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TITLE PAGE

1. Title: Changes in bone mineral density in the year after critical illness

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10. **"At a Glance Commentary"**
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    - **What This Study Adds to the Field:** We report a significant decrease in bone density in the year after ICU admission compared with controls, 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.

11. **"This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org"**

ABSTRACT (249 words)

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

Objectives: To measure the change in bone mineral density and bone turnover markers in patients one year after critical illness compared to population-based controls.

Methods: We studied adult patients admitted to a tertiary intensive care unit (ICU) and requiring mechanical ventilation for at least 24 hours. We measured clinical characteristics, bone turnover markers and bone mineral density during admission and one year after ICU discharge. We compared change in bone mineral density to age and sex-matched controls from the Geelong Osteoporosis Study.

Measurements and Main Results: Sixty-six patients completed bone mineral density testing. Bone mineral density decreased significantly in the year after critical illness at both femoral neck and anterior-posterior spine site. The annual decrease was significantly greater in the ICU cohort compared to matched controls (anterior-posterior spine -1.59%, 95% CI -2.18, -1.01, p< 0.001, femoral neck -1.20%, 95% CI -1.69, -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).

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

The pattern of bone resorption markers was consistent with accelerated bone turnover.

Conclusions: Critically ill patients experience a significantly greater decrease in bone mineral density in the year after admission compared to population-based controls. Their bone turnover biomarkers pattern is consistent with increased rate of bone loss.

Keywords: Critical illness, long-term outcomes, osteoporosis, fracture, bone loss, bone mineral density, bone turnover markers.

INTRODUCTION

Compared to their pre-illness status and general population controls, 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 where 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 wrist fracture is 30-40% in developed countries, and the lifetime risk of hip fracture is one in six in white females[18], with significant associated health burden of mortality, morbidity, and cost[19,20]. However, as few as 13-27% of patients with osteoporosis are treated following a fragility fracture, suggesting osteoporosis remains an under diagnosed 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 fracture 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

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describe the changes in BMD and bone turnover markers (BTMs) in men and women in the year after critical illness, compared to population based age and sex controls.

MATERIALS AND 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. Prior to commencement approval was obtained from the Barwon Health Human Research Ethics Committee. Written informed consent was obtained prior to inclusion in the study. Participants were compared to matched population based controls from the Geelong Osteoporosis Study.

Study population and controls

Adult (age greater than 20 years) patients admitted to the ICU during the study period, and with duration of mechanical ventilation greater than 24 hours were considered eligible for enrolment in the study. The control population was obtained from the Geelong Osteoporosis Study (GOS)[35], a random population-based sample from the Australian Commonwealth Electoral Rolls. Additional details on exclusion criteria and GOS are provided in an online data 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 (EQ VAS; EuroQol visual analogue scale where own health 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 detail on the measurement of serum BTMs and BMD are provided in an online data 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, accommodation). Details of operating procedure are provided in Appendix 1.

BMD was presented as an absolute value (g/cm^2), annualised percentage (difference between BMDs divided by initial 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 [37] with cut-off 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 World Health Organization (WHO)[40] that combines clinical risk factors with or without femur BMD to estimate 10-year probability of hip and major osteoporotic fracture.

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Outcomes

The primary outcome was annualised percentage change in BMD (lumbar spine and dual femoral neck) for the year after ICU discharge compared to matched population controls. 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 controls by age, sex, and BMI, in a one-to-four fashion using Mahalanobis weights, without replacement. Continuously normally distributed data were reported as mean (±standard deviation), whereas non-parametric data were reported using median (interquartile range [IQR]) or frequency distribution. 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. The primary outcome was analysed using Analysis of Covariance, and a two-sided p-value of 0.05 was considered to be statistically significant. Additional information relating to statistical analyses are provided in an online data supplement.

RESULTS

Patient enrolment

We screened 686 patients 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%) for

analysis (Figure 1).

Baseline characteristics

Baseline patient 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%) of patients having at least one factor.

In the 66 patients 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 females at both sites, and in males at femoral neck only (Table 2). The mean baseline BMD of the ICU cohort and controls were similar after matching at both dual femur and anterior-posterior spine (supplement table 1).

Primary outcome

The annual decrease in BMD was significantly greater in the ICU cohort compared to matched controls (Table 3). The annual decrease in BMD in the year after ICU was 1.48 $\pm$ 4.37% (anterior-posterior spine) and 1.72 $\pm$ 4.37% (femoral neck), compared to an increase of 0.11$\pm$ 1.12% (anterior-posterior spine) and a decrease of 0.53 $\pm$ 1.07% (femoral neck) in controls. This represents a difference in annual change in BMD of -1.59% (95% CI -2.18, -1.01, $p < 0.001$) at anterior-posterior spine, and -1.20% (95% CI -1.69, -0.70, $p < 0.001$) at femoral neck in the ICU cohort compared to controls. This

