this analysis is that no report changes in BMD during or following critical illness. A single study reported an increased in fragility fracture risk in older females following ICU compared to population controls, and although this was a large study with a high methodological quality score, it was limited by its retrospective design [18]. There are no studies reporting the use of bone histomorphometric analysis of bone biopsies or other methods for assessing bone microarchitecture (high-resolution CT or MRI imaging) in critically ill patients. Bone histomorphometry could provide information regarding the effects of critical illness on microarchitectural deterioration, mineralisation and dynamic indices of bone resorption, and formation.

Bone turnover markers were the outcome measured in 10 of the 11 studies identified in this review. BTMs are an important tool to assess progression of osteoporosis, fracture risk, and treatment response [29, 32, 33]. Overall, BTMs are separated into markers of bone resorption (PyD, DpD, *B*-CTX/ICTP) and bone formation (ALP, BALP, OC, P1CP, P1NP) [34]. However, BTM levels are affected by a number of factors, requiring more complex interpretation. Osteocalcin is a marker of osteoblast function and bone formation, but smaller fragments are derivatives of bone resorption and included in assay. The bone formation markers P1NP and P1CP are both procollagen terminal extension peptides, but P1NP is more specific for bone formation. Also, a number of BTMs are affected by biological factors including age, gender, co-existing disease, and medications [34]. Examples include decreased excretion of *B*-CTX in renal failure [34] and sensitivity of OC to glucocorticoid exposure [35].

The studies identified in this review consistently described changes in BTMs during critical illness suggestive of an increased osteoclastic bone resorption (increased urinary DpD and PyD, serum *B*-CTX/ICTP), an increase in immature osteoblast number and activity (serum P1CP and P1NP), and a reduced activity of mature osteoblasts (serum OC and ALP). The increase in bone resorption markers described in these studies is of the magnitude described in postmenopausal females, or metabolic bone disease [33, 36, 37], and has been likened to other metabolic bone disorders, such as Paget's disease, where uncoupling of bone osteoclast and osteoblast activity is described [26].

A limitation of the studies using BTMs to assess bone turnover in this analysis was the short duration of follow-up, ranging from 1 to 26 days, and a lack of premorbid assessment of bone turnover or skeletal health. When BTMs are used to assess treatment effect of anti-resorptive agents, an interval of 3–6 months is normally recommended [35]. Although these studies are not designed to assess the effect of anti-resorptive agents on bone turnover, the short duration of follow-up decreases the ability to establish a causal relationship between critical illness and bone turnover.

An important limitation of the evidence identified by this review is the limited analysis of the effect of possible confounding variables on the association between critical illness and altered bone turnover. Although six of the studies provided an age- and gender-matched assessment of a control group, the effects of other known causes of osteoporosis and variables, known to affect the metabolism of BTMs (including menopausal status, renal failure, liver disease, diabetes, thyroid disease, and medications) [36, 37], were not consistently addressed. These variables are likely to occur in critically ill patients, leaving the possibility that altered metabolism of BTMs or known risk factors for osteoporosis are partly or wholly responsible for the observed increase in bone turnover.

The studies in this analysis do provide some information about the relationship between critical illness duration, inflammation, immunomodulation, endocrine dysfunction, and increased bone turnover. Higher levels of bone resorption markers were observed in ICU patients with a length of stay of greater than 5 days compared to less than 5 days [28]. This may indicate a relationship between duration of critical illness and bone resorption, although the lack of adjustment for confounders, including co-morbid illness such as renal failure, prevents the nature of this relationship being established.

Vitamin D deficiency with resultant secondary hyperparathyroidism and prolonged immobilisation may increase the risk of excessive bone resorption; however, a range of metabolic abnormalities characterised as primary hyperparathyroidism, secondary hyperparathyroidism, and mixed disorder were described in critically ill patients with elevated bone resorption markers [19]. Two studies report the effects on bone turnover of treating vitamin D deficiency in critically ill patients. The interventional data from one study in this analysis described the effect of parenteral vitamin D 200 IU or 500 IU daily in long-term surgical ICU patients receiving parenteral nutrition. Higher dose vitamin D was associated with a relatively small increase in serum OC, and a decrease in serum B-CTX, but did not affect other BTMs. In addition, the decrease in inflammatory markers interleukin-6 and C-reactive protein over time was more pronounced with the higher dose vitamin D [26]. However, treating vitamin D deficiency with calcitriol did not lead to a reduction in bone resorption markers, suggesting that vitamin D deficiency was not the mechanism for accelerated bone turnover [21].

A positive relationship between inflammation and increased bone turnover was present in a number of studies [22–24, 26], and was unrelated to severity of illness, type of illness, age, or outcome [26]. Systemic inflammation has been identified as a marker for increased fracture risk in non-critically ill patients, with a 23 % increase risk of fracture associated with each standard deviation increase in the inflammatory marker high-sensitivity C-reactive protein [38]. Inflammatory cytokines such as TNF-α and IL-6 are potent stimulators of RANK ligand-mediated activation of osteoclastogenesis and direct activation of osteoclast precursors [26]. Ongoing bone resorption did not correlate with inflammatory markers, which may reflect the influence of other mechanisms, a prolonged effect of cytokines through osteoclast activation factors that increases maturation and lifespan of osteoclasts, or a direct effect of cytokines on osteoclast precursors. In one of the studies, concomitant treatment with glucocorticoids, thyroid hormones, or any other ICU medication did not significantly affect markers of bone turnover at any of the studied time points [26].

A series of studies included in this review by Van den Berghe et al. [22–24] described changes to the somatotrophic, thyrotrophic, and gonadotrophic axes in prolonged critical illness, and included bone markers as a part of measures of target tissue effects. The studies describe a positive correlation between inflammatory cytokines and osteoclastic and osteoblastic activity, with variable effects of restoration of somatotrophic, thyrotrophic, and gonadotrophic axes on BTMs. The administration of growth hormone-releasing peptide (GHRP) alone led to reactivation of pulsatile GH secretion in critically ill patients, but no changes in BTMs [22]. The addition of thyroid releasing hormone (TRH) led to increased osteocalcin, suggesting impaired maturation of osteoblasts may be explained by a suppressed thyroid axis [22]. Finally, the addition of gonadotropin-releasing hormone (GnRH) led to a further increase in osteocalcin [22, 23]. This complex relationship between sex hormones and altered bone turnover markers in critical illness is not surprising given the increasingly complicated interaction between these regulators of osteoclast differentiation and activity [39].

In vitro experiments have shown that compared to healthy controls, critically ill patients' peripheral blood mononuclear cells (PBMCs) responded to the presence of osteoclastic activation factors with an increased number and activity of mature osteoclasts [25]. In addition, exposure of PBMCs to critically ill patient sera resulted in an increased formation of mature osteoclasts, whereas a model of bone formation showed a reduction in angiogenesis factor expression, and reduced vascularity and maturity of bone formation.

This systematic review provides moderate evidence of a relationship between critical illness and increased bone turnover. Increased bone turnover may lead to impaired fracture healing or post-ICU fragility fractures, with their associated morbidity and mortality. Increased bone turnover is associated with mortality in elderly patients [40] and patients with cardiovascular disease [41]. If future studies find that survivors of critical illness are at high risk of subsequent fragility fracture, target interventions to prevent or attenuate acute bone loss such as the early administration of anti-resorptive therapies may be assessed as a broader fracture prevention strategy.

There is limited evidence examining the efficacy of bisphosphonates in this setting. A randomised controlled trial identified in the search strategy and excluded from this analysis reported a transient decrease in serum *B*-CTX in chronic critically ill patients receiving a single intravenous dose of ibandronate compared to placebo [42]. In addition, the decrease in the bone turnover marker (serum OC) observed in postmenopausal women receiving ibandronate [43] was not observed in this study, supporting the theory that bone formation and resorption is uncoupled in critical illness. Although limited by small sample size, short follow-up, and limited extent and duration of effect, this study provides evidence that suppression of excessive bone resorption in critical illness is possible.

The higher-quality cohort and case–control studies provide moderate evidence for an association between critical illness requiring intensive care admission, and increased bone turnover. A prospective observational study evaluating BMD changes in the year after critical illness, with comparisons to age- and gender-matched population controls, and adjustment for known risk factors and possible critical illness factors is now required.

Acknowledgments SLB is supported by the NHMRC Early Career Fellowship (1012472).

Conflicts of interest None.

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Chapter 3: Skeletal morbidity among survivors of critical illness

This chapter presents the evidence for fragility fracture in critical illness survivors compared to age and gender matched controls from the Geelong Osteoporosis Study. Published in Critical Care Medicine in 2011, it presents data obtained through linkage of the Geelong ICU electronic database with the regional fracture databases, to ascertain fracture post ICU using the same method as GOS. The overall fracture rate, and the significant increase in fragility fracture in older female survivors of critical illness compared to population controls, provides the first evidence of possible morbidity from previously described increase in bone turnover markers during critical illness. Although this study is included in the systematic review, from a narrative perspective it is included at this point.

The associated editorial in *Critical Care Medicine* observed this was the first paper to examine fracture incidence in survivors of critical illness. It also stated the need for prospective cohort studies to confirm whether critical illness increased the risk of subsequent osteoporosis-related fractures, and to determine factors associated with increased bone loss during critical illness.

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Bone loss during critical illness: A skeleton in the closet for the intensive care unit survivor?*

"I have no history but the length of my bones."—Robin Skelton

As more patients receive multiple organ support in critical care units, many of whom are elderly and/or have significant comorbidities, attention is turning toward an ever increasing population of intensive care unit (ICU) survivors (1). Critical illness is recognized to result in a "post-ICU syndrome," which can occur whatever the original presenting illness and result in cognitive, neurologic, and physical function impairments, which significantly affect patients' quality of life for many months or years (2). These impairments and disabilities also place a heavy burden on healthcare systems and caregivers (3). In recent years, our knowledge of the prevalence of psychologic and physical problems has improved through cohort studies, and research is beginning to explore the risk factors, mechanisms, and possible treatments that may affect the severity and duration of issues ranging from psychologic conditions such as posttraumatic stress disorder to physical problems such as fatigue and breathlessness. Until now, very little work has specifically investigated the affect of critical illness on the skeleton, having focused mainly on neuromuscular dysfunction.

Osteoporosis is a major public health issue that has been estimated to affect 55% of Americans aged ≥50 yrs, of whom 80% are women (4). It is responsible for millions of fractures annually, mostly involving the lumbar vertebrae, hip, and wrist. The World Health Organization defines osteoporosis as a bone mineral density that is >2.5 SDs below the mean bone mineral density of young adult women (5). The disease can be classified as primary type 1, primary type 2, or secondary. Primary type 1 or postmenopausal osteoporosis is the form most common in women after menopause, whereas primary type 2 osteoporosis occurs after age 75 yrs and is seen in both females and males at a ratio of 2:1. Secondary osteoporosis may arise at any age and affects men and women equally. This form of osteoporosis results from chronic predisposing medical problems or disease or prolonged use of medications such as glucocorticoids.

A significant proportion of patients admitted to ICUs will possess strong risk factors for osteoporosis such as female gender, older age, a positive family history, low body mass index, and white origin. Many will also be smokers, have a history of prior corticosteroid use, chronic inflammatory disease, or reduced mobility (6). Although no studies have formally quantified the prevalence of osteoporosis among patients admitted to critical care units, it is likely that many have this condition. Given the potential for osteoporosis-related fractures to impact on long-term quality of life, together with the availability of potential treatments, it is relevant to understand whether an episode of critical illness increases its severity or rates of disease progression and complications.

In this issue of *Critical Care Medicine*, Orford and colleagues (7) address this issue. They are the first to examine fracture incidence in patients who survive critical illness. The authors estimated fracture incidence for both men and women cared for in a major Australian ICU who required >48 hrs of mechanical ventilation and were able to compare the female cohort with age-matched control subjects from a high-quality prospective population-based osteoporosis study from the same region. Fracture incidence was assessed in the cohort of patients discharged after critical illness by searching electronic radiology reports for a median follow-up time of 3.7 yrs for females and 4 yrs for men. They found that 14% of female and 10% of male survivors who had been ventilated for >48 hrs sustained fractures in the follow-up period. In female survivors, the overall incidence trended to a higher fracture rate over the follow-up period than was present in the population control subjects, but this was not statistically significant (hazard ratio, 1.20; 95% confidence interval, 0.84–1.71; $p = .31$). Interestingly, when older female patients (aged ≥60 yrs) were analyzed as an age-matched subgroup, there was a statistically significant increase in fracture rate suggesting clinically important increases in fracture rates (hazard ratio, 1.65; 95% confidence interval, 1.08–2.52; $p = .02$). Because older women are more likely to have coexisting osteoporosis when they have their critical illness and/or are more prone to developing it, this observation raises the possibility that critical illness itself may accelerate osteoporosis development and increase the chance of fracture.

The study was not prospective such that fracture detection relied on patients having undergone imaging in the radiology departments included in the region. It is possible that fracture underdetection occurred in both critically ill and control populations, and ascertainment bias resulting from imbalance between the groups cannot be excluded. The excess of fractures in the female ICU survivors was attributable largely to vertebral fractures. These comprised a much higher proportion of the fractures in the ICU cohort

***See also p. 1295.**

Key Words: critical care; intensive care; bone and bones; bone resorption; osteoporosis; survivors; quality of life

The authors have not disclosed any potential conflicts of interest.

DOI: 10.1097/CCM.0b013e318215beb4

Table 1. Potential risk factors for bone loss during critical illness (12)

Risk Factor	Mechanism
Immobility (13, 14)	Increased calcium resorption inhibits parathyroid hormone and 1,25 dihydroxy vitamin D formation
Inflammatory cytokines (12)	Stimulate osteoclast formation and differentiation Stimulate mature osteoclasts Inhibit osteoblast formation
Endocrine dysfunction (12)	Increased cortisol Depressed growth hormone and insulin growth factor-1 Decreased thyroid-stimulating hormone and T4
Vitamin D deficiency (11)	Disturbance in calcium homeostasis
Glucocorticoids (15)	Decrease in osteoblastic activity

(41.7%) than the population control cohort (17.4%) and could also be a form of ascertainment bias perhaps attributable to increased imaging in post-ICU patients, for example, chest radiography for respiratory symptoms. As pointed out by the authors, the retrospective design also makes it difficult to disentangle preexisting risk factors from the effects of critical care, and confounding factors may not have been adequately controlled for. Despite these limitations, the findings raise the possibility that critical illness increases the risk of subsequent osteoporotic fractures.

Bone turnover can be assessed in patients using various biochemical markers (8). These have been broadly categorized as collagenous bone resorption markers, osteoclast regulatory proteins, and bone formation markers. Peptide fragments from the breakdown of mature collagen are the most commonly used measures of bone resorption and include the pyridinolines (pyridoniline and deoxypyridinoline), which can be detected in the serum and the urine (8). Increased bone turnover in critically ill patients, particularly those who require multiple organ support for prolonged periods, has been reported in the literature for well over a decade (9–11). Shapses and colleagues (9) compared bone turnover in a small sample of critically ill patients after gastrointestinal surgery, all of whom were receiving parenteral nutrition, with age-matched healthy volunteers. Excretion of pyridinium crosslinks was increased in the critically ill sample when compared with healthy volunteers and was more pronounced in patients who had a longer ICU stay. Smith et al (10) reported increased bone resorption compared with healthy control subjects in 23 patients with sepsis and trauma measured using pyridinoline/creatinine and deoxypyridinoline/creatinine ratios. The authors found this was particularly pronounced in the subgroup of septic patients who had a tenfold increase in pyridinoline/creatinine ratio and a sixfold increase in deoxypyridinoline/creatinine ratio. Serum markers of osteoblast activity were increased at ICU admission in Van Den Berghe's study of vitamin D in critically ill patients compared with healthy control subjects. This was accompanied by a similar increase in urinary deoxypyridinoline and pyridinoline implying upregulation of both osteoclast and osteoblast activity but with an imbalance in favor of bone resorption (11). These studies all suggest that critical illness is associated with changes to normal bone metabolism, which most likely favor bone breakdown and demineralization.

Although the impact of critical illness on bone mineralization is ill defined, much can be inferred from other settings and the known pathophysiological processes that occur. Factors known to cause bone loss are summarized in Table 1 and have been recently reviewed by Via and colleagues (12). The multiple potential mechanisms whereby critical illness could result in excessive osteoclast activity, bone loss, and demineralization provide a strong biologic plausibility for increased risk of subsequent osteoporosis, especially after prolonged critical illness.

Although the study by Orford and colleagues requires validation in prospective, adequately controlled studies with a low risk of bias, their findings are particularly interesting because potential therapies exist to prevent or minimize the detrimental effects of critical illness on bone metabolism. These include vitamin D and biphosphonate therapy. Biphosphonates in particular are well-established effective treatments for osteoporosis, bone metastases, and other bone diseases. They act by promoting osteoclast apoptosis, thereby reducing bone loss. Some small studies have used both vitamin D and biphosphonates in critically ill patients and demonstrated biochemical evidence of reduced bone resorption (11, 16). The overall excellent safety profile of biphosphonates make them a potentially attractive therapeutic option for the chronically critically ill, although caution is required in patients with renal failure and they have also been associated with fever and atrial fibrillation, both of which could have adverse effects in frail patients.

Orford et al have opened a new avenue of research into the consequences of critical illness. Their data support the need for well-designed prospective cohort studies to confirm whether critical illness increases the risk of subsequent osteoporosis-related fractures together with further well-designed studies to determine the factors that increase bone loss during intensive care. Clinical trials of biphosphonates and/or vitamin D to determine the risk-to-benefit profile of these agents in patients with organ dysfunction are needed. However, the ready availability of these agents raises true hope that intervening in the right patients at the right time during critical illness might result in long-lasting benefits to patients' subsequent quality of life.

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Skeletal morbidity among survivors of critical illness*

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Objectives: **To describe the incident fracture rate in survivors of critical illness and to compare fracture risk with population-matched control subjects.**

Design: **Retrospective longitudinal case–cohort study.**

Setting: **A tertiary adult intensive care unit in Australia.**

Patients: **All patients ventilated admitted to intensive care and requiring mechanical ventilation for ≥48 hrs between January 1998 and December 2005.**

Interventions: **None.**

Measurements and Main Results: **New fractures were identified in the study population for the postintensive care unit period (intensive care unit discharge to January 2008). The incident fracture rate and age-adjusted fracture risk of the female intensive care unit population were compared with the general population adult females derived from the Geelong Osteoporosis Study. Over the 8-yr period, a total of 739 patients (258 women, 481 men) were identified. After a median follow-up of 3.7 yrs (interquartile range, 2.0–5.9 yrs) for women and 4.0 yrs (interquartile range, 2.1–6.1 yrs) for men, incident fracture rates (95% confidence interval) per 100 patient years were 3.84 (2.58–5.09) for females 2.41 (1.73–3.09) for males. Compared with an age-matched random population-based sample of women, elderly women were at increased risk for sustaining an osteoporosis-related fracture after critical illness (hazard ratio, 1.65; 95% confidence interval, 1.08–2.52; $p = .02$).**

Conclusions: **The increase in fracture risk observed in postintensive care unit older females suggests an association between critical illness and subsequent skeletal morbidity. The explanation for this association is not explored in this study and includes the effects of pre-existing patient factors and/or direct effects of critical illness. Prospective research evaluating risk factors, the relationship between critical illness and bone turnover, the extent and duration of bone loss, and the associated morbidity in this population is warranted. (Crit Care Med 2011; 39:1295–1300)**

KEY WORDS: critical illness; long-term outcomes; osteoporosis; fracture; bone loss

Intensive care patients face health issues that extend beyond their critical illness, including ongoing mortality, reduced physical and mental quality of life, greater dependence on others, reduced return to work, and ongoing economic and social costs to families and caregivers when compared with preillness and general population controls (1–7). Despite an increasing awareness of these long-term outcomes of critical illness, the identification of specific pathophysiologies amenable to intervention remains elusive.

The skeletal consequences of critical illness remain largely unexplored. Hypercalcemia, an increase in bone turnover markers, reduced bone density, and osteoporotic fracture have been reported in small cohorts of critically ill patients. Case studies of patients with prolonged immobilization and ventilation associated with Guillain-Barré syndrome have reported a biochemical response to antiresorptive therapy (8–13). The combination of risk factors for low bone mass present in an aging population and an acute increase in bone turnover during critical illness may predispose patients to bone loss with associated adverse skeletal outcomes, including increased risk of fragility fractures.

Assessment of the relationship between critical illness and skeletal outcomes requires further examination of the effect of critical illness on bone mineral density and fracture outcomes with comparisons to the general population. In this study, we document the fracture incidence in men and women after critical illness and compare fracture risk in women after critical illness with a general population sample from the Geelong Osteoporosis Study (GOS).

MATERIALS AND METHODS

Study Design and Participants

Control Subject. The GOS was a recruited random population-based sample of women (ages 20–94 yrs) from the Commonwealth Electoral Rolls between 1994 and 1997 for an area surrounding Geelong in southeastern Australia called the Barwon Statistical Division. Because voting is compulsory in Australia, the electoral roll provides a comprehensive listing of all adults (age >18 yrs). The sample was age-stratified with a minimum of 100 in each 5-yr age stratum between ages 20 and 69 yrs and a minimum of 200 in the age 70- to 79-yr group and the >80-yr group.

Cases. The study was conducted in The Geelong Hospital intensive care unit (ICU), a 19-bed tertiary, adult, mixed medical, surgical, cardiothoracic ICU. Adult patients (age ≥18 yrs) were included in the study if they were admitted to the ICU between January 1998 and December 2005, required invasive mechanical ventilation for ≥48 hrs, and survived to ICU discharge. Patients were identified retrospectively using the ICU electronic database. Comparison to the control group (GOS) was limited to females aged 20–94 yrs.

Ethics approval was obtained from the Barwon Health Human Research Ethics Committee before start of the study.

*See also p. 1554.

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The authors have not disclosed any potential conflicts of interest.

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DOI: 10.1097/CCM.0b013e318211ff3d

Data Collection

Baseline information collected from the database included age, gender, Acute Physiology and Chronic Health Evaluation II score, admission diagnosis, ICU length of stay, duration of ventilation, hospital length of stay, and hospital outcome. Patients who had multiple ICU admissions were only included for their first ICU stay.

Time of follow-up for the post-ICU period was defined as the period from ICU discharge to January 2008 or date of death or fracture. Deaths of the patients during the study period were confirmed by application to the Office of Births, Deaths, and Marriages. Because the earliest patient enrollment occurred in 1998, the maximum follow-up period was 10 yrs.

A new fracture was defined as a fracture reported for the first time in the radiology report between ICU discharge and January 1, 2008. Fractures were identified by key word search of the Barwon Health, Lake Imaging, and Colac Hospital electronic radiologic records, the three major radiologic centers in the region during the study period. Date of fracture was recorded as the date of initial radiograph demonstrating a new fracture. Patients who sustained fractures on multiple occasions were included only for the first fracture for the ascertainment period. Fractures that occurred as a result of a known pathologic cause other than osteoporosis (metastatic cancer, Paget disease, and multiple myeloma) were not included. This method of fracture ascertainment is the GOS-validated fracture ascertainment method (14).

Statistical Analysis

Statistical packages used for analysis were Minitab (Minitab Inc., State College PA) and SPSS (SPSS Inc, Chicago, IL). Demographic and interventional data are presented as frequency or median. Comparisons between fracture and fracture-free groups were performed using Mann-Whitney test or chi-square test, as appropriate. A p value $<.05$ was considered significant.

Fracture risk was defined as new fracture per 100 person-years with time to fracture measured from ICU discharge date and from baseline appointment for GOS participants. The risk of fracture for the female patients post-ICU and for the female general population from the GOS sample was compared using the Cox proportional hazard models after adjustment for age.

RESULTS

A total of 9,008 patients were admitted to the ICU during the during the 8-yr study period. Of these, 929 patients were ventilated for >48 hrs and had a higher median Acute Physiology and Chronic Health Evaluation II score, a higher proportion of medical admissions, longer ICU and hospital length of stay, and higher mortality compared with the whole ICU population. A total of 739 patients (median age, 68 yrs; range, 16–93 yrs; 481 males) survived to ICU discharge and were eligible for this study. Descriptive characteristics of all ICU admissions and admissions requiring ventilation for ≥48 hrs are displayed in Table 1. Descriptive statistics by gender of ICU survivors with survival to 5-yrs post-ICU discharge are displayed in Table 2.

Thirty-six adult females (14.2%) and 48 adult men (10%) sustained a fracture during the post-ICU time period with the distribution of fractures by age shown in Tables 3 and 4. The majority of fractures occurred in those aged ≥60 yrs (males 70.8%, females 91.7%). The median time to follow-up for women was 3.7 yrs (interquartile range, 2.0–5.9 yrs) and men 4.0 yrs (interquartile range, 2.1–6.1 yrs). The overall fracture incident rates per 100 patient-years (95% confidence interval) for females were 3.84 (2.58–5.09) and males 2.41 (1.73–3.09).

In the female post-ICU population, the fracture group were significantly older (72.2 yrs, 67.1 yrs; $p = .01$) with a longer hospital length of stay (30.0 vs. 23.0 days; $p = .02$) compared with the fracture-free group (Table 5). There were no significant differences observed in the male population (Table 6).

The population-based sample (GOS) included 1494 participants (median age, 54 yrs; interquartile range, 37–71

Table 1. Descriptive characteristics of all ICU admissions and admissions ventilated for ≥48 hrs during 8-yr study period (data are shown as median [IQR] or no. [%])

Variable	All Admissions	All ICU Patients Ventilated ≥48 hrs	Survived ICU Ventilated >48 hrs
Number	9008	929	739
Age, yrs	67 (54–75)	68 (58–75)	68 (57–75)
APACHE II	13 (10–17)	20 (16–25)	20 (15–24)
Category, %			
Medical	2931 (33)	460 (50)	333 (44)
Surgical	3304 (37)	223 (24)	185 (25)
Cardiothoracic	2773 (31)	246 (26)	221 (31)
Ventilation, days	0.6 (0.3–1.5)	5.0 (3.3–9.9)	4.8 (3.1–9.7)
ICU stay, days	1.8 (0.9–2.9)	7.8 (5.0–13.6)	7.8 (5.1–13.8)
Hospital stay, days	10 (6–18)	20.0 (12.0–36.0)	22.0 (14.0–40.0)
ICU mortality, %	565 (6.3)	190 (20.4)	Not applicable
Hospital mortality, %	969 (10.8)	248 (26.7)	59 (8.0)

ICU, intensive care unit; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation.

Table 2. Descriptive data of ICU survivors with ≥48 hrs ventilation by gender (as above median [IQR] or no. [%])

Variable	Females	Males
Number, %	258 (35%)	481 (65%)
Age, yrs	68.0 (58.1–75.5)	68.1 (57.1–75.1)
APACHE II	20 (16–24)	19 (15–24)
Category, %		
Medical	124 (48)	209 (43)
Surgical	64 (25)	121 (26)
Cardiothoracic	70 (27)	151 (31)
Ventilation, days	4.8 (3.2–9.7)	4.9 (3.1–9.7)
ICU stay, days	7.7 (5.1–13.4)	7.9 (5.0–14.0)
Hospital stay, days	24.0 (14.0–45.0)	21.0 (13.0–39.0)
Survival, %		
Hospital	234/258 (91)	446/481 (93)
28 days	228/258 (90)	441/481 (92)
90 day	226/258 (88)	436/481 (86)
1 yr	212/258 (84)	414/481 (86)
2 yrs	207/257 (80)	391/480 (82)
5 yrs	106/181 (59)	198/339 (58)

ICU, intensive care unit; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation.

Table 3. Number of fractures post-ICU by age in adult males[a]

Age Group, yrs	Patient No.	Fractures	Fracture Site								
			Hip	Upper Arm	Vertebral	Pelvis	Lower Leg	Rib	Wrist	Hand	Face
<20	3	0									
20–29	15	2							1		1
30–39	18	0									
40–49	42	4			1				2	1	
50–59	67	8		2			2	3		1	
60–69	119	9		1	4		1	2	1		
70–79	178	22	5		11	1		3	1	1	
80+	39	3		1	1	1					
Total	478	48	5	4	17	2	3	8	5	3	1

ICU, intensive care unit.
[a]Fracture rate (fractures/100 patient-years) = 2.41 (1.73–3.09).

Table 4. Number of fractures in adult females by age in post-ICU and GOS samples

Age Group, yrs	Patient No.	Fractures	Fracture Site							
			Hip	Upper Arm	Vertebral	Pelvis	Lower Leg	Rib	Wrist	Other
20–29	9	0								
30–39	11	1		1						
40–49	16	1					1			
50–59	31	1						1		
60–69	77	10	2	1	6			1		
70–79	89	20	6	3	8	1		1		
80+	21	3	1	1	1					
Total post-ICU	254	36	9	6	15	1	1	3	1	0
GOS	1494	281	37	22	49	14	22	17	33	87

ICU, intensive care unit; GOS, Geelong Osteoporosis Study.

Table 5. Comparison of female ICU survivors by post-ICU fracture status (data are shown as median [IQR] or no. [%])

Variable	Post-ICU Fracture	No Post-ICU Fracture	*p*
Number	37	221	
Age, yrs	72.2 (65.4–76.4)	67.1 (56.3–75.0)	.01
APACHE II	20 (17–24)	20 (16–25)	.98
Category, %			
Medical	18 (49)	106 (48)	
Surgical	9 (24)	54 (24)	
Cardiothoracic	10 (27)	61 (28)	1.00
Ventilation, days	4.6 (3.5–9.7)	4.8 (3.2–9.7)	.94
ICU stay, days	8.5 (6.0–13.7)	7.6 (5.1–13.0)	.27
Hospital stay, days	30.0 (20.0–55.0)	23.0 (14.0–40.0)	.02

ICU, intensive care unit; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation.

yrs; range, 20–94 yrs) with 281 fractures in total (Table 4) (15). The risk of fracture for the entire adult (20–94 yrs) female ICU cohort, expressed as a hazards ratio, was 20% higher than the population based sample (hazards ratio, 1.20; 95% confidence interval, 0.84–1.71; $p = .31$) but did not achieve statistical significance (Table 7). Analysis limited to adult females aged ≥60 yrs where the highest number of fractures occurred and specifically investigating fractures more likely to be related to osteoporosis (wrist, vertebral, hip, or humerus) resulted in a significantly increased risk of fracture for those in the ICU group (hazards ratio, 1.65; 95% confidence interval, 1.08–2.52; $p = .02$) (Table 7; Fig. 1). The comparison of male ICU survivor fracture incidence to random population-based control subjects was not performed, because this is currently not available in the control population.

DISCUSSION

The description of fracture incidence after critical illness and comparison of incident rate with a large, well-defined, age- and gender-matched general population control group have not been reported previously. The observed increase in fragility fracture risk in older female ICU survivors compared with age- and gender-matched population control subjects suggests an association between critical illness and subsequent skeletal morbidity in this population. In this study, the possible explanations for this association were not defined but could include an increased prevalence of known risk factors for low bone mass or bone loss in the critically ill patient population and/or a direct effect of critical illness resulting in accelerated bone loss.

Older females who survive critical illness may represent a population at high risk of subsequent fragility fracture, a complication with established health and economic impacts (16–19). An increased risk of fracture in survivors of prolonged critical illness would contribute significantly to their health burden, because fragility fractures are associated with increased morbidity and mortality (16, 20). Hip fracture, regarded as the most serious fragility fracture, is associated with increased mortality in men and women with a substantial proportion of survivors losing their independence (21, 22). Furthermore, the average cost of hip fracture in Australia is estimated at AU $16,000 with an average length of hospital stay of 13 days (23). Survival to hospital discharge (92.2%) of 2 yrs (males 82%, females 80%) was high in this study, suggesting identification and prevention of long-term sequelae in critical illness is important.

An increased prevalence of known risk factors for low bone mass or bone loss may explain the increase in fracture risk observed in older females who survive critical illness. The concept of increasing fracture risk related to the presence of risk factors exists in validated fracture assessment tools such as FRAX (University of Sheffield, South Yorkshire, UK) (24–26). This tool permits an estimate of future fracture probability, dependent on the number of risk factors present, with

Table 6. Comparison of male ICU survivors by post-ICU fracture status (data are shown as median [IQR] or no. [%])

Variable	Post-ICU Fracture	No Post-ICU Fracture	
Number, %	48	433	
Age, yrs	71.0 (56.8–75.2)	67.9 (57.2–75.0)	.51
APACHE II	19.5 (15.0–24.3)	19.0 (15.0–24.0)	.91
Category, %			
Medical	18 (38)	191 (44)	
Surgical	14 (29)	107 (25)	
Cardiothoracic	16 (33)	135 (31)	.66
Ventilation, days	5.0 (3.4–9.9)	4.9 (3.1–9.5)	.74
ICU stay, days	7.4 (5.1–12.8)	7.9 (5.0–14.2)	.83
Hospital stay, days	22.5 (16.3–32.3)	21.0 (13.0–40.0)	.64

ICU, intensive care unit; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation.

Table 7. Unadjusted and adjusted fracture rates and hazard ratios for females (20–94 yrs of age) post-ICU compared with population-based females (GOS)

Variable	Post-ICU Fracture Rate (95% CI)	GOS Fracture Rate (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI, p)
All ages, all fracture	3.84 (2.58–5.09)	2.01 (1.76–2.25)	1.63 (1.14–2.32)	1.20 (0.84–1.71, $p = .31$)
>60 yrs of age, osteoporotic fracture	4.33 (2.72–5.93)	2.81 (2.33–3.28)	1.48 (0.98–2.25)	1.65 (1.08–2.52, $p = .02$)

ICU, intensive care unit; GOS, Geelong Osteoporosis Study; CI, confidence interval; HR, hazards ratio.

