AWARD NUMBER: W81XWH-14-2-0136

TITLE: Vitamin D Supplementation for Prevention of Post-Traumatic Osteoarthritis: Evaluation in Animal and Clinical Models

PRINCIPAL INVESTIGATOR: Jennifer Moriatis Wolf, MD

CONTRACTING ORGANIZATION: University of Connecticut
Farmington, CT 06032

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
**Title and Subtitle:** Vitamin D Supplementation for Prevention of Post-Traumatic Osteoarthritis: Evaluation in Animal and Clinical Models

**Authors:** Jennifer Wolf

**Performing Organization:**
- University of Connecticut
  - Farmington, CT 06032

**Sponsoring Agency:**
- U.S. Army Medical Research and Materiel Command
  - Fort Detrick, Maryland 21702-5012

**Availability Statement:** Approved for Public Release; Distribution Unlimited

**Abstract:** Attached
Abstract

The goals of this translational study are to create an animal model of joint injury and evaluate the impact of Vitamin D in prevention and progression of PTOA. Concurrently, we will evaluate a clinical cohort of USMA cadets treated for ACL tear, with pre- and post-injury serum 25-hydroxy-Vitamin D levels and correlation with joint space narrowing and biomarkers of cartilage injury. If Vitamin D supplementation can prevent the onset of often rapid joint destruction that occurs with PTOA, this simple and safe intervention could potentially translate to pre-emptive treatment in high-risk military occupations. In addition, Vitamin D could be used at the time of injury to possibly mitigate ongoing articular cartilage damage.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>4</td>
</tr>
<tr>
<td>2. Keywords</td>
<td>4</td>
</tr>
<tr>
<td>3. Overall Project Summary</td>
<td>4</td>
</tr>
<tr>
<td>4. Key Research Accomplishments</td>
<td>13</td>
</tr>
<tr>
<td>5. Conclusion</td>
<td>13</td>
</tr>
<tr>
<td>7. Inventions, Patents and Licenses</td>
<td>13</td>
</tr>
<tr>
<td>8. Reportable Outcomes</td>
<td>13</td>
</tr>
<tr>
<td>9. Other Achievements</td>
<td>13</td>
</tr>
<tr>
<td>10. References</td>
<td>13</td>
</tr>
<tr>
<td>11. Appendices</td>
<td>14</td>
</tr>
</tbody>
</table>
Introduction

The purpose of this study is to create an animal model of joint injury and evaluate the impact of Vitamin D supplementation in prevention and progression of post-traumatic osteoarthritis (PTOA). Concurrently, this funding supports an add-on study at the United States Military Academy, to evaluate a clinical cohort of USMA cadets treated for anterior cruciate ligament (ACL) tear, with pre- and post-injury serum 25-hydroxy-Vitamin D levels and correlation with joint space narrowing and biomarkers of cartilage injury. If Vitamin D supplementation can prevent the onset of often rapid joint destruction that occurs with PTOA, this simple and safe intervention could potentially translate to pre-emptive treatment in high-risk military occupations. In addition, Vitamin D could be used at the time of injury to possibly mitigate ongoing articular cartilage damage.

Keywords

Murine, post-traumatic osteoarthritis, military, ACL, knee, medial meniscus, femoral, tibial, 25-hydroxy-Vitamin D, supplementation

Overall Project Summary

This report represents the first annual summary of work for the 2014-15 year of funding for this project. Reporting will be organized by task as noted in the Statement of Work.

Specific Aim 1: to evaluate the impact of systemic Vitamin D supplementation on the initiation and development of surgically induced OA in a murine model

Objectives: Vitamin D Supplementation and Rodent Surgery

Imaging/Tissue Analysis of Surgical Model

Progress

• Institutional IACUC and federal ACURO applications were submitted and approved, respectively, in May and June of 2014.
• We initiated the animal model using C57-Bl6 mice fed with four types of supplemented chow:
  o 1.5IU/kg Vitamin D (minimal Vitamin D - control)
  o 1,500 IU/kg Vitamin D (regular food)
  o 5,000 IU/kg Vitamin D
  o 10,000 IU/kg Vitamin D
• We pretreated mice with these feeding regimens for 2 weeks and obtained blood for 25-hydroxy-Vitamin D analysis.
• We then performed destabilization of the medial meniscus (DMM)\(^1\) surgery (with sham surgery on the opposite leg as control) to surgically initiate osteoarthritis in each feeding subgroup, and sacrificed mice at 4, 8, 12, and 16 weeks.
We also obtained blood for 25-hydroxy-Vitamin D analysis in each group prior to sacrifice.

We then performed histology, faxitron Xray imaging, and selected micro-CT analysis of the murine. We chose to perform histology as opposed to immunohistochemistry as the first round was to determine optimal timepoints and surgical technique to initiate OA reliably.

A group of experienced animal histology investigators performed a blinded rating of the degree of osteoarthritis of the murine knee histology using the Glasson scale.²

**Results**

Using ELISA, we evaluated differential levels of circulating 25-hydroxy-Vitamin D in each of the 4 groups of mice fed different levels of Vitamin D in feed over time, and noted initial increase in circulating 25-hydroxy-Vitamin D levels that differed by feeding dose, with metabolic equilibration over time. While high doses of Vitamin D have been previously shown to be well-tolerated in mice,³ the findings of metabolic equilibration over time have not been previously reported. In males, the dose-response from minimal to high levels was shown best at 2 and 4 weeks; we did not have data on females in this group at 2 weeks.

We also tested Vitamin-D binding protein (DBP), which binds Vitamin D metabolites in plasma up to a certain species-specific level. It has been shown that free Vitamin D metabolites are active, and thus once DBP binding is maximized, the free metabolite levels will increase.⁴ Our results showed the highest levels of DBP in the mice given minimal Vitamin D, with DBP decreasing as supplementation increased.
Histology imaging with Glasson grading\textsuperscript{2} showed minimal signs of osteoarthritis overall, but particularly at the 4 and 8 weeks timepoints. This calls into question the reliability of DMM alone to induce osteoarthritis.

- At 12 and 16 weeks, we noted more consistent signs of osteoarthritis.
Both views above in 16 week mice, top 1.5IU Vitamin D; bottom 1500 IU Vitamin D supplementation.

Faxitron imaging showed progressive signs of osteoarthritis over time, but again increased most at 12 and 16 weeks:

4 and 8 week views with DMM (surgical) limb on left, sham surgery on right; for comparison, all are of male mice with 1500 IU (normal) feed levels.
12 and 16 week radiographs on left and right respectively. Note the osteophytes on both left murine legs in these radiographs, as shown in this magnified example:
In performing Glasson rating of osteoarthritis across all four groups with Vitamin D supplementation, results showed the following:

- Overall minimal induction of osteoarthritis in the earlier timepoints
- No correlation between Vitamin D supplementation and osteoarthritis in male or female mice at 4 or 8 weeks.