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difference was significantly greater in females at both AP spine and femoral neck, while in males it was significantly greater at 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 [397,894] vs 306 ng/L [202,554]; p<0.001), and a significant increase in serum type 1 N-terminal procollagen (median 31.0 ug/L [20.5, 49.8] vs 44.0 ug/L [31.2,73.9]; p=0.04) (Table 4). When stratified by sex and compared to population reference levels, the median collagen type 1 cross-linked c-telopeptide levels exceeded the 3rd interquartile reference values for females (ICU 654.0 ng/L [IQR 478.5, 1165] vs GOS 338 ng/L [IQR 212, 499] and males (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 males and females (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 [31.0,52.8] vs 55.0 mmol/L [46.0,71.0], p<0.001]), elevated median parathyroid hormone levels at baseline that did not significantly change (8.1 pmol/L [4.0,13.8] vs 5.4 pmol/L[4.2,9.4], p=0.15), and a significant increase in median adjusted calcium levels (2.0 mmol/L [1.8,2.1]) vs 2.2 {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 one-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 females and 44.1% of males were classified as either osteoporotic or osteopenic at 1-year, and the 10-year fracture risk was highest in females.

DISCUSSION

Key findings

We performed a prospective observational study in critically ill mechanically ventilated patients to measure the changes in BMD and bone turnover markers in the year after ICU admission. We found that these patients experienced a significant decrease in BMD in the year after ICU admission, and this was significantly greater than matched controls. Moreover, they carried a significantly increased estimated risk of future fracture. These clinical events were associated with a specific pattern of bone turnover markers. 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 ICU described a significant decrease in calcaneal BMD over a 10-day period in patients with Acute Respiratory Distress Syndrome (ARDS) compared to ventilated non-ARDS patients[33] . However, BMD undergoes relatively small changes over time of a magnitude similar to measurement error therefore

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

follow-up over longer periods is recommended, making a one year change in BMD the standard for interventional research studies[41-45]. This makes our observations relevant to potential intervention in the future.

Bone turnover markers 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 bone turnover markers 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 reporting increased bone resorption markers [24,26,29-31,48,49], increased serum osteoclast precursors[32], increased bone formation, and decreased osteocalcin during critical illness compared to controls [26,28,29,49]. Overall these findings suggest changes associated with critical illness of a magnitude similar to that described in postmenopausal females, or metabolic bone disease [29,47,50,51]. The interpretation of bone turnover markers during critical illness is complex, however as levels are affected by a number of factors including age, gender, co-existing disease, inflammatory markers, and medications, particularly glucocortocoids [26,28,49,52,53]. This study was unable to analyse 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 [54-64]. We observed a pattern of vitamin D insufficiency, increased parathyroid hormone release, and hypocalcaemia, during critical illness and normalisation at one-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 bone turnover markers, suggesting that accelerated bone turnover is multifactorial [29,48].

Over half of survivors in our study had BMD in the osteopenic or osteoporotic range, higher than local population levels, with the Geelong Osteoporosis Study reporting one-fifth of females greater than fifty years of age have BMD in the osteopenic range, and 1 in 6 have osteoporosis[65]. Although the proportion of patients 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, as over half of fragility fractures arise from the population of individuals with osteopenia, rather than the higher risk but smaller population with osteoporosis[65-67]. 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 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 females

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

following critical illness compared to population-based controls [14].

Study implications

This study implies that critical illness is associated with increased bone turnover, loss of BMD and increased risk of fragility fractures for up to one year after ICU discharge. Such reduced bone mass and increased bone loss in the year following critical illness, particularly in female survivors, has not been previously described and further implies that such patients may be at particular risk. Moreover, in such female patients it also implies that anti-resorptive 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 towards, the design of pilot interventional trials aimed at decreasing bone resorption using BMD at one year as the primary outcome. The notion that intervention may be effective is supported by short term studies such as a retrospective case series describing a reduction in urine N-telopeptide in long-term ventilated patients treated with pamidronate[48], and a randomised trial of 20 chronic critically ill postmenopausal females reporting a decrease in serum collagen type 1 cross-linked c-telopeptide and increase in osteocalcin with ibandronate compared to placebo[68]. In addition multimodal therapies, including physical therapies and rehabilitation warrant investigation in this population[69].

Strengths and limitations

Our study has several strengths. For the first time, we collected detailed information, including risk factors, relating to bone mass in a cohort of ICU survivors over a prolonged period. This is important as 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 patients for one 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 females. Finally, and also for the first time, we compared our cohort with age and sex matched controls 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 restricts 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 patients to a population-based control group rather than a hospital-based control group means we cannot exclude that hospitalisation per se (instead of ICU admission alone) may be associated with increased loss of BMD. However, the decrease in BMD seen in these patients constitutes the necessary initial evidence to conduct such larger studies. Measurement of BMD at ICU

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discharge and again at one-year does not allow for the observation of non-linear changes in bone density, for example it is possible an early rapid loss of bone density occurred in the months after critical illness, with subsequent recovery. However one year change in BMD is the standard for interventional research studies[41-45].