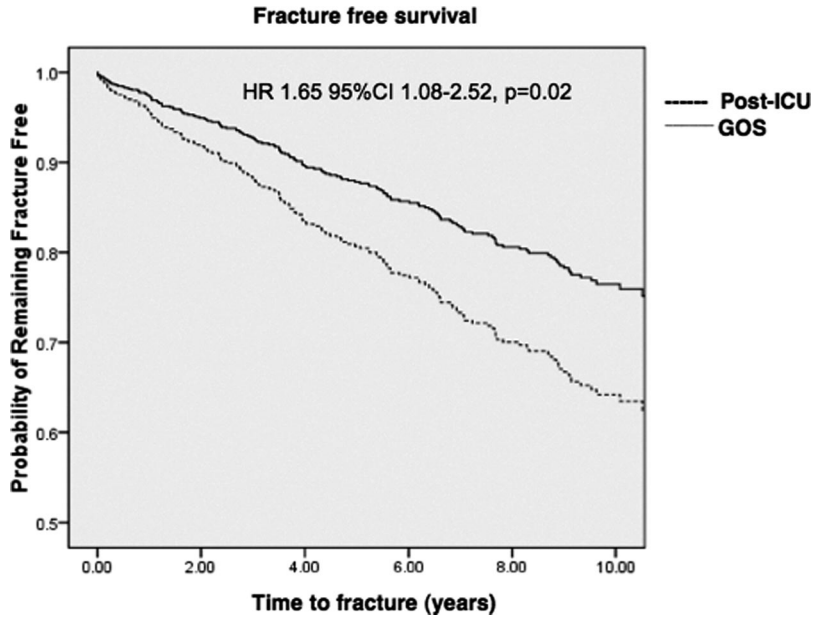

Figure 1. Time to fracture of the wrist, hip, humerus, or vertebral fracture after intensive care unit (*ICU*) compared with the random population-based sample in older age group (≥ 60 yrs) females. *HR*, hazard ratio; *CI*, confidence interval; *GOS*, Geelong Osteoporosis Study.

or without the addition of bone mineral density. The fracture probability increases in a dose-dependent manner with the presence of additional risk factors (24, 27). The risk factors used by FRAX include age, low body mass index, parental history of hip fracture, prior fragility fracture, smoking, long-term use of glucocorticoids, rheumatoid arthritis, daily alcohol consumption of three or more units, and other secondary causes of osteoporosis to calculate future fracture risk. There is currently no data relating to the skeletal health of patients before ICU admission or the prevalence of known risk factors for low bone mass or bone loss and fragility fracture. This study was unable to measure known risk factors for bone loss, leaving the effect of premorbid patient health on fracture risk unknown.

In addition to premorbid risk factors for low bone mass or increased bone loss, it is possible that critical illness-related factors directly result in accelerated bone loss and microarchitectural deterioration that contribute to an increase in future fracture risk. There is limited evidence for an acute increase in bone turnover in critical illness. Normal bone turnover requires osteoclast and osteoblast activity to be tightly coupled with regulation by mechanical, nutritional, immune, endocrine, paracrine, and autocrine factors (8–10). In critical illness, there is biochemical evidence of an uncoupling of bone formation and resorption. Urinary excretion of bone collagen markers increases up to four times the levels seen in postmenopausal women, reflecting increased bone resorption (8–10). Circulating biochemical markers suggest an excess of immature osteoblasts, producing mainly alkaline phosphatase and collagen, resembling the abnormal matrix production observed in Paget disease (8). Numerous etiologies have been suggested for this increased bone resorption in critical illness, including prolonged immobility, secondary hyperparathyroidism, renal insufficiency, vitamin D deficiency, hypogonadism, hypercortisolism, and hyposomatotropism (8–10, 28, 29). A gradual and sustained increase in biochemical markers of osteoclastic activity resulting from cytokine-induced activation and reduction in apoptosis has also been suggested (8) with a multifactorial etiology supported by the outcomes of previous attempts to reverse increased bone resorption.

The relative contributions of premorbid patient health and critical care-related factors to fracture risk are not measured by this study, a limitation present in critical care long-term research, as a result of the difficulty identifying patients before critical illness (6). The observation that females with fractures had a longer hospital length of stay than frac-

ture-free females may be explained by a population that presents to ICU with worse premorbid health and an associated reduction in bone mass or a population with more complex critical illness, prolonged recovery, and an associated increase in bone loss. Identifying the relative impact of premorbid patient health and critical care-related factors could be compared with the model of osteoporotic fractures and FRAX.

Excess mortality and morbidity is well described after hip and clinical vertebral fracture, and despite improvements in care, the increased risk of death after hip fracture compared with the general population remains. This excess mortality is most marked in the year after fracture and is contributed to by the presence of significant comorbidities and factors relating to the hip fracture itself (17, 18, 30, 31). Similarly, despite improvements in critical illness care, survivors of critical illness have an increased mortality compared with the general population, particularly in the first years after critical illness (2, 32–35). In both cases, there is an associated long-term mortality resulting from premorbid patient health with possible direct effects related to the acute injury or insult. Recent evidence indicates that in a high-risk population, men and women with hip fracture, osteoporosis interventions can reduce mortality (22). Improvement of long-term outcomes after critical illness, including prevention of bone loss, is likely to require identification and management of contributing factors.

The comparison of fracture distribution in females after critical illness compared with the population control subjects is important, because the increase in fracture rate after critical illness could be attributed to the incidental detection of vertebral fractures when imaging is performed in the immediate period after critical illness. In this study, vertebral fractures accounted for 41.6% of all fractures detected in females after critical illness compared with 17.4% in the GOS population. This apparent increase in vertebral fracture incidence in female critical illness survivors may represent an increase in clinical vertebral fractures or an ascertainment bias toward detection of fractures in this population. Clinical vertebral fracture rates underestimate fracture incidence because approximately two-thirds of vertebral fractures are not associated with acute fracture-related back pain (36). The 15 vertebral fractures detected in females after critical illness occurred at a median time of 6.37 yrs (interquartile range, 4.23–7.25) after ICU discharge, making incidental detection of fractures from imaging related to critical illness unlikely. However, improved detection of vertebral fractures may occur in populations with increased comorbidities (18).

There are a number of limitations to this study. As discussed previously, the inability to adjust fracture risk for known risk factors for low bone mass or bone loss prevents this study from identifying critical illness as an additional risk factor. Second, a comparison of pre-ICU and post-ICU fracture rates for ICU survivors would have provided an assessment of the change in fracture risk resulting from critical illness. This could not be achieved reliably as a result of the loss of electronic records from one of the radiology providers for a period of 4 yrs in the early 1990s. Finally, this study focused on patients considered to be at a higher risk of bone loss resulting from prolonged critical illness, defined as duration of mechanical ventilation of $\geq$48 hrs. Although not precisely defined, prolonged critical illness is generally defined by a requirement for mechanical ventilation for a period ranging from 2 days to 4 wks (32, 37, 38). However, it is possible that patients with shorter ventilation duration are also at increased risk of fracture and have not been identified.

The increased fracture risk in older female ICU survivors reported in this study suggests an adverse long-term outcome after critical illness with significant morbidity and mortality. The relative contributions of premorbid patient health, and the direct effects of critical illness on skeletal fragility remain to be defined. The authors are currently undertaking a prospective study evaluating bone mineral density changes in the year after critical illness with comparisons to population control subjects, including assessment of known risk factors. If survivors of critical illness represent an additional population at high risk of subsequent fragility fracture, target interventions to prevent or attenuate acute bone loss such as the early administration of antiresorptive therapies could be assessed as a broader fracture prevention strategy.

ACKNOWLEDGMENTS

We thank Barwon Medical Imaging, Lake Imaging, Colac Imaging, and Geelong Medical Imaging for access to their radiologic databases. We thank Dr. Marissa Daniels for her assistance with data collection, Ms. Sharyn Milnes for manuscript comments, and Professor Rinaldo Bellomo for his advice and comments.

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Chapter 4: Changes in Bone Mineral Density in the Year after Critical Illness

This chapter, published in *American Journal of Respiratory and Critical Care Medicine* in 2016, represents the major body of work for this thesis. Prior to this study there had been no prospective data describing longitudinal changes in BMD following critical illness, and comparing these changes to age and gender matched population controls. The finding of a significant decrease in BMD at both femoral neck and anterior-posterior spine sites, as well as a significantly greater loss of bone density in the ICU cohort compared to controls, was unique. The article was accompanied by an editorial discussing these findings, and the need for further exploration of critical illness related bone loss.

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Bone Loss in the Intensive Care Unit

It is universally accepted that a reduction in bone mineral density (BMD) is a powerful risk factor for the fragility fracture (1). This fact has led to the widespread use of BMD in the assessment of skeletal health and forms the basis for a diagnostic classification of osteoporosis; namely, the *t*-score (2). Many conditions are associated with bone loss in addition to aging and menopause. These factors include smoking, excessive alcohol intake, glucocorticoids, immobilization, and certain diseases such as rheumatoid arthritis and chronic obstructive pulmonary disease (3, 4). In the setting of critical illness, bone loss has also been described along with an increased risk of fracture (5, 6). The bone loss associated with a critical illness is likely to be multifactorial and explained, in part, by immobilization, the underlying disease, and the use of glucocorticoids. In fact, glucocorticoids can be associated with rapid bone loss, literally within months of their introduction (7). In this issue of the *Journal*, Orford and colleagues (pp. 736–744) follow BMD and bone turnover markers, for the first time in a prospective manner, in a cohort of patients hospitalized in the intensive care unit (ICU) with a requirement for at least 24 hours of mechanical ventilation (8). Patients who completed the 1-year follow up all were functional, arguing that their ICU admission was acute and reversible and not associated with major long-term morbidity. In addition, by the FRAX risk assessment tool, both at baseline and at 1 year, these subjects were not at high risk for an osteoporotic fracture. Nevertheless, after 1 year, in the 66 subjects who completed the study, significant reductions in lumbar spine and femoral neck BMD were measured in the women and at the femoral neck in the men. The reductions were greater than the rate of bone loss in a convenience sample from the Goolong Osteoporosis Study (GOS). At baseline, the bone resorption marker CTX was increased, again in comparison to the GOS normative dataset; it fell significantly at the 1-year point in both men and women. The bone formation marker P1NP, which was below the mean of the GOS data set, increased significantly overall, but not when the men and women were analyzed separately.

This article adds new prospective information about the dynamics of bone loss in the setting of an illness or procedure for which ICU and brief ventilatory assistance is needed. It calls attention to situations not so much related to the one presented in this paper, namely, a short ICU stay, but, rather, for subjects who require an ICU setting for more prolonged periods when, presumably, bone loss might be even greater.

This important article should be interpreted with several points in mind. The study cohort was heterogeneous, with some admitted after cardiothoracic (22%) or general (20%) surgery, whereas others were admitted for issues that might have been much more serious, such as respiratory failure (8%) or sepsis (28%). The small numbers in each subgroup precluded a stratified analysis on the basis of these baseline admission criteria. Heterogeneity was evident, as well, in the percentage of subjects (34%) who were treated with glucocorticoids. Neither the dose nor range of glucocorticoids is given. Unfortunately, again because of the small cohort size, it was not possible to analyze these subjects separately. This point looms large because the dynamics of bone turnover described in this article are not different from expected findings in subjects who are immobilized or begin glucocorticoid therapy, in whom an increase in bone resorption and a reduction in bone formation are commonly observed (9). The data for the entire cohort could have been driven, at least in part, by those who were treated with glucocorticoids.

The baseline bone turnover marker data reported in this article, high CTX and low-normal P1NP levels, are likely to reflect the rapid changes that were already occurring in these subjects as a result either of the effect of glucocorticoids or immobilization or of both. Although the reductions in BMD were significant, the average reduction was small and well within the least significant change of the measurement. This means that for a given patient, the small reduction in BMD at 1 year might not be statistically significant. To be confident of a statistically significant reduction in BMD at the 95% confidence level (10), the changes in an individual patient would have had to be 1.77% at the lumbar spine and 4.43% at the femoral neck (multiply the precision of the measurement, as reported by the authors, of 0.6% at the lumbar spine and 1.6% at the femoral neck by a factor of 2.77). As reported in the article, the standard deviation of these mean reductions is large, suggesting some would have experienced significant reductions in BMD during this period. It would have been interesting to know whether those with the greatest reductions were those who were treated with glucocorticoids. It is also important to point out that the reduction at the 1 year point may not reflect a true decline during that entire period. In this setting, particularly if the data are driven by immobilization and those who were treated with glucocorticoids, there might have been more rapid declines at the 3 month and 6 month points. The 1 year measurement might reflect partial recovery of more significant short-term reductions in BMD.

This article is a successful exploration into the chronology and dynamics of bone loss in the ICU setting. We look forward to additional work to elucidate further the basis for these observations. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Cryobiopsy in the Diagnosis of Interstitial Lung Disease
A Step Forward or Back?

If you don't know where you're going, you might not get there.
— Yogi Berra

Diagnosing hypertension is simple. We estimate brachial artery systolic and diastolic pressure using a sphygmomanometer and then compare these pressures with agreed-upon upper limits of normal (1). In contrast, diagnosing interstitial lung disease (ILD) is difficult. Once the presence of ILD is detected (a challenge unto itself), health care providers lack agreed-upon diagnostic approaches and gold standards for most forms of ILD. The exception may be idiopathic pulmonary fibrosis (IPF), for which guidance exists (2). Yet even this gold standard is flawed, recommending "careful exclusion of alternative etiologies" without specific guidance on how to achieve this goal and relying heavily on interpretation of high-resolution computed tomography scans (which has only fair interobserver agreement among community-based radiologists) (3) and surgical lung biopsies.

In recent years, the cryobiopsy has been proposed as a less invasive alternative to surgical lung biopsy in patients with ILD, partly because of the perception that it is a safer procedure (4). The technique, which has its origins in the treatment of endobronchial tumors, uses compressed gas to cool lung parenchyma at the site of a cryoprobe, which is then retracted with an attached tissue specimen. At least seven previous case series totaling more than 340 adults with ILD describe obtaining larger biopsy specimens with less crush artifact than traditional transbronchial biopsy

Changes in Bone Mineral Density in the Year after Critical Illness

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Abstract

Rationale: Critical illness may be associated with increased bone turnover and loss of bone mineral density (BMD). Prospective evidence describing long-term changes in BMD after critical illness is needed to further define this relationship.

Objectives: To measure the change in BMD and bone turnover markers (BTMs) in subjects 1 year after critical illness compared with population-based control subjects.

Methods: We studied adult patients admitted to a tertiary intensive care unit (ICU) who required mechanical ventilation for at least 24 hours. We measured clinical characteristics, BTMs, and BMD during admission and 1 year after ICU discharge. We compared change in BMD to age- and sex-matched control subjects from the Geelong Osteoporosis Study.

Measurements and Main Results: Sixty-six patients completed BMD testing. BMD decreased significantly in the year after critical illness at both femoral neck and anterior–posterior spine sites. The annual decrease was significantly greater in the ICU cohort compared with matched control subjects (anterior–posterior spine, −1.59%; 95% confidence interval, −2.18 to −1.01; $P < 0.001$; femoral neck, −1.20%; 95% confidence interval, −1.69 to −0.70; $P < 0.001$). There was a significant increase in 10-year fracture risk for major fractures (4.85 ± 5.25 vs. 5.50 ± 5.52; $P < 0.001$) and hip fractures (1.57 ± 2.40 vs. 1.79 ± 2.69; $P = 0.001$). The pattern of bone resorption markers was consistent with accelerated bone turnover.

Conclusions: Critically ill individuals experience a significantly greater decrease in BMD in the year after admission compared with population-based control subjects. Their bone turnover biomarker pattern is consistent with an increased rate of bone loss.

Keywords: critical illness; long-term outcomes; osteoporosis; bone loss; bone mineral density

Compared with their pre-illness status and general population control subjects, survivors of critical illness face increased mortality (1–4), physical (1, 5–7), and cognitive impairment (8–10), and psychological distress (11–13). A specific area in which critical illness may adversely affect the well-being of survivors relates to an increased risk of fragility fracture (14, 15). However, unlike other aspects of post-critical illness recovery, this risk has not been repeatedly explored.

Osteoporosis is a chronic progressive disease and major public health issue (16) characterized by low bone mass, micro-architectural bone disruption, and skeletal fragility, leading to fracture (17). The lifetime risk of osteoporotic spine, hip, or

(Received in original form August 2, 2015; accepted in final form November 11, 2015)

Supported by an unrestricted grant from the Intensive Care Foundation.

Authors Contributions: Substantial contributions to the conception: N.R.O., R.B., D.J.C., and M.A.K. Contributions to the design: N.R.O., M.B., J.A.P., R.B., D.J.C., and M.A.K. Acquisition, analysis, or interpretation of data: N.R.O., S.E.L., M.B., J.A.P., C.C., T.E., S.L.B.-O., R.B., D.J.C., and M.A.K. Drafting the work or revising it critically for important intellectual content: N.R.O., S.E.L., M.B., J.A.P., C.C., T.E., S.L.B.-O., R.B., D.J.C., and M.A.K. Final approval of the version to be published: N.R.O., S.E.L., M.B., J.A.P., C.C., T.E., S.L.B.-O., R.B., D.J.C., and M.A.K. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: N.R.O., S.E.L., M.B., J.A.P., C.C., T.E., S.L.B.-O., R.B., D.J.C., and M.A.K.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 193, Iss 7, pp 736–744, Apr 1, 2016

Originally Published in Press as DOI: 10.1164/rccm.201508-1514OC on November 11, 2015
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Current evidence suggests critical illness, with its associated immobilization, inflammation, and endocrine dysfunction, is associated with increased bone turnover, loss of bone mineral density, and an increased risk of fragility fracture. However, prospective evidence describing the long-term relationship between critical illness and bone loss is needed to establish the rationale for intervention.

What This Study Adds to the Field: We report a significant decrease in bone density in the year after intensive care unit admission compared with control subjects, a significantly increased risk of future fracture, and a typical pattern of bone turnover consistent with accelerated resorption. Our findings suggest that critical illness may trigger increased bone resorption and bone loss, and provide impetus for future interventional studies aimed at decreasing such loss.

wrist fracture is 30 to 40% in developed countries, and the lifetime risk of hip fracture is one in six in white women (18), with a significant associated health burden of mortality, morbidity, and cost (19, 20). However, as few as 13 to 27% of patients with osteoporosis are treated after a fragility fracture, suggesting that osteoporosis remains an underdiagnosed disease (21, 22).

Critical illness, with its associated immobilization, inflammation, and endocrine dysfunction, may be associated with increased bone turnover (23–32), loss of bone mineral density (BMD) (33), and an increased risk of fragility fracture (14, 15). Critical illness associated increase in bone turnover and subsequent fragility fractures could contribute to long-term morbidity and mortality, and is a potential target for intervention (34). However, prospective evidence describing the relationship between critical illness and bone loss is needed to establish the rationale for intervention. Accordingly, the aim of this study was to describe the changes in BMD and bone turnover markers (BTMs) in men and women in the year after critical illness, compared with an age- and sex-matched control population.

Methods

Design, Ethics, and Consent

We performed a prospective observational cohort study in a tertiary regional intensive care unit (ICU) in Geelong, Australia, between February 2010 and September 2014. Before commencement, approval was obtained from the Barwon Health Human Research Ethics Committee. Written informed consent was obtained before inclusion in the study. Participants were compared with matched population-based control subjects from the GOS (Geelong Osteoporosis Study).

Study Population and Control Subjects

Adult (age >20 yr) subjects admitted to the ICU during the study period, and who were on mechanical ventilation for more than 24 hours were considered eligible for enrollment in the study. The control population was from the GOS (35), a random population-based sample from the Australian Commonwealth Electoral Rolls. Additional details on exclusion criteria and GOS are provided in the online supplement.

Data Collection

Data collected included demographics, osteoporosis risk factors, information relating to critical illness and ICU interventions, ICU and hospital length of stay, survival, discharge destination, quality of life (EuroQol visual analog scale [EQ VAS], in which own health is rated "today" on a scale from 0 [worst imaginable health] to 100 [best imaginable health]) (36), serum biochemistry, serum bone formation marker (type 1 N-terminal procollagen), serum bone resorption marker (collagen type 1 cross-linked c-telopeptide), and BMD. Additional details on the measurement of serum BTMs and BMD are provided in the online supplement.

Data were collected at three separate time points: ICU baseline (demographic data, clinical information, BTMs); post-ICU discharge (BMD); and 1-year post-ICU discharge (repeat BMD, BTMs, biochemistry, EQ VAS, and accommodation). Details of the operating procedure are provided in the online supplement.

BMD was presented as an absolute value (grams per square centimeter), annualized percentage (difference between BMDs divided by initial BMD calculated as an annualized rate), and categorized as normal (t score > -1.0), osteopenic (t score -2.5 to -1.0), or osteoporotic (t score < -2.5). The t score is the number of SDs above or below the young adult mean, based on World Health Organization (WHO) criteria (37), with cutoff values calculated from the Australian reference ranges (38, 39). Fracture risk assessment was performed for each ICU participant who completed both BMD studies using the Australian version of the FRAX fracture risk assessment tool, an algorithm developed by the WHO (40) that combines clinical risk factors with or without femur BMD to estimate 10-year probability of hip and major osteoporotic fracture.

Outcomes

The primary outcome was the annualized percentage change in BMD (lumbar spine and dual femoral neck) for the year after ICU discharge compared with matched population control subjects. Secondary outcomes were restricted to the ICU cohort and included osteoporosis classification, fracture risk assessment, change in BTMs, and analysis of factors associated with change in BMD.

Statistical Analysis

ICU patients who completed both BMD measurements were matched to GOS control subjects by age, sex, and body mass index, in a one-to-four fashion using Mahalanobis weights, without replacement. Continuous normally distributed data were reported as mean $\pm$ SD, whereas nonparametric data were reported using median (interquartile range [IQR]) or frequency distribution. Results were calculated as total and percentage changes, with the difference in change and 95% confidence intervals (CIs) calculated using profile likelihood methods, and P values calculated from the likelihood ratio test. The primary outcome was analyzed using the analysis of covariance, and a two-sided P value of 0.05 was considered statistically significant. Additional information related to statistical analyses is provided in the online supplement.

Results

Patient Enrollment

We screened 686 subjects and enrolled 138. Of these, 48 (34.8%) withdrew before completing the 1-year BMD measurement, and 24 (17.4%) died before the 1-year BMD measurement, leaving a final cohort of 66 (47.8%) subjects for analysis (Figure 1).

Baseline Characteristics

Baseline subject characteristics, including osteoporosis risk factors, critical illness severity, biochemistry, BTMs, key interventions, and major outcomes, are presented in Table 1. Across all three groups, osteoporotic risk factors were relatively common with 58 (42.0%) subjects having at least one factor.

In the 66 subjects assessed, we found a significant decrease in BMD in the year after ICU discharge. This decrease was present at both femoral neck and anterior–posterior spine assessment (Table 2). When stratified by sex, a significant decrease in BMD was observed in women at both sites and in men at the femoral neck site only (Table 2). The mean baseline BMD of the ICU cohort and control subjects were similar after matching at both the dual femur and anterior–posterior spine (*see* Table E1 in the online supplement).

Primary Outcome

The annual decrease in BMD was significantly greater in the ICU cohort compared with the matched control subjects (Table 3). The annual decrease in BMD in the year after ICU was 1.48 ± 4.37% (anterior–posterior spine) and 1.72 ± 4.37% (femoral neck) compared with an increase of 0.11 ± 1.12% (anterior–posterior spine) and a decrease of 0.53 ± 1.07% (femoral neck) in the control subjects. This represents a difference in the annual change in BMD of −1.59% (95% CI, −2.18 to −1.01; $P < 0.001$) at the anterior–posterior spine and −1.20% (95% CI, −1.69 to −0.70; $P < 0.001$) at the femoral neck in the ICU cohort compared with the control subjects. This difference was significantly greater in women at both the anterior–posterior spine and femoral neck, whereas the difference in men was significantly greater at the femoral neck only (Table 3).

Secondary Outcomes

Overall, in the year after ICU, there was a significant decrease in serum collagen type 1 cross-linked c-telopeptide (median, 579 ng/L [IQR, 397–894] vs. 306 ng/L [IQR, 202–554]; $P < 0.001$) and a significant increase in serum type 1 N-terminal procollagen (median, 31.0 μg/L [IQR, 20.5–49.8] vs. 44.0 μg/L [IQR, 31.2–73.9]; $P = 0.04$) (Table 4). When stratified by sex and compared with population reference levels, the median collagen type 1 cross-linked c-telopeptide levels exceeded the third interquartile reference values for women (ICU 654.0 ng/L [IQR, 478.5–1,165] vs. GOS 338 ng/L [IQR, 212–499] and men (ICU 483.0 ng/L [IQR, 382.0–851.0] vs. GOS 328 ng/L [IQR, 235–459]), and returned to normal by 1 year. In contrast, type 1 N-terminal procollagen significantly increased, but remained within normal levels for men and women (Table 4). Over the year from critical illness to follow-up, there was a significant increase in median vitamin D concentration (43.0 nmol/L [IQR, 31.0–52.8] vs. 55.0 mmol/L [IQR, 46.0–71.0]; $P < 0.001$]), elevated median parathyroid hormone levels at baseline that did not significantly change (8.1 pmol/L [IQR, 4.0–13.8] vs. 5.4 pmol/L [IQR, 4.2–9.4]; $P = 0.15$), and a significant increase in median adjusted calcium levels (2.0 mmol/L [IQR, 1.8–2.1]) vs. 2.2 mmol/L [IQR, 2.2–2.3]; $P < 0.0001$).

The percentage of patients with an osteoporotic or osteopenic T-score did not change significantly from baseline to 1 year after ICU discharge (45.3% vs. 54.7%; $P = 0.08$), although the estimated 10-year fracture risk for both all major fractures (4.85 ± 5.25 vs. 5.50 ± 5.52; $P < 0.001$) and hip fractures specifically (1.57 ± 2.40 vs. 1.79 ± 2.69; $P = 0.001$) significantly increased (Table 4). Finally, 66.7% of women and 44.1% of men were classified as either osteoporotic or osteopenic at 1 year, and the 10-year fracture risk was highest in women.

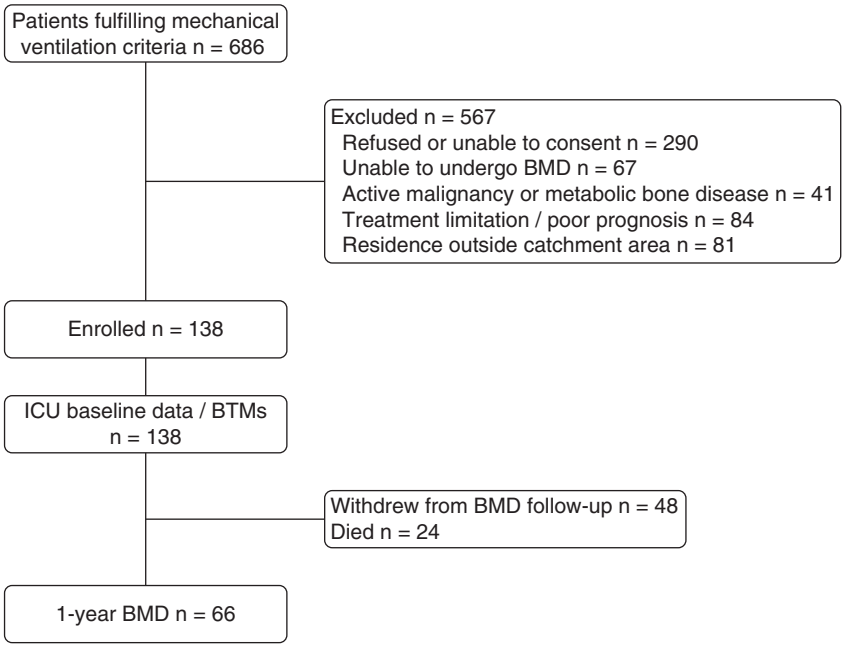

Figure 1. Summary of eligibility, enrollment, and long-term follow-up for study procedures. BMD = bone mineral density; BTM = bone turnover marker; ICU = intensive care unit.

Discussion

Key Findings

We performed a prospective observational study in critically ill, mechanically ventilated patients to measure the changes in BMD and BTMs in the year after ICU admission. We found that these subjects experienced a significant decrease in BMD in the year after ICU admission, and this was significantly greater than that in the matched subjects. Moreover, they carried a significantly increased estimated risk of future fracture.

Table 1. Demographic, Clinical Characteristics, Baseline Bone Turnover Markers, Biochemistry, Interventions, and Outcomes by Study Completion Status

Variable	All (*N* = *138*)	Assessed (*n* = *66*)	Not Assessed (*n* = *72*)
Age, yr	68.8 (59.8–76.3)	68.7 (61.1–74.5)	70.3 (58.7–77.6)
Female	69 (50.0)	31 (47.0)	38 (52.8)
BMI, kg/m^2	26.7 (23.8–30.2)	27.0 (24.3–30.5)	25.2 (23.1–27.7)
Osteoporosis risk factors			
Parent hip fracture	12 (8.7)	5 (7.6)	7 (9.7)
Previous fragility fracture	8 (5.8)	3 (4.5)	5 (6.9)
BMI $<$20 kg/m^2	7 (5.1)	2 (3.0)	5 (6.9)
Rheumatoid arthritis	3 (2.2)	2 (3.0)	1 (1.4)
Alcohol $>$ 3 U/d	14 (10.1)	4 (6.1)	10 (13.9)
Smoker (current)	28 (20.3)	11 (16.7)	17 (23.6)
Secondary osteoporosis	8 (5.8)	2 (3.0)	6 (8.3)
Corticosteroids (current)	6 (4.3)	2 (3.0)	4 (5.6)
Bisphosphonate (current)	12 (8.7)	5 (7.6)	7 (9.7)
At least 1 osteoporosis risk factor	58 (42.0)	23 (34.8)	35 (48.6)
Comorbidity			
Renal	14 (10.1)	5 (7.6)	9 (12.5)
Cardiovascular	63 (45.7)	31 (47.0)	32 (44.4)
Respiratory	33 (23.9)	15 (22.7)	18 (25.0)
Diabetes mellitus	25 (18.1)	12 (18.2)	13 (18.1)
APACHE III score	69.5 (56.0–96.5)	66.0 (56.0–92.8)	72.0 (59.0–102.2)
ICU admission category			
Cardiac failure	21 (15.2)	11 (16.7)	10 (13.9)
Cardiothoracic surgery	30 (21.7)	15 (22.7)	15 (20.8)
General surgery	27 (19.6)	11 (16.7)	16 (22.2)
Other	10 (7.2)	5 (7.6)	5 (6.9)
Respiratory failure	11 (8.0)	5 (7.6)	6 (8.3)
Sepsis	39 (28.3)	19 (28.8)	20 (27.8)
Biochemistry and biomarkers			
Albumin, g/L	23.0 (19.0–27.0)	24.0 (20.0–28.0)	22.0 (18.0–27.0)
Calcium adj, mmol/L	2.0 (1.8–2.2)	2.0 (1.8–2.1)	2.0 (1.8–2.2)
Creatinine, μmol/L	128.5 (89.0–181.8)	121.0 (85.5–178.8)	136.5 (94.0–198.0)
Vitamin D, nmol/L	41.0 (31.0–52.0)	43.0 (31.0–52.8)	40.0 (31.0–51.0)
Phosphate, mmol/L	0.7 (0.5–1.0)	0.7 (0.5–1.0)	0.7 (0.5–1.1)
PTH, pmol/L	7.0 (3.8–10.9)	8.1 (4.0–13.8)	6.3 (3.7–10.3)
CTx, ng/L	660.0 (418.0–953.0)	579.0 (396.5–893.5)	738.0 (487.0–969.0)
P1NP, μg/L	23.0 (19.0–27.0)	24.0 (20.0–28.0)	22.0 (18.0–27.0)
Interventions/outcomes			
Ventilation duration, h	96.0 (47.2–218.8)	87.0 (48.0–144.0)	117.5 (47.5–283.2)
Corticosteroid	47 (34.3)	21 (32.3)	26 (36.1)
CRRT	28 (20.3)	11 (16.7)	17 (23.6)
ICU LOS, d	7.0 (4.2–13.0)	6.5 (4.0–9.0)	7.5 (5.0–15.0)
Hospital LOS, d	19.0 (12.0–31.8)	16.5 (11.0–31.0)	21.5 (12.0–32.0)
ICU survival	129 (93.5)	66 (100)	63 (87.5)
Hospital survival	124 (89.9)	66 (100)	58 (80.6)
1-yr status			
Survival	114 (82.6)	66 (100)	48 (66.7)
Living at home	—	60 (95.2%)	—
EQ VAS	—	80 (70–90)	—

Definition of abbreviations: adj = adjusted; APACHE = Acute Physiology and Chronic Health Evaluation; BMI = body mass index; CRRT = continuous renal replacement therapy; CTX = collagen type 1 cross-linked c-telopeptide; EQ VAS = EuroQol visual analog scale; ICU = intensive care unit; LOS = length of stay; P1NP = type 1 N-terminal procollagen; PTH = parathyroid hormone.
Data are shown as median (interquartile range) or number (%). Reference ranges: vitamin D: $<$25 nmol/L, deficient; 25–50 nmol/L, insufficient; $>$50 nmol/L, sufficient; PTH: range, 1.6–6.9 pmol/L.

These clinical events were associated with a specific pattern of BTMs. This pattern was suggestive of increased bone resorption with no commensurate response in bone formation during critical illness, followed by normalization of resorptive activity, but no compensatory increased formation activity a year later.

Relationship to Previous Studies

To our knowledge, the only previous report of BMD changes after an ICU stay described a significant decrease in calcaneal BMD over a 10-day period in subjects with acute respiratory distress syndrome (ARDS) compared with ventilated subjects without ARDS (33). However, BMD undergoes relatively small changes over time of a magnitude similar to measurement error; therefore, follow-up over longer periods is recommended, making a 1-year change in BMD the standard for interventional research studies (41–45). This makes our observations relevant to potential interventions in the future.

Table 2. Changes in Bone Mineral Density at 1-Year Follow-up after Critical Illness

	BMD (g/cm^2)		
	Baseline	1 yr	*P* Value
All (n = 66)			
Dual femur	0.958 ± 0.192	0.940 ± 0.193	<0.001
AP spine	1.226 ± 0.232	1.205 ± 0.241	0.007
Women (n = 31)*			
Dual femur	0.892 ± 0.165	0.872 ± 0.161	0.02
AP spine	1.183 ± 0.223	1.142 ± 0.223	0.001
Men (n = 35)*			
Dual femur	1.015 ± 0.197	0.999 ± 0.202	0.002
AP spine	1.264 ± 0.236	1.260 ± 0.246	0.74

Definition of abbreviations: AP = anteroposterior; BMD = bone mineral density.
Data are shown as mean ± SD.
*Thirty-five men completed both AP spine BMD tests, and 34 completed both femur BMD tests. Thirty-one women completed both AP spine BMD tests, and 30 completed both femur BMD tests.

BTMs are an important tool to assess progression of osteoporosis, fracture risk, and treatment response (46, 47). We measured type 1 N-terminal procollagen, a bone formation marker synthesized by osteoblasts and released during the processing of type I procollagen into collagen, and the bone resorption marker collagen type 1 cross-linked c-telopeptide, a product of collagen degradation. Both markers correlate with corresponding histomorphometric parameters of bone formation and resorption, and have been identified as the most promising BTMs by the Joint International Bone Markers Standards Working Group (46). We observed a change from increased bone resorption during critical illness, to normal bone resorption with increasing bone formation over the subsequent year, consistent with previous studies that reported increased bone resorption markers (24–27, 29–31), increased serum osteoclast precursors (32), increased bone formation, and decreased osteocalcin during critical illness compared with controls (26–29). Overall, these findings suggest changes associated with critical illness of a magnitude similar to that described in postmenopausal women or in those with metabolic bone disease (29, 47, 48, 49). The interpretation of BTMs during critical illness is complex; however, levels are affected by a number of factors, including age, sex, coexisting disease, inflammatory markers, and medications, particularly glucocortocoids (26–28, 50, 51). This study was unable to analyze these relationships due to its limited sample size.