- In female mice at 12 and 16 weeks, ratings showed decreased OA histologically on the tibial side at 12 weeks and on both the tibial and femoral sides at 16 weeks.
We did not observe this effect in male mice, as shown below:
Micro-CT analysis was performed only in the 16 week subset to evaluate the qualitative degree of osteoarthritis visible. This modality is highly sensitive, as it shows osteophytes and joint irregularity clearly:

Accomplishments

- Established animal model with successful supplementation of Vitamin D via feed – but need to refine surgical technique and timepoints to most accurately induce and measure osteoarthritis at baseline. We have submitted a proposal to IACUC and ACURO to drop the 4 and 8 week timepoints and use 12, 16, and 20 weeks; and to add ACL transection to a subgroup of mice (to be evaluated at 8 weeks) in addition to DMM technique.
- Established reliable histology and Glasson measurement techniques
• We have some exciting potential evidence of Vitamin D mitigation of OA in female animals.

Specific Aim 2: To evaluate the serum 25-hydroxy-Vitamin D status of military cadets before and after ACL injury and reconstruction and correlate these findings with biomarkers of articular cartilage injury as well as radiographic joint space narrowing.

Objectives: Initiation of Add-on to Existing Study
Subject Enrollment/Specimen and Data Collection

Progress

• We obtained Keller Army Hospital and UConn Institutional Review Board (IRB) approval in October 2014 to add-on to the existing study of ACL tears in United States Military Academy (USMA) cadets and biomarkers for initiation of PTOA. Our IRB approval allows us to also measure 25-hydroxy-Vitamin D levels in pre-injury, at-injury, at-surgery, and post-surgical serum samples from USMA subjects.
• To date, study participation is as follows per Dr. Cameron (USMA PI):
  o 63 ACL injured cadets screened
  o 36 ACL injured cadets enrolled in study; this is on target for 90-100 cadets to be enrolled over three years.
    o Matched control subjects are also enrolled for each ACL injured case.
• We will not perform Vitamin D testing until we have reached target enrollment, both for reliability of testing (batched testing is much more comparable) and budget costs.

Results/Accomplishments reporting is deferred pending further enrollment for this segment of the study.

Problems/Changes

• Based on the observations of minimal osteoarthritis in the early timepoints, we plan to address this with two changes:
  o Investigation of the impact of adding anterior cruciate ligament (ACL) transection to the DMM model on development of OA – to be evaluated at 8 weeks
  o Deleting early timepoints of 4 and 8 weeks, and addition of one additional timepoint in order to evaluate mice at 12, 16, and 20 weeks after surgical induction of osteoarthritis.
  o We have received institutional IACUC approval for these changes
  o This proposed amendment has been submitted to ACURO and is pending approval.
Key Research Accomplishments

- The main accomplishment thus far is the preliminary finding of a correlation between increased Vitamin D supplementation and decreased OA histologically in the murine model. It is interesting to note that this was only seen in females, implying a possible sex-differential effect. Van Grootheest et al showed in a recent epidemiological study in the Netherlands that circulating Vitamin D levels were higher in women than men, particularly in the group under 35 years. In contrast, Rabenberg et al showed no sex differences in 25-hydroxy-Vitamin D levels in an adult census study. In our second and third rounds of the animal study, we can evaluate whether this is a consistent effect and whether the sex difference is still present.

Conclusion

This combined animal and clinical study is making progress on both fronts. We have successfully established a mouse model of surgical induction of PTOA and have demonstrated that we can effectively supplement with dietary Vitamin D in varying doses. Additionally, we have obtained some interesting preliminary evidence that Vitamin D in supraphysiologic doses mitigates the degree of PTOA in female animals. This will be further investigated as we refine the animal models in the upcoming rounds of animal surgery.

Our add-on to the clinical study has been successfully initiated and over one-third of subjects has been enrolled. Data from these subjects will be available for analysis once we perform batched serum Vitamin D testing, at the end of enrollment.

Publications, Abstracts, and Presentations - Nothing to report

Inventions, Patents, and Licenses – Nothing to report

Reportable Outcomes – Nothing to report

Other Achievements - Nothing to report

References


6. Rabenberg M, Scheidt-Nave C, Busch MA, Rieckmann N, Hintzpeter B, Mensink GB. Vitamin D status among adults in Germany--results from the German Health Interview and Examination Survey for Adults (DEGS1). BMC Public Health 2015;15:641.

Appendix

- PI CV

- Relevant clinical research from PI – recent publication about Vitamin D and bone turnover levels in patients with distal radius fractures
CURRICULUM VITAE
Jennifer Moriatis Wolf, MD

University of Connecticut
New England Musculoskeletal Institute/Department of Orthopaedic Surgery
Medical Arts and Research Building
263 Farmington Avenue
Farmington, CT 06030-4037
Phone: 860-679-6655
Fax: 860-679-6649
Email: jmwolf@uchc.edu

EDUCATION

1987-1991 University of Maryland
College Park, MD
B.A., magna cum laude with General Honors

1991-1996 University of Pennsylvania School of Medicine
Philadelphia, PA
M.D., May 21, 1996

POST-DOCTORAL EDUCATION

1996-1997 Brown University Department of Surgery - Internship
Providence, RI
Director: Kirby I. Bland, MD

1997-2001 Brown University Department of Orthopaedic Surgery - Residency
Providence, RI
Director: Michael G. Ehrlich, MD

2001-2002 Brown University Division of Orthopaedic Trauma, Department of Orthopaedics – Orthopaedic Trauma Fellowship
Providence, RI
Director: Peter G. Trafton, MD

2002-2003 Mayo Clinic Division of Hand Surgery, Department of Orthopaedics – Hand Surgery Fellowship
Rochester, MN
Director: Robert D. Beckenbaugh, MD/Richard A. Berger, MD, PhD

CERTIFICATION

2005/2013 Board Certified (Diplomate) - American Board of Orthopaedic Surgery (Chicago, Illinois)

2006/2013 Certificate of Added Qualification (Hand Surgery) - American Board of Orthopaedic Surgery (Chicago, Illinois)
LICENSURE
Licensed in Connecticut, Colorado, Minnesota, Illinois and Georgia

ACADEMIC APPOINTMENTS

2003 – 2009  Assistant Professor, Department of Orthopaedic Surgery, University of Colorado Health Sciences Center
2009-2010  Associate Professor, Department of Orthopaedic Surgery, University of Colorado-Denver
2010-2015  Associate Professor, Department of Orthopaedic Surgery, University of Connecticut
2015-present  Professor, Department of Orthopaedic Surgery, University of Connecticut

TEACHING/EDUCATIONAL APPOINTMENTS

University of Colorado School of Medicine
  Co-Director, Musculoskeletal Block (required 3rd-year course) (2007-2010)
  Director, Orthopaedic Medical Student Courses/Sub-Internships (2007-2010)
University of Connecticut School of Medicine
  Curriculum Reform Clinical Education Committee (2015-2016)
  Instructor, Musculoskeletal Block (2010-present)

HOSPITAL APPOINTMENTS

2003-2010  University of Colorado Hospital
2004-2010  Denver Veterans Administration Medical Center
2004-2010  Denver Health Medical Center
2004-2010  The Children’s Hospital of Denver
2005-2010  Rose Hospital (Denver)
2010-present  John Dempsey Hospital
2014-present  Connecticut Children’s Medical Center