Also, we have demonstrated an association between critical illness and greater decline in BMD, but 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 patients, and are associated with trajectory and degree of recovery [70-73]. The matching of the ICU cohort and the GOS controls by gender and age resulted in similar baseline BMDs, refuting the notion that the ICU cohort had lower bone density than controls at the time of admission. This may be explained by the characteristics of the cohort that completed the study. By definition they had 100% one-year survival, reported high quality of life, and 95% lived independently one-year after ICU discharge. This pattern is most consistent with a minimal disability functional trajectory, or a non-frail population [70,71]. Finally although long-term ICU recovery trials would ideally include prospectively collected pre-ICU trajectory data [73-75], critical illness may act as a marker for under-diagnosed disease burden irrespective of causality [73].

Conclusions

We performed a prospective observation study in critically ill mechanically ventilated patients to measure the changes in BMD and bone turnover

markers in the year after ICU admission. We found that these patients experienced a significant decrease in bone density in the year after ICU admission compared with controls; 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.

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

REFERENCE

1. Chelluri L, Im KA, Belle SH, et al. Long-term mortality and quality of life after prolonged mechanical ventilation*. *Critical Care Medicine*. 2004;32(1):61-69. doi:10.1097/01.CCM.0000098029.65347.F9.

2. Garland A, Olafson K, Ramsey CD, Yogendran M, Fransoo R. Distinct determinants of long-term and short-term survival in critical illness. *Intensive Care Medicine*. 2014;40(8):1097-1105. doi:10.1007/s00134-014-3348-y.

3. Williams TA, Dobb GJ, Finn JC, et al. Determinants of long-term survival after intensive care*. *Critical Care Medicine*. 2008;36(5):1523-1530. doi:10.1097/CCM.0b013e318170a405.

4. Lown DJ, Knott J, Rechnitzer T, Maclsaac C. Predicting short-term and long-term mortality in elderly emergency patients admitted for intensive care. *Crit Care Resusc*. 2013;15(1):49-55.

5. Needham DM, Wozniak AW, Hough CL, et al. Risk Factors for Physical Impairment after Acute Lung Injury in a National, Multicenter Study. *American Journal of Respiratory and Critical Care Medicine*. 2014;189(10):1214-1224. doi:10.1164/rccm.201401-0158OC.

6. Dowdy DW, Eid MP, Sedrakyan A, et al. Quality of life in adult survivors of critical illness: A systematic review of the literature. *Intensive Care Medicine*. 2005;31(5):611-620. doi:10.1007/s00134-005-2592-6.

7. Herridge MS, Cheung AM, Tansey CM, et al. One-year outcomes in survivors of the acute respiratory distress syndrome. *N Engl J Med*. 2003;348(8):683-693. doi:10.1056/NEJMoa022450.

8. Cuthbertson BH, Scott JE, Strachan M, Kilonzo M, Vale L. Quality of life before and after intensive care. *Anaesthesia*. 2005;60:332-339.

9. Niskanen M, Ruokonen E, Takala J, Rissanen P, Kari A. Quality of life after prolonged intensive care. *Crit Care Med*. 1999;27:1132-1139.

10. Pandharipande PP, Girard TD, Jackson JC, et al. Long-Term Cognitive Impairment after Critical Illness. *N Engl J Med*. 2013;369(14):1306-1316. doi:10.1056/NEJMoa1301372.

11. Bienvenu OJ, Colantuoni E, Mendez-Tellez PA, et al. Cooccurrence of and remission from general anxiety, depression, and posttraumatic stress disorder symptoms after acute lung injury: a 2-year longitudinal study. *Crit Care Med*. 2015;43(3):642-653. doi:10.1097/CCM.0000000000000752.

12. Jackson JC, Hart RP, Gordon SM, Hopkins RO, Girard TD, Ely EW. Post-traumatic stress disorder and post-traumatic stress symptoms

following critical illness in medical intensive care unit patients: assessing the magnitude of the problem. *Crit Care*. 2007;11(1):R27. doi:10.1186/cc5707.

13. Bienvenu OJ, Colantuoni E, Mendez-Tellez PA, et al. Depressive symptoms and impaired physical function after acute lung injury: a 2-year longitudinal study. *Am J Respir Crit Care Med*. 2012;185(5):517-524. doi:10.1164/rccm.201103-0503OC.

14. 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.

15. 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.

16. Nguyen TV, Center JR, Eisman JA. Osteoporosis: underrated, underdiagnosed and undertreated. *Med J Aust*. 2004;180(5 Suppl):S18-S22.

17. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. *The Lancet*. 2002;359(9321):1929-1936. doi:10.1016/S0140-6736(02)08761-5.