Abnormalities in the vitamin D–parathyroid-calcium axis during critical illness and the association with increased illness severity, length of stay, and mortality, and the effects of vitamin D supplementation on inflammatory markers and outcomes have been described (52–62). We observed a pattern of vitamin D insufficiency, increased parathyroid hormone release, and hypocalcaemia during critical illness and normalization at 1-year follow-up. Vitamin D deficiency with resultant secondary hyperparathyroidism and prolonged immobilization may increase the risk of excessive bone resorption; however, treating critically ill patients with vitamin D resulted in inconsistent effects on BTMs, which suggested that accelerated bone turnover is multifactorial (25, 29).

Over half of survivors in our study had BMD in the osteopenic or osteoporotic range, which was higher than local population levels, with the GOS reporting one-fifth of women older than 50 years of age having BMD in the osteopenic range

Table 3. Annualized Change in Bone Mineral Density after Critical Illness Compared with Matched Geelong Osteoporosis Study Control Subjects

Variable	ICU	GOS	Difference (95% CI)	*P* Value
All	n = 66	n = 256		
Total change AP spine, g/cm^2	−0.018 ± 0.055	0.001 ± 0.013	−0.019 (−0.026 to −0.012)	<0.001
Percent change AP spine	−1.48 ± 4.37	0.11 ± 1.12	−1.59 (−2.18 to −1.01)	<0.001
Total change femur, g/cm^2	−0.016 ± 0.032	−0.005 ± 0.010	−0.011 (−0.016 to −0.007)	<0.001
Percent change femur	−1.72 ± 3.43	−0.53 ± 1.07	−1.20 (−1.69 to −0.70)	<0.001
Women	n = 31*	n = 120		
Total change AP spine, g/cm^2	−0.035 ± 0.050	−0.002 ± 0.012	−0.033 (−0.042 to −0.023)	<0.001
Percent change AP spine	−2.85 ± 4.05	−0.18 ± 1.08	−2.67 (−3.49 to −1.86)	<0.001
Total change femur, g/cm^2	−0.018 ± 0.037	−0.006 ± 0.008	−0.013 (−0.020 to −0.005)	0.001
Percent change femur	−1.96 ± 4.03	−0.65 ± 0.98	−1.31 (−2.10 to −0.51)	0.001
Men	n = 35*	n = 136		
Total change AP spine, g/cm^2	−0.003 ± 0.055	0.005 ± 0.014	−0.007 (−0.018 to 0.003)	0.16
Percent change AP spine	−0.28 ± 4.34	0.36 ± 1.10	−0.64 (−1.45 to 0.17)	0.12
Total change femur, g/cm^2	−0.015 ± 0.027	−0.004 ± 0.011	−0.010 (−0.016 to −0.005)	0.001
Percent change femur	−1.52 ± 2.85	−0.42 ± 1.13	−1.10 (−1.71 to −0.49)	<0.001

Definition of abbreviations: AP = anteroposterior; BMD = bone mineral density; CI = confidence interval; GOS = Geelong Osteoporosis Study; ICU = intensive care unit.
Data are shown as mean ± SD unless otherwise indicated.
*Thirty-five men completed both AP spine BMD tests, and 34 completed both femur BMD tests. Thirty-one women completed both AP spine BMD tests, and 30 completed both femur BMD tests.

Table 4. Changes in Biochemistry and Bone Turnover Markers, *t* Score, and Fracture Risk, at 1-Year Follow-up after Critical Illness

Variable	All (*N* = 66)			Female Cohort (*n* = 31)			Male Cohort (*n* = 35)		
	Baseline	1 yr	*P* Value	Baseline	1 yr	*P* Value	Baseline	1 yr	*P* Value
Biochemistry and BTMs*									
Calcium adj, mmol/L	2.0 (1.8–2.1)	2.2 (2.2–2.3)	<0.001	2.0 (1.7–2.2)	2.3 (2.2–2.4)	0.001	2.0 (1.9–2.1)	2.2 (2.2–2.3)	<0.001
Creatinine, μmol/L	121 (86–179)	91 (77–116)	<0.001	119 (84–203)	78.0 (67.0–92.0)	<0.001	126 (99–165)	100 (88–120)	<0.001
Vitamin D, nmol/L	43.0 (31.0–52.8)	55.0 (46.0–71.0)	<0.001	47.0 (33–55)	55.0 (48.0–69.0)	0.02	37.0 (30.0–49.5)	53.5 (39.5–74.5)	0.003
Phosphate, mmol/L	0.7 (0.5–1.0)	1.1 (1.0–1.2)	0.002	0.7 (0.4–1.0)	1.1 (1.0–1.3)	0.04	0.7 (0.6–0.9)	1.1 (0.9–1.2)	0.03
PTH, pmol/L	8.1 (4.0–13.8)	5.4 (4.2–9.4)	0.15	6.9 (2.9–11.4)	5.1 (4.5–8.2)	0.58	8.3 (5.2–14.4)	7.0 (3.8–10.4)	0.10
CTx, ng/L	579 (397–894)	306 (202–554)	<0.001	654 (479–1,165)	315 (162–592)	0.001	483 (382–851)	305 (230–542)	0.002
P1NP, μg/L	31.0 (20.5–49.8)	44.0 (31.2–73.8)	0.04	29.0 (17.5–46.0)	47.0 (35.0–77.2)	0.10	32.0 (23.0–58.0)	41.0 (29.2–69.8)	0.11
Dual femur *t* score†									
Osteoporosis/ osteopenia	29 (45.3)	35 (54.7)	0.08	17 (56.7)	20 (66.7)	0.37	12 (35.3)	15 (44.1)	0.25
Normal	35 (54.7)	29 (45.3)		13 (43.3)	10 (33.3)		22 (64.7)	19 (55.9)	
FRAX 10-yr risk†‡									
Major fracture	4.85 ± 5.25	5.20 ± 5.52	<0.001	6.81 ± 6.83	7.34 ± 7.17	0.004	3.12 ± 2.26	3.31 ± 2.29	<0.001
Hip fracture	1.57 ± 2.40	1.79 ± 2.69	0.001	2.13 ± 3.12	2.47 ± 3.53	0.01	1.07 ± 1.39	1.19 ± 1.43	<0.001

Definition of abbreviations: adj = adjusted; BMD = bone mineral density; BTM = bone turnover marker; CTx = collagen type 1 cross-linked c-telopeptide; FRAX = Fracture Risk Assessment Tool; IQR = interquartile range; P1NP = type 1 N-terminal procollagen; PTH = parathyroid hormone.
Data are shown as mean ± SD, median (IQR), or number (%). Reference ranges: vitamin D: <25 nmol/L, deficient; 25–50 nmol/L, insufficient; >50 nmol/L, sufficient; PTH: range, 1.6–6.9 pmol/L.
*Geelong Osteoporosis Study reference values (74): female median P1NP 37 μg/L (IQR, 26–51), CTx 338 ng/L (IQR 212–499); male median P1NP 37 μg/L (IQR 27–49), CTx 328 ng/L (IQR 235–459).
†Thirty-four males completed both femur BMD tests, and 30 females completed both femur BMD tests.
‡Ten-year probability of fracture, given as a percentage.

and one in six having osteoporosis (63). Although the proportion of subjects with BMD below normal values did not change significantly during the year after critical illness, the use of BMD category alone has limitations for fracture prediction, because more than one-half of fragility fractures arise from the population of individuals with osteopenia, rather than the higher risk but smaller population with osteoporosis (63–65). This has led to the development of fracture risk prevention models that incorporate clinical factors and BMD, including the FRAX fracture risk assessment tool (40). In this regard, we observed a significant increase in the 10-year probability of hip and major osteoporotic fracture in the year after critical illness, consistent with a previous observation of increased fracture prevalence in older women after critical illness compared with population-based control subjects (14).

Study Implications

This study implies that critical illness is associated with increased bone turnover, loss of BMD, and an increased risk of fragility fractures for up to 1 year after ICU discharge. Such reduced bone mass and increased bone loss in the year after critical illness, particularly in female survivors, has not been previously described and further implies that such individuals may be at particular risk. Moreover, in such women, it also implies that antiresorptive therapy to prevent bone loss, reduce fracture risk, and possibly improve survival should be carefully considered. Finally, this study provides a rationale for, and essential information toward, the design of pilot interventional trials aimed at decreasing bone resorption using BMD at 1 year as the primary outcome. The notion that intervention may be effective is supported by short-term studies such as a retrospective case series that describe a reduction in urine N-telopeptide in long-term ventilated patients treated with pamidronate (25), and a randomized trial of 20 chronic critically ill postmenopausal women that reported a decrease in serum collagen type 1 cross-linked c-telopeptide and an increase in osteocalcin with ibandronate compared with placebo (66). In addition, multimodal therapies, including physical therapies and rehabilitation, warrant investigation in this population (67).

Strengths and Limitations

Our study has several strengths. For the first time, we collected detailed information, including risk factors that were related to bone mass in a cohort of ICU survivors over a prolonged period. This is important because establishing the presence of bone loss after critical illness requires extended observation. We measured biomarkers of bone turnover to establish broad relevance to similar biomarker studies. For the first time, we followed up a cohort of critically ill subjects for 1 year to establish the long-term changes in BMD. Moreover, we were able to identify clear and logical changes in BMD over time, which were particularly striking in women. Finally, and also for the first time, we compared our cohort with age- and sex-matched control subjects from a well-described, population-based sample from the same community (35).

There are limitations to this study. The loss of over half of the participants enrolled due to withdrawal or death made it impossible to develop a robust predictive model for specific additional risk factors, and restricted generalizability of the findings. However, as expected, female sex was clearly associated with greater loss of

BMD. Furthermore, despite a higher than expected rate of death and loss to follow-up, the greater than expected decline in BMD resulted in sufficient data to demonstrate differences in the primary outcome that were statistically significant and clinically relevant. A larger cohort would provide the ability to identify further risk factors, but such demanding work can only be justified if studies such as ours provide sufficient preliminary data to support its conduct. Comparison of BMD in ICU subjects to a population-based control group rather than a hospital-based control group means we could not exclude that hospitalization *per se* (instead of ICU admission alone) might be associated with increased loss of BMD. However, the decrease in BMD seen in these subjects constitutes the necessary initial evidence to conduct such larger studies. Measurement of BMD at ICU discharge and again at 1 year did not allow for the observation of nonlinear changes in bone density; for example, it was possible an early rapid loss of bone density occurred in the months after critical illness, with subsequent recovery. However, a 1-year change in BMD is the standard for interventional research studies (41–45).

We also demonstrated an association between critical illness and greater decline in BMD, but we were unable to separate the effects of pre–critical illness factors from critical illness *per se*. Pre–critical illness chronic disease, frailty, and worsening functional trajectory are common in critically ill individuals, and are associated with trajectory and degree of recovery (68–71). The matching of the ICU cohort and the GOS control subjects by sex and age resulted in similar baseline BMDs, refuting the notion that the ICU cohort had lower bone density than control subjects at the time of admission. This may be explained by the characteristics of the cohort that completed the study. By definition, they had 100% 1-year survival, reported a high quality of life, and 95% lived independently 1 year after ICU discharge. This pattern is most consistent with a minimal disability functional trajectory, or a nonfrail population (68, 69). Finally, although long-term ICU recovery trials would ideally include prospectively collected pre-ICU trajectory data (71–74), critical illness may act as a marker for underdiagnosed disease burden, irrespective of causality (71).

Conclusions

We performed a prospective observation study in critically ill, mechanically ventilated subjects to measure the changes in BMD and BTMs in the year after ICU admission. We found that these subjects experienced a significant decrease in bone density in the year after ICU admission compared with control subjects; they carried a significantly increased risk of future fracture and had a typical pattern of bone turnover consistent with accelerated resorption. Our findings suggest that critical illness may trigger increased bone resorption and faster loss of BMD and provide impetus for future interventional studies aimed at decreasing such loss. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Appendix / Supplement Files

Supplementary Methods

Exclusion criteria: Exclusion criteria included active malignancy, existing neurological illness with impaired weight bearing, inability to lie flat, metabolic bone disease, pregnancy, weight greater than 120 kilograms, and considered unlikely to survive by the treating intensivist. Patients who had multiple ICU admissions during the study period were included for their first ICU admission only. Patients not enrolled in the study by day 7 of mechanical ventilation were no longer considered for inclusion in the study

Geelong Osteoporosis Study description: The Geelong Osteoporosis Study is a random population-based sample from the Australian Commonwealth Electoral Rolls recruited between 1993 and 1997 (women) and 2003 to 2008 (men), for a geographically well defined region, surrounding Geelong, in south-eastern Australia called the Barwon Statistical Division. As voting is compulsory in Australia, the electoral roll provides a comprehensive listing of adults (age > 18 years). Age-stratified random samples of 1494 women (age range 20-94 years) and 1540 men (age range 20-97 years) were enrolled, with a minimum of 100 in each 5-year age stratum between ages 20 and 69, and a minimum of 200 in the age 70-79 year group, and the over 80 year group. In this control population, BMD measurements are performed second yearly in the female cohort and five yearly in the male cohort.

Bone mineral density and serum bone turnover marker measurement description: BMD was measured by dual energy x-ray absorptiometry (DXA) (Lunar; GE Healthcare, Madison, Wis, USA), at the proximal femur and lumbar spine. Short-term precision in vivo was 1.6% for the femoral neck and 0.6% for the lumbar spine[1]. The serum bone turnover markers collagen type 1 cross-linked c-telopeptide and type 1 N-terminal procollagen were collected the morning after enrolment with routine early morning blood tests, and measured using the automated Roche Modular Analytics E170 analyser. Serum collagen type 1 cross-linked c-telopeptide limit of detection was 10 ng/L with inter-assay coefficient of variations (CVs) of 6.5% at 361 ng/L, 3.8% at 816 ng/L and 3.4% at 3304 ng/L (n = 10). Serum type 1 N-terminal procollagen inter-assay CVs were 4.9% at 73 µg/L, 2.6% at 392 µg/L, and 2.1% at 768 µg/L (n = 10) with a limit of detection of 5 µg/L. Bone turnover markers were compared to reference ranges derived from an Australian population sample[2].

Statistical analysis description: ICU patients who completed both BMD measurements were

matched to GOS controls by age, sex, and BMI, in a one-to-four fashion using Mahalanobis weights, without replacement. Mahalanobis weighting was chosen to account for correlation [3]. The average treatment effect for those participants admitted to the ICU was estimated via linear regression including the covariates used for matching, an indicator variable for whether the participant was admitted to the ICU or was a (GOS) population control, and a random effect to account for correlation induced by the matching. Ninety-five percent confidence intervals (95% CIs) were calculated for the difference by profile likelihood, with p-values from the likelihood ratio test.

The annual decline in lumbar spine in the GOS population is normally distributed, with a standard deviation of 0.06% at the lumbar spine in males, and 0.25% at the lumbar spine in females. In the absence of any existing data describing long-term BMD changes following critical illness, a global effect size of 50% of one standard deviation was chosen to be clinically significant as it equates to a 12% difference across the range of each variable. In order to have a 90% power (2-sided p-value of 0.05) to detect an effect size of 50% of one standard deviation, 84 subjects are required. Allowing for potential deaths (20%) and dropouts (20%), 138 subjects were recruited.

Data were analysed using R version 3.1.3 (R Core Team 2015) and a two-sided p-value of 0.05 was considered to be statistically significant. Continuously normally distributed data were reported as mean (±standard deviation), whereas non-parametric data were reported using median (IQR) or frequency distribution. The primary outcome was analysed using Analysis of Covariance. Results were calculated as total and percentage change, with the difference in change and 95% CIs calculated using profile likelihood methods, and p-values calculated from the likelihood ratio test.

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Appendix 1: Study operating procedures

Softer Study Procedures	
>24 hrs to <168 hrs duration of mechanical ventilation	
Enrolment	Inclusion criteria met, consent obtained
Study procedures	Baseline and demographic data
	Biochemistry and BTM (serum PINP, CTx, Vit D, PTH, albumin, calcium, phosphate, creatinine)
ICU discharge (ICU discharge to 1-month)	
Study procedure	BMD #1
1 year follow-up (1 year post-ICU discharge)	
Study procedure	Contact participant
	BMD #2
	Biochemistry and BTMs (serum PINP, CTx, vitamin D, PTH, albumin, calcium, phosphate, creatinine)
	Questionnaire (EQ VAS)
Vitamin D / calcium / anti-resorptive therapy will be offered to participants in accordance with current guidelines and review of results and risk factors by an endocrinologist	

Supplement Table 1: Comparison of baseline demographics of ICU and GOS cohorts before and after matching

Variable	ICU (n=64)[2]	Before Matching		After Matching	
		GOS (n=3277)	SMD	GOS (n=256)	SMD
Female	30 (47%)	1533 (47%)	0.53	120 (47%)	0.00
Age	65.03 (± 13.65)	51.80 (16.44)	9.62	65.00 (± 13.54)	0.02
BMI	27.82 (± 5.07)	26.98 (4.41)	1.63	27.42 (± 4.34)	0.77
Weight	77.00 (± 16.02)	77.65 (13.84)	0.40	76.88 (± 13.37)	0.07
Height	1.66 (± 0.10)	1.70 (0.09)	3.26	1.67 (± 0.09)	1.2
AP Spine baseline BMD	1.225 (± 0.226)	1.25 (0.18)	1.23	1.229 (± 0.220)	0.17
Dual Femur baseline BMD	0.958 (± 0.190)	0.98 (0.15)	1.25	0.928 (± 0.158)	1.56

1. Data are shown as mean (±SD), median [IQR] or no.(%) or standardised mean difference (SMD)
2. 64 participants had both spine and femur BMD measurements performed at follow-up

Chapter 5: The association of time and medications with changes in BMD in the 2 years after critical illness

This chapter, published in *Critical Care* in 2017, is an extension of the previous prospective observational study of change in BMD in the year after critical illness, and describes the association of time and medication use on change in BMD over 2-years after ICU. In women a greater loss of spine BMD was observed in the first year after critical illness, and anti-fracture therapy use was associated with an increase in BMD. In men BMD loss increased in the second year after critical illness. In a smaller cohort of men and women who did not receive either glucocorticoids or ant-fracture medication, a decrease in bone mass was observed for both years after critical illness.

Orford et al. Critical Care (2017) 21:69
DOI 10.1186/s13054-017-1657-6

Critical Care

RESEARCH Open Access

The association of time and medications with changes in bone mineral density in the 2 years after critical illness

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Abstract

Background: Critical illness is associated with increased risk of fragility fracture and loss of bone mineral density (BMD), although the impact of medication exposures (bone anti-fracture therapy or glucocorticoids) and time remain unexplored. The objective of this study was to describe the association of time after ICU admission, and post-ICU administration of bone anti-fracture therapy or glucocorticoids after critical illness, with change in BMD.

Methods: In this prospective observational study, conducted in a tertiary hospital ICU, we studied adult patients requiring mechanical ventilation for at least 24 hours and measured BMD annually for 2 years after ICU discharge. We performed mixed linear modelling to describe the association of time, and post-ICU administration of anti-fracture therapy or glucocorticoids, with annualised change in BMD.

Results: Ninety-two participants with a mean age of 63 (±15) years had at least one BMD assessment after ICU discharge. In women, a greater loss of spine BMD occurred in the first year after critical illness (year 1: -1.1 ± 2.0% vs year 2: 3.0 ± 1.7%, $p = 0.02$), and anti-fracture therapy use was associated with reduced loss of BMD (femur 3.1 ± 2.4% vs -2.8 ± 1.7%, $p = 0.04$, spine 5.1 ± 2.5% vs -3.2 ± 1.8%, $p = 0.01$). In men anti-fracture and glucocorticoid use were not associated with change in BMD, and a greater decrease in BMD occurred in the second year after critical illness (year 1: -0.9 ± 2.1% vs year 2: -2.5 ± 2.1%, $p = 0.03$).

Conclusions: In women a greater loss of spine BMD was observed in the first year after critical illness, and anti-fracture therapy use was associated with an increase in BMD. In men BMD loss increased in the second year after critical illness. Anti-fracture therapy may be an effective intervention to prevent bone loss in women after critical illness.

Keywords: Critical illness, Long-term outcomes, Osteoporosis, Fracture, Bone loss, Bone mineral density, Bone turnover marker

Background

Over the last two decades the focus of research on intensive care outcomes has broadened from survival to include morbidity and quality of life [1–10]. To date, interventions aimed at improving recovery after critical illness have had a functional focus - physical therapy programs [11, 12], mental health support [13], and follow-up clinics [14–16] - but met with limited success [17]. However, there has been little focus on post-intensive care unit (ICU) bone loss, a potentially treatable condition.

In recent years, an association between critical illness and accelerated bone turnover has been described, including an increase in bone turnover markers (BTM) during critical illness [18], accelerated loss of bone mineral density (BMD) in the year following critical illness [19], and increased fragility fractures in survivors of critical illness [20]. This association was, as expected, most pronounced in older women [19, 20]. The annual change in femur and spine BMD in women that survived critical illness was -1.96% and -2.85%, compared to -0.65% and

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-0.18% in age-matched community controls [19]. The risk of fragility fracture for women greater than 60 years of age was significantly higher following critical illness than age-matched controls [20].

However the duration of this effect, and the potential impact of medications that are known to adversely (such as glucocorticoids [21, 22]) or positively (anti-fracture therapy) affect BMD and fracture risk, are not fully elucidated, and no long-term prospective studies have described the BMD outcome association after critical illness.

The aim of this study was to describe the association of time, post-ICU administration of bone anti-fracture therapy and glucocorticoids on change in BMD over a 2-year period in survivors of critical illness.

Methods

Design, ethics and consent

We conducted a prospective observational cohort study of longitudinal changes in BMD for a 2-year period after critical illness. Prior to commencement, approval was obtained from the Barwon Health Human Research Ethics Committee. Written informed consent was obtained from surrogate decision-makers and patients for inclusion for the first year of the study [19]. Subsequent consent was obtained from patients to extend follow-up to 2 years post critical illness.

Study population

Adult (age greater than 20 years) patients admitted to a tertiary, mixed medical, surgical, and cardiac surgical ICU during the study period, and with duration of mechanical ventilation greater than 24 hours were eligible for enrolment in the study. Exclusion criteria included active malignancy, existing neurological illness with impaired weight bearing, inability to lie flat, metabolic bone disease, pregnancy, weight greater than 120 kilograms, and considered unlikely to survive by the treating intensivist. Patients with multiple ICU admissions during the study period were included for the first ICU admission only.

Data collection

Data collected included demographics, osteoporosis risk factors [parental history of hip fracture, previous fragility fracture, body mass index (BMI) less than 20, current smoking, use of glucocorticoids, rheumatoid arthritis, alcohol consumption of three units daily or greater, or secondary causes of osteoporosis], information relating to critical illness and ICU interventions, ICU and hospital length of stay, survival, serum biochemistry, serum bone formation marker: type 1 N-terminal procollagen (P1NP), serum bone resorption marker: collagen type 1 cross-linked c-telopeptide (CTX), and BMD. BMD was measured by dual-energy X-ray absorptiometry (DXA) (Lunar; GE Healthcare, Madison, WI, USA), at the proximal femur (femoral neck) and lumbar spine. Short-term precision in vivo was 1.6% for the femoral neck and 0.6% for the lumbar spine. Details on the measurement of serum BTMs are provided in Additional file 1. Medication history included medications taken by participants prior to critical illness, during critical illness, and during follow-up periods. Use of anti-fracture therapy was defined as use of a bisphosphonate, strontium ranelate, teriparatide, denosumab, or raloxifene, in the previous year. Use of glucocorticoids was defined as greater than 3 months' use in the previous year at a dose of prednisolone of 5 mg daily or more (or equivalent dose of other glucocorticoids).

Data were collected at ICU baseline (demographic data, clinical information, BTMs), post-ICU discharge (BMD), 1-year post-ICU discharge (BMD, BTMs, clinical information), and 2-year post-ICU discharge (BMD, clinical information). Details of the study operating procedure are provided in Additional file 2. BMD was presented as an absolute value (g/cm^2), annualised percentage change (difference between BMDs divided by initial post-ICU discharge BMD calculated as an annualised rate), and categorised as normal (T-score > -1.0), osteopenic (T-score -2.5 to -1.0), or osteoporotic (T-score < -2.5). The T-score is the number of standard deviations above or below the young adult mean, based on WHO criteria [23] with cutoff values calculated from the Australian reference ranges [24, 25].

Outcomes

The outcomes of the study were annualised percentage change compared to baseline BMD (lumbar spine and dual femoral neck) for each of the 2 years after ICU discharge. The effect of the post-ICU variables including year post-ICU discharge, anti-fracture therapy use, and glucocorticoid use, on annual percentage change in BMD were also assessed.

Statistical analysis

All data were initially assessed for normality. Group comparisons were performed using chi-square tests for equal proportion, Student t tests for normally distributed data and Wilcoxon rank sum tests otherwise, with results reported as number (%), mean (standard deviation) or median (interquartile range) respectively. Mixed linear modelling was used to explore the nature of the relationship between anti-fracture therapy use, glucocorticoid use, and the mean annualised change in bone mineral density using all available data. Given the known differences in BMD between men and women, all results have been stratified by sex. To account for potential survival bias due to participant drop-out, additional sensitivity analysis was conducted considering only patients that completed all three BMD measurements over the 2-year study period. Finally,

to further establish the duration and magnitude of change in BMD after critical illness in the absence of known modifiers, a final subgroup of completers excluding those with post-ICU glucocorticoid or anti-fracture therapy use was considered. All modelling results are reported as least square means ± standard errors and a two-sided p value of 0.05 was used to indicate statistical significance. Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), with figures produced using Graphpad Prism 7.0 © (GraphPad Software, San Diego, CA, USA).

Results

Patient enrolment

A total of 92 of 138 patients enrolled in the study during their ICU stay underwent initial BMD assessment following ICU discharge and were eligible for this study. Of the 92 subjects, 66 had two BMD assessments, and 48 had all three BMD assessments over the 2-year study period (Fig. 1).

Baseline characteristics

Baseline characteristics are presented in Table 1 stratified by BMD assessment status. Overall 40 (44%) of participants had at least one osteoporosis risk factor, 29 (32%) received glucocorticoids during critical illness, median ICU length of stay was 6 days [IQR 4,9], and hospital length of stay was 16 days [IQR 11,30]. Mortality at 1 year was 9%, and at 2 years was 11%. The 48 participants that completed all BMD assessments and were included in the sensitivity analysis were compared to the 44 participants that withdrew or died prior to completion of all BMD assessments. The groups had similar characteristics, except for an increased prevalence of osteoporosis risk factors (57% vs 31%, $p = 0.02$) in the group that withdrew or died prior to completion of all assessments.

Change in BMD and association with time, anti-fracture therapy and glucocorticoids

Over the 2-year post-ICU period 92 participants underwent a total of 114 measurements of annual change in BMD (post-ICU year 1 $n = 66$, post-ICU year 2 $n = 48$) (Table 2). Over the 2-year period ten participants were prescribed anti-fracture therapies (six women, four men), including alendronate (five participants), denosumab (two participants), strontium ranelate (two participants), and risedronate (one participant). Three (10%) women and one (3%) man received anti-fracture therapies in year 1 post-ICU, and six (27%) women and four (15%) men received anti-fracture therapies in year 2. Glucocorticoids were received by two (7%) women and one (2%) man in year 1 post-ICU, and five (23%) women in year 2.

In 44 women with 53 measurements of annual change in BMD over the 2-year period, a significantly greater decrease in BMD was observed in post-ICU year 1 compared to year 2 for spine BMD (year 1: -1.1 ± 2.0% vs year 2: 3.0 ± 1.7%, $p = 0.02$), but not femur BMD (year 1:

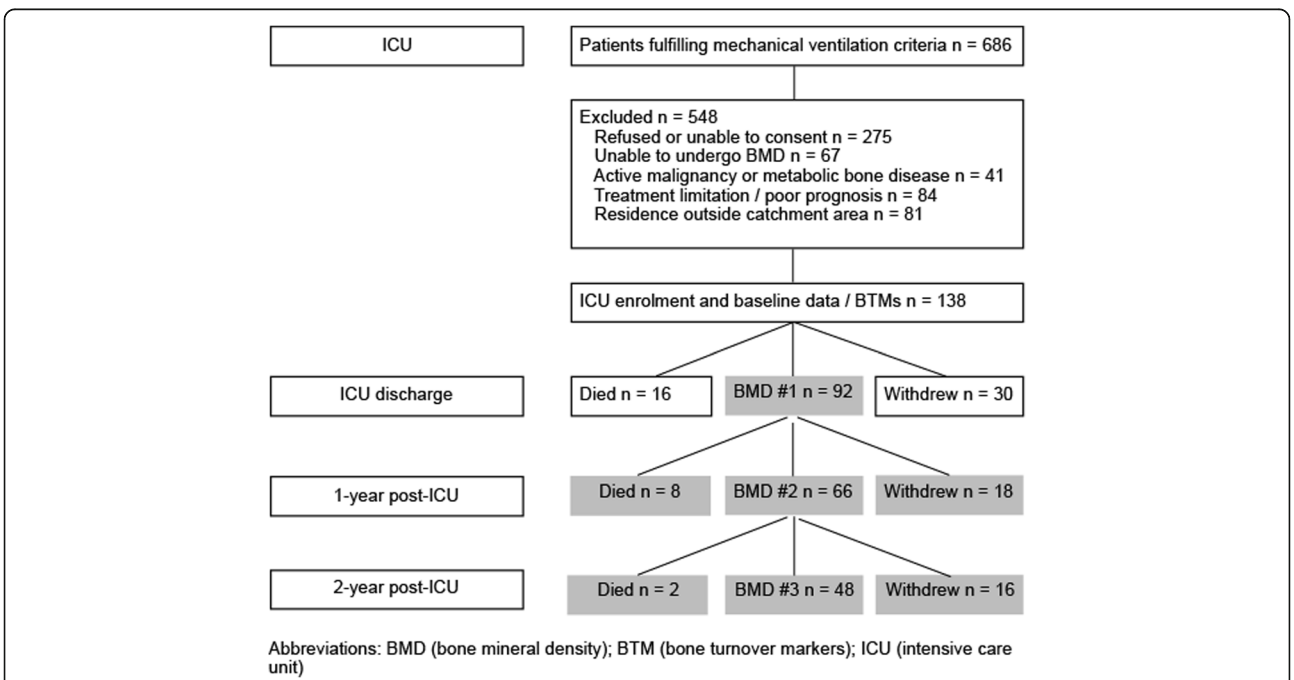

Fig. 1 Summary of eligibility, enrolment, and follow-up of patients undergoing BMD assessment following intensive care. *Abbreviations*: *BMD* bone mineral density, *BTM* bone turnover markers, *ICU* intensive care unit

Table 1 Demographic, clinical characteristics, baseline bone turnover markers, biochemistry, and outcomes by bone mineral density assessments at 2-year follow-up

Variable	All ($n = 92$)	Completed three BMD assessments ($n = 48$)	Completed one to two BMD assessments ($n = 44$)	p value
Age (yrs)	63.4 (±14.7)	65.8 (±11.4)	60.8 (±17.4)	0.1
BMI	27.1 (±5.1)	27.2 (4.4)	27.1 (5.8)	0.9
Women	44 (47.8)	22 (45.8)	22 (50.0)	0.8
Any osteoporosis risk factor	40 (43.5)	15 (31.3)	25 (56.8)	0.02
Co-morbidity				
Renal	7 (7.6)	4 (8.3)	3 (6.8)	1.0
Cardiovascular	40 (43.5)	22 (45.8)	18 (40.9)	0.7
Respiratory	22 (23.9)	8 (16.7)	14 (31.8)	0.1
APACHE III score	74.4 (±29.5)	76.3 (±29.3)	72.4 (±30.0)	0.5
ICU admission category				
Medical	54 (58.7)	26 (54.2)	28 (63.6)	
Cardiothoracic surgery	16 (17.4)	10 (20.8)	6 (13.6)	
General surgery	22 (23.9)	12 (25.0)	10 (22.7)	
ICU biochemistry and biomarkers				
Albumin (g/L)	23.9 (±5.8)	23.6 (±5.9)	24.1 (±5.7)	0.6
Calcium adj (mmol/L)	1.99 (±0.33)	2.01 (±0.32)	1.96 (±0.33)	0.5
Creatinine (umol/L)	116 [85, 178]	125 [85, 175]	109 [89, 196]	0.7
Vitamin D (nmol/L)	44.2 (±20.2)	43.4 (±20.0)	45.1 (±20.7)	0.7
Phosphate (mmol/L)	0.70 [0.52, 1.00]	0.67 [0.49, 1.04]	0.72 [0.52, 0.96]	0.7
PTH (pmol/L)	9.29 (±7.51)	9.88 (±7.94)	8.65 (±7.05)	0.4
CTX (ng/L)	581 [400, 851]	581 [386, 884]	581 [414, 837]	1.0
P1NP (ug/L)	31.5 [22.0, 60.0]	30.5 [22.0, 46.0]	32.5 [22.5, 87.0]	0.2
Hospital interventions/outcomes				
Ventilation duration (hrs)	86.0 [47.4, 146.0]	80.4 [43.9, 118.0]	91.9 [52.3, 215.0]	0.1
Glucocorticoid	29 (31.5)	16 (33.3)	13 (29.5)	0.8
CRRT	15 (16.3)	6 (12.5)	9 (20.5)	0.3
ICU LOS (days)	6 [4, 9]	7 [4, 8]	6 [4, 11]	0.9
Hospital LOS (days)	16 [11, 30]	15 [11, 28]	17 [10, 32]	0.7
Baseline BMD (post-ICU discharge)				
ICU admit to BMD (days)	33 [13,58]	36 [14, 63]	33 [12,56]	
T-score femur	-0.8 (±1.5)	-1.0 (±1.4)	-0.7 (±1.5)	0.3
Absolute femur (g/cm^3)	0.956 (±0.197)	0.941 (±0.183)	0.974 (±0.212)	0.4
T-score AP spine	0.1 [-1.4, 1.0]	-0.1 [-1.6, 0.8]	0.1 [-1.3, 1.2]	0.4
Absolute AP spine (g/cm^3)	1.207 (±0.242)	1.200 (±0.228)	1.210 (±0.261)	0.7
Mortality				
1-year	8 (8.7)	0 (0)	8 (18.2)	
2-year	10 (10.9)	0 (0)	10 (22.7)	

Data are shown as mean (±standard deviation), median [interquartile range] or number (%). Complete BMD follow-up defined as all three post-ICU measurements performed during the 2-year period. Incomplete BMD follow-up defined as one or two post-ICU measurements performed. Reference ranges: vitamin D (<25 nmol/L = deficient, 25–50 nmol/L insufficient, >50 nmol/L sufficient), PTH (range 1.6–6.9 pmol)

Abbreviations: *BMD* bone mineral density, *BMI* body mass index, *APACHE* Acute Physiology and Chronic Health Evaluation, *ICU* intensive care unit, *PTH* parathyroid hormone, *CTX* collagen type 1 cross-linked c-telopeptide, *P1NP* type 1 N-terminal procollagen, *CRRT* continuous renal replacement therapy, *LOS* length of stay, *AP* anterioposterior

Table 2 Bone mineral density assessments performed and results for entire cohort by gender

	All ($n = 92$[a])			Women ($n = 44$[a])			Men ($n = 48$[a])		
Variable	Baseline	1 year	2 years	Baseline	1 year	2 years	Baseline	1 year	2 years
BMD studies performed	92	66	48	44	31	22	48	35	26
Anti-fracture therapy in prior year	-	4 (6.1)	10 (20.8)	-	3 (9.7)	6 (27.3)	-	1 (2.9)	4 (15.4)
Glucocorticoid in prior year	-	3 (4.5)	5 (10.4)	-	2 (6.5)	5 (22.7)	-	1 (2.9)	0 (0)
BMD measurement									
Femur T-score	- 0.8 (±1.5)	-1.0 (±1.4)	- 1.1 (±1.3)	- 1.2 (±1.4)	- 1.3 (±1.2)	- 1.4 (±0.9)	- 0.5 (±1.4)	- 0.7 (±1.5)	- 1.0 (±1.6)
Femur absolute (g/cm^3)	0.956 (±0.197)	0.940 (±0.193)	0.923 (±0.178)	0.876 (±0.176)	0.872 (±0.161)	0.871 (±0.126)	1.028 (±0.187)	0.999 (±0.202)	0.964 (±0.203)
AP spine T-score	- 0.2 (±1.9)	- 0.2 (±1.9)	- 0.1 (±1.9)	- 0.7 (±1.8)	- 0.6 (±1.6)	- 0.5 (±1.2)	0.3 (±1.8)	0.2 (±2.0)	0.3 (±2.2)
AP spine absolute (g/cm^3)	1.207 (±0.242)	1.205 (±0.241)	1.211 (±0.231)	1.135 (±0.250)	1.142 (±0.223)	1.151 (±0.173)	1.273 (±0.217)	1.260 (±0.246)	1.262 (±0.264)

Data are shown as mean (±standard deviation) or number (%)
Abbreviations: *BMD* bone mineral density, *AP* anterioposterior
[a]At baseline femur BMD not measured in three participants (one woman, two men). At 1 year femur BMD not measured in two participants (one woman, one man), at 2 years femur BMD not measured in one participant (one woman)

-0.3 ± 1.9% vs year 2: 0.6 ± 1.7%, $p = 0.6$) (Fig. 2a). The use of anti-fracture therapy associated with a significant difference in post-ICU annual change of BMD, with an increase in BMD in participants who received anti-fracture medication compared to a decrease in those that did not (femur 3.1 ± 2.4% vs -2.8 ± 1.7%, $p = 0.04$, spine 5.1 ± 2.5% vs -3.2 ± 1.8%, $p = 0.01$). In women use of glucocorticoids was not associated with a difference in annual change in BMD compared to no use (femur -0.2 ± 2.7% vs 0.5 ± 1.6%, $p = 0.8$, spine 0.5 ± 2.9% vs 1.4 ± 1.6%, $p = 0.8$).