AWARDS & HONORS

2014  Connecticut Technology Council Women of Innovation Award
2013  American British Canadian Traveling Fellowship – American Orthopaedic Association
2010  Sterling Bunnell Traveling Fellowship – American Society for Surgery of the Hand
2008  Clinician Scientist Award – Orthopaedic Research and Education Foundation
2008  Leadership Fellows Program – American Academy of Orthopaedic Surgeons
2006  American Society for Surgery of the Hand – Young Member Leadership Program
2006  Alexandra Kirkley Traveling Fellowship - Ruth Jackson Orthopaedic Society
2005  United States Bone and Joint Decade Young Investigator
2001  Haffenreffer Award for Resident Research
1996  William G. Munn Memorial Prize for Promise in Orthopaedics
1995  Alpha Omega Alpha Medical Honor Society
1990  Phi Beta Kappa
1987  Chancellor’s Scholar  (full four-year college merit scholarship)

PROFESSIONAL SOCIETY MEMBERSHIP

American Society for Surgery of the Hand (Active Member, 2007 - present)
American Academy of Orthopaedic Surgeons (Fellow, 2007 – present)
American Orthopaedic Association (Member, 2012-present)
American Association of Hand Surgeons (Member, 2003-present)
Orthopaedic Leadership Institute (2010-present)
Ruth Jackson Orthopaedic Society (2002-present)
Rocky Mountain Orthopaedic Society (2003-present)
Connecticut Orthopaedic Society (2010-present)
New England Orthopaedic Society (2015-present)

JOURNAL REVIEW

Deputy Editor, Journal of Hand Surgery (2011-present)
Associate Editor, Scientific – Journal of Hand Surgery (2009-present)

Associate Editor, Hand and Microsurgery, Journal of Bone and Joint Surgery Reviews (2013-preset)

Editorial Board, Orthopedics (2003-present)

Web Updates Editor, Skeletal Trauma (2008-2015)


Consultant Reviewer
   Journal of Bone and Joint Surgery – British (2009-present)
   Clinical Orthopaedics and Related Research (2007-present)
   Orthopedics (2003-present)
   Hand (2010-present)
   British Journal of Sports Medicine (2013-present)
   International Journal of Sports Medicine (2012-present)

Editor, Hand Module, Orthopaedic Hyperguide (2008-2011)

COMMITTEES/SERVICE

American Society for Surgery of the Hand
   Council Member at Large (2014-2017)
   Program Co-Chair, Annual Meeting (2014)
Commercial Support Committee (2012-2015)
Touching Hands Project (2012-2015)
Bunnell Traveling Fellows Committee (2010-2014)
Products and Publications Committee (2005-2011)
Annual Meeting Scientific Displays Committee (Member, 2006-2015; Chair, 2009-2012)
Mentoring Task Force (2006)
Resident Education Committee (2007-2010)
Crucial Elements of Hand Surgery Committee (2007-2008)
Courses and Meetings Advisory Committee (2007-2010)
Young Members Steering Committee (Member, 2008-2010; Chair 2010-2011)
Diversity Committee (2008-2011)
Membership Task Force (2009)

American Foundation for Surgery of the Hand
Board Member-at-Large (2012-2014)
Complus Manus Committee (2012-2014)
Nominating Committee (2012-2013)
Touching Hands Project (2012-2013)

American Academy of Orthopaedic Surgeons
Chair, Residents, Fellows, and Candidate Members Subcommittee (2008-2011)
Member (2006-2009)
Co-Editor, Residents’ Monthly E-Newsletter (2007-2009)
Co-Chair, Leadership Development Endowment Fund Meeting Committee (2010-2012)

American Board of Orthopaedic Surgeons/National Board of Medical Examiners
Joint Committee for CAQ Question-Writing Task Force (2011-2015)

Orthopaedic Research and Education Foundation
Grant Reviewer (2010-present)

Ruth Jackson Orthopaedic Society Governing Board
President (2014-2015)
Vice-President (2013-2014)
Secretary (2011-2013)

Orthopaedic Leadership Institute
Inaugural Meeting Program Coordinator (2011)

American Association of Hand Surgery
Research Committee (2008-2011)

Board of Directors, Rocky Mountain Hand Surgery Society (2008-2011)
Secretary/Treasurer (2008-2009)
Vice President (2009-2010)

New England Hand Society (2011-present)

Department of Orthopaedic Surgery, University of Connecticut
Research Committee (2011-present, Chair 2012-present)
Admissions Committee member (2010-present)
OR Lean Committee (2014-15)
Colorado Multiple Institutions Review Board (IRB) reviewer, 2004-2008

Faculty Advisor, Orthopaedic Student Interest Group, University of Colorado School of Medicine, 2008-2010

Department of Orthopaedics, University of Colorado
Finance Committee member, 2006-2010
Academic Council member, 2007-2010
Curriculum Committee member, 2006-2010

University of Colorado Hospital Trauma Committee member, 2004-2010

Active Women’s Health Initiative, University of Colorado Hospital, 2004-2010

PEER-REVIEWED PUBLICATIONS


46. Wolf JM, Cameron KL, Clifton K, Owens BD. Serum relaxin values in young athletic males are similar to females. *Orthopedics* 36(2):128-31, 2013.


NON-PEER REVIEWED PUBLICATIONS


ELECTRONIC MEDIA


TEXTBOOK CHAPTERS


**TEXTBOOKS**


**RESEARCH SUPPORT**

**PEER-REVIEWED**

**CURRENT**

1. Wolf (PI) 9/1/14-8/31/15 $20,000 3% effort  
   **American Foundation for Surgery of the Hand**  
   Conditional Deletion of Relaxin Receptor in Ligament: In Vivo Model  
   We will create a transgenic mouse with inducible deletion of relaxin receptor at the level of tendon and ligament using a cross of relaxin null and scleraxis-Cre mice.

2. Wolf (PI) 10/7/14-10/6/17 $750,000 10% effort  
   **Department of Defense/Congressionally Directed Medical Research Program**  
   Supplementation of Vitamin D in Prevention of Post-Traumatic Osteoarthritis: Animal and Clinical Models  
   This project will study the impact of oral Vitamin D in prevention of surgically induced arthritis in a murine model, as well as evaluate Vitamin D levels in military cadets prior to and after ACL injury.
3. Wolf (PI) 7/1/14-6/30/15 $50,000 5% effort
**Orthopaedic Research and Education Foundation/Goldberg Arthritis Grant**
Animal Model of Vitamin D Supplementation for Prevention of Osteoarthritis
This project evaluates the potentially preventive impact of Vitamin D oral supplementation on the initiation and development of surgically induced osteoarthritis in mice.
_Awarded but declined due to overlap with DOD/CDMRP grant above_

4. Wolf (PI) 9/14/13-09/13/15 $20,000 3% effort
**American Foundation for Surgery of the Hand**
Impact of local and systemic relaxin in a murine osteoarthritis model
This study uses a murine model to examine the impact of locally and systemically delivered relaxin on the development of surgically induced osteoarthritis.

