Karen B. King, Ph.D.
Associate Professor
Department of Orthopaedics, Division of Bioengineering
University of Colorado Denver

Contact Information for PI:
Mail Stop 8343
RC1 North, 12800 E. 19th Ave, Room 2103
Aurora, CO 80045
Telephone: 303-724-1596
Fax: 303-724-0394
Email: Karen.King(a~UCDenver.edu

In Vivo Rabbit Model of Finger Musculoskeletal Disorders
Date of Report: November 20, 2008

Award Institution:
University of Colorado at Denver & Health Sciences Center
Mail Stop F428, Anschutz Medical Campus, Bldg 500 Rm
W1 126, 13001 East 17th Place, PO Box 6508
Aurora, CO 80045-0507

Principal Investigator: Karen B. King, PhD (from 09/03/03 to present) Co-Investigator: David Rempel, MD (09/03/03 - 06/30/06)

National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention 5 R01
OH007786
From: 09/03/03
Through: 08/31/08

Note: An application for competitive continuation/renew is pending NIOSH council review for continuation of this project.

Table of Contents

List of Terms and Abbreviations

CT	computed tomography
DIP	distal interphalangeal
FTIR	Fourier transform infrared
Hz	hertz
MCP	metacarpophalangeal
MMP	matrix metalloproteinases
MPa	megapascal
MRI	magnetic resonance imaging
N	newton
NORA	National Occupational Research Agenda
OA	osteoarthritis
TIMP	tissue inhibitors of metalloproteinases
TGFI3	transforming growth factor beta

Abstract

The goal of this project was to develop and validate a method for testing the biomechanical risk factors for occupational hand injury due to overuse. This project focused on the risk factors of force and frequency in order to determine how hard and fast a worker can perform a repeating hand task without injury. This is an important problem for the United States workforce because manual handling and similar tasks are on the rise along with upper musculoskeletal disorders. Even some jobs considered white collar such as dental hygiene are seeing increasing hand injuries which may be due to forceful and repetitive hand tasks like periodontal scaling.

The design approach of this project was to measure the biological changes in finger joint tissues following repeated grip-like motions with groups of experiments testing different force levels and different frequency levels. Because measuring biological effects of joint tissues requires destructive methods (biochemistry, histology, etc.), it was necessary to first create an animal model for cyclical finger joint loading. Thus, the first aim of the project was to create an in *vivo* model that would simulate hand tasks using occupationally relevant loads and speeds. The second and third aims of the project were to measure the structural and biochemical changes resulting from the loading and to determine if these changes are co-localized. The fourth aim of the project was to test different force levels and different frequency levels to determine if thresholds of loading exist.

The key accomplishment of this project is the development of the in vivo rabbit model for testing force and frequency of finger joint loading. The rabbit has similar joint, muscle, tendon and ligament anatomy with only a few exception (e.g., the 1st digit is not opposable like the human thumb), but these do not affect the use and value of the model. This new animal model applies precise loads to the digit tip at precise frequencies. At the end of each experiment, the loaded joint and the control non-loaded joint of the opposite limb are measured for structural and biochemical changes such as the amount of specific proteins required for joint function. Previous experimental animal models of loading have been developed by others; however, most of those models either rely on behavioral modification and/or training of the animal which do not provide consistant force or frequency levels or the model includes surgical alteration of the joint at the beginning of the experiment which doesn't address typical workplace issues. Prior to this project [5 R01 OH007786], no methodology existed for in vivo testing of force and frequency at levels of cyclical joint loading that were reasonable for understanding the biological effects of occupational hand tasks. Thus, the successful development of this experimental model is an important accomplishment in occupational safety research.

Other major accomplishments of this project are 1) identifying a low frequency threshold i. e., the speed at which no changes (good or bad) occur, 2) identifying some proteins that are increased with loading, 3) identifying some proteins that are decreased with loading, and 4) identifying the effect of different force levels on these key proteins. These findings and future findings regarding the changes in proteins due to repetitive hand tasks are critical to identifying and understanding the biological mechanisms that connect specific levels of force and frequency of repetitive hand tasks to occupational injury. By understanding these mechanisms and their force and frequency threshold levels, logical interventions can be developed to prevent injury while maintaining productivity at work.