In 48 men with 61 measurements of annual change in BMD over the 2-year period, a greater annual decrease in femur BMD was observed in post-ICU year 2 compared to year 1 (year 1: -0.9 ± 2.1% vs year 2: -2.5 ± 2.1%, $p = 0.03$), with no difference in annual change of spine BMD (year 1: 0.9 ± 4.0% vs year 2: 2.1 ± 4.0%, $p = 0.45$). In men no association between anti-fracture therapy use and annual change in BMD was observed (femur -0.4 ± 2.5% vs -3.0 ± 2.0%, $p = 0.1$, spine 2.4 ± 4.8% vs 0.7 ± 3.6%, $p = 0.6$) (Fig. 2b). As only one male participant received glucocorticoids, analysis was not performed.

The sensitivity analysis for the 48 participants who completed all three BMD assessments is presented in Additional files 3 and 4. For women the percentage of participants with osteoporosis or osteopenia was 59% at ICU discharge, 68% at year 1, and 59% at year 2. In men the proportion was 39% at ICU discharge, 50% at year 1, and 54% at year 2. The results of sensitivity analysis for this group are presented in Additional file 4, and are consistent with the primary analysis.

Annual change in BMD in participants not receiving glucocorticoids or anti-fracture therapy

The annual change in BMD in the first and second years after ICU discharge in the cohort of participants who did not receive either glucocorticoids or anti-fracture therapies are presented by sex in Fig. 3a and b. In women an annual decrease in femur and spine BMD was observed for both year 1 and 2, with no significant change over the 2-year period (femur -2.8 ± 1.3% vs -1.9 ± 0.7, $p = 0.6$, spine -4.8 ± 1.4% vs -1.3 ± 1.8%, $p = 0.08$). In men the annual decrease in femur BMD was significantly greater in year 2 than year 1 (femur -1.9 ± 0.7% vs -3.2 ± 0.7%, $p = 0.03$), with no difference in annual spine BMD change between year 1 and year 2 (spine 0.0 ± 1.2% vs 0.9 ± 1.5%, $p = 0.6$).

Discussion

Key findings

We studied the association between time, post-ICU administration of bone anti-fracture therapy and glucocorticoids, and annual change in BMD over a 2-year period after critical illness. In women a significantly greater loss of spine BMD was observed in the first year after ICU compared to the second. In women who did not receive anti-fracture therapy or glucocorticoids, a decrease in BMD was observed in both years after ICU discharge. However, post-ICU administration of anti-fracture therapy was associated with an increase in BMD, compared to a decrease in women who did not. In men, loss of femur BMD was significantly greater in the second year after ICU discharge. There was no association between use of anti-fracture therapy or glucocorticoids and change in BMD, although only a small number of men received post-ICU treatment.

Relationship to previous studies

Loss in BMD following critical illness has been reported in two previous studies. A significant decrease in calcaneal BMD was observed over 10 days in patients with

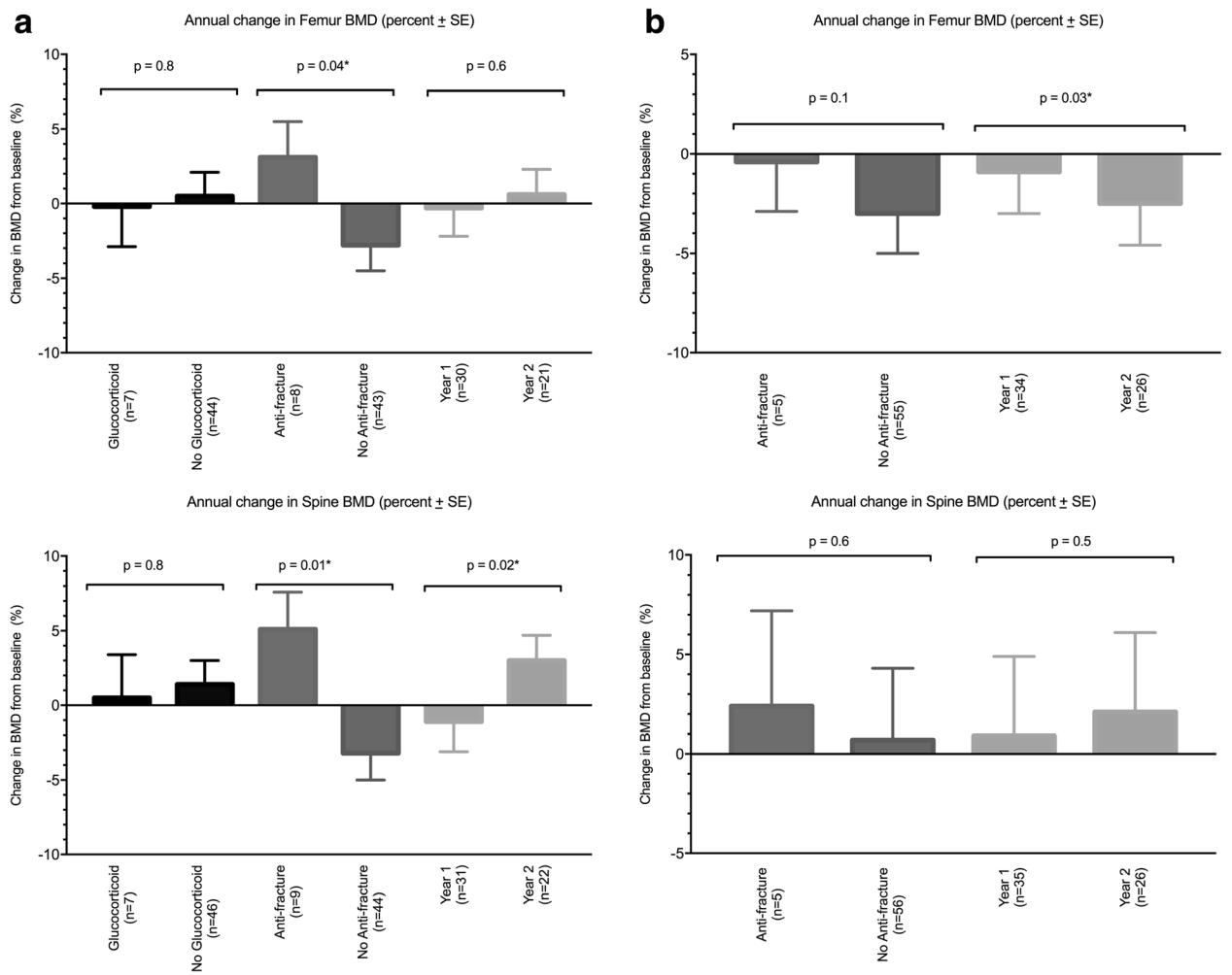

Fig. 2 a RMANOVA assessment of annual BMD change in women. *Abbreviations*: *BMD* bone mineral density, *RMANOVA* repeat measure analysis of variance, *SE* standard error. **b** *RMANOVA* assessment of annual BMD change in men. *Abbreviations*: *BMD* bone mineral density, *RMANOVA* repeat measure analysis of variance, *SE* standard error

acute respiratory distress syndrome [26], although this result is limited by precision error of portable BMD devices and short time frame [27]. We described a significant decrease in spine and femur BMD in the first year after ICU admission, greater than age- and gender-matched community controls, in the initial cohort from this study [19]. In addition a number of studies have described abnormal BTMs during and after critical illness, of a magnitude similar to that described in postmenopausal women's or metabolic bone disease [19, 28–34]. High bone turnover and bone loss, due to negative remodelling balance at the basic multicellular unit, has been described as an independent risk factor for fracture [29, 35]. An increased fracture risk in older women after intensive care compared to matched population controls has been described [20].

The extension of BMD assessment to 2 years after critical illness in this study adds important information about the time course and magnitude of changes in BMD following critical illness [19, 26]. In women we observed a loss in femur and spine BMD in the first 2 years after critical illness, with recovery of BMD observed in women receiving anti-fracture therapy. The reported change in BTMs after critical illness describes increased resorption markers during and after ICU [19, 28–34], followed by increased formation markers and normalisation of resorption markers by 1 year [19]. The magnitude of this decrease was greater than we have previously observed in community controls [19], supporting the hypothesis that factors associated with critical illness contribute to an increase in bone loss, and that administration of anti-fracture therapy is a major determinant of BMD recovery after critical illness. The different pattern of BMD loss in men compared to women following critical illness is also of interest. The observed decrease in femur BMD is consistent with our

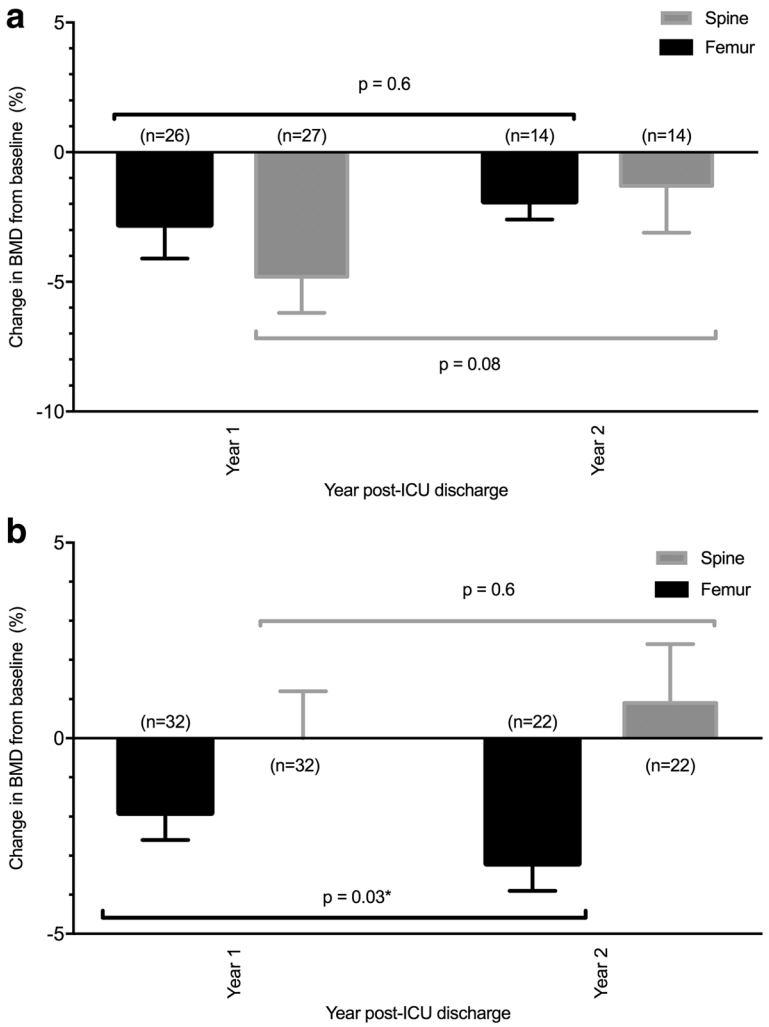

Fig. 3 **a** Annual change in BMD in women not receiving anti-fracture or corticosteroid medications. **b** Annual change in BMD in men not receiving anti-fracture or corticosteroid medications. *Abbreviations*: *BMD* bone mineral density, *ICU* intensive care unit, *SE* standard error

previous study of change in BMD after ICU compared to community controls [19]. The significantly greater loss in femur BMD in the second year after ICU discharge, the high proportion of men with osteoporosis and osteopenia at 2 years post-ICU discharge, and the low rate of post-ICU anti-fracture treatment, suggest further investigation of risk factors and consequences of bone loss in men is warranted.

The current literature regarding the relationship between anti-fracture therapy use and change in BMD following critical illness is limited. A small study reported a transient decrease in bone resorption markers after administration of intravenous ibandronate [36], and a retrospective propensity-matched cohort study described an association between pre-ICU bisphosphonate use and reduced mortality [37]. In addition, serial computed tomography (CT) assessment of vertebral BMD revealed bisphosphonate users had lower baseline bone density and an attenuated decrease in BMD during critical illness. This study is the first to prospectively describe an association between anti-fracture therapy use and change in BMD over a prolonged period following critical illness. The observed increased proportion of anti-fracture therapy use in women is expected, based on lower measured BMDs in the years after critical illness. The observed positive association between anti-fracture therapy use and BMD provides support for future interventional studies in this population.

The observation that use of glucocorticoid, a known risk factor for osteoporosis, was not associated with an increase in annual change in BMD was interesting, although limited by small sample size and the risk of type II error. More prospective data on the relationship between BMD changes following critical illness and the effect of known osteoporosis factors, including medications administered before and after critical illness, are required to further elucidate these relationships.

Study implications

This study implies that critical illness is associated with prolonged and sustained loss of BMD, with variable effects on femur and spine in women and men. Although recovery of BMD occurs overall in women, this may be associated with the use of anti-fracture therapy in the post-ICU period. This implies that anti-resorptive therapy may be an effective intervention to prevent bone loss in women with critical illness as has been shown in other at-risk patients.

Strengths and limitations

Our study has several strengths. It is the first study to collect prospective data on bone density using DXA, the gold standard for BMD assessment, over a 2-year period after critical illness. This is important because the previously described changes in bone mineral density that occur immediately after critical illness may be attenuated over time. Moreover, understanding of the natural history of these changes can be used to guide the need and design of interventional trials. In addition, the collection of post critical illness medication history allows assessment of factors that are known to modify bone turnover, over a time frame required to assess this effect.

There are limitations to this study. The loss of a large proportion of patients prior to the 2-year follow-up due to death or withdrawal introduces limitations due to small sample size, including ability to assess the impact of multiple risk factors on post critical illness change in BMD, perform subgroup analysis, and introduces the possibility of type II error. However, the ability to assess the effect of anti-fracture therapy and glucocorticoids, although limited by numbers, provides unique and valuable information about feasibility and design of an interventional study. Also, the assessment of glucocorticoid use following critical illness was defined as use for greater than 3 months in the previous year, and it is possible that shorter duration of glucocorticoids during critical illness or recovery were associated with a change in BMD that was not captured. However, glucocorticoids are a known risk factor for loss of BMD, and a much larger study would be required to assess the effect of glucocorticoids administered before, during, and after ICU. Also, data relating to a number of variables associated with BMD was not collected, including other medications that affect bone turnover, nutrition, falls, and fractures. However, given the small sample size, analysis of the relationship between these factors and BMD would not have been possible. Finally, anti-fracture medications were clinician-initiated rather than randomised, introducing selection bias into the results. However, anti-fracture therapies are initiated in the highest risk patients with the lowest BMD, with the effect observed in this study likely to underestimate that observed in a mixed population of critically ill patients.

Conclusions

We performed a prospective observational study of changes in BMD in critically ill, mechanically ventilated subjects, and observed a high prevalence of osteopenia and osteoporosis at 2 years post-ICU discharge. In women participants, a greater loss of spine BMD was observed in the first year after critical illness, with anti-fracture therapy use associated with an increase in BMD compared to a decrease in BMD in those that did not receive such therapy. In men BMD loss increased in the second year after critical illness, and there was no association between use of anti-fracture therapy or glucocorticoids and change in BMD, although only a small proportion of men received post-ICU bone-related medications. These findings suggest anti-fracture therapy may be an effective intervention to prevent bone loss in women with critical illness, and prospective trials investigating this effect are warranted.

Additional files

Additional file 1: Measurement of bone turnover markers. Details of BTMs measurement. (DOC 22 kb)

Additional file 2: Study operating procedures. Details of study procedure and data collection time points from enrolment to completion. (DOCX 12 kb)

Additional file 3: Bone mineral density and T-score for the 2 years after critical illness in participants that completed all bone mineral density assessments. Bone mineral density and T-score at enrolment, 1 year, and 2 years after critical illness, presented overall and stratified by gender, for the 47 participants who completed all assessments. (DOCX 13 kb)

Additional file 4: Sensitivity analysis of annual BMD change in women and men. The sensitivity analysis of annual change in BMD compared to baseline for women and men who completed all three BMD assessments, with repeat measure analysis of variance to explore the relationship between anti-fracture use, glucocorticoid use, and time after ICU discharge. (DOCX 666 kb)

Abbreviations

AP: Anterioposterior; APACHE: Acute Physiology and Chronic Health Evaluation; BMD: Bone mineral density; BMI: Body mass index; BTM: Bone turnover marker; CRRT: Continuous renal replacement therapy; CT: Computed tomography; CTX: Collagen type 1 cross-linked c-telopeptide; DXA: Dual-energy X-ray absorptiometry; ICU: Intensive care unit; LOS: Length of stay; P1NP: Type 1 N-terminal procollagen; PTH: Parathyroid hormone

Acknowledgement

Funding

This study was supported by an unrestricted grant from the Intensive Care Foundation. SBO is supported by a National Health and Medical Research Council (NHMRC) of Australia Career Development Fellowship (1107510).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NO, RB, DC, MB, JP and MK made substantial contributions to the conception or design of the work; the acquisition, analysis, or interpretation of data for the

work; drafting the work or revising it critically for important intellectual content; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. CC, TE and SB-O made substantial contributions to the acquisition, analysis, or interpretation of data for the work; drafting the work or revising it critically for important intellectual content; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Ethics approval was obtained from the Barwon Health Research Ethics Committee prior to commencement of the study. Individual consent was obtained from all participants.

Declarations
The authors have nothing to disclose.

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Received: 5 December 2016 Accepted: 28 February 2017
Published online: 21 March 2017

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critical illness evokes superior endocrine and metabolic effects compared to treatment with GHRP-2 alone. Clin Endocrinol (Oxf). 2002;56(5):655–69.
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Additional Files

Additional File 1: Measurement of Bone Turnover Markers

The serum bone turnover markers CTX and P1NP were collected the morning after enrolment with routine early morning blood tests, and measured using the automated Roche Modular Analytics E170 analyser. Serum CTX limit of detection was 10 ng/L with inter-assay coefficient of variations (CVs) of 6.5% at 361 ng/L, 3.8% at 816 ng/L and 3.4% at 3304 ng/L (n = 10). Serum P1NP inter-assay CVs were 4.9% at 73 µg/L, 2.6% at 392 µg/L, and 2.1% at 768 µg/L (n = 10) with a limit of detection of 5µg/L.

Additional Figure 1: Study Operating Procedures

Softer Study Procedures	
>24 hrs to <168 hrs duration of mechanical ventilation	
Enrolment	Inclusion criteria met, consent obtained
Study procedures	Baseline and demographic data
	Biochemistry and BTM (serum PINP, CTx, Vit D, PTH, albumin, calcium, phosphate, creatinine)
ICU discharge (ICU discharge to 1-month)	
Study procedure	BMD #1
1 year follow-up (1 year post-ICU discharge)	
Study procedure	Contact participant
	BMD #2
	Biochemistry and BTMs (serum PINP, CTx, vitamin D, PTH, albumin, calcium, phosphate, creatinine)
	Medication history
2 year follow-up (2 year post-ICU discharge)	
Study procedure	Contact participant
	BMD #3
	Medication history
Vitamin D / calcium / anti-resorptive therapy will be offered to participants in accordance with current guidelines and review of results and risk factors by an endocrinologist	

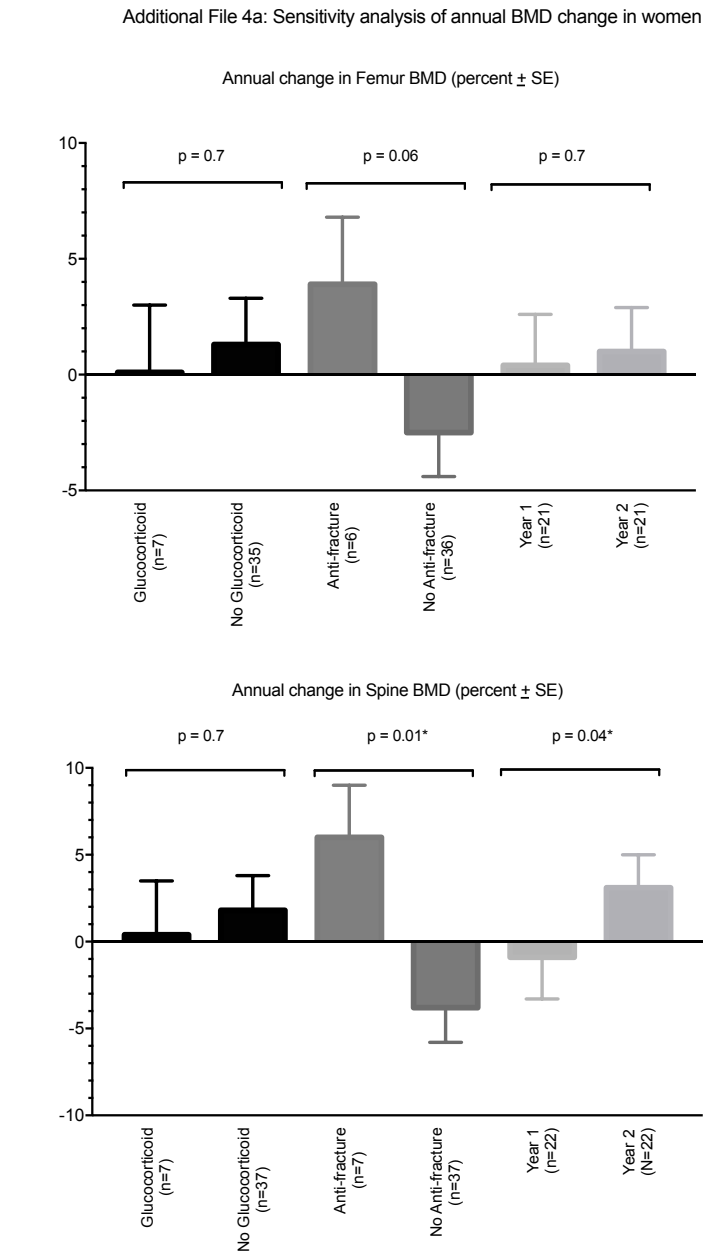
1. Abbreviations: BMD (bone mineral density), AP (anteroposterior)

Additional Table 1: Bone mineral density and T-score for the 2-years after critical illness in participants that completed all Bone Mineral Density Assessments.

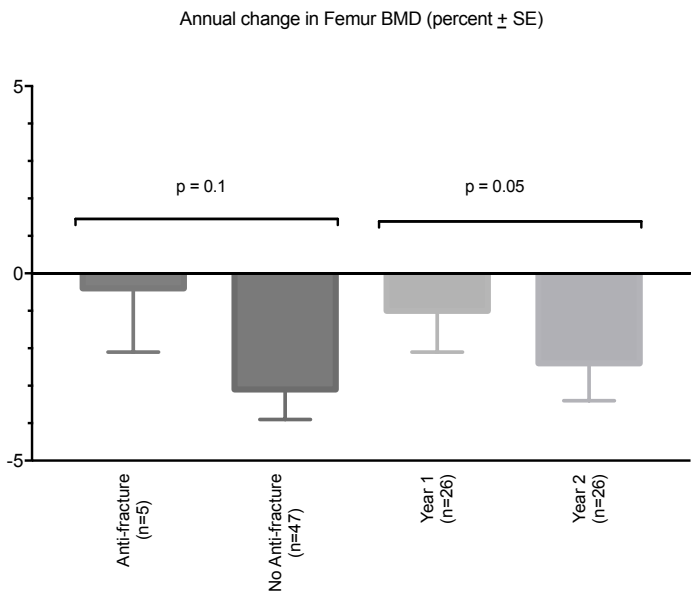
Variable	Baseline	1-year	2-year	P-value
All (n=48)				
BMD (g/cm2)				
Dual Femur *	0.941 (±0.183)	0.922 (±0.181)	0.923 (±0.178)	0.006
AP Spine	1.200 (±0.228)	1.182 (±0.242)	1.211 (±0.231)	0.04
T score				
Osteoporosis / osteopenia	23 (47.9)	28 (53.1)	27 (56.3)	
Normal	25 (52.1)	20 (41.7)	21 (43.8)	
Women (n=22)*				
BMD (g/cm2)				
Dual Femur*	0.880 (±0.143)	0.862 (±0.135)	0.871 (±0.126)	0.4
AP Spine	1.146 (±0.193)	1.100 (±0.190)	1.151 (±0.173)	0.006
T score				
Osteoporosis / osteopenia	13 (59.1)	15 (68.2)	13 (59.1)	0.77
Normal	9 (40.9)	7 (31.8)	9 (40.9)	
Men (n=26)				
BMD (g/cm2)				
Dual Femur	0.990 (±0.198)	0.971 (±0.201)	0.964 (±0.203)	0.0002
AP Spine	1.247 (±0.248)	1.251 (±0.263)	1.262 (±0.264)	0.6
T score femur				
Osteoporosis / osteopenia	10 (38.5)	13 (50.0)	14 (53.8)	0.5
Normal	16 (61.5)	13 (50.0)	12 (46.2)	

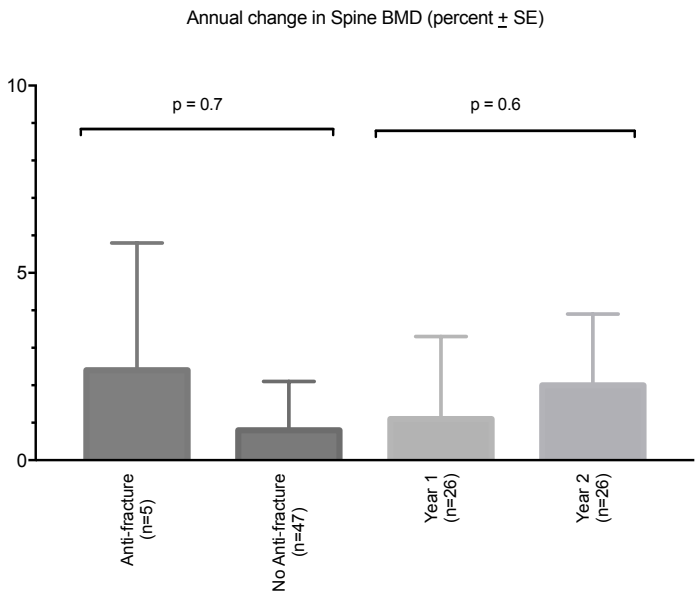
Data are shown as mean (±standard deviation) or number (%)
Abbreviations: BMD (bone mineral density), AP (anteroposterior)
* At baseline femur BMD not measured in 1 woman, at 1-year femur BMD not measured in 1 woman, at 2-year femur BMD not measured in 1 woman.

Additional Figure 2: Sensitivity analysis of annual BMD in women and men.



Additional File 4b: Sensitivity analysis of annual BMD change in men





Abbreviations: BMD (bone mineral density); RMANOVA (repeat measure analysis of variance); SE (standard error)

Chapter 6: Changes in Bone Mineral Density in Women before Critical Illness compared to Population Matched Controls – A Nested Cohort Study

The contribution of pre-critical illness factors to the increased bone loss observed after critical illness is not clear. This chapter aimed to compare the trajectory of BMD changes in women prior to critical illness with the trajectory of age and medication matched women who did not become critically ill, by performing a nested cohort study through linkage of the Geelong Osteoporosis Study and University Hospital Geelong ICU databases. We hypothesised the long-term trajectory of bone density in the years prior to critical illness in women would be consistent with a pattern of lower bone mass and increased rate of bone loss, with further accelerated bone loss in the years immediately prior to critical illness, compared to women who did not become critically ill. Analysis of BMDs performed over a 15-year period, found no difference overall in absolute bone density, and a greater rate of decline in AP spine bone density, in critically ill women compared to community-based controls. Surprisingly, we found femoral neck bone mass increased, and no difference in AP spine bone mass, in the two-years prior to critical illness compared to age and medication matched controls.

The Journal of Clinical Endocrinology & Metabolism

Changes in Bone Mineral Density in Women before Critical Illness: A Matched Controls Nested Cohort Study

--Manuscript Draft--

Manuscript Number:	
Article Type:	Clinical Research Article
Full Title:	Changes in Bone Mineral Density in Women before Critical Illness: A Matched Controls Nested Cohort Study
Short Title:	Changes in Bone Density before Critical Illness
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Section/Category:	Parathyroid, Bone, and Mineral Metabolism
Manuscript Classifications:	Bone / Mineral Metabolism; Metabolic bone disease; Osteoporosis; Surgery
Keywords:	Critical illness; long-term outcomes; osteoporosis; bone loss; bone mineral density
Abstract:	Context: The relative importance of pre-critical illness related factors, to bone loss occurring after critical illness is unknown. Objective: To compare trajectory of bone mineral density (BMD) in women before critical illness, to women who did not become critically ill. Design: Prospective, nested, age and medication matched, case-control study. Setting: Tertiary adult Intensive Care Unit (ICU) in Australia and the Geelong Osteoporosis Study (GOS). Patients: Women recruited into GOS and aged over 40 years, requiring admission to ICU between June 1998 and March 2016, were compared to GOS women not admitted to ICU. Interventions: None Main Outcome Measure: Age and medication use adjusted change in BMD. Results: A total of 52 GOS women were admitted to ICU during the study period. A greater age-adjusted mean annual rate of decline was observed for pre-ICU compared to no-ICU at AP spine BMD (-0.010 + 0.002 g/cm2 vs -0.005 +¬ 0.002 g/cm2, p=0.01) for the entire 15-year study period. In a cohort of 15 participants with multiple BMDs within two years of critical illness, there was a significantly greater increase in femoral neck BMD compared to matched controls (difference in BMD, ICU vs no-ICU = 0.037 + 0.013 g/cm2, p=0.006). Conclusion: Despite greater overall loss of bone mass at AP spine than femoral neck in the 15-years prior to critical illness, bone health prior to critical illness was comparable to controls, with a relative increase in femoral neck bone mass in a matched sub-group in the two-years prior to critical illness.
Funding Information:	
Author Comments:	NO, MB, RB, JP, DJC, MK have nothing to declare.
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Opposed Reviewers:	
Additional Information:	
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PRECIS: The precis is a brief description of your paper that will appear on the Table of Contents underneath your article title, should your paper be accepted (see the	This study compared pre-critical illness bone density in women to non critically ill women. Overall bone density was similar, although femoral neck bone mass increased just prior to critical illness.

current issue of JCEM for examples). The description should be **no longer than 200 characters, including spaces,** and should briefly explain what was done in your study and what was concluded. Please ensure that the precis does not simply repeat the article title.	
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SPECIAL REQUESTS: Enter specific comments or requests to the editors here.	

TITLE PAGE

1. Title: Changes in Bone Mineral Density in Women before Critical Illness: A Matched Controls Nested Cohort Study

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1. Substantial contributions to the conception (NO, RB, DC, MB, JP, MK) or design (NO, MB, JP, RB, DC, MK) of the work; or the acquisition, analysis, or interpretation of data (NO, MB, JP, RB, DC, MK) for the work
2. Drafting the work or revising it critically for important intellectual content (NO, MB, JP, RB, DC, MK)
3. Final approval of the version to be published (NO, MB, JP, RB, DC, MK)
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. (NO, MB, JP, RB, DC, MK)

6. Key words: Critical illness, long-term outcomes, osteoporosis, fracture, bone loss, bone mineral density

7. Disclosure summary: NO, MB, RB, JP, DJC, MAK have nothing to declare.

8. Word count: 2941

ABSTRACT

Context: The relative importance of pre-critical illness related factors, to bone loss occurring after critical illness is unknown.