5. Chung (PI) 06/01/2011-05/30/2016 $22,500 3% effort
**NIH/NIAMS RO1. WRIST Study Group**
A clinical trial for the surgical treatment of elderly distal radius fractures
This multicenter randomized trial compares 3 different methods of fixation in surgically treated distal radius fractures in elderly patients.
Role: Co-investigator, PI on subcontract

**COMPLETED**

1. Rozental (PI) 05/01/2012-04/30/2013 $45,000 3% effort
**Orthopaedic Research and Education Foundation/RJOS/DePuy**
Markers of bone turnover and Vitamin D in patients with distal radius fractures
This study expands the smaller pilot study to evaluate biomarkers of bone turnover and 25-hydroxy-Vitamin D in patients with distal radius fractures, compared to controls.
Role: Co-Investigator

2. Wolf (co-PI) 09/01/11-08/31/12 $20,000 3% effort
**American Foundation for Surgery of the Hand**
25-Hydroxy-Vitamin D and bone turnover marker levels in patients with distal radius fractures
This study will evaluate Vitamin D and biomarkers of bone turnover in patients with wrist fractures and controls.
Role: co-PI

3. Wolf (PI) 08/20/10-06/01/11 $20,000 3% effort
**University of Connecticut GCRC/CICATS Pilots and Feasibility Funds-2010**
Correlation of serum relaxin with joint mobility and ligament injury and analysis for gender differences
This study will correlate serum relaxin with a prospective injury database in military cadets.
Role: PI

4. Wolf (PI) 09/01/08-08/31/10 $20,000 3% effort
**American Foundation for Surgery of the Hand**
Effect of relaxin on gender differences in laxity and arthritis of the thumb base
This study will evaluate hormonal effects on gender differences in thumb laxity and osteoarthritis.
Role: PI

5. Wolf (PI) 07/01/08-06/30/11 $300,000 15% effort
Orthopaedic Research and Education Foundation Clinician-Scientist Award
Does relaxin mediate gender differences in joint laxity and osteoarthritis of the thumb carpo-metacarpal joint?
This study's goal is to correlate serum relaxin levels and joint laxity in normal subjects as well as to evaluate this relationship in patients with surgically treated thumb CMC osteoarthritis.
Role: PI

6. Wolf (PI) 10/01/06-09/30/08 $20,000 3% effort
American Foundation for Surgery of the Hand
A prospective, randomized, controlled trial of autologous blood injection vs. corticosteroid injection for the treatment of lateral epicondylitis.
This is a prospective, blinded, multicenter trial to evaluate the efficacy of autologous blood injection for lateral epicondylitis.
Role: PI

7. Dawson (PI) 2/01/08-1/31/09 $1000 2% effort
Southwest Orthopaedic Trauma Association
Incidence of scaphoid fractures in a young, active population.
This study uses a military database of healthcare visits coded by ICD-9 to calculate the incidence of scaphoid fracture in a young, active population as well as analyze potential demographic risk factors for this injury.
Role: Co-investigator

8. Sobky (PI) 07/01/04-06/30/05 $5,000
Department of Orthopaedics, University of Colorado Health Sciences Center
Comparison of bending strength and load to failure of multiple volar plates.
This was a biomechanical study of the strength and stiffness of multiple plates used for fixation in distal radius fractures.
Role: Co-investigator

9. Wolf (PI) 07/01/94-06/30/95 $20,000
American Heart Association
Sequencing of bone morphogenetic proteins and effects on human osteoblast-like cells.
This was a project to evaluate the effect of BMP-2 and BMP-4 on osteoblasts in culture.
Role: PI

NON-PEER-REVIEWED

1. Wolf (PI) 01/01/04-04/01/06 $20,000
Orthologic, Inc., Phoenix, Arizona
A double-blind, randomized, placebo-controlled Phase III study to evaluate the efficacy and safety of Chrysalin on the rate of healing in distal radius fractures.
This was a multicenter trial of an injectable substance with the goal to increase healing in distal radius fractures.
Role: PI
INVITED PRESENTATIONS and LECTURES (National/International)


2. Trapeziometacarpal Arthritis and Other Degenerative Arthropathies of the Hand: *Evidence-Based Treatment.* Instructional Course Lecture, ASSH Annual Meeting, September 2007, Seattle, WA.


28. Acute and Chronic Scapholunate Ligament Injury. Invited Speaker, Department of Orthopaedic Surgery, Landspitalinn Hospital/University of Iceland, June 8, 2011, Reykjavik, Iceland.


NATIONAL/INTERNATIONAL PRESENTATIONS

1. **Wolf JM;** Gannon FH; Shore EM; Bilker W; Zasloff MA; Kaplan FS: The prevalence, natural history, and pathogenesis of limb swelling in patients who have fibrodysplasia ossificans progressiva. Adult Bone and Mineral Working Group, American Society for Bone and Mineral Research Annual Meeting; September 10, 1995, Baltimore, Maryland. (podium)


34. Wolf JM, Scott F, Williams AE, Delaronde S, King KB. Serum Relaxin is Correlated with Relaxin Receptors and MMP-1 in the Anterior Oblique Ligament. 2012 World Congress on Osteoarthritis, Barcelona, Spain, April 26-29, 2012. (poster)


40. Webber T, Patel SP, Pensak M, Fajolu O, Rozental TD, **Wolf JM.** Correlation between distal radius cortical thickness and bone mineral density. Hand Wrist Biomechanics International Meeting, Milan, Italy, June 16, 2015. (podium)

41. Rohde RS, **Wolf JM,** Adams JE. Where are the Women in Orthopaedic Surgery? Special Interest Poster, American Orthopaedic Association Annual Meeting, Providence, Rhode Island, June 24-27, 2015. (poster)


**COURSE FACULTY**


15. Co-Chair, Interactive Case Reviews, American Society for Surgery of the Hand, October 2013, San Francisco, California.


17. Program Co-Chair, Annual Meeting, American Society for Surgery of the Hand, September 2014, Boston, Massachusetts.


20. Faculty, 2nd Annual Course on Wrist Arthroscopy and Arthroplasty, October 10-12, Arezzo, Italy.

REGIONAL/LOCAL PRESENTATIONS


3. Osteoporosis and Orthopaedics. Sargent School of Physical Therapy, Boston University, November 6, 2001, Boston, Massachusetts.

4. Foot and Ankle Injuries. Sargent School of Physical Therapy, Boston University, November 13, 2001, Boston, Massachusetts.


25-Hydroxyvitamin-D and Bone Turnover Marker Levels in Patients with Distal Radial Fracture

Tamara D. Rozental, MD, Lindsay M. Herder, BA, Kempland C. Valley, BSc, David Zurakowski, PhD, Kathleen Coyle, RN, BSN, Mary L. Bouxsein, PhD, and Jennifer M. Wolf, MD

Investigation performed at the Department of Orthopaedic Surgery, Beth Israel Deaconess Medical Center, Boston, Massachusetts, and the Department of Orthopaedic Surgery, University of Connecticut Health Center, Farmington, Connecticut

Background: Fragility fractures are a major public health issue with substantial socioeconomic cost. Vitamin-D deficiency and increased bone turnover are associated with higher rates of bone loss and an increased risk of fracture. We hypothesized that patients with a distal radial fracture would have lower levels of 25-hydroxyvitamin D (25(OH)D) and increased levels of serum bone turnover markers than controls without a fracture.

Methods: Postmenopausal women with a recent distal radial fracture (fracture group, n = 105) were prospectively recruited and were compared with individuals without a fracture (control group, n = 150). Outcome variables included serum levels of 25(OH)D and markers of bone formation, including N-terminal extension propeptide of type-I collagen (P1NP), parathyroid hormone (PTH), bone-specific alkaline phosphatase (BSAP), and osteocalcin, as well as a marker of resorption (C-terminal telopeptide of type-I collagen [CTX-1]). Bone mineral density was measured with dual x-ray absorptiometry.