18. Sambrook P, Cooper C. Osteoporosis. *The Lancet*. 2006;367(9527):2010-2018. doi:10.1016/S0140-6736(06)68891-0.

19. Pasco JA, Sanders KM, Hoekstra FM, Henry MJ, Nicholson GC, Kotowicz MA. The human cost of fracture. *Osteoporosis International*. 2005;16(12):2046-2052. doi:10.1007/s00198-005-1997-y.

20. Abimanyi-Ochom J, Watts JJ, Borgström F, et al. Changes in quality of life associated with fragility fractures: Australian arm of the International Cost and Utility Related to Osteoporotic Fractures Study (AusICUROS). *Osteoporosis International*. June 2015:1-10. doi:10.1007/s00198-015-3088-z.

21. Andrade SE, Majumdar SR, Chan A, et al. Low Frequency of Treatment of Osteoporosis Among Postmenopausal Women Following a Fracture. *Archives of internal medicine*. 2003;163:2052-2057.

22. Vestergaard P, Rejnmark L, Mosekilde L. Osteoporosis is markedly underdiagnosed: a nationwide study from Denmark. *Osteoporosis International*. 2004;16(2):134-141. doi:10.1007/s00198-004-1680-8.

23. Nierman DM. Bone Hyperresorption Is Prevalent in Chronically Critically Ill Patients. *Chest*. 1998;114(4):1122. doi:10.1378/chest.114.4.1122.

24. Lind L, Carlstedt F, Rastad J, et al. Hypocalcemia and parathyroid

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

hormone secretion in critically ill patients. *Crit Care Med*. 2000;28(1):93-99.

25. Nierman DM. Biochemical Response to Treatment of Bone Hyperresorption in Chronically Critically Ill Patients*. *Chest*. 2000;118(3):761. doi:10.1378/chest.118.3.761.

26. Van den Berghe G, Baxter RC, Weekers F, et al. The combined administration of GH-releasing peptide-2 (GHRP-2), TRH and GnRH to men with prolonged critical illness evokes superior endocrine and metabolic effects compared to treatment with GHRP-2 alone. *Clin Endocrinol (Oxf)*. 2002;56(5):655-669.

27. Five-Day Pulsatile Gonadotropin-Releasing Hormone Administration Unveils Combined Hypothalamic-Pituitary- Gonadal Defects Underlying Profound Hypoandrogenism in Men with Prolonged Critical Illness*. June 2001:1-10.

28. Van den Berghe G, Wouters P, Weekers F, et al. Reactivation of Pituitary Hormone Release and Metabolic Improvement by Infusion of Growth Hormone- Releasing Peptide and Thyrotropin-Releasing Hormone in Patients with Protracted Critical Illness*. *J Clin Endocrinol Metab*. 1999;84:1311-1323.

29. Van den Berghe G. Bone Turnover in Prolonged Critical Illness: Effect of Vitamin D. *Journal of Clinical Endocrinology & Metabolism*. 2003;88(10):4623-4632. doi:10.1210/jc.2003-030358.

30. Smith LM, Cuthbertson B, Harvie J, Webster N, Robins S, Ralston SH. Increased bone resorption in the critically ill: association with sepsis and increased nitric oxide production. *Crit Care Med*. 2002;30(4):837-840.

31. Shapses SA, Weissman C, Seibel MJ, Chowdhury HA. Urinary pyridinium cross-link excretion is increased in critically ill surgical patients. *Crit Care Med*. 1997;25(1):85-90.

32. Owen HC, Vanhees I, Solie L, et al. Critical illness-related bone loss is associated with osteoclastic and angiogenic abnormalities. *J Bone Miner Res*. 2012;27(7):1541-1552. doi:10.1002/jbmr.1612.

33. Rawal J, McPhail MJ, Ratnayake G, et al. A pilot study of change in fracture risk in patients with acute respiratory distress syndrome. *Crit Care*. 2015;19(1):165. doi:10.1186/s13054-015-0892-y.

34. Hollander JM, Mechanick JI. Bisphosphonates and metabolic bone disease in the ICU. *Current Opinion in Clinical Nutrition and Metabolic Care*. 2009;12(2):190-195. doi:10.1097/MCO.0b013e328321cda6.

35. Pasco JA, Nicholson GC, Kotowicz MA. Cohort profile: Geelong Osteoporosis Study. *International Journal of Epidemiology*. 2012;41(6):1565-1575. doi:10.1093/ije/dyr148.

36. Janssen MF, Pickard AS, Golicki D, et al. Measurement properties of the EQ-5D-5L compared to the EQ-5D-3L across eight patient groups: a multi-country study. *Qual Life Res*. 2013;22(7):1717-1727. doi:10.1007/s11136-012-0322-4.

37. Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: Synopsis of a WHO report. *Osteoporosis International*. 1994;4(6):368-381. doi:10.1007/BF01622200.