Objective: To compare trajectory of bone mineral density (BMD) in women before critical illness, to women who did not become critically ill.

Design: Prospective, nested, age and medication matched, case-control study.

Setting: Tertiary adult Intensive Care Unit (ICU) in Australia and the Geelong Osteoporosis Study (GOS).

Patients: Women recruited into GOS and aged over 40 years, requiring admission to ICU between June 1998 and March 2016, were compared to GOS women not admitted to ICU.

Interventions: None

Main Outcome Measure: Age and medication use adjusted change in BMD.

Results: A total of 52 GOS women were admitted to ICU during the study period. A greater age-adjusted mean annual rate of decline was observed for pre-ICU compared to no-ICU at AP spine BMD (-0.010 $\pm$ 0.002 g/cm^2 vs -0.005 $\pm$ 0.002 g/cm^2, p=0.01) for the entire 15-year study period. In a cohort of 15 participants with multiple BMDs within two years of critical illness, there was a significantly greater increase in femoral neck BMD compared to matched controls (difference in BMD, ICU vs no-ICU = 0.037 $\pm$ 0.013 g/cm^2, p=0.006).

Conclusion: Despite greater overall loss of bone mass at AP spine than femoral neck in the 15-years prior to critical illness, bone health prior to critical illness was comparable to controls, with a relative increase in femoral neck bone mass in a matched sub-group in the two-years prior to critical illness.

PRECIS

This study compared bone density in women before critical illness to matched women who did not become critically ill. Overall bone density was similar, although femoral neck bone mass increased in the two years before critical illness.

INTRODUCTION

In recent years, an association between critical illness and accelerated bone turnover has been described, including an increase in bone turnover markers (BTM) during critical illness[1]; accelerated bone loss in the year following critical illness[2]; an increase in bone mineral density (BMD) after critical illness in women receiving anti-fracture therapy compared to a decrease in those that did not[3], and increased fragility fractures in older women after critical illness[4]. However, whether this increase in bone loss is the result of critical illness related factors, or represents the continuation and acceleration of disease and morbidity that precede critical illness, is yet to been defined.

The uncertainty regarding the relative importance of pre-critical illness disease and critical illness-related factors, to post-critical illness health trajectory is a common limitation of long-term intensive care outcome research. However, in recent years prospective population based studies have described the characteristics and outcomes of nested cohorts of participants requiring intensive care unit (ICU) admission after study enrolment, making it possible to obtain valuable information about the relative impact of the pre-ICU clinical and functional trajectory on outcomes after critical illness[5]. One such population-based study is the Geelong Osteoporosis Study (GOS)[6], combined with the University Hospital Geelong (UHG) ICU database.

The aim of this study was to use the above databases to compare the trajectory of BMD changes in women prior to critical illness with the trajectory of age and medication matched women who did not become critically ill. We hypothesised the long-term trajectory of bone density in the years prior to critical illness in women would be consistent with a pattern of lower bone mass and increased rate of bone loss, with further accelerated bone loss in the years immediately prior to critical illness, compared to women who did not become critically ill.

MATERIAL AND METHODS

Study Design: We performed an observational, nested case-control study in women aged 40 years or older participating in GOS. We compared GOS participants admitted to ICU (ICU cohort) to a control population of GOS participants not admitted to ICU (no-ICU cohort). Ethics approval was obtained from the Barwon Health Human Research Ethics Committee.

Controls: Beginning in 1993, the GOS recruited a random population-based sample of 1725 women (ages 20-94 years) from the Commonwealth Electoral Rolls for an area surrounding Geelong in south-eastern Australia called the Barwon Statistical Division[6]. As voting is compulsory in Australia the electoral roll provides a comprehensive listing of all adults (age ≥ 18 years). The sample was age-stratified, with a minimum of 100 patients in each 5-year age stratum between ages 20 and 69 years, and a minimum of 200 in the age 70-79 year group, and the over 80 year group. As part of the study, BMD measurements were performed two to five yearly. In this study the cohort was restricted to women 40 years of age or older, to remove the effect of increasing BMD prior to the third decade of life[7-9], and to assess a control population of an age group relevant to that of the ICU population (median age greater than 65 years)[2].

Cases: Participants from GOS admitted to UHG ICU between 1st June 1998 and 30th March 2016, were identified by data linkage of the GOS and ICU electronic databases. The UHG ICU is a level III adult and paediatric ICU, and the only tertiary ICU for the region of South Western Victoria. Where multiple ICU admissions occurred, the first ICU admission in the study period was used.

Study-period: Enrolment in GOS commenced in 1993, while the ICU electronic database commenced in 1998. To reduce the potential confounding from temporal changes in BMD measurement, participants with all BMD assessments completed before 1998 were excluded.

Data Collection: Baseline information collected from the ICU electronic database included age, sex, Acute Physiology and Chronic Health Evaluation II score, admission diagnosis, ICU length of stay, duration of ventilation, hospital length of stay, and hospital outcome. Information collected from the GOS database included date and results of scheduled GOS BMD appointments from baseline to 15-

year follow-up, and use of anti-fracture medications (bisphosphonate, strontium ranelate, teriparatide, denosumab, or raloxifene), gonadal hormones, or glucocorticoids, in the 2-years prior to each BMD study visit. BMD was recorded as the absolute value (g/cm^2), and annual change in BMD (g/cm^2/year). Details of BMD measurement are provided in Additional File 1.

Outcomes: The outcomes were absolute BMD and annual change in BMD (anterior-posterior lumbar spine and femoral neck) for measurements performed in the pre-ICU period, compared to population-based controls. Outcomes were reported for two time periods. The first was for BMD measurements performed for the entire 15-year study period. The second was restricted to participants with multiple BMD measurements performed in the two-years prior to critical illness, to assess if accelerated bone loss occurred in the immediate pre-ICU period.

Statistical Analysis: A preliminary analysis of the relationship between age and change in BMD for the entire GOS cohort of women was performed using locally weighted polynomial regression (LOWESS), to determine whether a linear relationship between age and BMD could be assumed for women over forty years of age.

Univariate and multivariate analyses were performed to describe the relationship between BMD measurements at femoral neck and AP spine, with age as a continuous variable, anti-fracture therapy, glucocorticoids, and hormone therapy as discrete variables (never, ever, current), and intensive care as discrete variables (ICU or no-ICU). The ICU variable included all GOS scheduled BMD assessments performed in women in the years prior to ICU admission. The no-ICU variable included all GOS scheduled BMD assessments performed in women not admitted to ICU. The relationship between BMD and patient age was determined by using longitudinal mixed linear modelling with each patient treated as a random effect. To determine if the nature of the relationship between BMD and age differed according to the previously described covariates, interaction terms with patient age were fitted to the model. The analysis of rate of change of BMD in the period immediately preceding critical illness was performed by identifying patients from the ICU cohort with multiple BMD measurements in the 2-year period prior to ICU admission, matching these on age, anti-fracture medications,

glucocorticoids, and hormone therapy to no-ICU controls at a ratio of 9:1, and comparing total change in BMD.

All data were initially assessed for normality. Group comparisons were performed using chi-square tests for equal proportion, student t-tests for normally distributed data and Wilcoxon rank sum tests otherwise, with results reported as percentage (%), mean (standard deviation) or median (interquartile range) respectively. All modelling results are reported as least square means $\pm$ standard errors and a two-sided p-value of 0.05 was used to indicate statistical significance. Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), with figures produced using Graphpad Prism 7.0 ©.

RESULTS

Of the 1725 women participating in GOS, a total of 968 were 40 years of age or over and had at least one BMD assessment after 1998. Of this cohort, 52 (4.8%) were admitted to ICU during the study period and were included in the primary analysis (Figure 1). Analysis of the relationship between age and change in BMD for the entire GOS cohort of women confirmed a linear relationship between age and decrease in BMD for women over forty years of age (Additional File 2).

The overall GOS characteristics of the ICU and no-ICU cohorts, and ICU characteristics of the ICU cohort are presented in Table 1. The ICU cohort were older at entry into GOS (ICU 69 [IQR 63,73] vs no-ICU 60 [48,72], $p<0.001$), but the proportions of participants receiving glucocorticoids, anti-fracture therapy, and hormone therapy were similar. Completion rates of scheduled GOS BMD measurements were similar for years 0 to 10 of the GOS study, with a significant decrease in completion of the 15-year BMD measurement in the ICU cohort (25% vs 47%, $p=0.002$). With regards to the critical care characteristics of the ICU cohort, the median age at ICU admission was 78 years, with predominantly surgical admissions. The major comorbidities were cardiovascular, respiratory, cancer, and frailty or dementia. Almost half required mechanical ventilation, median ICU length of stay was 2 days, and hospital length of stay 11 days. Intensive care and hospital mortality were 9.6% and 17% respectively.

The mean AP spine and femoral neck BMD values for the ICU and no-ICU cohorts at each GOS scheduled measurement time are presented in Figure 2. Compared to the no-ICU cohort, the ICU cohort had lower AP spine BMD values at years 6 and 10, and lower femoral neck BMD values at years 6,8,10, and 15`. The proportion of participants in each cohort receiving anti-fracture therapy, glucocorticoids, and hormone therapy, at each measurement time are presented in Additional File 3.

After adjustment for age and medication use, multivariate analysis found no difference in mean AP spine or femoral neck BMD values when comparing the ICU cohort to the no-ICU cohort for the entire 15-year period(Table 2). However, anti-fracture medication was associated with significantly higher BMD values at both AP spine and femoral neck, glucocorticoid use was associated with significantly lower femoral neck BMD, and hormone therapy was associated with significantly higher femoral neck BMD.

Mixed linear modelling of the age adjusted interaction of annual change in BMD with ICU admission and medication use, for the 15-year GOS study period, are presented in Table 3. A significantly greater annual rate of decline was observed for the ICU compared to no-ICU cohort at AP spine BMD, but not femoral neck BMD. The use of anti-fracture medication was associated with a significant reduction in rate of decline of BMD at AP spine, but not femoral neck, and glucocorticoid use was associated with significantly greater decline in femoral neck BMD.

The analysis of BMD in the 2-year period immediately preceding critical illness is presented in Figure 3. A subset of 15 participants had two BMD measurements performed in the 2-years prior to ICU admission, and were matched 1 to 9 by age, anti-fracture medication, glucocorticoid use, and hormone therapy use, to no-ICU controls (Additional File 4). The mean BMD of the ICU cohort and the matched no-ICU cohort were similar at femoral neck and AP spine for both measurement time-points. However, a difference in change in BMD was observed for femoral neck BMD, with a significantly greater increase in BMD in the 2-years prior to ICU admission for the ICU cohort compared to matched no-ICU controls (difference in BMD, ICU vs no-ICU = 0.037 $\pm$ 0.013 g/cm^2, p=0.006). There was no difference in change in BMD at AP spine.

DISCUSSION

Key findings

We analysed bone density measurements performed over a 15-year period as part of a large population based osteoporosis study, and compared the trajectory of bone density in women prior to critical illness to non-critically ill controls. Contrary to our hypothesis, although we found no difference overall in absolute bone density, and a greater rate of decline in AP spine bone density, in critically ill women compared to community-based controls in the 15 years prior to ICU admissions, we also found that femoral neck bone mass increased, and that there was no difference in AP spine bone mass, in the two-years prior to critical illness compared to age and medication matched controls.

Relationship to Previous Studies

Previous studies have reported increased bone turnover markers during critical illness[2,10-22], accelerated bone loss, and increase in fragility fractures following critical illness[2-4,23,24]. Although these studies consistently demonstrate an increase in bone loss associated with critical illness, the relative contribution and timing of pre-critical illness related factors remains unknown. Our comparison of bone density trajectory over a 15-year period, between women who became critically ill and those who did not, provides new insights into bone health prior to critical illness.

We hypothesised the long-term trajectory of bone density in the years prior to critical illness in women would be consistent with a pattern of lower bone mass and increased rate of bone loss, particularly in the years immediately prior to critical illness, compared to women who did not become critically ill. Our findings were contrary to this hypothesis, with no difference in absolute bone mass between critically ill women and controls for the entire 15-year period, and a relative increase in femoral neck bone mass in the two-years before critical illness compared to matched controls. Moreover, although we observed a greater rate of decline in AP spine bone mass in critically ill women compared to controls for the entire 15-year period, there was no difference was observed in the two years prior to critical illness.

The observation of no difference in absolute bone mass, and a relative increase in femoral neck bone mass in the two-years prior to critical illness compared to matched controls is surprising, as we expected bone mass to reflect the high prevalence of comorbid disease, frailty, and functional decline in critically ill patients [5,25]. Instead, these findings imply that critically ill women may have bone health that is comparable to non-critically ill women, that their bone loss does not accelerate in the years immediately before critical illness, and that pre-critical illness factors may not be the major determinant of increased bone loss observed after critical illness [1,2,21,23,26].

A relatively greater loss of bone mass at AP spine was observed for the entire study period, compared to femoral neck bone mass. This is consistent with the trajectory of post critical illness bone loss, with women losing an average of 2.7% more bone mass at AP spine than controls, compared to 1.3% more bone mass at femoral neck than controls, in the year after critical illness[2]. This may lead to an increased risk of vertebral fracture, a pattern observed after critical illness in women, with vertebral fractures accounting for 42% of fragility fractures, compared to 17% in community-based controls [4].

Although not specific to critical illness, it is important to note the expected relationship between glucocorticoid use, anti-fracture medication use, and absolute BMD was observed. Anti-fracture medications are prescribed for low BMD and fragility fracture, and a significantly lower BMD was observed in participants who received anti-fracture medications. In addition, glucocorticoid use, a known risk factor for bone loss, was associated with lower BMD.

Study implications

These findings suggest bone health prior to critical illness is comparable to the underlying population, and is not responsible for the accelerated bone loss observed after critical illness. In addition, the relatively greater loss of bone mass at AP spine in the years prior to critical illness is consistent with the pattern of post-ICU bone loss, and may contribute to an increased risk of vertebral fracture.

Establishing the relative contribution of premorbid and critical illness related factors to subsequent bone loss is important, as suppression of bone turnover during critical illness could result in improved

skeletal outcomes, as well as non-skeletal outcomes. There is evidence of increased risk of cardiovascular disease, immune dysfunction, endocrine dysfunction, and death related to increased bone turnover [27-31], an association between anti-fracture therapies and reduced mortality in clinical trials [32], and an association between prior bisphosphonate use and reduced mortality in critical illness[24]. This study provides a rationale to further investigate bone turnover, bone loss and fragility fracture after critical illness, and to test the value of anti-fracture interventions in critically ill populations.

Strengths and Limitations

Our study has several strengths. It is the first study to describe the trajectory of BMD prior to critical illness. The linkage of two prospective population based databases in a single geographical region allowed detailed description of the trajectory of both AP spine and femoral neck BMD in a critically ill population compared to a community based control population matched for age and the confounding effect of medications, over a 15-year period.

There are limitations to this study. The relative increase in femoral neck BMD in the two-years prior to critical illness, and the overall similarity in trajectory of bone health when comparing critically ill to non-critically ill women, may reflect a study cohort that is community based, with an ICU population not sufficiently representative of critically ill women. However, the demographics of the population identified in this study are similar to those we described in a recent prospective study of BMD changes in a cohort of critically patients ventilated for greater than 24 hours[2]. Also, the sample size of patients in the two-years prior to critical illness was small, however the p-value for change in femoral neck BMD was low, making a type I error unlikely and making accelerated bone loss in the two years prior to ICU admission even more unlikely. The limited number of participants admitted to ICU, and the lack of information about comorbid disease and functional trajectory, reduced the ability to identify specific risk factors for bone loss prior to critical illness. However, a much larger cohort is required to identify such factors, and studies such as ours provide preliminary data to support and inform their design. Finally, the lack of post-ICU BMD assessments in GOS participants precluded analysis of the

relationship between pre-ICU and post-ICU BMD. However, we have previously reported changes in BMD in the 2-years after critical illness in a prospective cohort of patients [2,3].

Conclusions

This study describes the trajectory of bone density in women who became critically ill compared to those who did not, through use of a large population based osteoporosis study. Overall the findings suggest bone health prior to critical illness was comparalble to the underlying population, with a relative increase in femoral neck bone mass in the two-years prior to critical illness compared to matched controls. In addition, we observed a greater overall loss of bone mass at AP spine than femoral neck prior to critical illness.

ACKOWLDEGEMENT

- Not applicable

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LEGENDS FOR FIGURES AND TABLES

Figure 1
Title: Flow diagram of patient identification for women in GOS and ICU
Content: Description of flow of participants through the study.
Legend: BMD (Bone Mineral Density), ICU (Intensive Care Unit), GOS (Geelong Osteoporosis Study)

Figure 2a
Title: AP Spine BMD over time (mean ± SD)
Content: Average BMD measurements at scheduled GOS assessments, comparing ICU to no-ICU women
Legend: BMD (Bone Mineral Density), GOS (Geelong Osteoporosis Study), ICU (Intensive Care Unit), SD (standard deviation)

Figure 2b
Title: Femoral Neck BMD over time (mean ± SD)
Content: Average BMD measurements at scheduled GOS assessments, comparing ICU to no-ICU women
Legend: BMD (Bone Mineral Density), GOS (Geelong Osteoporosis Study), ICU (Intensive Care Unit), SD (standard deviation)

Figure 3a
Title: Change in Femoral Neck BMD relative to ICU admission
Content: Rate of change in BMD in the two-years prior to ICU admission compared to matched no-ICU controls
Legend: AP (anterior-posterior), BMD (Bone Mineral Density), ICU (Intensive Care Unit), Δ (Change)

Figure 3b
Title: Change in AP Spine BMD relative to ICU admission
Content: Rate of change in BMD in the two-years prior to ICU admission compared to matched no-ICU controls
Legend: AP (anterior-posterior), BMD (Bone Mineral Density), ICU (Intensive Care Unit), Δ (Change)

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Title: Critical care characteristics, interventions, and outcomes of the total GOS ICU cohort
Content: Characteristics of the ICU and non-ICU cohorts compared in the study, including bone assessment and critical care characteristics.
Legend: BMD= bone mineral density; GOS =Geelong Osteoporosis Study; ICU = intensive care unit; GOS = Geelong Osteoporosis Study; MV=mechanical ventilation; RRT = renal replacement therapy.

Table 2
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Content: Age-adjusted multivariate analysis of BMD measurements performed for the entire 15-year study period, categorised by ICU admission, and medication use.
Legend: BMD = bone mineral density; ICU = intensive care unit.

Table 3
Title: Age adjusted interaction of ICU status and medication use with annual change in Spine and Femur BMD
Content: Age-adjusted multivariate analysis of annual rate of change of BMD for the entire 15-year study period, categorised by ICU admission, and medication use.
Legend: BMD = bone mineral density; ICU = intensive care unit.

TABLES

Table 1: Critical care characteristics, interventions, and outcomes of the total GOS ICU cohort

GOS characteristics	ICU cohort (n=52)	No-ICU cohort (n=916)	P-value
Age at GOS enrolment	69.0 [62.5, 73.0]	59.5 [47.7,72.2]	<0.0001
Any risk factor exposure			
Glucocorticoids	4 (7.7)	56 (6.1)	0.65
Anti-fracture therapy	6 (11.5)	73 (8.0)	0.36
Hormone therapy	8 (15.4)	218 (23.8)	0.16
BMD assessments by study year			
Year 0	52 (100)	915 (100)	1.0
Year 2	49 (94.2)	849 (92.8)	1.0
Year 4	38 (73.1)	688 (75.2)	0.74
Year 6	40 (76.9)	754 (82.4)	0.35
Year 8	11 (21.2)	266 (29.1)	0.27
Year 10	29 (55.8)	595 (65.0)	0.18
Year 15	13 (25.0)	430 (46.9)	0.002
Critical Care characteristics			
Age at ICU admission	77.6 (±8.8)	-	-
APACHE III	64.1 (±23.3)	-	-
Admission category			
Medical	13 (25.0)	-	-
Surgical	39 (75.0)	-	-
Comorbidity			
Renal	1 (1.9)	-	-
Cardiovascular	37 (71.2)	-	-
Respiratory	12 (23.1)	-	-
Neurological	4 (7.7)	-	-
Gastrointestinal	3 (5.8)	-	-
Cancer	11 (21.2)	-	-
Frailty/dementia	9 (17.3)	-	-
ICU interventions			
MV Number	24 (46.2)	-	-
MV Duration (hrs)	12 [8,43]	-	-
RRT	2 (3.8)	-	-
ICU length of stay	1.7 [0.9, 2.8]	-	-
Hospital length of stay	11.2 [7.0,19.1]	-	-
ICU mortality	5 (9.6%)	-	-
Hospital mortality	9 (17.3%)	-	-

1. Abbreviations: BMD= bone mineral density; GOS =Geelong Osteoporosis Study; ICU = intensive care unit; GOS = Geelong Osteoporosis Study; MV=mechanical ventilation; RRT = renal replacement therapy.
2. Data are shown as mean (± standard deviation), number (percentage), or median [interquartile range)

Table 2: Multivariate analysis of all scheduled GOS BMD measurements categorised by study variables

Variable	AP Spine BMD		Femoral Neck BMD	
	BMD (g/cm^2)	P-value	BMD (g/cm^2)	P-value
ICU				
No-ICU	1.085 (0.019)	0.36	0.840 (0.008)	0.93
ICU	1.069 (0.019)		0.837 (0.014)	
Glucocorticoid				
Current	1.076 (0.024)	0.38	0.813 (0.017)	0.02
Ever	1.108 (0.020)		0.861 (0.015)	
Never	1.090 (0.015)		0.838 (0.011)	
Anti-fracture				
Current	1.050 (0.022)	<0.001	0.805 (0.016)	<0.001
Ever	1.044 (0.021)		0.811 (0.015)	
Never	1.181 (0.015)		0.895 (0.011)	
Hormone				
Current	1.104 (0.018)	0.09	0.849 (0.013)	0.03
Ever	1.078 (0.017)		0.828 (0.013)	
Never	1.093 (0.016)		0.834 (0.012)	

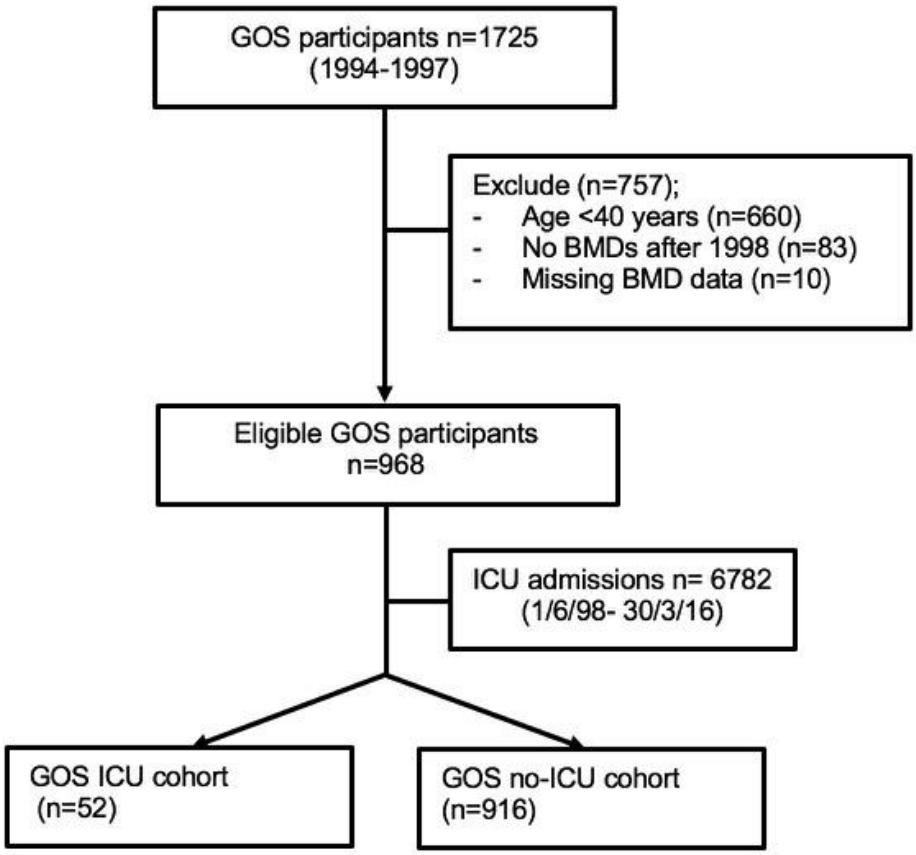
1. Abbreviations: BMD = bone mineral density; ICU = intensive care unit.
2. Absolute BMD (g/cm^2) adjusted for age, ICU status, anti-fracture medication, glucocorticoid, hormone therapy, at time of measurement.
3. All data are shown as mean (standard deviation)

Table 3: Age adjusted interaction of ICU status and medication use with annual change in Spine and Femur BMD

AP Spine	Annual Change in BMD	p-value for interaction	Femoral Neck	Annual Change in BMD	p-value for interaction
ICU			ICU		
ICU	-0.010 (0.002)	0.01	ICU	-0.009 (0.001)	0.055
No-ICU	-0.005 (0.002)		No-ICU	-0.006 (0.001)	
Glucocorticoid			Glucocorticoid		
Current	-0.012 (0.002)	0.1	Current	-0.008 (0.001)	0.047
Ever	-0.012 (0.001)		Ever	-0.011 (0.001)	
Never	-0.010 (0.002)		Never	-0.009 (0.001)	
Anti-fracture			Anti-fracture		
Current	-0.004 (0.002)	<0.001	Current	-0.007 (0.001)	0.1
Ever	-0.007 (0.001)		Ever	-0.007 (0.001)	
Never	-0.010 (0.001)		Never	-0.009 (0.001)	
Hormone			Hormone		
Current	-0.010 (0.001)	0.7	Current	-0.009 (0.001)	0.7
Ever	-0.010 (0.001)		Ever	-0.008 (0.001)	
Never	-0.010 (0.002)		Never	-0.009 (0.001)	

1. Abbreviations: BMD = bone mineral density; ICU = intensive care unit.
2. Annual change in BMD (g/cm^3) adjusted for age, ICU status, anti-fracture medication, glucocorticoid, hormone therapy, at time of measurement.
3. All data are shown as mean (standard deviation)

Figure 1: Flow diagram of patient identification for women in GOS and ICU



Abbreviation: BMD (Bone Mineral Density), ICU (Intensive Care Unit), GOS (Geelong Osteoporosis Study)

Figure 2a: AP Spine BMD over time (mean ± SD)

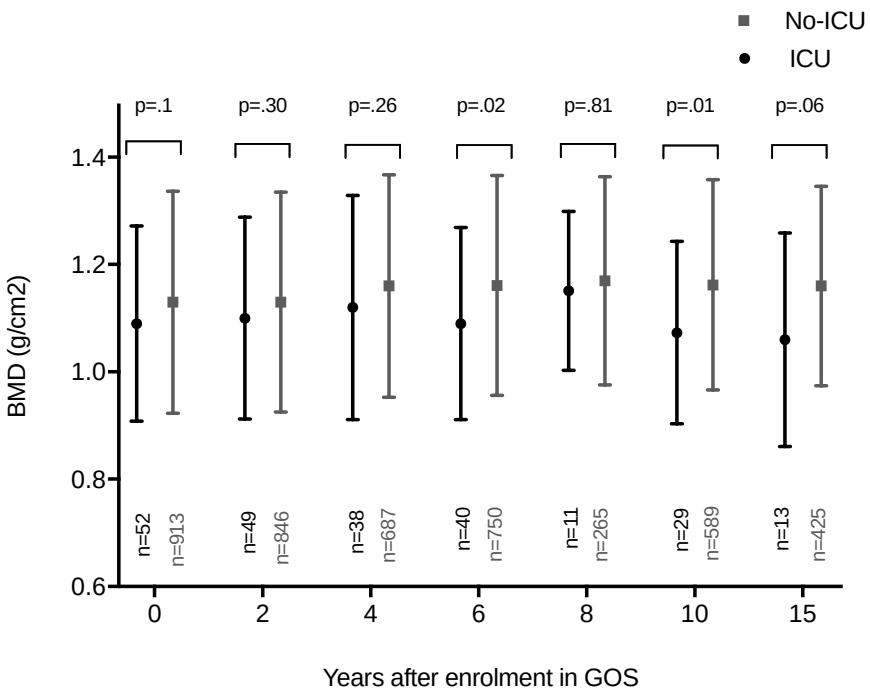
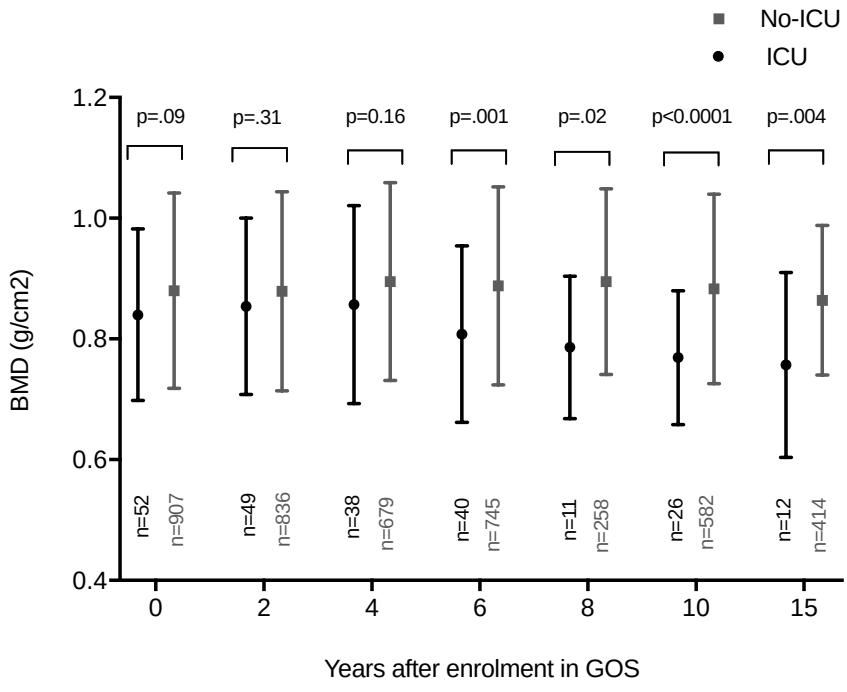


Figure 2b: Femoral Neck BMD over time (mean ± SD)



Abbreviation: BMD (Bone Mineral Density), GOS (Geelong Osteoporosis Study), ICU (Intensive Care Unit), SD (standard deviation)

Figure 3a: Change in Femoral Neck BMD relative to ICU admission

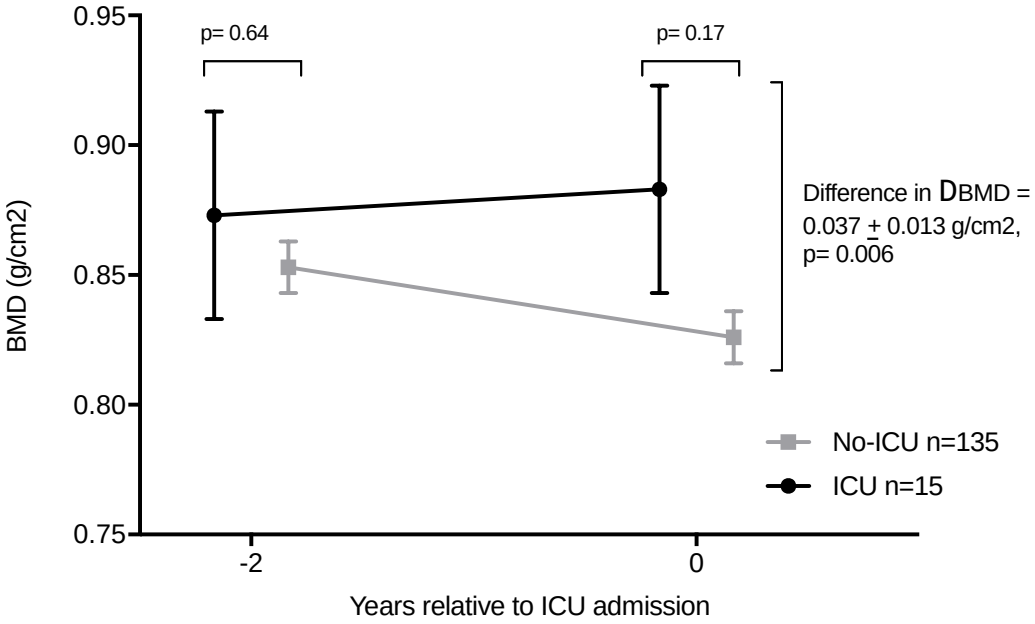
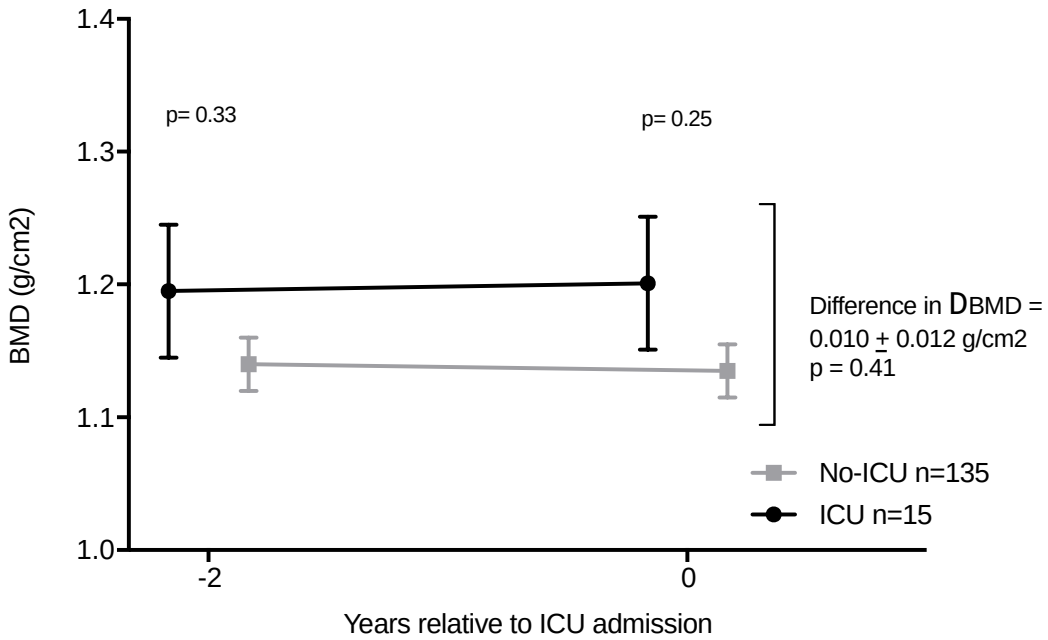