Results: The fracture group was slightly older than the control group (mean ± SD, 66.8 ± 10.8 years versus 63.3 ± 9.0 years, p = 0.008), had a lower body mass index (26.4 ± 5.9 kg/m² versus 28.0 ± 6.2 kg/m², p = 0.05), and more commonly had a prior fracture (52% versus 31%, p < 0.001). Bone mineral density at the hip was lower in the fracture group than in the control group (0.831 ± 0.130 g/cm² versus 0.917 ± 0.139 g/cm², p < 0.001). The mean 25(OH)D levels were similar in the fracture and control groups (44.4 ± 14.6 ng/mL versus 41.3 ± 14.5 ng/mL, p = 0.08). Levels of serum markers of bone formation were significantly higher in the fracture group than in the control group (P1NP: 70.4 ± 33.2 ng/mL versus 53.2 ± 25.6 ng/mL, p < 0.001; osteocalcin: 22.3 ± 9.9 ng/mL versus 20.2 ± 9.2 ng/mL, p = 0.017). Levels of BSAP, PTH, and CTX-1 were similar in the two groups. Multivariable logistic regression showed independent associations between a distal radial fracture and low total hip bone mineral density (odds ratio [OR] = 2.02 for each decrease of 1 SD, 95% confidence interval [CI] = 1.38 to 3.01, p < 0.001) and a high P1NP level (OR = 2.17 for each 1-SD increase, 95% CI = 1.52 to 3.06, p < 0.001).

Conclusions: In this cohort, 25(OH)D levels were not associated with distal radial fracture and do not appear to affect the risk assessment for distal radial fracture in postmenopausal women. Patients with a distal radial fracture, however, had increased bone turnover as evidenced by high P1NP and osteocalcin levels. Women with both a high P1NP level and low bone mineral density were at particularly high risk for fracture.

Level of Evidence: Prognostic Level III. See Instructions for Authors for a complete description of levels of evidence.

Disclosure: One or more of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of an aspect of this work. In addition, one or more of the authors, or his or her institution, has had a financial relationship, in the thirty-six months prior to submission of this work, with an entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. Also, one or more of the authors has had another relationship, or has engaged in another activity, that could be perceived to influence or have the potential to influence what is written in this work. The complete Disclosures of Potential Conflicts of Interest submitted by authors are always provided with the online version of the article.
50% of those with a fragility fracture do not have osteoporosis as demonstrated by bone mineral density testing\textsuperscript{5-7}. Recent efforts have thus focused on other means of identifying patients who are at greatest risk for future fracture.

Low serum levels of vitamin D are associated with higher rates of bone loss and increased risk of fracture\textsuperscript{8-12}. Circulating levels of serum 25-hydroxyvitamin D (25(OH)D) are currently considered the most reliable marker for vitamin-D status\textsuperscript{13}. The optimal level of 25(OH)D has not yet been established, although several thresholds have been suggested\textsuperscript{14}. The Institute of Medicine defines vitamin-D deficiency as a serum level of 25(OH)D of <25 ng/mL\textsuperscript{15,16}.

High bone turnover has also been associated with increased rates of bone loss\textsuperscript{17-19} and with an increased risk of fracture independent of bone mineral density\textsuperscript{18,20-22}. The most commonly used bone turnover markers are those that reflect bone formation, including N-terminal extension propeptide of type-I collagen (P1NP), bone-specific alkaline phosphatase (BSAP), and osteocalcin, and those that reflect bone resorption, including C-terminal telopeptide of type-I collagen (CTX-1)\textsuperscript{19}.

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Characteristic & Distal Radial Fracture (N = 105) & Controls (N = 150) & P Value \\
\hline
Age* (yr) & 66.8 ± 10.8 & 63.3 ± 9.0 & 0.008\dagger \\
Race (no. [%]) & & & 0.16 \\
Caucasian & 91 (87%) & 124 (83%) & \\
African American & 5 (5%) & 19 (13%) & \\
Hispanic & 7 (7%) & 6 (4%) & \\
Asian & 2 (2%) & 1 (1%) & \\
Weight* (kg) & 68.8 ± 15.6 & 73.0 ± 17.2 & 0.05\dagger \\
BMI* (kg/m\textsuperscript{2}) & 26.4 ± 5.9 & 28.0 ± 6.2 & 0.05\dagger \\
Hand dominance (no. [%]) & & & 0.63 \\
Left & 8 (8%) & 16 (11%) & \\
Right & 97 (92%) & 134 (89%) & \\
Age at menarche* (yr) & 12.9 ± 1.5 & 12.8 ± 1.4 & 0.89 \\
Gravida\textsuperscript{‡} & 2 (1-4) & 2 (1-3) & 0.55 \\
Para\textsuperscript{‡} & 1 (0-3), n = 77 & 2 (0-3), n = 96 & 0.58 \\
History of fracture (no. [%]) & 55 (52%) & 47 (31%) & <0.001\dagger \\
Smoking (no. [%]) & 4 (4%) & 8 (5%) & 0.77 \\
Calcium supplements (no. [%]) & 52 (50%) & 91 (61%) & 0.10 \\
Vitamin-D supplements (no. [%]) & 50 (48%) & 66 (44%) & 0.93 \\
Alcohol (no. [%]) & & & 0.24 \\
0 drinks/wk & 54 (51%) & 75 (50%) & \\
1-3 drinks/wk & 23 (22%) & 47 (31%) & \\
4-7 drinks/wk & 19 (18%) & 21 (14%) & \\
≥8 drinks/wk & 9 (9%) & 7 (5%) & \\
Caffeinated beverages (no. [%]) & & & 0.95 \\
0 cups/day & 19 (18%) & 28 (19%) & \\
1 cup/day & 32 (30%) & 50 (33%) & \\
2-3 cups/day & 46 (44%) & 62 (41%) & \\
≥4 cups/day & 8 (8%) & 10 (7%) & \\
Physical activity level (no. [%]) & & & 0.27 \\
Inactive & 11 (10%) & 26 (17%) & \\
Active & 82 (78%) & 118 (79%) & \\
Unknown & 12 (11%) & 6 (4%) & \\
\hline
\end{tabular}
\caption{Demographic Characteristics and Lifestyle Activities of the Two Study Groups}
\label{table:1}
\end{table}

\*The values are given as the mean and standard deviation. †A significant difference between groups. ‡The values are given as the median with the interquartile range in parentheses.
TABLE II Univariate Comparison of Bone Density Between the Fracture and Control Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Distal Radial Fracture* (N = 105)</th>
<th>Controls* (N = 150)</th>
<th>Area Under Curve</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.976 ± 0.187</td>
<td>1.108 ± 0.814</td>
<td>0.602</td>
<td>0.11</td>
</tr>
<tr>
<td>Bone mineral density T-score</td>
<td>−1.15 ± 1.18</td>
<td>−0.56 ± 1.25</td>
<td>0.632</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Femoral neck</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.756 ± 0.153</td>
<td>0.798 ± 0.261</td>
<td>0.621</td>
<td>0.18</td>
</tr>
<tr>
<td>Bone mineral density T-score</td>
<td>−1.48 ± 1.06</td>
<td>−0.81 ± 1.08</td>
<td>0.694</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Total hip</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td>0.831 ± 0.130</td>
<td>0.917 ± 0.139</td>
<td>0.679</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Hip bone mineral density T-score</td>
<td>−1.12 ± 1.06</td>
<td>−0.45 ± 1.10</td>
<td>0.680</td>
<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

*The values are given as the mean and standard deviation. †A significant difference between groups as shown by the Student t test.