38. Henry MJ, Pasco JA, Pocock NA, Nicholson GC, Kotowicz MA. Reference ranges for bone densitometers adopted Australia-wide: Geelong osteoporosis study. *Australas Radiol*. 2004;48(4):473-475. doi:10.1111/j.1440-1673.2004.01351.x.

39. Henry MJ, Pasco JA, Korn S, Gibson JE, Kotowicz MA, Nicholson GC. Bone mineral density reference ranges for Australian men: Geelong Osteoporosis Study. *Osteoporos Int*. 2009;21(6):909-917. doi:10.1007/s00198-009-1042-7.

40. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX™ and the assessment of fracture probability in men and women from the UK. *Osteoporosis International*. 2008;19(4):385-397. doi:10.1007/s00198-007-0543-5.

41. Cawthon PM, Ewing SK, Mackey DC, et al. Change in hip bone mineral density and risk of subsequent fractures in older men. *J Bone Miner Res*. 2012;27(10):2179-2188. doi:10.1002/jbmr.1671.

42. Berger C, Langsetmo L, Joseph L, et al. Association Between Change in BMD and Fragility Fracture in Women and Men*. *J Bone Miner Res*. 2009;24(2):361-370. doi:10.1359/jbmr.081004.

43. Bruyere O, Varela AR, Adami S, et al. Loss of hip bone mineral density over time is associated with spine and hip fracture incidence in osteoporotic postmenopausal women. *Eur J Epidemiol*. 2009;24(11):707-712. doi:10.1007/s10654-009-9381-4.

44. Nguyen TV, Center JR, Eisman JA. Femoral Neck Bone Loss Predicts Fracture Risk Independent of Baseline BMD. *J Bone Miner Res*. 2005;20(7):1195-1201. doi:10.1359/JBMR.050215.

45. Hillier TA, Stone KL, Bauer DC, et al. Evaluating the value of repeat bone mineral density measurement and prediction of fractures in older women: the study of osteoporotic fractures. *Archives of internal medicine*. 2007;167(2):155-160. doi:10.1001/archinte.167.2.155.

46. for the IOF-IFCC Bone Marker Standards Working Group, Vasikaran S, Eastell R, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*. 2010;22(2):391-420.

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

doi:10.1007/s00198-010-1501-1.

47. Nishizawa Y, Ohta H, Miura M, et al. Guidelines for the use of bone metabolic markers in the diagnosis and treatment of osteoporosis (2012 edition). *J Bone Miner Metab*. 2012;31(1):1-15. doi:10.1007/s00774-012-0392-y.

48. Nierman DM, Mechanick JI. Biochemical response to treatment of bone hyperresorption in chronically critically ill patients. *Chest*. 2000;118(3):761-766.

49. Van den Berghe G, Weekers F, Baxter RC, et al. Five-Day Pulsatile Gonadotropin-Releasing Hormone Administration Unveils Combined Hypothalamic-Pituitary- Gonadal Defects Underlying Profound Hypoandrogenism in Men with Prolonged Critical Illness*. *J Clin Endocrinol Metab*. 2001;86(7):3217-3226.

50. Vasikaran S, Cooper C, Eastell R, et al. International Osteoporosis Foundation and Interational Federation of Clinical Chemistry and Laboratory Medicine Position on bone marker standards in osteoporosis. *Clin Chem Lab Med*. 2011;49(8):1271-1274.

51. Delmas PD. *The Use of Biochemical Markers of Bone Turnover in the Management of Post-Menopausal Osteoporosis*. 2000.

52. Hlaing TT, Compston JE. Biochemical markers of bone turnover - uses and limitations. *Annals of Clinical Biochemistry: An international journal of biochemistry and laboratory medicine*. 2014;51(2):189-202. doi:10.1177/0004563213515190.

53. Kotowicz MA, Hall S, Hunder GG, Cedel SL, Mann KG, Riggs BL. Relationship of glucocorticoid dosage to serum bone Gla-protein concentration in patients with rheumatologic disorders. *Arthritis Rheum*. 1990;33(10):1487-1492.

54. Hu J, Luo Z, Zhao X, et al. Changes in the Calcium-Parathyroid Hormone-Vitamin D Axis and Prognosis for Critically Ill Patients: A Prospective Observational Study. Slominski AT, ed. *PLoS ONE*. 2013;8(9):e75441-e75445. doi:10.1371/journal.pone.0075441.

55. Nair P, Lee P, Reynolds C, et al. Significant perturbation of vitamin D–parathyroid–calcium axis and adverse clinical outcomes in critically ill patients. *Intensive Care Medicine*. 2012;39(2):267-274. doi:10.1007/s00134-012-2713-y.

56. Mata-Granados JM, Vargas-Vasserot J, Ferreiro-Vera C, de Castro MDL, Pavón RG, Gómez JMQ. Journal of Steroid Biochemistry and Molecular Biology. *Journal of Steroid Biochemistry and Molecular Biology*. 2010;121(1-2):452-455. doi:10.1016/j.jsbmb.2010.03.078.