Figure 3b: Change in AP Spine BMD relative to ICU admission



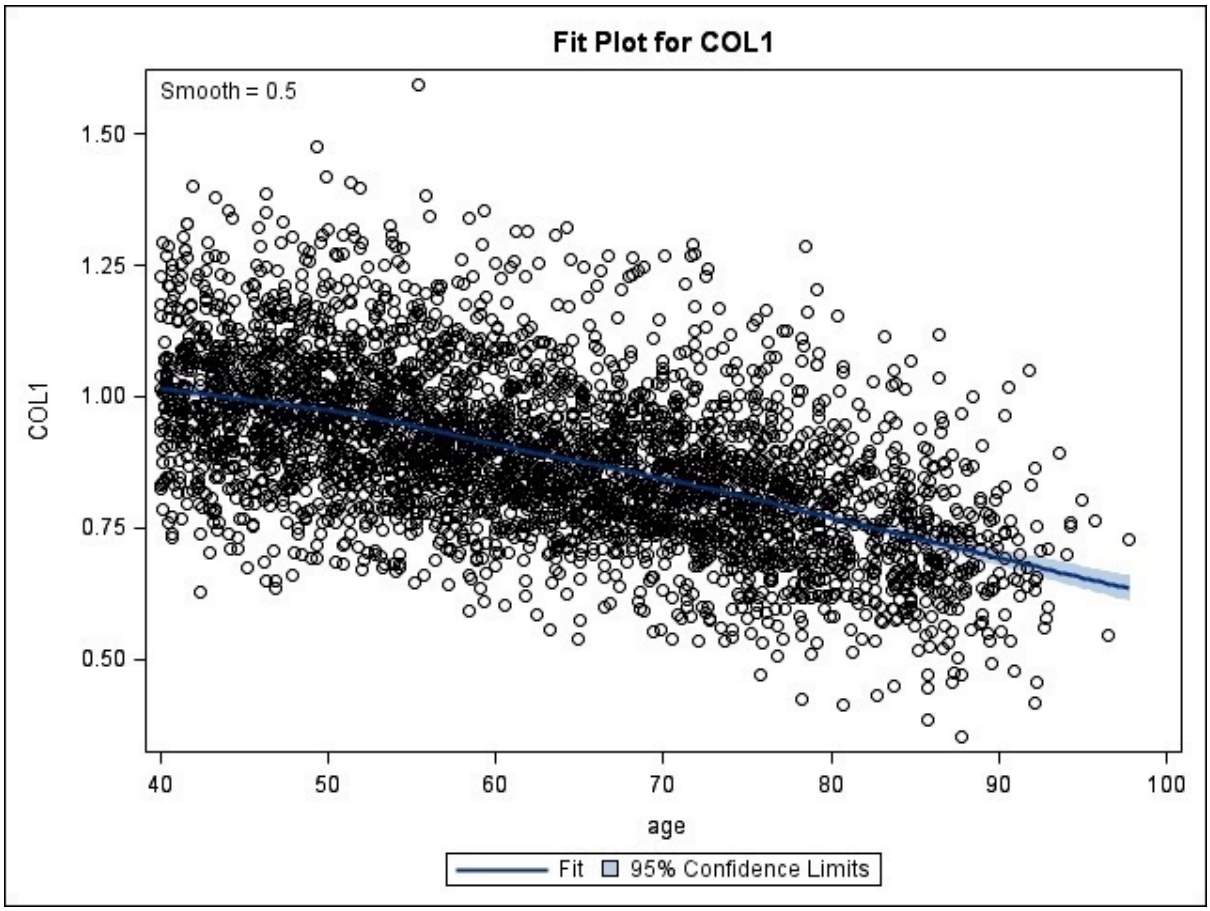
Abbreviation: AP (anterior-posterior), BMD (Bone Mineral Density), ICU (Intensive Care Unit), Δ (Change)

ADDITIONAL FILES

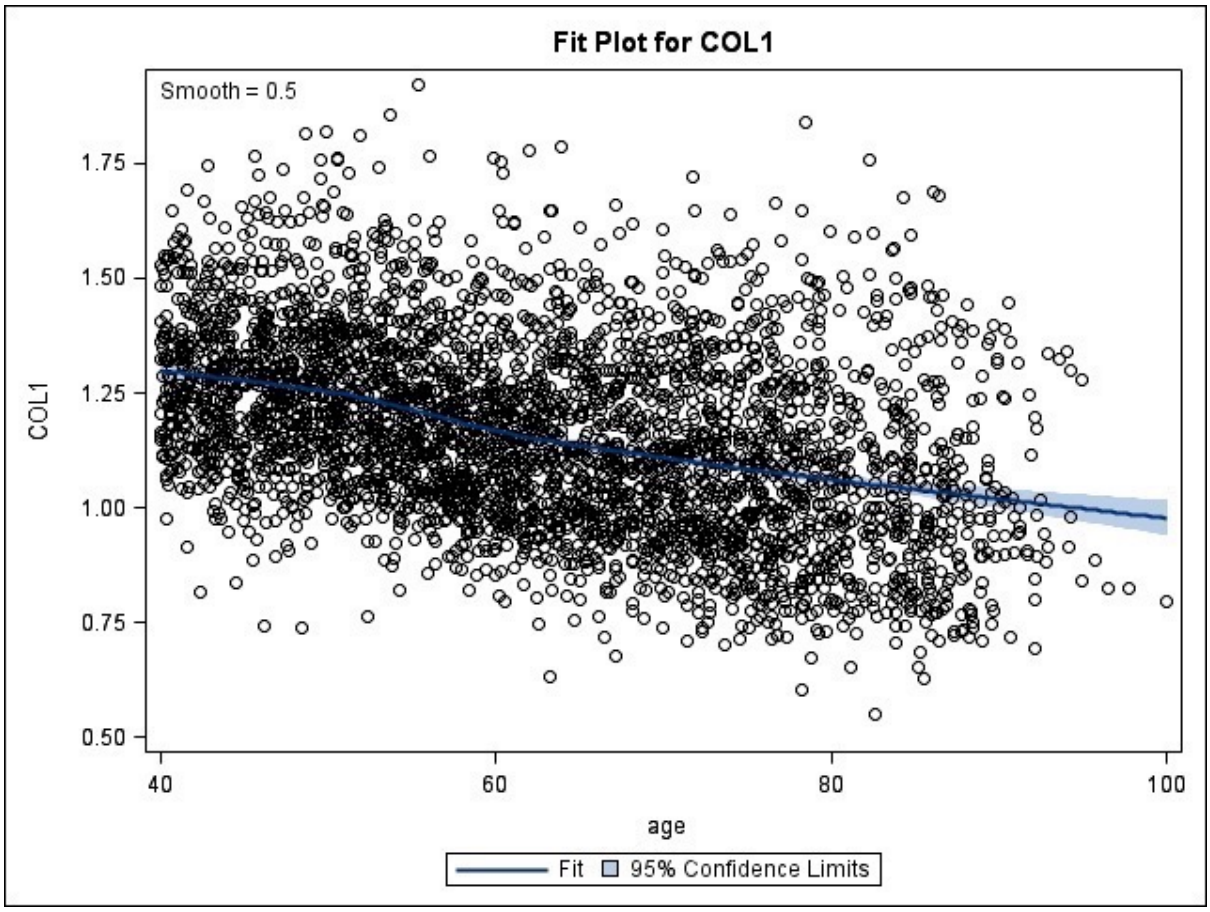
Additional File 1 – Additional Methods

Lumbar spine (L2-4), and proximal femur (femoral neck, total hip, trochanter, Ward's triangle) BMD was quantified with dual energy X-ray absorptiometry (DXA) initially using a Lunar DPX-L (software version 1.31; Lunar, Madison, WI, USA) and subsequently, a GE-Lunar Prodigy (Prodigy; GE Lunar, Madison, WI, USA) when the DPX-L became outmoded. Cross calibration of the two scanners was performed prior to decommissioning of the DPX-L, and no significant differences in lumbar spine or femoral neck BMD were seen in dual scans performed on 40 subjects aged 21 to 82 years. Long-term stability of both machines was confirmed by scanning an anthropomorphic phantom (Hologic) three times a week.

Additional File 2a: Regression analysis of Femoral Neck BMD against age for all Geelong Osteoporosis Study Women



Additional File 2b: Regression analysis of AP Spine BMD against age for all Geelong Osteoporosis Study Women



Additional File 3: Baseline bone mineral density and risk factor characteristics of ICU vs no-ICU women cohorts from Geelong Osteoporosis Study

Variable	ICU	No-ICU	P-value
Age at Year 0	69.0 [62.5, 73.0]	59.5 [47.7,72.2]	<0.0001
Year 0 (no.)	52	915	
AP Spine BMD	1.090 (±0.182)	1.130 (±0.207)	0.1
Femoral Neck BMD	0.840 (±0.142)	0.880 (±0.162)	0.09
Anti-fracture	1 (1.9)	16 (1.7)	0.93
Hormone	6 (11.5)	142 (15.5)	0.45
Glucocorticoid			
Year 2 (no.)	49	849	
AP Spine BMD	1.100 (±0.188)	1.130 (±0.205)	0.30
Femoral Neck BMD	0.854 (±0.146)	0.879 (±0.165)	0.31
Anti-fracture	4 (7.7)	26 (2.8)	0.05
Hormone	7 (13.5)	146 (15.9)	0.63
Glucocorticoid	2 (3.8)	27 (2.9)	0.71
Year 4	38	688	
AP Spine BMD	1.120 (±0.209)	1.160 (±0.207)	0.26
Femoral Neck BMD	0.857 (±0.164)	0.895(±0.164)	0.16
Anti-fracture	2 (3.8)	20 (2.2)	0.44
Hormone	3 (5.8)	136 (14.8)	0.07
Glucocorticoid	2 (3.8)	13 (1.4)	0.17
Year 6	40	754	
AP Spine BMD	1.090 (±0.179)	1.161(±0.205)	0.02
Femoral Neck BMD	0.808 (±0.146)	0.888 (±0.164)	0.001
Anti-fracture	1 (1.9)	26 (2.8)	0.70
Hormone	3 (5.8)	110 (12.0)	0.17
Glucocorticoid	2 (3.8)	19 (2.1)	0.39
Year 8	11	266	
AP Spine BMD	1.151 (±0.148)	1.170 (±0.194)	0.81
Femoral Neck BMD	0.786 (±0.118)	0.895 (±0.154)	0.02
Anti-fracture	-	-	
Hormone	-	-	
Glucocorticoid	-	-	
Year 10	29	595	
AP Spine BMD	1.073 (±0.170)	1.162 (±0.196)	0.01
Femoral Neck BMD	0.769 (±0.111)	0.883 (±0.157)	<0.0001

Anti-fracture	0 (0)	30 (3.3)	0.19
Hormone	1 (1.9)	59 (6.4)	0.19
Glucocorticoid	0 (0)	15 (1.6)	0.35
Year 15	13	430	
AP Spine BMD	1.060 (±0.199)	1.160 (±0.186)	0.06
Femoral Neck BMD	0.757 (±0.153)	0.864 (±0.124)	0.004
Anti-fracture	2 (3.8)	22 (2.4)	0.52
Hormone	0 (0)	24 (2.6)	0.24
Glucocorticoid	2 (3.8)	21 (2.3)	0.48

1. Abbreviations: BMD = bone mineral density; ICU = intensive care unit.
2. BMD units are g/cm^3.
3. Data shown as mean (±standard deviation), or number (percentage).

Additional File 4: Comparison of characteristics of matched ICU and non-ICU cohort

Variable	ICU cohort (n=15)	No-ICU cohort (n=135)	P-value
Age at first BMD	69.4 (±10.8)	69.7 (±12.8)	0.92
Exposures in period prior to BMD #1			
Glucocorticoids	0 (0)	0 (0)	1.0
Anti-fracture therapy	0 (0)	0 (0)	1.0
Hormone therapy	1 (7)	9 (7)	1.0
Exposures in period prior to BMD #2			
Glucocorticoids	0 (0)	0 (0)	1.0
Anti-fracture therapy	0 (0)	0 (0)	1.0
Hormone therapy	1 (7)	9 (7)	1.0

1. Abbreviations: BMD = bone mineral density; ICU = intensive care unit.
2. Data shown as mean (±standard deviation), or number (percentage).

Chapter 7: Effect of denosumab on bone turnover markers in critically ill women - A safety and feasibility, randomised, placebo, controlled trial

This chapter presents a trial protocol for a phase II safety and efficacy RCT of the effect of denosumab vs placebo on bone turnover in post menopausal female intensive care patients requiring greater than 24 hours of mechanical ventilation. The rationale for this study is the evidence to date of increased been turnover, the increased effect observed in older females, and the known risk of accelerated bone loss in this population. The use of denosumab, a RANK decoy, has not been described in critical illness before. Therefore a safety and efficacy study has been developed, with 28-day change in BTMs as the primary outcome, to provide evidence for the feasibly of a larger study with 1-year change in BMD as the primary outcome.

CLINICAL TRIAL PROTOCOL

Skeletal Outcomes Following Intensive Care

(SOFter)

Effect of denosumab on bone turnover markers in critically ill women - A safety and feasibility, randomised, placebo controlled trial

BH REGI No. 17/12
WHO UTN: U1111-1194-4990
ACTRN: 12617000545369p

TABLE OF CONTENTS

1. GENERAL INFORMATION

Protocol Title: Effect of denosumab on bone turnover markers in critically ill women - A safety and feasibility, randomised, placebo, controlled trial

BH REGI No. 17/12

Protocol version: 6

Protocol Date: 17th Juy 2017

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2. SYNOPSIS

Background: Intensive care patients face health issues that extend beyond their critical illness. The current evidence indicates an association between critical illness and skeletal morbidity. This includes increased loss of bone mineral density (BMD), increased bone turnover markers (BTMs), increased fracture risk, and an increased rate of fragility fracture compared to matched community controls. This is most pronounced in older female survivors of critical illness. Bone antiresorptive therapies are effective at reducing bone loss, decreasing fracture risk, and may reduce mortality in patients with osteoporosis. A recent retrospective cohort study described an association between concurrent antiresorptive therapy and reduced mortality in critical illness[1]. Denosumab is a human monoclonal antibody directed against RANKL, a central stimulator of osteoclast activity, and is effective for prevention of fractures and bone loss in osteoporosis, and malignancy, with evidence of superiority compared to bisphosphonates. It is metabolised by intracellular mechanisms, with no adjustment necessary in renal dysfunction. No prospective randomised controlled studies have described the effect of antiresorptive therapies on long-term bone or mortality outcomes in critically ill patients.

Hypotheses: The administration of denosumab to critically ill postmenopausal women will safely and effectively attenuate critical illness associated bone loss.

Objectives:

- **Primary Objective:** Assess the efficacy and safety of subcutaneous denosumab in postmenopausal intensive care patients requiring longer than 24 hours of mechanical ventilation
- **Secondary Objectives:** Obtain early feasibility and biochemical efficacy data for a subsequent phase IIb study

Methods: A prospective, randomised, controlled, trial of denosumab (60mg sc 6-monthly) compared to placebo, in post-menopausal female intensive care patients requiring longer than 24 hours of mechanical ventilation. A sample size of 18 participants has been chosen to determine a clinically significant effect on bone turnover markers.

Significance: The role of antiresorptive therapies, including denosumab, in survivors of critical illness, to prevent bone loss, fracture, or death, requires an initial program of testing for safety and efficacy. The evidence from this trial will be used to inform progress to larger trials with bone mineral density, fracture, and mortality as the primary outcome.

3. BACKGROUND AND RATIONALE

3.1 Introduction

Intensive care patients face health issues that extend beyond their critical illness. Compared to their pre-illness status and general population controls, survivors of critical illness face increased mortality[2-5], physical[2,6-8] and cognitive impairment[9-11], and psychological distress[12-14]. A specific area where critical illness may adversely affect the well-being of survivors relates to an increased risk of fragility fracture due to accelerated bone loss[15-18]. Osteoporosis is a chronic progressive disease and major public health issue[19], characterized by low bone mass, micro-architectural bone disruption, and skeletal fragility leading to fracture[20]. The lifetime risk of osteoporotic spine, hip, or wrist fracture is 30-40% in developed countries, and the lifetime risk of hip fracture is one in six in white females[21], with significant associated health burden of mortality, morbidity, and cost[22,23]. However, as few as 13-27% of patients with osteoporosis are treated following a fragility fracture, suggesting osteoporosis remains an under diagnosed disease[24,25].

3.2 Pathophysiology of osteoporosis

Normal bone turnover requires osteoclast and osteoblast activity to be tightly coupled, with regulation by mechanical, nutritional, immune, paracrine, autocrine and endocrine factors [9,7,8]. This modelling and remodelling results in changes to the size and contours of bone internally and externally, a normal process that establishes bones peak strength during growth, and works to maintain it during aging. Remodelling, resorption, then replacement, occurs asynchronously through the skeleton, and involves 5-10% of the skeleton per year[21]. The replication, differentiation, activity, and lifespan of osteoclast and osteoblast progenitors are determined by growth factors from matrix, cytokines, circulating hormones, soluble and membrane-bound products of osteoclasts and their precursors, signals from osteocytes, and immune cells from osteoblast lineage. Osteoclasts are derived from haemopoietic precursors from the capillary blood supply and marrow, and are closely related to macrophages. Differentiation from osteoclast precursor to mature osteoclast requires signals from macrophage-colony-stimulating factor (M-CSF), receptor activator of nuclear factor-κB ligand (RANKL), and vascular endothelial growth factor (VEGF). RANKL is abundantly expressed by osteoblasts, bone marrow stromal cells, and T and B-lymphocytes, and binds to RANK receptor on osteoclasts, stimulating activity. Osteoblasts also release osteoprogeretin, a RANKL decoy/ antagonist. Osteoblasts are stimulated by vitamin D, parathyroid hormone, and the development of mature osteoblasts is promoted by growth factors released from bone matrix during resorption, and produced by osteoblasts themselves. Many of these local factors also contribute to osteoblast and osteoclast apoptosis. Uncoupling of bone resorption and formation occurs in numerous conditions, including menopause, myeloma, rheumatoid arthritis, bone metastases, suppression of sex hormones (androgen suppression therapy for prostate cancer in men, aromatase inhibitor therapy for breast cancer in women), and in the presence of pro-inflammatory cytokines (IL-1, TNF)[26]. Oestrogen deficiency increases the rate of remodelling and the volume of bone resorption by prolonging the life span of osteoclasts, and decreasing the life span of

osteoblasts. This leads to trabecular thinning, loss of connectivity between trabeculae, cortical thinning, and increased cortical porosity. As a result, bone fragility is more common in women than men, partly because the production of sex hormones does not decrease rapidly in men, with no subsequent increase in remodelling rate. The bone fragility and fractures observed in osteoporosis vary in pathogenesis, with some related to reduced bone mineral density, others a reduced density of osteocytes, and high, normal, or low rates of remodelling.

3.3 Assessment of Bone

Bone Mineral Density

The measurement of BMD by dual energy x-ray absorptiometry (DXA) at the proximal femur and lumbar spine forms the basis of assessment and treatment of osteoporosis, with change in BMD estimated to account for 60-80% of variance in bone strength[19], and is the central component of internationally agreed definitions of osteoporosis [27]. BMD values in individuals are expressed as an absolute value (g/cm^2), and in relation to a reference young adult population in standard deviation (SD) units, the T-score. The T-score is the number of standard deviations above or below the young adult mean, with cut-off values calculated from the Australian reference ranges[28,29]. The WHO operational definition[30] of osteoporosis includes normal (T-score > -1.0), osteopaenia (T-score -2.5 to -1.0), or osteoporotic (T-score <-2.5). Established osteoporosis is defined as a T-score below -2.5 in the presence of one or more fragility fractures [20]. BMD measurement is also used to estimate fracture risk, providing a continuous relationship with no absolute cut-off threshold that discriminates who will and will not fracture. Individuals with a 1SD decrease in BMD compared to their age-matched peers will have an approximate 2-fold increase risk of fractures in their remaining lifetime. This increases to 4-fold increase in fracture risk for a T-score of -2.5 [18]. In addition to categorisation of osteoporosis, BMD is used to assess response to treatment, and as a surrogate outcome in trials of antiresorptive agents. Change in BMD over one year is the standard for interventional research studies[31-35], as BMD undergoes relatively small changes over time, of a magnitude similar to measurement error (short-term precision in vivo for Lunar DXA (GE Healthcare, Madison, USA) is 1.6% for the femoral neck and 0.6% for the lumbar spine[1]).

Bone Turnover Markers

Biochemical markers of bone turnover also have a role in the assessment of bone loss. Although the diagnosis of osteoporosis is not based on evaluation of biochemical markers, they are used in predicting the rate of bone loss and subsequent fracture risk[36,37]. Overall BTMs are separated into markers of bone resorption and bone formation [38]. The bone resorption markers include urinary collagen type 1 cross-linked N-telopeptide (NTX), pyridinoline (Pyd) or deoxypyridinoline (Dpd), carboxy-terminal cross-linked telopeptide of type 1 collagen (ICTP/CTX). Bone formation markers include skeletal alkaline phosphatase (SALP), osteocalcin (OC), procollagen type 1 C peptide (P1CP) and procollagen type 1 N peptide (P1NP). The cytokine receptor osteoprotegerin (OPG), a member of the TNF receptor superfamily, acts as a decoy receptor for receptor activator of nuclear factor kappa B ligand (RANKL), and prevents RANK mediated regulation of inflammation, innate immunity, apoptosis, and blocking maturation and activity of osteoclast

precursors. Although divided into formation and resorption markers, BTM levels are affected by several factors, requiring more complex interpretation. The bone formation markers P1NP and P1CP are both procollagen terminal extension peptides, but P1NP is more specific for bone formation. Also, a number of BTMs are affected by biological factors including age, gender, co-existing disease, and medications. Examples include decreased excretion of CTX in renal failure and sensitivity of OC to glucocorticoid exposure [38]. Markers for bone turnover are generally higher in those with osteoporosis compared to healthy controls, although there is considerable overlap. The combined use of BMD measurement and biochemical markers may be helpful in risk assessment, especially in those women who are not identified as at risk by BMD measurement alone [23]. Levels of bone markers decrease rapidly with antiresorptive therapies, with 30-60% decreases after 3-6 months. The short-term decrease in bone markers predicts the effects of antiresorptive agents on bone mass and fracture risk over the subsequent 2-year, thus providing a useful measure of treatment efficacy [24].

3.4 Consequences of osteoporosis

The consequences of fragility fractures are devastating in terms of mortality, morbidity, and cost[22,23]. Three-quarters of women with hip, pelvis, or lower limb fractures are confined to the home, or could walk only short distances for several weeks. After a year, nearly one-half have not regained pre-fracture mobility. One-seventh of women with upper-limb fractures did not venture outside the home for at least 6 weeks. After 6 months, 3.4% of all patients, 19.6% of hip, 12.8% of humeral, and 4.7% of spine fracture patients required assistance with bathing and showering. After a year, more than half of the hip fracture cases remained restricted regarding housework, gardening, and transport. In summary, a fracture, regardless of site, has a major impact on a woman's lifestyle and well-being for at least a year [22]. Despite the known consequences, as few as 13-27% of patients with osteoporosis are treated following a fragility fracture, suggesting osteoporosis remains an under diagnosed disease[24,25].

The consequences of osteoporosis extend to mortality. Between 10 to 20% of people who sustain a hip fracture die within one year[21], the risk highest in the first six-months and decreases over time. However, the relative contribution of fracture, comorbidity, or other mechanisms to subsequent mortality is disputed [21]. In addition, this association is strengthened by the relationship between osteoporosis treatments and reduced mortality. A meta-analysis of RCTs of studies investigating approved doses of medication with proven efficacy in preventing vertebral and non-vertebral fractures, with a duration of at least 12 months and reporting mortality, identified eight studies of four agents (risedronate, strontium ranelate, zoledronic acid, and denosumab), providing data of over 1400 deaths in approximately 40,000 subjects. Overall osteoporosis treatment was associated with an 11% reduction in mortality (RR 0.89, 95%CI 0.80-0.99, p=0.036)[39]. Meta-regression analyses revealed mortality reduction was not related to mean age, incidence of hip or non-vertebral fracture in the placebo group, or non-vertebral fracture risk reduction, but was associated with the baseline mortality rate of the placebo group (P=0.03). In the four studies where the placebo mortality rate was greater than 10 per 1000 patient years (range 13.9-70.2 deaths per 1000 patient-years), there was a significant reduction in mortality (RR 0.83; 95% CI 0.72-0.94, p=0.0052), compared to no reduction in

mortality in studies where placebo mortality rate was less than 10 per 1000 years (RR 1.01, 95% CI 0.87-1.19, p=0.86) [39]. The mortality effect appeared to be similar across the different classes of agents in the study.

3.5 Bone loss following critical illness

The current evidence of association between critical illness and accelerated bone loss includes changes in bone mineral density (BMD), bone turnover markers (BTMs), fracture risk, and fragility fracture rate.

Bone turnover markers and critical illness

A number of studies have identified a relationship between critical illness requiring mechanical ventilatory support and increased bone turnover, summarised in a recent systematic review[16] . Increased osteoclastic bone resorption (increased urinary DpD and PyD, serum CTX/ICTP), an increase in immature osteoblast number and activity (serum P1CP and P1NP), and reduced activity of mature osteoblasts (serum OC and ALP), of the magnitude described in postmenopausal females, or metabolic bone disease have been described[17,37,40,41]. Higher levels of bone resorption markers were observed in ICU patients with a length of stay of greater than 5-days, and a positive relationship between inflammation and increased bone turnover was present in a number of studies and was unrelated to severity of illness, type of illness, age or outcome.

There is limited evidence describing the effect of known osteoporosis risk factors and critical illness related factors on BTMs in critical illness, with the exception of age and gender. Higher levels of bone resorption markers were observed in ICU patients with a length of stay of greater than 5-days [42], although the lack of adjustment for confounders, including co-morbid illness such as renal failure, prevents the nature of this relationship being established. A positive relationship between inflammation and increased bone turnover was present in a number of studies [40,43-45], and was unrelated to severity of illness, type of illness, age or outcome. Systemic inflammation has been identified as a marker for increased fracture risk in non-critically ill patients [46], however ongoing bone resorption did not correlate with inflammatory markers, which may reflect the influence of other mechanisms, a prolonged effect of cytokines through osteoclast activation factors that increase maturation and lifespan of osteoclasts, or a direct effect of cytokines on osteoclast precursors. In one of the studies, concomitant treatment with glucocorticoids, thyroid hormones, or any other ICU medication did not significantly affect markers of bone turnover at any of the studied time points [43-45]. A series of studies by Van den Berghe et al [43,44] described changes to the somatotrophic, thyrotrophic, and gonadotrophic axes in prolonged critical illness, and included bone markers as a part of measures of target tissue effects. The studies describe a positive correlation between inflammatory cytokines and osteoclastic and osteoblastic activity, with variable effects of restoration of somatotrophic, thyrotrophic, and gonadotrophic axes on BTMs [47]. In-vitro experiments have shown that compared to healthy controls, critically ill patients peripheral blood mononuclear cells (PBMCs) responded to the presence of osteoclastic activation factors with an increased number and activity of mature osteoclasts [18]. In addition, exposure of PBMCs to critically ill patient sera resulted in an increased formation of mature osteoclasts, whereas a model of bone formation showed a reduction in angiogenesis factor expression, and reduced vascularity and maturity of

bone formation.

Bone mineral density assessment and critical illness

To date there are two prospective observational studies describing longitudinal changes in BMD in survivors of critical illness. The first described changes in calcaneal BMD over 10-days in 46 adult patients expected to be ventilated for over 48 hours and remain in ICU for over 7-days. They reported a decrease in BMD ARDS patients compared to ventilated non-ARDS patients (-2.81% vs +2.40%, p=0.03)[18], and an increase in fracture risk of 19.4% in ARDS compared to 9.35% in non-ARDS patients (p=0.012). The use of calcaneal BMD limited by precision issues, the short measurement period, and small numbers are major limitations to this study.

The second study describes the change in BMD in the year after critical illness in 66 adult patients ventilated for greater than 24 hours who survived to ICU discharge[17]. The annual decrease in BMD in critical illness was significantly greater than age and gender matched population controls[48] (Table 2). When analysed by gender, the difference was significantly greater in females at both AP spine and femoral neck, while in males it was significantly greater at femoral neck only. This study also reported the percentage of patients with an osteoporotic or osteopaenia T-score and fracture risk. The proportion of patients with abnormal T-score at 1-year post ICU (females 66.7%, males 44.1%) were higher than local population levels, with the Geelong Osteoporosis Study (GOS) reporting one-fifth of females greater than fifty years of age have BMD in the osteopaenia range, and 1 in 6 with osteoporosis[49].

Table 1: Annualised change in bone mineral density in women after critical illness compared to matched Geelong Osteoporosis Study controls (Data are shown as mean (±standard deviation))

Variable	ICU (n=31)	GOS (n=120)	Difference (95% CI)	P-value
Total change AP spine	-0.035 (0.050)	-0.002 (0.012)	-0.033 (-0.042, -0.023)	< 0.001
Percent change AP spine	-2.85 (4.05)	-0.18 (1.08)	-2.67 (-3.49, -1.86)	< 0.001
Total change Femur	-0.018 (0.037)	-0.006 (0.008)	-0.013 (-0.020, -0.005)	0.001
Percent change Femur	-1.96 (4.03)	-0.65 (0.98)	-1.31 (-2.10, -0.51)	0.001

This study also calculated fracture risk using the Australian version of the FRAX® fracture risk assessment tool, an algorithm developed by the World Health Organization (WHO)[50]. The estimated 10-year fracture risk for both all major fractures (4.85±5.25 vs 5.50±5.52, p<0.001) and hip fractures specifically (1.57±2.40 vs 1.79±2.69, p=0.001) significantly increased, and was highest in females.

Fragility fractures in survivors of critical illness

The major sequelae of increased bone turnover, and accelerated bone loss, is an increased risk of fragility fracture. The fragility fracture rate following critical illness, and comparison to age and gender matched population controls, has been described in one retrospective observational case-cohort study [15]. The radiological databases of 739 adult patients that were ventilated for greater than 24 hours and survived to ICU discharge, were assessed for evidence of fragility fracture using the same ascertainment period as the

control population, the GOS [48]. In the ICU survivor cohort followed for a median of 3.7 years, thirty-six women (14.2%) and 48 men (10.0%) sustained a fracture during the post-ICU time period, and incident fracture rate of 3.84 and 2.41 per 100 patient-years respectively. The over 60-year female ICU survivor cohort were compared to the GOS gender and age matched controls, with a significant increase in fracture, and shorter time to fracture observed in in the ICU group (HR 1.65 95%CI 1.08-2.52) (p = 0.02).

Figure 1: Unadjusted and adjusted fracture rates and hazard ratios for females (20-94 yrs of age) post-ICU compared with population-based females (GOS)

Variable	Post-ICU Fracture Rate (95% CI)	GOS Fracture Rate (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI, *p*)
All ages, all fracture	3.84 (2.58–5.09)	2.01 (1.76–2.25)	1.63 (1.14–2.32)	1.20 (0.84–1.71, $p = .31$)
>60 yrs of age, osteoporotic fracture	4.33 (2.72–5.93)	2.81 (2.33–3.28)	1.48 (0.98–2.25)	1.65 (1.08–2.52, $p = .02$)

ICU, intensive care unit; GOS, Geelong Osteoporosis Study; CI, confidence interval; HR, hazards ratio.

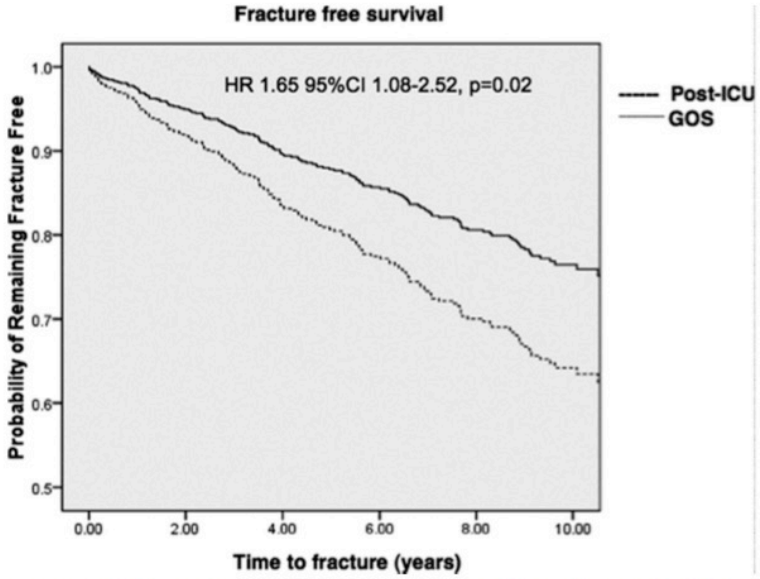

Figure 1. Time to fracture of the wrist, hip, humerus, or vertebral fracture after intensive care unit (*ICU*) compared with the random population-based sample in older age group (≥ 60 yrs) females. *HR*, hazard ratio; *CI*, confidence interval; *GOS*, Geelong Osteoporosis Study.

3.6 Prevention of critical illness related bone loss

The evidence to date supports the hypothesis that bone loss is increased during critical illness, resulting in an increased risk of fracture in survivors. This would contribute significantly to their health burden; with the average cost of hip fracture in Australia is estimated at $16,000, with an average length of hospital stay of thirteen days [10]. Furthermore, fragility fractures are associated with excess mortality, pain, immobility, and reduced functional capacity resulting in significant quality of life issues [12 16 17 11]. To date there is no evidence

of an association between accelerated bone turnover and increased mortality after critical illness. The availability of target interventions to prevent or attenuate acute bone loss following critical illness provides the incentive to further explore this area of clinical research. The management of osteoporosis can be classified into non-pharmacological options, with pharmacological treatments classified as ant-resorptive and anabolic.

Non-pharmacologic options – Physical Activity and Modifiable Risk Factors

Physical activity, including resistance and weight-bearing exercise, can increase muscle mass and transiently improve BMD [51], and regular physical activity may result in beneficial effects on skeletal microarchitecture [52]. The relationship between falls and fractures is well described, with falls, and fractures from falls, increasing with age. Exercise and balance programs that result in reduced falls may be of benefit. Other measures that may be of benefit are reductions in known risk factors for reduced BMD, ie alcohol, smoking.