Vitamin D and bone turnover markers have not been extensively studied in patients with fracture of the distal part of the radius. To our knowledge, no studies have explored the association between vitamin-D levels and wrist fracture in a North American population. Similarly, although prior studies have demonstrated that increases in levels of bone turnover markers would have lower circulating levels of 25(OH)D and higher levels of 25(OH)D and bone turnover markers. We hypothesize that postmenopausal women with a distal radial fracture would have lower circulating levels of 25(OH)D and higher levels of bone turnover markers than women of similar age with no fracture.

Materials and Methods

Patient Identification

Following approval by our institutional review boards, postmenopausal women over the age of fifty years were recruited at Beth Israel Deaconess Medical Center (Boston, Massachusetts) and University of Connecticut Health Center (Hartford, Connecticut) by their treating orthopaedic surgeon. Consecutive patients with a distal radial fracture were screened for inclusion. All subjects gave written informed consent prior to participation. Subjects were eligible for inclusion into our fracture group if they had a history of a distal radial fracture within three weeks before presentation for treatment. Only fractures occurring from low-energy falls were included. Patients with no history of fractures in adulthood who were presenting for treatment of other conditions were screened, 407 were eligible for inclusion. Seventy-two patients were lost to follow-up, twenty-four patients refused to participate, and fifty-six patients either withdrew consent or elected not to participate in at least one study component.

Demographic Information and Medical and Medication History

At the time of enrollment, standardized questionnaires were used to record prior fractures; reproductive, menstrual, and smoking history; alcohol and caffeine intake; physical activity; and calcium/vitamin D supplementation.
TABLE IV Independent Multivariable Risk Factors Associated with Distal Radial Fracture in Women Fifty Years of Age and Older

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.05</td>
<td>1.02-1.08</td>
<td>0.03*</td>
</tr>
<tr>
<td>BMI</td>
<td>1.00</td>
<td>0.94-1.05</td>
<td>0.97</td>
</tr>
<tr>
<td>History of fracture</td>
<td>3.00</td>
<td>1.51-5.78</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Calcium supplements</td>
<td>0.40</td>
<td>0.23-0.85</td>
<td>0.014*</td>
</tr>
<tr>
<td>25(OH)D (per 1-SD decrease)</td>
<td>0.88</td>
<td>0.65-1.25</td>
<td>0.48</td>
</tr>
<tr>
<td>CTX-1 (per 1-SD increase)</td>
<td>0.80</td>
<td>0.47-1.24</td>
<td>0.39</td>
</tr>
<tr>
<td>P1NP (per 1-SD increase)</td>
<td>2.17</td>
<td>1.52-3.06</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Osteocalcin (per 1-SD increase)</td>
<td>1.14</td>
<td>0.78-1.77</td>
<td>0.50</td>
</tr>
<tr>
<td>Total hip bone mineral density (per 1-SD decrease)</td>
<td>2.02</td>
<td>1.38-3.01</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Significant independent predictor of fracture in multivariable logistic regression analysis with backward selection with use of the likelihood ratio test to assess significance.

Vitamin-D Levels and Bone Turnover Markers

To minimize the potential effect of fracture-healing on bone metabolism, levels of serum markers were assessed at three months after injury. Blood was drawn in the morning after an overnight fast to reduce the effects of diurnal variation and eating. Markers of bone formation included P1NP, BSAP, and osteocalcin. P1NP reflects an early phase of bone formation. BSAP is present in pre-osteoblasts and osteoblasts, and osteocalcin is made by mature osteoblasts. CTX-1 was used to measure bone resorption and is most reliable for this purpose. PTH levels were measured to identify secondary hyperparathyroidism associated with vitamin-D deficiency. Serum samples were kept frozen at -70°C, and assays were performed in batch by the Maine Medical Center Research Institute (Scarborough, Maine). Assays were analyzed with the IDS-iSYS automated analyzer (Immunodiagnostic Systems [IDS]) with detectable ranges of 25(OH)D, P1NP, BSAP, osteocalcin, PTH, and CTX-1 were 8.3%, 2.9%, 1.6%, 2.5%, 2.0%, and 3.2%, respectively. Hand dominance, mechanism of injury, and type of treatment (surgical or nonsurgical) were tabulated.

Bone Mineral Density

Areal bone mineral density (g/cm²) of the spine, total hip, and femoral neck was measured with dual x-ray absorptiometry (QDR 4500; Hologic, Waltham, Massachusetts) in the array (fan beam) mode.

Statistical Methods

The distal radial fracture and control groups were compared by using Pearson chi-square and Fisher exact tests for proportions and the Student t test for normally distributed continuous data. All serum biomarker values were found to be normally distributed and were not subject to log transformation. The area under the curve was calculated as a measure of discrimination between fractures and controls. Non-parametric data were compared by using the Mann-Whitney U test and were presented as the median and interquartile range (IQR). Power analysis indicated that the sample sizes targeted provided 90% power (α = 0.05, β = 0.10) to detect 10% mean differences in 25(OH)D levels, bone turnover marker levels, and bone mineral density between groups with use of a two-tailed Student t test (version 7.0; nQuery Advisor, Statistical Solutions, Boston, Massachusetts). Multivariable logistic regression analysis of ten candidate variables was applied to determine independent factors associated with fracture. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were derived as measures of association with biomarkers expressed per unit change in standard deviation (SD)²°. The relationship between the probability of fracture and levels of predictive biomarkers was estimated by maximum likelihood with precision based on 95% CIs. Statistical analysis was performed with use of IBM SPSS Statistics version 22.0 (IBM, Armonk, New York). A two-tailed p < 0.05 was considered significant.

Source of Funding

This work was supported by the Orthopaedic Research and Education Foundation and the American Foundation for Surgery of the Hand.

Results

Patient Characteristics

One hundred and five patients with a distal radial fracture (fracture group) and 150 patients without a fracture (control group) were prospectively enrolled. The fracture-group patients were slightly older (66.8 ± 10.8 years versus 63.3 ± 9.0 years, p = 0.008), had a lower body mass index (BMI) (26.4 ± 5.9 kg/m² versus 28.0 ± 6.2 kg/m², p = 0.05), and had more commonly sustained a prior fracture (52% [n = 55] versus 31% [n = 47], p < 0.001). The race distribution, percentage of patients who smoked and took calcium and vitamin-D supplements, caffeine and alcohol consumption, and physical activity level were similar in the two groups (Table I).

Thirty-eight patients sustained a fracture of the dominant extremity. Fifty-five fractures were treated with a cast and fifty, with operative fixation with a volar plate. All fractures healed without complications.