57. Lee P, Eisman JA, Jacqueline R Center. Vitamin D Deficiency in

Critically Ill Patients. *N Engl J Med*. 2009;360(18):1912-1914.

58. Lucidarme O, Messai E, Mazzoni T, Arcade M, Cheyron D. Incidence and risk factors of vitamin D deficiency in critically ill patients: results from a prospective observational study. *Intensive Care Med*. 2010;36(9):1609-1611. doi:10.1007/s00134-010-1875-8.

59. Braun AB, Litonjua AA, Moromizato T, Gibbons FK, Giovannucci E, Christopher KB. Association of low serum 25-hydroxyvitamin D levels and acute kidney injury in the critically ill*. *Crit Care Med*. 2012;40(12):3170-3179. doi:10.1097/CCM.0b013e318260c928.

60. Quraishi SA, Bittner EA, Blum L, McCarthy CM, Bhan I, Camargo CA Jr. Prospective Study of Vitamin D Status at Initiation of Care in Critically Ill Surgical Patients and Risk of 90-Day Mortality*. *Critical Care Medicine*. 2014;42(6):1365-1371. doi:10.1097/CCM.0000000000000210.

61. Braun AB, Gibbons FK, Litonjua AA, Giovannucci E, Christopher KB. Low serum 25-hydroxyvitamin D at critical care initiation is associated with increased mortality*. *Crit Care Med*. 2012;40(1):63-72. doi:10.1097/CCM.0b013e31822d74f3.

62. Matthews LR, Ahmed Y, Wilson KL, Griggs DD, Danner OK. Worsening severity of vitamin D deficiency is associated with increased length of stay, surgical intensive care unit cost, and mortality rate in surgical intensive care unit patients. *Am J Surg*. 2012;204(1):37-43. doi:10.1016/j.amjsurg.2011.07.021.

63. Amrein K, Schnedl C, Holl A, et al. Effect of High-Dose Vitamin D 3on Hospital Length of Stay in Critically Ill Patients With Vitamin D Deficiency. *JAMA*. September 2014. doi:10.1001/jama.2014.13204.

64. Quraishi SA, De Pascale G, Needleman JS, et al. Effect of Cholecalciferol Supplementation on Vitamin D Status and Cathelicidin Levels in Sepsis. *Critical Care Medicine*. 2015;43(9):1928-1937. doi:10.1097/CCM.0000000000001148.

65. Pasco JA, Seeman E, Henry MJ, Merriman EN, Nicholson GC, Kotowicz MA. The population burden of fractures originates in women with osteopenia, not osteoporosis. *Osteoporosis International*. 2006;17(9):1404-1409. doi:10.1007/s00198-006-0135-9.

66. Henry MJ, Pasco JA, Nicholson GC, Kotowicz MA. Prevalence of osteoporosis in Australian men and women: Geelong Osteoporosis Study. *Med J Aust*. 2011;195(6):321-322. doi:10.5694/mja11.10571.

67. Pasco JA, Lane SE, Brennan SL, et al. Fracture risk among older men: osteopenia and osteoporosis defined using cut-points derived from female versus male reference data. *Osteoporos Int*. 2014;25(3):857-862. doi:10.1007/s00198-013-2561-9.

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

68. Via MA, Potenza MV, Hollander J, et al. Intravenous Ibandronate Acutely Reduces Bone Hyperresorption in Chronic Critical Illness. *Journal of Intensive Care Medicine*. 2012;27(5):312-318. doi:10.1177/0885066611402156.

69. Bonaiuti D, Shea B, Iovine R, et al. *Exercise for Preventing and Treating Osteoporosis in Postmenopausal Women*. (Bonaiuti D, ed.). Chichester, UK: John Wiley & Sons, Ltd; 1996. doi:10.1002/14651858.CD000333.

70. Ferrante LE, Pisani MA, Murphy TE, Gahbauer EA, Leo-Summers LS, Gill TM. Functional Trajectories Among Older Persons Before and After Critical Illness. *JAMA Intern Med*. 2015;175(4):523-527. doi:10.1001/jamainternmed.2014.7889.

71. Bagshaw SM, Stelfox HT, Johnson JA, et al. Long-Term Association Between Frailty and Health-Related Quality of Life Among Survivors of Critical Illness. *Critical Care Medicine*. 2015;43(5):973-982. doi:10.1097/CCM.0000000000000860.

72. Puthucheary ZA, Denehy L. Exercise Interventions in Critical Illness Survivors: Understanding Inclusion and Stratification Criteria. *American Journal of Respiratory and Critical Care Medicine*. 2015;191(12):1464-1467. doi:10.1164/rccm.201410-1907LE.