Calcium and Vitamin D

The efficacy of calcium and vitamin D treatment for the prevention of osteoporotic fractures in controversial, with conflicting results from large trials, subgroup analyses, and meta-analyses. Standard recommendations for most postmenopausal women with osteoporosis suggest a total calcium intake of 1000-1500mg per day, and a total vitamin D intake of 600-800 IU per day [53].

The association between serum vitamin D levels and outcomes in critically ill patients has received attention since the publication in 2009 of a case series describing a high prevalence of hypovitaminosis D in 42 critically ill patients referred to an endocrinology service[54]. With an association between vitamin D deficiency and increased mortality present in the general community and specific disease cohorts[55,56], and a plausible mechanism for vitamin D to influence outcomes through its non-bone related activity in endothelial, immune, and cellular function [57-60], the links between vitamin D as both a prognostic marker and intervention in the critically ill population has been of increasing interest. Although there is debate regarding the threshold levels used to define insufficiency and deficiency, the proportion of critically ill patients with decreased vitamin D levels ranges from 42-97%[61-70 71]. A positive association between vitamin D deficiency during critical illness and increased mortality has been described in observational studies where cohorts of patients with vitamin D levels measured before or during critical illness were examined [62,66,69,72,73]. These studies consistently describe increased mortality rates in vitamin D deficient patients, but are limited by the selection bias created by enrolling patients in whom vitamin D levels were already ordered. In comparison, six prospective observational cohort studies enrolling patients with predicted or actual ICU length of stay of greater than 1 to 2 days have reported conflicting results. A positive association between vitamin D deficiency and increased 90-day mortality has been reported in two studies [61,74], while no association was found in four studies reporting ICU, hospital, or 28-day mortality [70,71,75,76]. These results, in combination with evidence that vitamin D deficiency during critical illness is associated with increasing age, seasonal variation, severity of illness, bacteraemia, sepsis, multi-organ failure, type of ICU and length of stay [61,63,66,69,74-7619], suggest the association between critical illness, vitamin D deficiency, outcomes, and the effect of other factors, is not

clear.

In terms of bone turnover, two studies report the effects on bone turnover of treating vitamin D deficiency in critically ill patients. One study described the effect of parenteral vitamin D 200 IU or 500 IU daily in long-term surgical ICU patients receiving parenteral nutrition, with higher dose vitamin D associated with a relatively small increase in serum OC, a decrease in serum B-CTX, but did not affect other BTMs. In addition the decrease in inflammatory markers interleukin-6 and C-reactive protein over time was more pronounced with the higher dose vitamin D[40]. However treating vitamin D deficiency with calcitriol did not lead to a reduction in bone resorption markers, suggesting that vitamin D deficiency alone was not the mechanism for accelerated bone turnover[77].

Antiresorptive agents – Bisphosphonates

Bisphosphonates inhibit bone resorption in a dose dependent manner, and result in an increase in bone mass. Large prospective trials of osteoporotic women demonstrated increases in lumbar spine and femoral BMD over 2-3 years, and reduced vertebral, wrist and hip fracture risk. Multiple agents are available including etidronate, alendronate, clodronate, pamidronate, and zoledronic acid. They have poor oral bioavailability, with 1-5% of the oral dose absorbed. PBS indications for bisphosphonates include treatment for osteoporosis in a patient aged 70 years of age or older with a T-score of -3.0 or less, and treatment for established osteoporosis in patients with fracture due to minimal trauma.

Common side effects of oral bisphosphonates include fatigue, anaemia, muscle aches, fever, swelling feet or legs, and oesophageal and upper gastrointestinal irritation. Flu-like symptoms are common after intravenous infusions in treatment naïve individuals and are thought to occur because of their potential to activate human gamma delta T cells. The association between bisphosphonates and renal dysfunction is well established. Acute tubular necrosis and collapsing focal segmental glomerulosclerosis have been implicated in the mechanism of renal toxicity, however the pathogenesis is poorly understood. A review of the FDA Adverse Event Reporting System identified 72 cases of renal failure associated with zoledronic acid. Indications for use were multiple myeloma (42), solid tumours (22), benign conditions (2), and unknown condition (6). Renal failure developed after an average of 56 days of use, in 25% of patients only one dose was received. The onset of renal failure and recovery of serum creatinine after drug discontinuation suggested a temporal relation to the use of zoledronic acid. The authors recommended renal function monitoring, adequate hydration, and discontinuation if renal function deteriorates.[27] A rare complication is osteonecrosis of the jaw, with an estimated incidence of <1:10,000 bisphosphonate users[53], and mainly observed in multiple myeloma patients with zoledronate who have had dental extractions where the rate may be as high as 1 in 10 [28].

There is limited experience with bisphosphonates in critical illness. Case reports and small studies [8] have reported the use of intravenous bisphosphonates to treat critically ill patients with biochemical evidence of bone resorption. A single randomised controlled trial reported a transient decrease in serum CTX in chronic critically ill patients receiving a single intravenous dose of ibandronate compared to placebo [78]. A single randomised controlled trial has reported the effect of a single intravenous dose of ibandronate compared to

placebo, on serum CTX and OC over 14-days, in 20 postmenopausal chronic critically ill women[78]. Although ibandronate was associated with a significant decrease in CTX from baseline at day-6 compared to placebo (-34% vs +13%, p=0.03), this effect had disappeared by day-11. In comparison there were no differences in OC levels between the groups. This suggests ibandronate had a significant but short-lived effect on osteoclast activation and bone resorption, but was ineffective at suppressing osteoblast activation and bone formation. This is different to the effect observed in post-menopausal women, where reduction of CTX and OC or P1NP is attributed to treatment resulting in coupling of resorption and formation [79].

A retrospective analysis compared 245 patients with an ICU length of stay of at least 24 hours receiving bisphosphonates within 5-years prior to admission, to propensity matched ICU controls, for the association between prior bisphosphonate use, mortality, and change in vertebral BMD assessed by serial CT scans. They reported recent bisphosphonate use in 3.1% of eligible patients, with a significantly reduced mortality in this group compared to matched controls (mortality RR 0.41, 95% CI 0.24-0.71, p<0.01). This relationship persisted after adjustment for known confounders of sex, age, premorbid disease burden, bisphosphonate route and time between ICU admission and bisphosphonate prescription. The only group in whom benefit disappeared were patients free of any comorbid disease. Serial CT assessment of vertebral BMD revealed lower baseline bone density in bisphosphonate users, with an attenuated decrease in BMD in users vs non-users (-3 $\pm$ 13% vs -15 $\pm$ 14% per week, p<0.01), over a short time period (11 $\pm$ 10 days).

Antiresorptive agents – Denosumab

Denosumab is a fully human monoclonal antibody directed against RANKL, a central stimulator of osteoclast activity. It is administered as a subcutaneous injection and is metabolised by intracellular mechanisms, with no adjustment necessary in renal dysfunction. Denosumab has been extensively trialed and shown to be effective at reducing loss of BMD and fracture prevention. It currently has indications for the prevention of skeletal-related events in bone metastases from solid tumors, treatment of androgen deprivation induced bone loss in men with prostate cancer, and treatment of aromatase inhibitor induced bone lose in women with breast cancer [80] [81] [82] [80] [83]. Although head-to-head trials of antiresorptive agents are lacking, denosumab appears to be at least as efficacious as other agents, and has the added advantage that is administered as a subcutaneous injection 6-monthly. This may improve compliance with antiresorptive therapy, a major issue for bisphosphonate therapy [84].

In clinical studies, treatment with 60 mg of denosumab resulted in reduction in the bone resorption marker CTX by 86% at 1-month post intervention compared to placebo. At 6-months, prior to the next scheduled dose, CTX reductions were partially attenuated with a mean reduction of 72% compared to placebo, reflecting the reversibility of the effects of denosumab on bone remodelling. These effects were sustained with continued treatment to 36-months [80]. In the same study P1NP was reduced 18% compared to placebo at 1-month, and 50% compared to placebo at 6-months, consistent with the physiological coupling of bone formation and resorption in skeletal remodelling.

Figure 2: Percent changes in BMD and Bone Turnover Markers for denosumab and placebo in post-menopausal women.

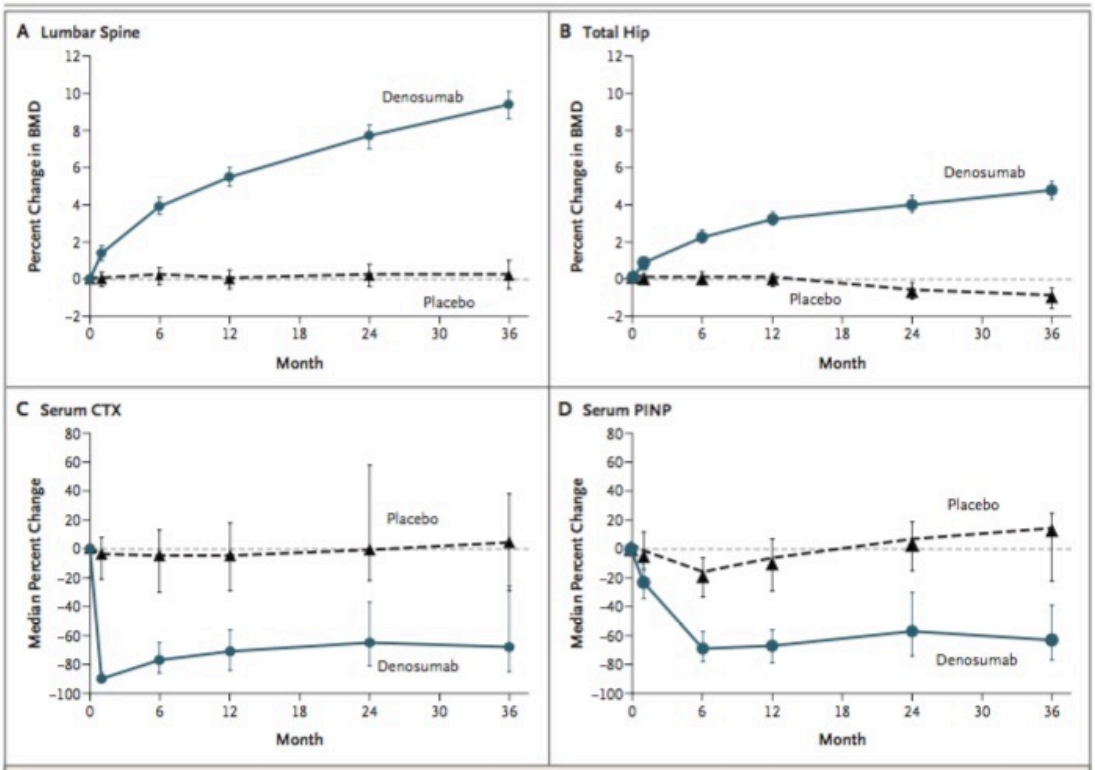

Figure 2. Percent Changes in Bone Mineral Density and Biochemical Markers of Bone Turnover.
Changes in mean bone mineral density (BMD) at the lumbar spine (Panel A) and total hip (Panel B) are shown for 441 subjects who were included in a substudy of measurements of bone mineral density. As compared with subjects in the placebo group, subjects in the denosumab group had a relative increase of 9.2% in bone mineral density at the lumbar spine and 6.0% at the total hip. Changes in mean values for serum C-telopeptide of type I collagen (CTX) (Panel C) and serum procollagen type I N-terminal propeptide (PINP) (Panel D) are shown for 160 subjects who were included in a substudy of bone-turnover markers. P<0.001 for all between-group comparisons at all time points on the basis of analysis-of-covariance (ANCOVA) models. For bone mineral density, the comparisons were adjusted for study group, baseline bone mineral density, type of machine used to analyze bone mineral density, and interaction between the type of machine and the baseline bone mineral density; for CTX and PINP, the comparisons were calculated with the use of the Wilcoxon rank-sum test.

(Cumming et al, NEJM, 2009;361(8);756-765)

Like all antiresorptive agents, adverse effects of denosumab include fatigue, headache, rash, musculoskeletal pain, hypocalcaemia, hypophosphatemia, and atypical fractures of the femoral shaft with long-term use. Hypocalcemia must be corrected prior to initiating therapy, and in patients predisposed to hypocalcemia and disturbances of mineral metabolism (e.g. history of hypoparathyroidism, thyroid surgery, parathyroid surgery, malabsorption syndromes, excision of small intestine, severe renal impairment [creatinine clearance < 30 mL/min] or receiving dialysis), clinical monitoring of calcium and mineral levels (phosphorus and magnesium) is highly recommended within 14 days of injection. Osteonecrosis of the jaw has been reported, but is rare, with no cases in 3420 cancer patients enrolled in a RCT [83]. Osteonecrosis of the jaw (ONJ), which can occur spontaneously, is generally associated with tooth extraction and/or local infection with delayed healing. A routine oral exam should be performed prior to initiation.

Perhaps the major concern about long-term use of denosumab relates to its possible effects on the immune system, since RANKL is expressed not just on bone cells but also on immune cells. In a clinical trial of over 7800 women with postmenopausal osteoporosis, the incidence of infections resulting in death was 0.2% in both treatment groups, and the incidence of nonfatal serious infections was 3.3% in the placebo and 4.0% in the denosumab groups. Hospitalizations due to serious infections in the abdomen (0.7% placebo vs. 0.9% denosumab), urinary tract (0.5% placebo vs. 0.7% denosumab), and ear (0.0% placebo vs. 0.1% denosumab) were reported. Endocarditis was reported in no placebo patients and 3 patients receiving denosumab. Skin infections, including erysipelas and cellulitis, were reported more frequently in patients treated with denosumab (< 0.1% placebo vs. 0.3% denosumab, p=0.002) [80].

3.7 Denosumab as trial intervention in critical illness

The experience of antiresorptive medications in the critical care setting is limited to case reports and small cohort studies. We have recently reported on the association between antiresorptive agents (including alendronate, denosumab, strontium ranelate, and risedronate) on annual change in BMD in a cohort of men and women in the 2-years after critical illness. In women participants, a greater loss of spine BMD was observed in the first year after critical illness, with antiresorptive medication use associated with an increase in BMD compared to a decrease in BMD in those that did not receive such therapy. In men BMD loss increased in the second year after critical illness, and there was no association between use of antiresorptive medications or glucocorticoids and change in BMD, although only a small proportion of men received post-ICU bone-related medications. These findings suggest anti-resorptive therapy may be an effective intervention to prevent bone loss in women with critical illness, and prospective trials investigating this effect are warranted[85].

Denosumab, with reduced renal effects, and efficacy, appears likely to be a more favourable target agent. Given the lack of experience in critical illness, the favourable characteristics of denosumab, and the existing evidence of accelerated bone loss in critical illness, this study proposes a safety and feasibility pilot, after which assessment of feasibility for a larger phase II trial could be considered.

This study proposes to enrol post-menopausal women requiring ventilatory support for greater than 24-hours, administer denosumab on day 3 in ICU, and again 6-months later. For this safety and exploratory study the primary outcome will be change in the bone turnover markers CTX and P1NP to study day-28. Secondary outcomes include change in bone mineral density and bone turnover markers at 1-year post ICU, and safety outcomes.

Administration of denosumab without prior BMD assessment

The indications for denosumab include postmenopausal women with osteoporosis at high risk of fracture, and treatment of bone loss in women receiving adjuvant aromatase inhibitor therapy for breast cancer. With regards to assessment of osteoporosis, DXA BMD testing cannot be performed in the ICU, because patients need sufficient mobility and cognitive function to transfer from a chair to a bed and lie still for the study. Our

experience is this occurs one to four weeks after ICU discharge. Therefore, the intervention options are to administer denosumab in ICU without BMD testing, or to delay administration to the post-ICU period after BMD testing has been performed. The rationale for administering denosumab during ICU is three-fold;

1. The available evidence for accelerated bone turnover associated with critical illness indicates bone turnover markers increase within 48-hours of ICU admission, suggesting earlier intervention is more likely to be effective.

2. Our observational data revealed that 67% of female survivors of critical illness able to complete the 1-year follow-up had osteopaenia or osteoporosis. The cohort that withdrew or died before this had higher BTMs during ICU, suggesting we observed cohort that completed the study where healthier with lower risk of accelerated bone loss. Given this, it is estimate that less than 1/3 of women enrolled will have normal bone mass. General population data tells us that only a quarter of fragility fractures occur in women with osteoporosis, with ¾ occurring in women with osteopaenia and normal bone mass[17,49].

3. The administration of denosumab to postmenopausal women with a risk factor for accelerated bone loss irrespective of BMD has been performed in a 3500 patient randomised trial of women commencing an aromatase inhibitor for the management of breast cancer. In this study 55% of women enrolled had a BMD $\geq$ -1.0, and a significant reduction in fracture was observed with denosumab equally for women with normal and osteopaenia BMD. In addition, the change in BMD observed in the first year of the study was -1.81% (placebo) vs + 3.94% (denosumab) at lumbar spine, and -1.08% vs +2.29% at femur[83]. In comparison to the placebo group in this trial, female ICU survivors have a change in BMD of -2.85 $\pm$ 4.05% at lumbar spine and -1.96 $\pm$ 4.03% at femur.

Administration of denosumab and possible immune modulation.

The major concern with the use of denosumab is the concern of immune modulation in critical illness. If present, this may be of no consequence, result in benefit through reduction in inflammatory response, or lead to unwanted effects. Although the evidence from antiresorptive trials and bisphosphonate users in critical illness suggest possible beneficial effects from these classes of agents, we have chosen a conservative approach to administration of denosumab in this study. The intervention will be delayed until infection has been treated (new sepsis or septic shock as defined by Sepsis-3 criteria [86]).

4. HYPOTHESIS AND OBJECTIVES

4.1 Hypothesis: The administration of denosumab to critically ill postmenopausal women will safely and effectively attenuate critical illness associated increase in bone turnover markers.

4.2 Objectives:

- **Primary Objective:** Assess the efficacy and safety of subcutaneous denosumab in postmenopausal intensive care patients requiring longer than 24 hours of mechanical ventilation

- **Secondary Objectives:** Establish whether a phase IIb trial in Australia and New Zealand is justified and feasible, and provide information regarding endpoints necessary in the design of such a trial.

5. STUDY DESIGN AND OUTCOMES

5.1 Design

A prospective, randomised, placebo-controlled, safety and feasibility trial to assess the effects of subcutaneous denosumab on bone mass in post-menopausal female intensive care patients expected to require greater than 24 hours of mechanical ventilation.

5.2 Study population

Inclusion criteria

1. Female
2. Age >50 years or postmenopausal (amenorrhea for greater than 6-months or serum FSH >40mIU/L) *or* age < 50 years with bilateral salpingo-oopherectomy
3. Expected duration of mechanical ventilation $\geq$ 24 hrs

Exclusion criteria

1. Unable to undertake BMD (weight >120kg, impaired mobility)
2. Active malignancy
3. Currently receiving immunosuppressive agents
4. Metabolic bone disease
5. Pregnancy
6. eGFR <30ml/min
7. Known contraindication to denosumab (previous reaction, osteonecrosis of the jaw, atypical femoral fracture)
8. Increased risk of osteonecrosis (poor dentition or oral hygiene, dental infection)
9. Hypocalcaemia (<0.9 mm/L ionized calcium)
10. Hypoparathyroidism
11. Malabsorption sydnromes / extensive small bowel resection
12. Neurological condition likely to prevent weight-bearing (eg severe traumatic brain injury, stroke with loss of mobility, degenerative neurological disease)

13. Current treatment with anti-fracture agent (bisphosphonate, denosumab, strontium, teriparatide, within previous 2 years)
14. Current indication for anti-fracture therapy (known BMD T-score < -2.5 and fragility fracture)
15. Treatment limitations in place

5.3 Screening, Enrolment, Randomisation, and Blinding

Patients in UHG ICU will be screened daily to determine eligibility for enrolment in the trial. If patients fulfil criteria the physician caring for the patient will be approached and asked if they consent to enrolment, after which the patient or surrogate decision-maker will be approached for consent. A randomisation table and allocation schedule will be created by computer software (i.e. computerised sequence generation) and used by a trials pharmacist at Barwon Health. All personnel, apart from the trial pharmacist, will be blinded to treatment allocation. Following patient randomisation, the trial pharmacist will dispense the trial drug (placebo or denosumab) in a blinded formulation, and the trial drug will then be administered by the ICU bedside nurse, or the trial nurse, according to the study treatment plan.

5.4 Outcome Measures

As this is a safety and feasibility trial the purpose is to establish a treatment effect of denosumab in the study population, and assess potential adverse effects. These results will determine the feasibility of a larger phase II, multi-centre study with change in BMD at 1-year as the primary outcome.

Primary Outcome

- Change in the bone turnover markers collagen type 1 cross-linked c-telopeptide (CTX) 28-days after administration of study drug dose 1.

Secondary Outcomes

- Bone turnover outcomes
    - Change in serum type 1 procollagen N-terminal (P1NP) 28-days after administration of study drug dose 1
    - Change in P1NP, CTX 1-year after administration of study drug dose 1
    - Annualised change in lumbar-spine and femur BMD in the year after critical illness
- Safety outcomes
    - Incidence of serious adverse events (severe hypocalcaemia, infection, osteonecrosis) 28-days after administration of study drug dose 1
    - Haematological, biochemical (urea, creatinine, calcium, liver function tests, white cell count, CRP)
- Patient-centred outcomes in the year after ICU
    - Fragility fracture
    - Mortality

Bone mineral density measurement

BMD measurements will occur at 2 separate time-points. The first is between ICU and hospital discharge, the second 1-year post-intervention. BMD will be measured by dual energy x-ray absorptiometry (DXA) (Lunar; GE Healthcare, Madison, Wis, USA), at the proximal femur and lumbar spine. Short-term precision in vivo is 1.6% for the femoral neck and 0.6% for the lumbar spine[1].

Serum bone turnover marker measurement

The serum bone turnover markers collagen type 1 cross-linked c-telopeptide (CTX) and type 1 N-terminal procollagen (P1NP) will be collected at five separate time-points, the day of the first study drug administration, and days 7, 28, 180, and 365 post initial study drug administration. Bone turnover markers will be measured using the automated Roche Modular Analytics E170 analyser. Serum collagen type 1 cross-linked c-telopeptide limit of detection was 10 ng/L with inter-assay coefficient of variations (CVs) of 6.5% at 361 ng/L, 3.8% at 816 ng/L and 3.4% at 3304 ng/L (n = 10). Serum type 1 N-terminal procollagen inter-assay CVs were 4.9% at 73 µg/L, 2.6% at 392 µg/L, and 2.1% at 768 µg/L (n = 10) with a limit of detection of 5 µg/L. Bone turnover markers will be compared to reference ranges derived from an Australian population sample[2].

5.5 Study Treatment Plan

Study plan during ICU admission

Enrolment

Enrolment

Following enrolment baseline demographic and clinical data will be collected, and baseline serum vitamin D assessed from the most recent routine blood test.

Standard Care

- Standard nutrition will be administered to participants per ICU feeding protocols, including dietician review and advice provided to participants in hospital.
- Vitamin D supplementation:
    - Following enrolment and randomisation, a serum vitamin D level will be collected and analysed. If the serum vitamin D level is < 50 mol/L, a single dose of 50,000 IU cholecalciferol will be administered via oral or enteral route.

Day 0 – day of study drug

Baseline investigations: Bone turnover markers, biochemistry and haematology. These blood tests will be collected as part of the routine morning blood collection in ICU patients, via existing vascular access (central venous line or intra-arterial line) when present, as is routine practice in ICU. All other ICU care will be carried out as per unit policy and standard practice.

Intervention

The intervention to be examined in this trial is the subcutaneous administration of denosumab 60mg compared to placebo (0.9% saline). The first dose of trial drug will be given on day 3 in ICU after vitamin D assessment has been completed and supplementation provided, and in the absence of untreated or new infection. The second dose of trial drug will be administered at the 6-month follow-up, after vitamin D assessment and supplementation as indicated.

The first dose of trial drug will be administered by an ICU registered nurse as a subcutaneous injection on study day 3 in ICU.

- Placebo:
    - Formulation: 0.9% Saline in a single-use pre-filled 1ml syringe
    - Administration: subcutaneous injection administered in upper arm, upper thigh, or abdomen.
- Denosumab:
    - Formulation: 60mg denosumab in a single-use pre-filled 1ml syringe
    - Administration: subcutaneous injection administered in upper arm, upper thigh, or abdomen.

- Following administration of the trial drug in ICU, monitoring for hypocalcaemia will occur a minimum of twice daily for 48-hours. Most patients will have intra-arterial and/or central venous vascular access, with regular blood gas measurement that include calcium performed. If routine testing provides twice-daily calcium additional testing will not be performed. Hypocalcaemia is defined as ionized calcium <0.9 mmol/L, based on ICU protocols for treatment of hypocalcaemia in other settings, ie citrate induced hypocalcaemia with the use of citrate for anticoagulation. Hypocalcaemia will be treated with parenteral calcium, as per hospital dosing and administration protocols, to maintain a target ionized calcium range of 0.9-1.1 mmol/L.

Day 7 and 28 follow-up

- Serum biochemical, haematological, and bone turnover marker testing: At day-7 and 28 participants will be asked to undergo serum biochemistry, haematology, and bone turnover marker tests. Where participants remain as in-patients in UHG or have been transferred to a subacute site, these tests will be collected as part of daily blood tests. Where participants have returned home, participants will be contacted by telephone and asked to undergo testing at their preferred place of pathology testing, or at Myers House, Barwon Health. Research staff will ensure pathology order forms are made available at the preferred site. Participants with serum vitamin D levels < 50 mol/L, will be offered a single dose of 50,000 IU cholecalciferol via oral or enteral route, either provided at UHG or by their local medical officer.

- Bone mineral density testing: The first BMD assessment will be performed between ICU discharge and day 28. This will be organised to occur either before hospital discharge, or at the day 7 or 28 follow-up, based on participant convenience.

6-month follow-up

- Serum biochemical, haematological, and bone turnover marker testing: At 6-months participants will be asked to undergo serum biochemistry, haematology, and bone turnover marker tests. Where participants remain as in-patients in UHG or have been transferred to a subacute site, these tests will be collected as part of daily blood tests. Where participants have returned home, participants will be contacted by telephone and asked to undergo testing at their preferred place of pathology testing, or at Myers House, Barwon Health. Research staff will ensure pathology order forms are made available at the preferred site. Participants with serum vitamin D levels < 50 mol/L, will be offered a single dose of 50,000 IU cholecalciferol via oral or enteral route, either provided at UHG or by their local medical officer.

- Trial drug: The second dose of trial drug will be administered by a registered nurse as a subcutaneous injection at 6-months post-ICU discharge.
    - Placebo:
        - Formulation: 0.9% Saline in a single-use pre-filled 1ml syringe
        - Administration: subcutaneous injection administered in upper arm, upper thigh, or abdomen.
    - Denosumab:
        - Formulation: 60mg denosumab in a single-use pre-filled 1ml syringe
        - Administration: subcutaneous injection administered in upper arm, upper thigh, or abdomen.

1-year follow-up and study completion:

- Serum biochemical, haematological, and bone turnover marker testing: At 1-year participants will be asked to undergo serum biochemistry, haematology, and bone turnover marker tests. Where participants remain as in-patients in UHG or have been transferred to a subacute site, these tests will be collected as part of daily blood tests. Where participants have returned home, participants will be contacted by telephone and asked to undergo testing at their preferred place of pathology testing, or at Myers House, Barwon Health. Research staff will ensure pathology order forms are made available at the preferred site. Participants with serum vitamin D levels < 50 mol/L, will be offered a single dose of 50,000 IU cholecalciferol via oral or enteral route, either provided at UHG or by their local medical officer.

- Bone mineral density testing: The first BMD assessment will be performed between ICU discharge and 28-day follow-up. This will be organised before hospital discharge, or at the 7 or 28-day follow-up, based on participant convenience. Research staff will accompany participants while they attend the UHG DEXA scan.

- At completion of the study continued treatment with vitamin D and antifracture agents will be offered to if an ongoing PBS indication is present. In addition, a letter with results and treatment recommendations will be provided to the participant and copied to their local medical officer.

5.6 Trial Schedule

Table 2: Softer Study Procedures	
Ventilation duration >24 hours to 7-days duration of mechanical ventilation	
D1 ICU Enrolment	Inclusion criteria met, consent obtained
	Baseline and demographic data
	Vitamin D level measured
Day 3 Intervention	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP
	Vitamin D supplement if level <50 nmol/L
	Denosumab 60mg sc vs Placebo administered if no new or untreated sepsis
Day 7 Post-intervention	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP
Day 7-28 Post-intervention	BMD #1
Day 28 Post-intervention	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP
Day 180 Post-intervention	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP
	Vitamin D supplement if level <50 nmol/L
	Denosumab 60mg sc vs Placebo
Day 365 Post-intervention	BMD #2
	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP

Close-out: Vitamin D / calcium / anti-resorptive therapy offered to participants in accordance with guidelines and review by an endocrinologist

5.7 Data collection schedule (Table 3)

Study ID	Enrolment	1st trial drug	7-day	28-day	6-month	1-year
Inclusion / exclusion	+	-	-	-	-	-
Date	+	+	+	+	+	+
DOB	+	-	-	-	-	-
UR	+	-	-	-	-	-
Sex	+	-	-	-	-	-
Level accom	+	+	+	+	+	+
Osteoporosis Risk Factors	+	-	-	-	-	-
Co-morbidity	+	-	-	-	-	-
Medication						
Glucocorticoids	+	+	+	+	+	+
Denosumab	+	-	-	-	+	+
Bisphosphonate	+	-	-	-	+	+
Teriparatide	+	-	-	-	+	+
Strontium Ranelate	+	-	-	-	+	+
Vitamin D	+	+	+	+	+	+
Calcium	+	+	+	+	+	+
Hospital						
Admission date	+	-	-	-	-	-
Discharge date	+	-	-	-	-	-
Discharge status	+	-	-	-	-	-
ICU						
Admission date	+	-	-	-	-	-
Diagnosis	+	-	-	-	-	-
Category	+	-	-	-	-	-
APACHE III	+	-	-	-	-	-
Ventilation duration	+	-	-	-	-	-
CRRT	+	-	-	-	-	-
Nutrition	+	-	-	-	-	-
Discharge date	+	-	-	-	-	-
Discharge status	+	-	-	-	-	-
Biochemistry / haem /BTM	-	+	+	+	+	+
BMD						
Height	-	-	-	+	-	+
Weight	-	-	-	+	-	+
Dual femur BMD	-	-	-	+	-	+
Dual femur T-score	-	-	-	+	-	+
AP spine BMD	-	-	-	+	-	+
AP spine T-score	-	-	-	+	-	+
Adverse events						
Hypcocalcaemia	-	+	+	+	+	+
Sepsis	-	+	+	+	+	+
Antibiotic duration	-	+	+	+	+	+
New infection	-	+	+	+	+	+
Osteonecrosis	-	+	+	+	+	+
GIT symptoms	-	+	+	+	+	+
Fragility fracture	+	-	-	-	+	+
Status	-	+	+	+	+	+

5.8 Timeline (Table 4)

Time	Event	Status
Jul 15 – Jul 16	Protocol development	Complete
Aug- Oct 16	Funding sourced Safety committee PICF / CTA	
Jun 2017	HREC submission	
Dec 2017	Commence enrolment UHG ICU	
Dec 2018	Complete enrolment	
Jan 2019	Primary outcome complete Initial BMDs complete	
Mar 2019	Data analysis **Primary manuscript preparation** Decision regarding expansion to stage 2 trial	
Aug 2019	Second dose intervention complete	
Jan 2020	Second BMD and BTM complete	

6. SAFETY OF SUBJECTS

As this is a pilot study, adverse events will be monitored throughout the trial by study investigators on a case-by-case basis. All adverse events and serious adverse events related to the trial intervention will be reported to the trial co-ordinating centre. Consistent with other studies in critically ill patients, adverse events already defined and reported as study outcomes will not be reported a second time as serious adverse events. Adverse events and serious adverse events;

Adverse events;

- General: Abdominal pain, arthralgia, back pain, pain in extremity
- Electrolyte disturbance: Hypocalcaemia
- Dermatological: Eczema, dermatitis, rash

Serious adverse events:

- Severe hypocalcaemia (ionized calcium < 0.90 mmol/L)
- Osteonecrosis of the jaw
- New Infections; Skin (erysipelas, cellulititis), abdominal, urinary tract, respiratory, bacteraemia, sepsis or septic shock.

7. DATA MANAGEMENT

Trained staff using a paper source document will collect all data. Data will be entered into a Barwon Health Redcaps database designed by the investigators. Randomised patients will be followed up to death or 12-months post-randomisation (whichever occurs first). Data collection will be restricted primarily to those variables necessary to define clinical patient characteristics including: baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events (SAE). Patients and/or their legal surrogate will be asked to provide three possible points of contact (home and close family contact details) to the research staff prior to hospital discharge. Full protocol data will be collected in all patients including those excluded at any stage.

8. SAMPLE SIZE AND STATISTICAL METHODS

Based on the fracture post-ICU and BMD post ICU studies, women aged 55yr and older are at risk of increased bone loss. UHG ICU admitted 6500 women, aged 55 yr and older, between 1998-2016. This represents an annual incidence of 0.1% of the total population. When extrapolated to Australia, this is 23,000 women per annum. Furthermore, emerging evidence suugests that anti resorptive therapy for osteoporosis is associated with a survival benefit. The Boland meta-analysis suggesting that the greatest benefit was among those with a baseline mortality rate of > 10: 1000 p-y, substantially less than the observed mortality rate among ICU survivors of 20% at one year. Within our prospective data, we have not been able to undertake further analysis, to identify a high risk subgroup because of sample size limitations, nor have we been able to identify any female participants aged 55 yr and older who did not experience accelarated bone loss.

The principal aim of this study is to detect the change in the bone resorption marker CTX in participants receiving denosumab compared to those receiving placebo. A prospective RCT conducted in 20 postmenopausal females with chronic critical illness administered 3mg ibandronate intravenously compared to placebo, and followed patients for 14-days. They observed a 34% decrease in serum CTX levels on day 6 compared to a 13% increase in the placebo group. By day 11 there was no difference [78]. A large RCT of denosumab for fracture prevention in women with osteoporosis reported a median decrease of serum CTX of 86% at 1-month compared to placebo [80]. In our prospective study of bone turnover markers and BMD in ICU survivors, we reported a median CTX of 654 [IQR 479–1165 ng/] at baseline, and 315 [162-592 ng/L] at 1-year in female participants, with a population median of 338 ng/L (IQR 212–499) [17].

Given these results we believe a clinically significant effect of denosumab is a 50% reduction in median serum CTX from baseline levels to day 28, compared to no change in the placebo group. A sample size of 7 patients per group will provide a 95% power (2 sided p-value of 0.05) to detect a difference in serum CTX from day 0 to day 28 equal to 2 standard deviations, and an 80% power (2 sided p-value of 0.05) to detect a difference equal to 1.5 standard deviations. With a predicted 20% rate of drop-out or death from enrolment to the 28-day primary outcome time-point, a sample size of 18 participants is required. This figure equates to

the anticipated enrolment over a 12-month period at the principal study site.