Bone Mineral Density

Bone mineral density as measured with dual x-ray absorptiometry was similar between the fracture and control groups at the lumbar spine and at the femoral neck but it was lower at the hip in the fracture group (0.831 ± 0.130 g/cm² versus 0.917 ± 0.139 g/cm², p < 0.001) (Table II). Thirty patients in the fracture group were classified as having osteopenia and...
independent factors associated with distal radial fracture after adjustment for age and BMI: a history of fracture (OR = 3.00, 95% CI = 1.51 to 5.78, p < 0.001), use of calcium supplements (OR = 0.40, 95% CI = 0.23 to 0.85, p = 0.014), elevated P1NP levels (OR = 2.17 for each 1-SD increase, 95% CI = 1.52 to 3.06, p < 0.001), and lower total hip bone mineral density (OR = 2.02 for each 1-SD decrease, 95% CI = 1.38 to 3.01, p < 0.001) (Table IV). Serum levels of 25(OH)D (p = 0.48), CTX-1 (p = 0.39), and osteocalcin (p = 0.50) were not associated with distal radial fracture.

Few (ten) patients in our cohort had a vitamin-D deficiency, and there was no association between vitamin-D deficiency (<25 ng/mL) and distal radial fracture (p = 0.74). When 25(OH)D levels were divided into quartiles, and with quartile 4 (highest 25(OH)D level) used as the reference, the odds of fracture were lower in quartiles 1, 2, and 3 but not significantly so (p = 0.12, p = 0.06, and p = 0.3, respectively) (Fig. 1).

Analysis of patients with a P1NP level higher than the reported reference median in premenopausal women (>37.3 ng/mL) revealed a significant association between that factor and distal radial fracture (p < 0.05). Quartile analysis with use of quartile 1 (lowest P1NP level) as the reference revealed a 5.5-fold higher risk of fracture in quartile 4 (OR = 5.51, 95% CI = 2.58 to 11.83, p < 0.001) and a twofold higher risk in quartile 3 (OR = 2.07, 95% CI = 1.20 to 4.19, p = 0.05). There was no significant difference in fracture risk between the two lowest quartiles (p = 0.52) (Fig. 1).

There was a significantly greater likelihood of distal radial fracture in women with lower bone mineral density at the hip (likelihood ratio test = 20.58, p < 0.001) and a higher serum P1NP level (likelihood ratio test = 20.67, p < 0.001) (Fig. 2). Furthermore, women with both low bone mineral density and a high P1NP level were at particularly high risk for fracture (Fig. 3). Women with low bone mineral density and a P1NP level in the highest quartile had a probability of fracture of >50% (Fig. 4).

25(OH)D and Bone Turnover Markers

Serum 25(OH)D levels were similar in the fracture and control groups after we controlled for age and BMI (44.4 ± 14.6 ng/mL versus 41.3 ± 14.5 ng/mL, p = 0.08) and after we excluded individuals who were taking over-the-counter supplementation (40.0 ± 14.8 ng/mL versus 40.1 ± 14.7 ng/mL, p = 0.97). After we adjusted for age and BMI, levels of markers of bone formation were higher in the fracture group than in the control group (P1NP: 70.4 ± 33.2 ng/mL versus 53.2 ± 25.6 ng/mL, p < 0.001; osteocalcin: 23.3 ± 9.9 ng/mL versus 20.2 ± 9.2 ng/mL, p = 0.017). Levels of BSAP, PTH, and CTX-1 were similar in the two groups (Table III). There was no significant correlation between the 25(OH)D and P1NP levels in the entire cohort (r = -0.3, p = 0.29) or in the fracture group (r = -0.06, p = 0.57). P1NP levels correlated with osteocalcin levels (r = 0.18, p = 0.004) in the entire cohort and in the fracture group (r = 0.65, p < 0.001). PTH levels correlated with osteocalcin levels (r = 0.18, p = 0.004) in the entire cohort and in the fracture group (r = 0.29, p = 0.003). There was no correlation among PTH levels, P1NP, and 25(OH)D levels.

Multivariable logistic regression analysis identified four independent factors associated with distal radial fracture after adjustment for age and BMI: a history of fracture (OR = 3.00, 95% CI = 1.51 to 5.78, p < 0.001), use of calcium supplements (OR = 0.40, 95% CI = 0.23 to 0.85, p = 0.014), elevated P1NP levels (OR = 2.17 for each 1-SD increase, 95% CI = 1.52 to 3.06, p < 0.001), and lower total hip bone mineral density (OR = 2.02 for each 1-SD decrease, 95% CI = 1.38 to 3.01, p < 0.001) (Table IV). Serum levels of 25(OH)D (p = 0.48), CTX-1 (p = 0.39), and osteocalcin (p = 0.50) were not associated with distal radial fracture.

Few (ten) patients in our cohort had a vitamin-D deficiency, and there was no association between vitamin-D deficiency (<25 ng/mL) and distal radial fracture (p = 0.74). When 25(OH)D levels were divided into quartiles, and with quartile 4 (highest 25(OH)D level) used as the reference, the odds of fracture were lower in quartiles 1, 2, and 3 but not significantly so (p = 0.12, p = 0.06, and p = 0.3, respectively) (Fig. 1).

Analysis of patients with a P1NP level higher than the reported reference median in premenopausal women (>37.3 ng/mL) revealed a significant association between that factor and distal radial fracture (p < 0.05). Quartile analysis with use of quartile 1 (lowest P1NP level) as the reference revealed a 5.5-fold higher risk of fracture in quartile 4 (OR = 5.51, 95% CI = 2.58 to 11.83, p < 0.001) and a twofold higher risk in quartile 3 (OR = 2.07, 95% CI = 1.20 to 4.19, p = 0.05). There was no significant difference in fracture risk between the two lowest quartiles (p = 0.52) (Fig. 1).

There was a significantly greater likelihood of distal radial fracture in women with lower bone mineral density at the hip (likelihood ratio test = 20.58, p < 0.001) and a higher serum P1NP level (likelihood ratio test = 20.67, p < 0.001) (Fig. 2). Furthermore, women with both low bone mineral density and a high P1NP level were at particularly high risk for fracture (Fig. 3). Women with low bone mineral density and a P1NP level in the highest quartile had a probability of fracture of >50% (Fig. 4).
The prevalence of distal radial fracture increases markedly with age, and independent predictors of distal radial fracture include decreased bone mineral density, a history of falls, and a prior fracture after the age of fifty years. Our study confirmed that a history of fracture and low bone mineral density are strongly associated with distal radial fracture. Although low bone mineral density has been associated with distal radial fracture, it does not explain all of the fracture risk. Øyen et al. noted that only one-third of men and half of women presenting with a low-energy distal radial fracture met the WHO criteria for osteoporosis. In our study, only 4% of the women with a distal radial fracture met the criteria for osteoporosis and 37%, for osteopenia. Clearly other factors besides low bone mineral density play a role in the etiology of these fractures.