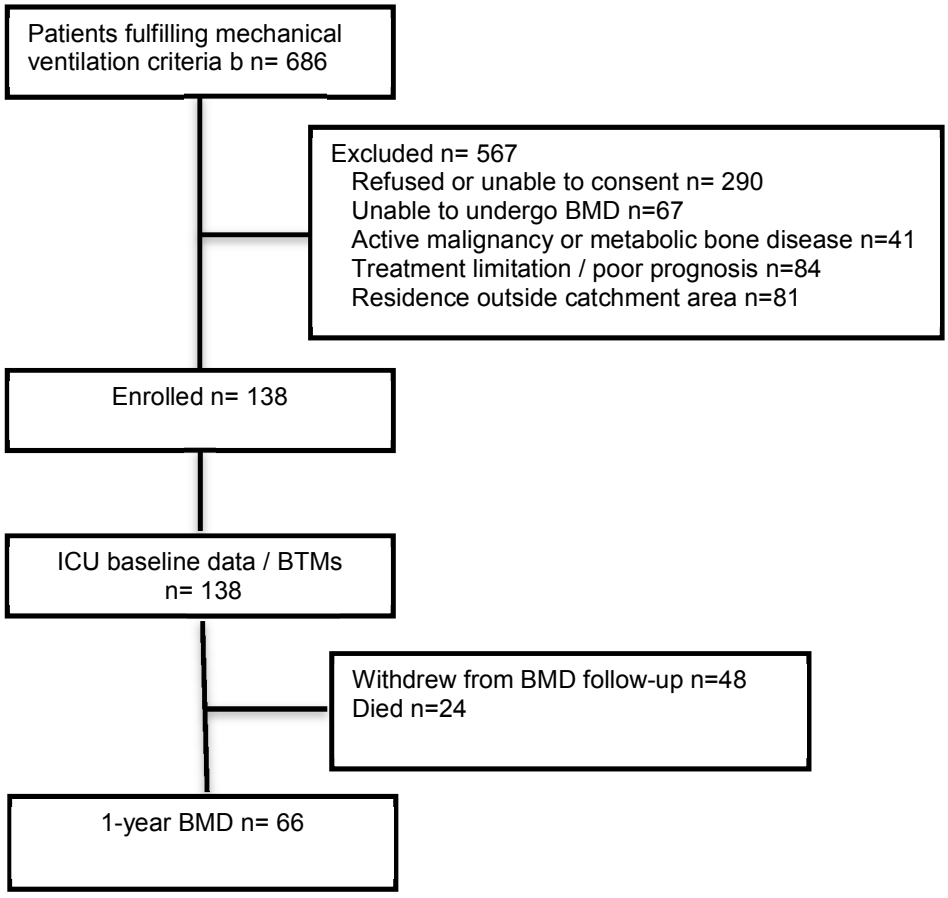
73. Iwashyna TJ, Netzer G, Langa KM, Cigolle C. Spurious inferences about long-term outcomes: the case of severe sepsis and geriatric conditions. *Am J Respir Crit Care Med*. 2012;185(8):835-841. doi:10.1164/rccm.201109-1660OC.

74. Iwashyna TJ. Trajectories of recovery and dysfunction after acute illness, with implications for clinical trial design. *Am J Respir Crit Care Med*. 2012;186(4):302-304. doi:10.1164/rccm.201206-1138ED.

75. Rubenfeld GD. Does the hospital make you older faster? *Am J Respir Crit Care Med*. 2012;185(8):796-798. doi:10.1164/rccm.201202-0267ED.

76. Jenkins N, Black M, Paul E, Pasco JA, Kotowicz MA, Schneider HG. Age-related reference intervals for bone turnover markers from an Australian reference population. *Bone*. 2013;55(2):271-276. doi:10.1016/j.bone.2013.04.003.

Figure 1: Summary of eligibility, enrolment, and long-term follow-up for study procedures



AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

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 (yrs)	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 (7.6)	2 (3.0)	5 (6.9)
Rheumatoid arthritis	3 (2.2)	2 (3.0)	1 (1.4)
Alcohol > 3 units/day	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 one osteoporosis risk factor	58 (42.0)	23 (34.8)	35 (48.6)
Co-morbidity			
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 (umol/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 (ug/L)	23.0 [19.0, 27.0]	24.0 [20.0, 28.0]	22.0 [18.0, 27.0]
Interventions/outcomes			
Ventilation duration (hrs)	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 (days)	7.0 [4.2, 13.0]	6.5 [4.0, 9.0]	7.5 [5.0, 15.0]
Hospital LOS (days)	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-year status			
Survival	114 (82.6)	66 (100)	48 (66.7)
Living at home	-	60 (95.2%)	-
EQ VAS	-	80 [70, 90]	-

1. Data are shown as mean (±standard deviation), median [interquartile range] or number (%).
2. Abbreviations: BMI (Body Mass index), APACHE = acute physiology and chronic health evaluation; CRRT (continuous renal replacement therapy), LOS (length of stay), PTH = parathyroid hormone, CTX = collagen type 1 cross-linked c-telopeptide, P1NP = type 1 N-terminal procollagen
3. Reference ranges: vitamin D (<25nmol/L = deficient, 25-50 nmol/L insufficient, >50 nmol/L sufficient), PTH (range 1.6-6.9 pmol/L