All data will be assessed for normality. Continuously normally distributed data will be reported as mean (±standard deviation), whereas non-parametric data will be reported using median (interquartile range [IQR]) or frequency distribution. Where normality exists, the primary and secondary outcomes will be analysed using paired t-tests, with a two-sided p-value of 0.05 considered to be statistically significant. Where changes in outcome are found to be non-symmetrical, Wilcoxon sign rank tests will be employed. Due to small sample size, multivariate analysis will not be performed.

9. ETHICAL CONSIDERATIONS

The observational component of the trial involves collection of bone turnover markers and BMD assessment. Patients will have initial blood tests performed while ventilated and sedated, while subsequent blood tests and both BMD assessments will be performed after participants have regained the ability to consent and understand the implications of enrolment. The interventional aspect of the trial has additional considerations. Firstly, patients with indications for antiresorptive agents will be excluded from the interventional arm and offered treatment according to current guidelines. The remaining population will be asked to participate in the intervention arm of the trial. The use of denosumab or placebo is justifiable as the consequences of accelerated bone loss in a high-risk population of ICU survivors are substantial. This is a study conducted in patients who are unconscious and unable to consent to participation; therefore, the patient's legal surrogate will be approached to provide consent for the patient. Patients who recover sufficient cognition to understand the explanation of the study will additionally be asked to consent to continue in the trial. Approval for this protocol will be sought from appropriate regulatory authorities, and from participating hospitals' human research ethics committees.

10. FEASIBILITY

The investigators have a track record in critical care and osteoporosis research, and have conducted the only long-term assessment of bone turnover in survivors of critical illness. We recruited 138 patients, including 69 females, into a prospective observational BMD study over a 4-year period, averaging approximately 16 female participants per year. Given this believe we will achieve enrolment over a 12-month period.

11. FUNDING

Funding for this trial has been obtained from two funding sources.

1. **Intensive Care Foundation Research Grant**: In October 2016, the study was successful in an application for **$14,638**
2. **UHG ICU Research Fund:** The UHG ICU will provide additional support for this study from operating budget.

Table 5: Study funding outline

Expenses	Per-patient	Pilot study
Enrolment		18
P1NP,CTx,VitD	$137	$2,466
FSH/LH	$140	$2,520
Week 1		
P1NP,CTx,VitD	$137	$2,466
Denosumab	$200	$1,800
ICU discharge/ day 7		
BMD1	$136	$2,448
1-month		
P1NP,CTx,VitD	$137	$1,918
6-months		
P1NP,CTx,VitD	$137	$1,370
Denosumab	$200	$1,800
1-year		10
P1NP,CTx,VitD	$137	$1,370
BMD2	$136	$1,360
Research Co-ord		
8 hrs per patient	$320	$5,760
Statistics	-	-
Pharmacy	-	-
Meetings/support		-
Total	**$2,517**	**$25,528**
Income		
ICF grant		$14,638
ICU research fund		$10,890
Total		**$25,528**

*Assumes 20% dropout/death from enrolment to 28-day primary outcome measure.

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Chapter 8: Conclusion – Current understanding of critical illness associated bone loss

8.1 Introduction

The aims of this thesis were to examine the evidence for accelerated bone turnover following critical illness, observe the change in bone mineral density and fracture risk following critical illness and, if justified, propose an interventional trial of anti-fracture therapy. This thesis has achieved these aims, and significantly added to the body of literature relating to critical illness associated bone loss that has emerged over the last decade. This conclusion will summarise the current evidence regarding critical illness associated bone loss, highlighting the relevant contribution of material from this thesis.

8.2 Bone turnover markers and critical illness

Bone metabolism occurs continuously, with a cycle of formation and resorption that is closely regulated and influenced by mechanical and biochemical factors[1]. Over the last two decades, the ability to measure the rate and direction of bone metabolic activity has become possible through the development of commercially available bone turnover marker (BTM) tests[2]. These tests are divided into two categories, measures of bone resorption and measures of bone formation, and provide a mechanism to predict the rate of bone loss, subsequent fracture risk, and treatment response in clinical trials [3,4].

The systematic review performed at the beginning of this thesis identified 10 studies that described BTMs as an outcome in critically ill adults ventilated for greater than 24-hours[3]. Overall these studies consistently report an association between critical illness and BTMs, including an increase in osteoclastic bone resorption markers, increase in immature osteoblast number and activity, and reduced activity of mature osteoblasts. The existing literature explored the relationship between duration of critical illness, sepsis, inflammation, loss of hypothalamic-pituitary axis pulsatility, and increase in BTMs. However, these relationships are only partially understood, with the confounding effects of premorbid disease, organ failure, and medications incompletely addressed [3].

A number of studies have since added to the evidence of association between critical illness and change in bone turnover markers. Our prospective observational study of critically ill adults described an increase in the bone resorption marker serum collagen type 1 cross-linked c-telopeptide (CTX) during ICU admission, with median levels greater than the upper quartile normal range, returning to normal by 1-year. In contrast the bone formation marker

type 1 N-terminal procollagen (P1NP), was within normal limits during ICU admission and at 1-year, although median levels significantly increased during this time [4]. The VITdAL-ICU study [5], an interventional vitamin D3 randomised controlled trial in critically ill adults, reported increased CTX levels from day 1 of enrolment in ICU to day 28, normalising by 6-months. Levels of osteocalcin (OC), a bone formation marker, were decreased during day 1 to 28, and normalized by 6-months. A prospective observational study of 28 adults with prolonged critical illness reported elevated CTX levels in 45% of patients at admission, increasing to over 80% of patients in week 1 and 2, and over 50% of patients at week 5. In contrast, P1NP levels were reduced in 55% of patients at admission to ICU, and 10% of patients by week 5[6]. A randomised controlled trial of ibandronate compared to placebo in 20 postmenopausal women with an ICU length of stay of greater than 5-days, reported increased serum CTX levels and reduced OC levels at entry into the study [7]. Finally, a prospective observational study reported serum sclerostin levels at admission and at 1-week, in 264 critically ill adults admitted to a medical ICU [8]. Sclerostin is a protein produced exclusively in the skeleton by osteocytes, and a key negative regulator of bone formation. It acts through inhibition of the Wnt pathway, inhibiting terminal differentiation of osteoblasts and promoting apoptosis[2]. Overall serum sclerostin levels were increased on admission to ICU compared to controls and increased further over the following week. Levels varied with severity of illness, with significantly higher levels in patient with an APACHE II score greater than 20 compared to less than 20. Increased levels were associated with the presence of liver cirrhosis and end stage renal disease.

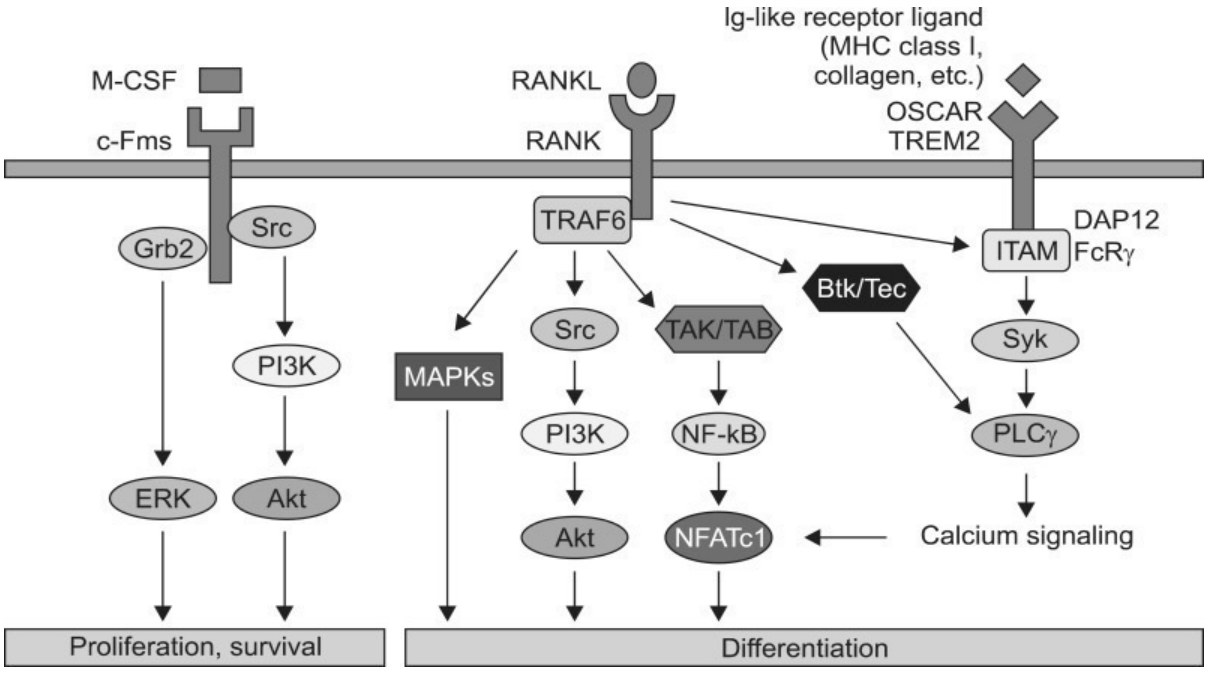
There are a number of questions remaining about the use and understanding of BTMs in critically ill populations. Firstly, the limitations to measurement and interpretation of BTMs in the critical care setting are unresolved. These include the effect of pre-analytic variation due to nutrition delivery, body fluid compartment changes, organ dysfunction, circadian rhythm, and changes to protein binding [2]. Secondly, the relationship between elevated BTMs and both subsequent fracture risk [2] and mortality [9,10], both described in non-critically ill patients, has not been established.

In summary the pattern of BTMs observed during and after critical illness is consistent with uncoupling of bone formation and resorption. This is characterised by accelerated bone loss beginning early in critical illness, persisting for weeks to months, and normalising over the following year. In contrast, bone formation remains within normal limits. Although there are associations with duration of critical illness, sepsis, and inflammation, more data is required to understand the magnitude and duration of change, effect of confounding factors, and effect of critical illness on measurement.

8.3 Pre-clinical studies

Over the last 5-years animal and in-vitro studies have begun to explore the mechanistic model of critical care associated bone loss. A human in vitro model described a number of critical illness related osteoclast and angiogenic abnormalities[10]. They reported an increase in peripheral blood mononuclear cells (PBMCs) primed to differentiate into mature osteoclasts in blood of critically ill patients. The activity of mature osteoclasts was dependent on the presence of the circulating humoral factors RANKL (receptor activator of nuclear factor kappa-B ligand) and M-CSF (macrophage colony-stimulating factor). This activity was not suppressed by anti-cytokine (IL-6 and TNF-α) antibodies, but was suppressed through blocking FcRIII, an immunomodulatory tyrosine-based activation motif (ITAM) receptor (Fig 1) [10,11]. This suggests immunomodulatory factors may have an important role in critical illness osteoclast differentiation through non-canonical pathways. Finally, in a murine bone model, critical illness was associated with reduced angiogenesis factor expression, reduced mature bone formation and reduced new vasculature formation.

Figure 1: Osteoclast differentiation pathways (*Kim JH and Kim N. CMJ 2016* [11])



The same authors reported the effects of a rabbit burn model of critical illness on bone biochemistry and histomorphometry[12]. Critical illness was associated with a reduction in OC, decreased bone formation, and decreased trabecular tibial bone content and density.

Surprisingly, there was no difference in number and activity of osteoclasts compared to healthy controls. There was a decrease in early and late markers of osteoblast differentiation, and decreased expression of angiogenesis markers. Assessment of osteoclastogenesis found significant decrease in gene expression in the canonical pathway of RANKL, a trend to decreased osteoprogeterin (OPG) gene expression, with an unchanged OPG/RANKL ratio. In addition, gene expression of the non-canonical ITAM signalling pathway FcRIII and DAP12 receptors was significantly increased. This supports the hypothesis that FcRIII positive monocytes, driven by circulating humoral factors and or IgG antibodies through non-canonical ITAM signalling pathways, are an important pathway for osteoclastogenesis in critical illness, rather than canonical RANKL/OPG pathways.

A final animal model of critical illness bone loss study reported the mechanical, microCT, and bone histomorphometry effects in a rat model of sepsis at 24 and 96 hours[13]. Bone mechanical testing revealed no difference in femoral shaft strength, with a significantly decreased femoral neck fracture load. A rapid decrease on collagen elastic modulus occurred, with slower decrease in mineral elastic modulus, was observed. Interestingly, bone architecture and bone mineral density were unchanged, as was morphometry. These findings suggest an early increase in bone fragility due to altered bone biochemistry, rather than osteoclast driven bone turnover.

Overall these studies provide evidence of the pathway for differentiation of PMNCs into mature active osteoclasts, and the relative role and interaction of canonical pathways, requiring the presence of RANKL or M-CSF, and non-canonical immunomodulatory ITAM pathways. They provide evidence of impaired angiogenesis and bone vascularisation, and varied observations of reduced bone strength and mass during critical illness. The exact role of biochemical changes to bone, changes to osteoclast and osteoblast number, maturation, and activity, and relative contributions of humoral and cytokine pathways, remains unresolved.

8.4 Bone mineral density and critical illness

The measurement of BMD by dual energy x-ray absorptiometry (DXA) at the proximal femur and lumbar spine forms the basis of assessment and treatment of osteoporosis, with change in BMD estimated to account for 60-80% of variance in bone strength, and the central component of internationally agreed definitions of osteoporosis[14]. BMD values in individuals are expressed as an absolute value (g/cm^2), and in relation to a reference young adult population in standard deviation (SD) units, the T-score[15], the basis of the WHO operational

definition[16] of osteoporosis (T-score <-2.5) and osteopaenia (T-score -2.5 to -1.0). In addition, BMD is used to assess response to treatment, and as an outcome in anti-fracture trials.

Over the last 5-years a number of studies have reported BMD measurements in 5 separate cohorts of critically ill patients. The prospective longitudinal cohort studied as part of this thesis remains the most comprehensive description of BMD trajectory after critical illness[4,17]. We reported significantly greater annual decrease in BMD in patients ventilated for greater than 24 hours who survived to ICU discharge, particularly women, compared to age and sex matched population controls[4] (Table 1). At ICU discharge, 45% of all patients were osteopaenic or osteoporotic, increasing to 55% at 1-year, with an increased proportion in women (ICU discharge 57%, 1-year 67%). The only other study to report T-score classification, the VITdAL-ICU study[5], found 55% of all patients were osteoporotic or osteopaenic at 6-month follow-up.

Table 1: Annualised percent change in bone mineral density after critical illness compared to matched Geelong Osteoporosis Study (GOS) controls [18]

Variable	ICU	GOS	Difference (95% CI)	P-value
All	n=66	n=256		
AP spine	-1.48 (4.37)	0.11 (1.12)	-1.59 (-2.18, -1.01)	<0.001
Femur	-1.72 (3.43)	-0.53 (1.07)	-1.20 (-1.69, -0.70)	<0.001
Women	n=31	n=120		
AP spine	-2.85 (4.05)	-0.18 (1.08)	-2.67 (-3.49, -1.86)	< 0.001
Femur	-1.96 (4.03)	-0.65 (0.98)	-1.31 (-2.10, -0.51)	0.001
Men	n=35	n=136		
AP spine	-0.28 (4.34)	0.36 (1.10)	-0.64 (-1.45, 0.17)	0.12
Femur	-1.52 (2.85)	-0.42 (1.13)	-1.10 (-1.7, -0.49)	<0.001

Data are shown as mean ± SD unless otherwise indicate

Participants not receiving anti-fracture treatment who completed two-year post-ICU follow-up experienced ongoing loss of bone mass. In women this was less in year 2 than year 1 at both sites (femur year 1 -2.8 ± 1.3% vs year 2 -1.9 ±0.7, p=0.6, spine year 1 -4.8±1.4% vs year 2 -1.3±1.8%, p=0.08). In men the annual decrease in femur BMD was significantly greater in year 2 than year 1 (femur year 1 -1.9 ± 0.7% vs year 2 -3.2 ± 0.7%, p = 0.03), with no difference in annual spine BMD change between year 1 and year 2 (spine year 1 0.0±

1.2% vs year 2 0.9 ± 1.5%, p = 0.6) [17].

No significant change in calcaneal BMD over a 10-day period was reported in a prospective study of critically ill patients expected to be ventilated for greater than 48 hours. However, a significant decrease in BMD was observed in patients with severe lung injury compared to ventilated control patients (-2.81% vs +2.40%, p=0.03)[19]. A large retrospective cohort study of critically ill patients with an ICU length of stay greater than 24 hours, that compared outcomes of patients who received bisphosphonate therapy prior to critical illness, reported an overall -13±19% decrease in BMD per week [20].

Finally, our nested cohort study of critically ill women in the Geelong Osteoporosis Study (GOS) [18], found bone health prior to critical illness was comparable to controls, despite greater overall loss of bone mass at AP spine than femoral neck. Surprisingly, there was a relative increase in femoral neck bone mass in a matched sub-group in the two-years prior to critical illness.

Overall these studies describe accelerated loss of bone mass during and after critical illness, with the effect persisting for up to 2-years. In addition, a high proportion of patients are ostepaenic or osteoporotic after ICU, suggesting a disease burden that may contribute to long-term morbidity and mortality. Finally, understanding the factors that influence the trajectory of bone mass before and after critical illness is not understood, partly due to the inherent difficulty performing long-term research in critically ill populations.

8.4 Critical illness associated fragility fracture

The major consequence of accelerated bone loss is increased risk of fragility fracture, and this has been described in two studies[21,22]. We described an increased risk of fragility fracture following critical illness, compared to age and gender matched population controls from the GOS [21]. The radiological databases of adult patients ventilated for greater than 24-hours who survived to ICU discharge were assessed for evidence of fragility fracture using the same ascertainment period as the GOS. In the ICU survivor cohort, followed for a median of 3.7 years, 36 women (14.2%) sustained a fracture during the post-ICU time period, and incident fracture rate of 3.84 (ICU survivors) and 2.41 (GOS controls) per 100 patient-years respectively. In older women who survived ICU a significant increase in fracture rate, and decreased time to fracture were observed compared to controls (HR 1.65 95%CI 1.08-2.52) (p = 0.02).

A retrospective study of patients admitted to ICU with a length of stay greater than 7-days, followed 178 patients to 2-year follow-up, and age and gender matched non-critically ill patients undergoing operations[22]. At 2-years the clinical fracture rate was 5% in the ICU group, compared to 3.4% in the control group, with all fracture associated with falls. The risk of new fracture was 50% higher in the ICU cohort, although this was not significant (OR 1.53, 95% CI 0.62,3.77, p=0.35). The major limitation was the fracture ascertainment method, which involved a phone call to the patient's local medical office, with no patient interview, or radiological ascertainment of morphological vertebral or clinical fractures.

In 2013, an analysis of annual osteoporosis and fracture rates was published in Osteoporosis Australia [23]. In the Australian community 71% of women aged 50 years or older were osteopaenic or osteoporotic, with an annual total fracture rate of 2.7%, vertebral fracture rate of 0.5%, and hip fracture rate of 0.4%. The Geelong ICU longitudinal BMD and fracture studies reveal 80% of women aged 50 years or older were osteopaenic or osteoporotic in the year after ICU, with a total fracture rate of 6.0%, vertebral fracture rate of 3.2%, and hip fracture rate of 0.9% (Table 2).

Table 2: Comparison of bone health and therapy in women aged ≥ 50 years after critical illness to Australian population [4,23] [21]

Variable	Post-ICU Year 1	Australia annual rate
T-score		
Osteoporosis	36%	23%
Osteopaenia	44%	48%
Normal	20%	29%
Cumulative annual fracture rate		
Hip	0.9%	0.4%
Vertebral	3.2%	0.5%
Wrist	0%	0.5%
Other	1.9%	1.2%
Total	6%	2.7%

In summary the evidence regarding fragility fracture rates after critical illness, is limited, although the higher quality study reports increased rates of fragility fracture, particularly in the highest risk group of older women. Confirmation of post-critical illness fracture rates through larger database linkages is needed.

8.5 Mortality associated with accelerated bone loss

Osteoporosis is associated with increased mortality, with evidence the common pathways shared by osteoporosis and atherosclerosis are associated with altered regulation of inflammation, innate immunity, apoptosis, and blocking of maturation and activity of osteoclast precursors, and increased all-cause and cardiovascular mortality [9,24-27]. The association between elevated bone turnover markers, bone loss, and mortality has been reported in varied populations, including cancer[9], older ambulatory women[28], and patients undergoing coronary angiogram[26,27]. Also, fragility fractures are associated with increased mortality. Age-adjusted mortality rates were increased for men and women for all ages and all fractures except for minor fractures, for which increased mortality was only apparent for those older than 75 years. Increased mortality persisted for 5-years for all fractures and up to 10-years for hip fractures. In women the increases in absolute mortality above expected levels ranged from 1.32 to 13.2 per 100 person-years[29].

This association is strengthened by evidence of reduced mortality associated with anti-fracture therapy. A meta-analysis of RCTs investigating anti-fracture agents for prevention of vertebral and non-vertebral fractures found treatment was associated with an 11% reduction in mortality in over 1400 deaths in approximately 40,000 subjects[30]. In studies with higher baseline mortality (greater than 10 per 1000 patient years), a 17% risk reduction was observed. This effect appeared to be similar across the different classes of agents in the study. In addition, a prospective cohort study reported a reduction in mortality rates in community-based women and men receiving bisphosphonates in propensity score adjusted analyses[31].

Currently there is no evidence of an association between abnormal bone turnover markers, reduced bone mass, or fragility fracture and increased mortality following critical illness. There is limited evidence describing an association between anti-fracture therapy and reduced mortality, and this will be discussed in the next section[20,32]. The intersection of inflammatory and immune disturbance, high baseline mortality, and retrospective evidence of mortality benefit in anti-fracture users, suggest accelerated bone turnover may be associated with increased mortality following critical illness.

8.6 Anti-fracture therapies and critical illness.

The use of anti-fracture therapy after critical illness is very uncommon, with only 4% of ICU

survivors treated at 1-year and 16% at 2-years, despite treating physician awareness of bone density results[29]. This combination of high prevalence and low treatment rates, suggests critically ill post-menopausal women are under-diagnosed, under-treated, and may benefit from anti-fracture therapy.

Three studies have reported the effects of treating vitamin D deficiency in critically ill patients on bone turnover. A comparison of parenteral vitamin D 200 IU or 500 IU daily in long-term surgical ICU patients receiving parenteral nutrition, found higher dose vitamin D was associated with a relatively small increase in serum OC, and a decrease in serum CTX, but did not affect other BTMs. In addition, the decrease in inflammatory markers IL-6 and C-reactive protein over time was more pronounced with the higher dose vitamin D [33]. However, in a cohort of 55 ventilator dependent chronic critically ill patients, treating vitamin D deficiency with calcitriol did not lead to a reduction in bone resorption markers [34]. Finally, the posthoc analysis of the VITdAL-ICU study[5] reported no effect of high-dose vitamin D3 (540,000 IU) compared to placebo on 6-month serum OC, sclerostin, or CTX in 289 adult critically ill patients.

Bisphosphonates bind to bone and suppress bone resorption by entering osteoclasts and inhibiting the enzyme farnesyl pyrophosphate synthase, resulting in disruption of osteoclast attachment to bone surface[30]. This class of agents are effective at reducing bone loss and vertebral and non-vertebral fractures, associated with reduced mortality, and recommended as first line agents in treatment of osteoporosis[31-33]. A retrospective survey described a significant reduction in urine NTX over an 18-day period in ventilator dependent chronic critically ill patients when oral pamidronate was added to calcitriol alone[34]. A prospective trial that randomised 20 postmenopausal women requiring greater than 5-days of mechanical ventilation to a single dose of intravenous ibandronate versus placebo, reported a significant decrease in bone resorption. However, this was transient, with serum CTX levels returning to baseline by day 11. There was no difference in serum OC levels between the ibandronate and placebo groups at day 11, although levels increased in both groups during the study. This suggests a lack of effect of ibandronate on bone formation, and a gradual increase in osteoblast activity in both groups [7].

Two studies have described an association between bisphosphonate use and mortality in critically ill patients. A retrospective case series of 148 patients with chronic critical illness compared outcomes of patients receiving pamidronate (n=118) to those who did not (n=30) [32]. A lower ICU (0% vs 19%, p=0.008) and 1-year mortality (20% vs 56%, p=0.004) was

reported with pamidronate, and this remained significant after adjustment for renal function and calcium levels. However, this study was limited by the single-centred, unblinded, retrospective design, and lack of information about confounders including pre-existing risk factors, ICU severity illness, and ICU interventions.

A retrospective propensity-matched cohort study described outcomes in 245 patients who had received bisphosphonates in the 5-years prior to an ICU admission of greater than 24-hour, compared to ICU patients who did not receive bisphosphonates. After matching for age, sex, comorbid disease, principal diagnosis, and year of admission, bisphosphonate use was associated with a significant decrease in hospital mortality rate ratio (MRR 0.39, 95% CI 0.22-0.67, $p<0.01$). In addition, a subgroup analysis of 37 patients from the bisphosphonate group who underwent serial CT scans were compared to 74 matched non-bisphosphonate patients. The bisphosphonate users had lower baseline bone density, with a significant attenuation of rate of vertebral bone loss compared to controls (-3 $\pm$ 13% vs -15 $\pm$ 14% per week, $p<0.01$) [20].

Finally, we reported the association between anti-fracture medications and bone loss in the two-years after critical illness [17]. Over the 2-year period after critical illness 11% of participants were prescribed anti-fracture therapies, including alendronate, denosumab, strontium ranelate, and risedronate. In women the use of anti-fracture therapy was associated with a significant difference in post-ICU annual change of BMD, with an increase in BMD in participants who received anti-fracture medication compared to a decrease in those that did not. In men no association between anti-fracture therapy use and annual change in BMD was observed.

Overall there is limited evidence suggesting benefit from bisphosphonates and other anti-fracture agents in terms of attenuated bone loss and mortality. Due to methodological limitations the overall benefit, as well as specific risk and benefit related to agent, dose, duration, timing, subgroups, and duration, remain unclear.

8.7 Next steps

Survivors of critical illness are at increased risk of accelerated bone loss, fragility fracture, and associated mortality. Currently there is no routine intervention provided to prevent this, and limited evidence that anti-fracture agents may be of benefit. The two anti-fracture agents best suited to administration in critical illness are zoledronic acid and denosumab.

The class effects of zoledronic acid, a bisphosphonate have been described in the previous section. Denosumab, a fully human monoclonal antibody directed against RANKL, is the first biologic therapy approved to treat osteoporosis. It is metabolised by organ-independent intra-cellular mechanisms, administered subcutaneously 6-monthly, a potent inhibitor of osteoclast activity, and effective at reducing bone loss and fragility fractures[31,32]. The indications for use include prevention of bone loss and fractures in osteoporosis, bone metastases from solid tumours, men with prostate cancer and androgen deprivation, and women with breast cancer receiving aromatase inhibitors [31-34]. The RANKL antagonist effects of denosumab may result in inflammatory, cardiovascular and cancer benefits, with associated reduction in mortality [35,36] and disease-free survival [36].

Both denosumab and zoledronic acid are suitable target interventions to study in critical illness. Both agents are likely to be effective at reducing bone resorption and preventing fragility fracture, and both agents show potential for extra-skeletal benefit including reduced mortality. The fracture reduction effect is likely to maximally benefit post-menopausal critically ill women, due to the high baseline risk. The immune, cardiovascular and mortality effects, if present, may benefit all critically ill patients, although there is currently insufficient evidence to justify administration for this purpose.

As part of this thesis we have designed and commenced a pilot safety and efficacy RCT of denosumab compared to placebo in post-menopausal critically ill women. Depending on the results of this study, a three-arm trial comparing denosumab, zoledronic acid, and placebo, with the primary aim to assess the effect of anti-fracture agents on vertebral fracture rate in the high-risk population of post-menopausal women with prolonged ventilation, may be warranted. A study design allowing for a secondary comparison of the two anti-fracture agents for superiority in fracture prevention, and health economic analysis comparing the two-agents to each other and to placebo would be ideal. Finally, comparison of the effects of the two agents on mortality would provide valuable high-quality data to justify and guide further RCTs of either agent in a broader ICU population, with mortality as a primary outcome.

8.8 Conclusion

We understand more about critical illness associated bone loss than we did a decade ago. There is increasing and consistent evidence of abnormal bone metabolism during critical illness, with a pattern of early increased bone resorption and suppression of bone formation that persists for up to a month, and changes to normal bone resorption and increased

formation over the subsequent year. There is evidence of skeletal impact of increased bone turnover associated with critical illness, with loss of bone mineral density and increased fracture risk in subsequent years. There is preliminary evidence that anti-fracture interventions may be effective at attenuating bone loss, and reducing mortality, after critical illness.

There are important gaps in our knowledge, questions we need to answer. The first is the contribution of pre-critical illness factors, critical illness factors, and recovery factors, to post critical illness bone health remains unclear. To separate out the major influences on critical illness related bone health will require larger participant cohorts, preferably with prospectively collected pre-critical illness data, a major challenge for critical care research in general. This is important, as identification of time and magnitude of skeletal insults may provide opportunities to intervene.

The second is our understanding of biological pathways involved in bone loss, the interaction of cytokine pathways with bone turnover, and the non-skeletal effects of activation of bone metabolism, are only beginning to be understood. Further investigation of these mechanisms, in both animal and human settings, may provide crucial information to guide interventions that alter both bone and inflammatory outcomes.

Finally, the prospect of reducing bone loss, fractures, and possibly mortality, in critically ill adults through ICU based anti-fracture interventions, is an intriguing area to investigate in future phase II and phase III randomised controlled trials. With careful design, these trials may also provide answers to the first two questions.

8.9 References

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Appendices - Ethics Approvals

FILE COPY

RESEARCH AND ETHICS ADVISORY COMMITTEE

[redacted]

Barwon Health

The Geelong Hospital,
Ryrie Street P.O. Box 281
Geelong Victoria 3220

ETHICS COMMITTEE APPROVAL STATEMENT

Project Number	**06/06**
Site	Barwon Health
Principal Investigator:	**Dr Neil Orford**
Title:	**Skeletal morbidity among survivors of critical illness**
Co investigators	A/Prof Mark Kotowicz, Dr peter Stow
Student names	Marissa Danials

Thankyou for submitting your application with the Research and Ethics Advisory Committee.

Full approval was granted on 17/05/2006

In addition any items approved in support of this project are listed below:

	Date Approved	*Item*		*Document Date*
1	17/05/2006	Participant Questionnaire: Protocol: Title: Long term skeletal sequelae of accelerated bone resorption in survivors of prolonged critical illness – A preliminary audit.		17/05/2006
2	17/05/2006	Participant Information & Consent	Ver 2	15/02/2006

Approval is granted on the basis that accurate documentation of the consent process is recorded in the partcipant's hospital history and that a photostated copy of the consent form is also placed in the hospital history.

I have attached a current list of the REAC Members at the date of the last meeting for your information. The Research and Ethics Advisory Committee is constituted and operates in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Research involving Humans.(1999).

All Research Projects approved by REAC must comply with the Section "Monitoring", which requires Human Research Ethics Committees (HRECs) to monitor research projects to which they have given ethical approval in order to ensure that they conform to the protocol approved.

The guidelines detailed below are the current methods and approach used by the REAC to monitor activities of research projects.

The Barwon Health Research and Ethics Advisory Committee (REAC) operates in accordance to guidelines established by the National Health and Medical Research Council, National Statement on Ethical Conduct in Research Involving Humans (June 1999).

02 March 2017

Attention:	Ms. Alison Bone ICU Clinical Research Nurse Intensive Care, Barwon Health
***HREC Number*:**	17/12
***Title*:**	Effect of denosumab on bone turnover markers in critically ill women - a safety and feasibility, randomised, placebo controlled trial
***Principal Investigator*:**	Associate Professor Neil Orford

Participant Information

Age:	≥50 years	**Gender:**	Female
Life expectancy:	> 5 years	**No. at Barwon Health:**	18

Duration of participation in research

Average Transit Time:	12 months
Maximum Transit Time:	12 months

The Radiation Safety Section, Victorian Department of Health & Human Service (DH&HS) stipulates compliance with the Code of Practice for the Exposure of Humans to Ionizing Radiation for Research Purposes[1] and the DH&HS guidance on risk statements[2]. For projects in which the research participant receives an exposure to ionising radiation beyond that considered normal care of the condition being treated, the Code requires an independent dose assessment be undertaken by a medical physicist.

This study is assessing whether the administration of denosumab to critically ill post-menopausal women will effectively attenuate bone loss associated with critical illness. The project requires that the volunteer participants have Bone Mineral Densitometry of the lumbar spine and femoral neck upon discharge from hospital and a repeat scan 12 months later.

It has been advised by the principal researcher that this imaging involves radiation exposure that is considered above standard care for these participants. The total effective dose received by each participant for imaging that is not part of their standard care will be approximately 0.25 millisieverts (mSv).

The Code specifies dose constraints, which should be met wherever possible, for radiation exposure that is **additional** to standard care. The total effective dose for adults should not exceed 5 mSv in any one year or 10 mSv over five years. In this project, all participants are listed as being over 50 years of age. The total effective dose of 0.25 mSv does **not** exceed the dose constraint of 5 mSv per year. This radiation dose falls within Category IIa, which represents a **very low** level of risk. The Code provides information on this risk category:

> *Category IIa (risk less than 1 in 10,000) represents a very low level of risk. The dose range of 0.2 to 2 mSv covers the allowable annual dose to the public from controlled sources. To justify risks in this category the benefit will probably be related to increases in knowledge leading to health benefit.*

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date:	**29 June 2011**
Project Number:	**2011000985**
Project Title:	**A prospective, observational study of bone mineral density following critical illness**
Chief Investigator:	**Dr Neil Orford**
Approved:	**From: 29 June 2011 To: 29 June 2016**

Terms of approval

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation. **Failure to provide permission letters to MUHREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research**.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. **Complaints:** The researchers are required to inform MUHREC promptly of any complaints made about the project, whether the complaint was made directly to a member of the research team or to the primary HREC.
6. **Amendments to the approved project (including changes in personnel):** Requires the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]

Professor Ben Canny
Chair, MUHREC

cc: Assoc Prof Mark Kotowicz, Assoc Prof Julie Pasco, Dr Margaret Henry, Prof Rinaldo Bellomo, Assoc Prof Michael Bailey, Prof Jamie Cooper

[redacted]