Vitamin-D levels have been explored as a risk factor for fracture, primarily at the hip. In one study, 96% of patients with a hip fracture were vitamin-D deficient. The Women’s Health Initiative reported that 25(OH)D levels of <8 ng/mL were associated with an increased risk of fracture. Another study showed that 25(OH)D levels in women with comorbid hip and upper-extremity fractures were significantly lower than those in women with an isolated hip fracture. Vitamin-D deficiency has not been extensively studied among patients with distal radial fracture. In one report, 49% of thirty-seven patients with a forearm fracture had vitamin-D deficiency, defined as <10 ng/mL. Another study showed that 43% of 100 patients with a wrist fracture had 25(OH)D levels of <12 ng/mL. In an analysis comparing 25(OH)D levels between patients with a distal radial fracture and non-fracture controls, Øyen et al. found that a level of 25(OH)D of <20 ng/mL was associated with an increased risk of fracture in women and men after they controlled for bone mineral density and BMI. They concluded that low 25(OH)D levels predicted fractures independently of bone mineral density. Contrary to these results, our study did not show an association between low 25(OH)D levels and distal radial fracture. There was no significant difference between groups after exclusion of women taking vitamin-D supplementation. Furthermore, our cohort did not display the high PTH values that are expected with vitamin-D deficiency. Together, these results suggest that low 25(OH)D levels are not associated with distal radial fracture in this cohort. Potential reasons are that our study exclusively enrolled postmenopausal women, whose awareness of the problem of vitamin-D deficiency is typically higher: 44% of the subjects enrolled and 48% of those with a fracture were taking supplementation prior to injury. Most notably, few patients in our cohort had a very low 25(OH)D level, likely reflecting recent attention given to treatment of this deficiency. We also applied a stringent set of inclusion criteria by excluding all patients taking prescription medications known to affect bone metabolism.
Our study revealed that patients with a fracture had higher levels of markers of bone formation than controls. Garnero et al. compared serum levels of bone turnover markers in healthy postmenopausal women with those in women with a fracture. After adjustment for bone mineral density, women with levels of resorption markers in the highest quartile had a twofold increase in hip fracture risk compared with women with levels in the lowest quartile. Sornay-Rendu et al. also found that markers of bone formation and resorption could be used to identify osteopenic women at a high risk for fracture. Similarly, others have documented that the association between fracture risk and increased levels of markers of bone formation is stronger than the association between fracture risk and increased levels of markers of bone resorption.

The effects of fracture repair must be distinguished from underlying abnormalities in bone metabolism. The Malmö Osteoporosis Prospective Risk Assessment (OPRA) study showed that fractures affect bone formation and levels of resorption markers up to one year following injury. In patients with a wrist fracture, however, levels of bone formation and resorption markers were unchanged immediately following fracture and four months postinjury. In contrast, a study of ankle fractures showed that P1NP and osteocalcin levels remained elevated up to a year after fracture whereas levels of resorption markers were stable.

We elected to examine bone turnover markers at three months after injury to minimize the effects of the fracture itself on bone turnover marker levels; however, it is possible that measurements performed at later time points would have yielded different results.

The mechanism by which increased bone turnover influences skeletal fragility is important to elucidate and may be related to altered bone microarchitecture. Deficits in trabecular structure have been documented in patients with higher levels of bone turnover markers. Similar to our results, a prior study of patients with a hip fracture showed a negative correlation between P1NP levels and bone mineral density and an increased risk of fracture with high levels of P1NP. At present, levels of bone turnover markers are not routinely used in the evaluation of osteoporosis and fracture risk. Our study revealed that the P1NP level may be of clinical relevance in identifying patients at risk for distal radial fracture. There are potential advantages to using P1NP levels in routine clinical analysis: measurements are not substantially altered by food intake or by circadian rhythms, and they are stable at room temperature and -70°C. P1NP may thus be a useful clinical marker of an increased fracture risk, particularly when combined with low bone mineral density.

Study limitations include a predominantly Caucasian patient population and a cross-sectional design, which does not allow prospective fracture risk prediction. We excluded patients with known comorbidities known to affect bone metabolism, which limits our ability to generalize results. We elected to obtain 25(OH)D and bone turnover marker levels at three months after injury to minimize the effects of fracture repair on measured bone formation and resorption. As a result, our study does not reflect 25(OH)D levels on the day of injury; however, no new vitamin-D supplementation was started until after the blood was drawn. When bone turnover markers are used to assess bone metabolism, consideration must be given to day-to-day variability; circadian rhythmicity; and, for CTX-1, considerable change in response to eating. To account for these factors, we collected all specimens after the patient had fasted overnight, and at the same time of day, and analyzed them together with the same reagents. We believe that these steps minimized potential measurement variations.

The study’s strength lies in its focus on distal radial fracture. Although central fractures are associated with greater morbidity, distal radial fractures are the earliest presenting fragility fractures in postmenopausal women and thus offer a unique opportunity to initiate treatment for underlying abnormalities in bone structure and metabolism. We were able to obtain all measurements relatively soon after fracture, and we limited the number of potential confounders by applying strict inclusion and exclusion criteria. Finally, this study is unique in that it focused on 25(OH)D and bone turnover markers specifically in postmenopausal women with a distal radial fracture.

In conclusion, we found that postmenopausal women with a distal radial fracture have similar vitamin-D levels but increased levels of bone formation markers when compared with women without a fracture. Women with a high P1NP level and low bone mineral density were at particularly high risk for fracture. Although limited by the cross-sectional study design and the fact that few subjects in our study had a low vitamin-D level, our results suggest that routine monitoring of vitamin-D levels in our region does not appear to be necessary in patients with a distal radial fracture. P1NP may be a useful clinical marker of bone fragility.

Note: The authors acknowledge Mark P. Cote, at the University of Connecticut Musculoskeletal Institute at UConn Health (Farmington, Connecticut) for his data and statistical analysis necessary for completing this work. They also acknowledge the Lowell P. Weicker, Jr. Clinical Research Center, UConn Health (Farmington, Connecticut).

Tamara D. Rozental, MD
Lindsay M. Herder, BA
Kempland C. Walley, BSc
Mary L. Boussein, PhD
Department of Orthopaedic Surgery,
Beth Israel Deaconess Medical Center,
330 Brookline Avenue,
Stoneman 10,
Boston, MA 02215

David Zurakowski, PhD
Departments of Anesthesia and Surgery,
Children’s Hospital,
300 Longwood Avenue,
Boston MA 02115

Kathleen Coyle, RN, BSN
Jennifer M. Wolf, MD
Department of Orthopaedic Surgery,
University of Connecticut Health Center,
263 Farmington Avenue,
Farmington, CT 06030
The purpose of this study is to evaluate the impact of Vitamin D in prevention and progression of post-traumatic osteoarthritis (PTOA). The animal portion of this study involves surgical induction of osteoarthritis in mice, with supplementation of varying levels of Vitamin D, and evaluation using histology and micro-CT. The clinical portion is an add-on study at the United States Military Academy, evaluating a clinical cohort of USMA cadets treated for anterior cruciate ligament (ACL) tear, with pre- and post-injury serum 25-hydroxy-Vitamin D levels and correlation with joint space narrowing and biomarkers of cartilage injury. Findings from the animal model show preliminary evidence that Vitamin D supplementation may decrease OA in female animals, with less severe histologic grading in animals given supraphysiologic doses of oral Vitamin D. In the clinical portion, we have enrolled 36/100 (36%) of the required patients for the clinical study, but will evaluate serum 25-hydroxy-Vitamin D once the entire cohort is enrolled. Our findings provide preliminary support for the concept that Vitamin D supplementation could prevent the onset of often rapid joint destruction that occurs with PTOA, with important implications for high-risk military occupations.