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

Table 2: Changes in bone mineral density at 1-year follow-up after critical illness

BMD (g/cm2)	Baseline	1-year	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
Female (n=31)[3]			
Dual Femur	0.892 (0.165)	0.872 (0.161)	0.02
AP Spine	1.183 (0.223)	1.142 (0.223)	0.001
Male (n=35)[3]			
Dual Femur	1.015 (0.197)	0.999 (0.202)	0.002
AP Spine	1.264 (0.236)	1.260 (0.246)	0.74

1. Data are shown as mean (±standard deviation),
2. Abbreviations: BMD = bone mineral density, AP = anteroposterior.
3. 35 males completed both AP spine BMDs, 34 completed both femur BMDs. 31 females completed both AP spine BMDs, 30 completed both femur BMDs.

Table 3: Annualised change in bone mineral density after critical illness compared to matched Geelong Osteoporosis Study controls

Variable	ICU	GOS	Difference (95% CI)	P-value
All	(n=66)[#]	(n=256)		
Total change AP spine	-0.018 (0.055)	0.001 (0.013)	-0.019 (-0.026, -0.012)	< 0.001
Percent change AP spine	-1.48 (4.37)	0.11 (1.12)	-1.59 (-2.18, -1.01)	< 0.001
Total change Femur	-0.016 (0.032)	-0.005 (0.010)	-0.011 (-0.016, -0.007)	< 0.001
Percent change Femur	-1.72 (3.43)	-0.53 (1.07)	-1.20 (-1.69, -0.70)	< 0.001
Female	(n=31)[3]	(n=120)		
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
Male	(n=35)[3]	(n=136)		
Total change AP spine	-0.003 (0.055)	0.005 (0.014)	-0.007 (-0.018, 0.003)	0.16
Percent change AP spine	-0.28 (4.34)	0.36 (1.10)	-0.64 (-1.45, 0.17)	0.12
Total change Femur	-0.015 (0.027)	-0.004 (0.011)	-0.010 (-0.016, -0.005)	0.001
Percent change Femur	-1.52 (2.85)	-0.42 (1.13)	-1.10 (-1.71, -0.49)	< 0.001

1. Data are shown as mean (±standard deviation)
2. Abbreviations: ICU = Intensive Care Unit, GOS = Geelong Osteoporosis Study, BMD = bone mineral density, AP = anteroposterior.
3. 35 males completed both AP spine BMDs, 34 completed both femur BMDs. 31 females completed both AP spine BMDs, 30 completed both femur BMDs.

Table 4: Changes in biochemistry and bone turnover markers, T-score, and fracture risk, at 1-year follow-up after critical illness

	All (n=66)			Female cohort (n=31)			Male cohort (n=35)		
Variable	Baseline	1-year	P-value	Baseline	1-year	P-value	Baseline	1-year	P-value
Biochemistry and BTMs[3]									
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 (umol/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, 1165]	315 [162, 592]	0.001	483 [382, 851]	305 [230, 542]	0.002
P1NP (ug/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[4]									
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-year risk[4]									
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

1. Data are shown as mean (±standard deviation), median [interquartile range] or number (%)
2. Abbreviations: BTM = bone turnover markers, BMD = Bone Mineral Density, PTH = parathyroid hormone, CTX = collagen type 1 cross-linked c-telopeptide, P1NP = type 1 N-terminal procollagen, FRAX®=Fracture risk assessment tool.
3. GOS Reference Values[76]: Female median P1NP 37ug/L (IQR 26, 51 ug/), CTx 338 ng/L (IQR 212, 499 ng/L), male median P1NP 37ug/L (IQR 27, 49 ug/L), CTx 328 ng/L (IQR 235, 459 ng/L)
4. Reference ranges: vitamin D (<25nmol/L = deficient, 25-50 nmol/L insufficient, >50 nmol/L sufficient), PTH (range 1.6-6.9 pmol/L)
5. 34 males completed both femur BMDs, 30 females completed both femur BMD

AJRCCM Articles in Press. Published on 11-November-2015 as 10.1164/rccm.201508-1514OC

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

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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.

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

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REFERENCES

1. Henry MJ, Pasco JA, Seeman E, et al. Assessment of fracture risk: value of random population-based samples--the Geelong Osteoporosis Study. *J Clin Densitom*. 2001;4(4):283-289.

2. Jenkins N, Black M, Paul E, Pasco JA, Kotowicz MA, Schneider HG. Age-related reference intervals for bone turnover markers from an Australian reference population. *Bone*. 2013;55(2):271-276.

3. Stuart EA. Matching methods for causal inference: A review and a look forward. *Stat Sci*. 2010;25(1):1-21.