Highlights/Significant Findings

The first significant finding of this project was the design and validation of an *in vivo* animal model for the study of injuries resulting from highly forceful and repetitive hand tasks in the workplace. This animal model allows the simulation of workplace hand motions with the benefit of having physiologically intact finger joints which better replicate the biomechanical and biochemical environment of the finger joint much more closely than cell culture or tissue explant culture. Using this model allows the study of articular joint tissues (cartilage and bone) that cannot be ethically removed from workers for biochemical studies.

The next significant finding was that there exists a biological response threshold to frequency of cyclical joint loading. We found that no cellular response (change in proteoglycan) occurs with a frequency of 10 load cycles per minute while several cellular responses (including increased proteoglycan content) occur with a frequency of 60 load cycles per minute.

Another significant finding is that there exists a biological response threshold to the force level of cyclical joint loading. We found that no cellular response (change in proteoglycan) occurs with a very low force level such as when fingers are flexed repetitively without any added load to the digit tip. However, with an added load that is approximately 17% of maximum muscle contraction there are several biological responses including increased proteoglycan content.

Related to the above highlights are recent findings that suggest a biological mechanism between cyclical finger joint loading and injury. Loading patterns that are above threshold for frequency and load result in alterations of matrix metalloproteinases (MMPs), the enzymes that break down extracellular matrix including proteoglycan. At the frequency level of 60 load cycles per minute and the force level of -17% maximum, the number of cells with MMP-2 (formerly known as gelatinase A) decreased while the content of proteoglycan increased. Since proteoglycan is important to cartilage homeostasis this may indicate that this particular loading pattern is beneficial to joint health. We are currently examining these same biological responses in loading patterns that have similar load frequencies but higher levels of force. Early data indicate that these higher forces elicit joint damaging biological responses. We are conducting data analysis on these higher forces to verify the MMP mechanism for repetitive load-induced injury and to determine the ratio between force level and MMP level. This approach will support the translation of our findings to usable guidelines for safe loading levels of hand tasks in the workplace.

Translation of Findings

Epidemiology has determined that in the workplace, hand tasks with high force and high frequency of repetition are associated with increased pain and injury. This project created an in *vivo* animal model to simulate hand tasks in the workplace. This project used the *in vivo* model to study the relationship between cyclical load and biological mechanisms that lead to joint injury. Several force and frequency levels were tested and higher force levels are currently being studied. Determining the dose-response relationship between biomechanical risk factors and injury will advance the development of hand task guidelines and other ergonomic tools with the support of scientific data that directly demonstrate the cause and effect of these occupational injuries. For example, these data could be used to design a pinch-grip equation analogous to the Revised NIOSH lifting equation that was created to reduce occupational back injury. Workplace interventions to decrease upper musculoskeletal disorders could potentially save both workers and employers significant costs in terms of lost wages and decreased productivity. Interventions using therapeutic/rehabilitative joint loading activities could potentially relieve pain and reduce disability of affected workers.

The National Occupational Research Agenda (NORA) initiative identifies work-related upper musculoskeletal disorders as a high priority area, specifically the pathophysiologic mechanisms of chronic musculoskeletal injury. This project addresses NORA in identifying dose-response relationships of force and frequency of finger joint loading and in identifying the ultrastructural injury and biochemical alterations associated with mechanical loading. Although it may be unrealistic to eliminate jobs with intensive hand tasks, with the appropriate mechanistic and threshold data the way such tasks are performed could be altered to decrease the risk of injury.

Outcomes/Relevance/Impact

The major impact of this project is the creation of an *in vivo* animal model for the study of occupational risk to an organ system (the diarthoidial joint) that cannot be studied in humans without the destruction of the system itself. Although there are non-destructive imaging modalities available for the study of joint disorders (e.g., MRI, microCT), these are not yet developed for in depth metabolic studies. Furthermore, the precise control of force and frequency (which is necessary for determining injury threshold) is difficult to achieve in human studies. Thus, an *in vivo* model is quite valuable to the study of biological mechanisms of occupational injury.

The development of an animal model for the study of force and frequency of cyclical joint loading is highly relevant to occupational health. Force and frequency are two biomechanical risk factors for injury that in most cases can be easily modified without great consequence to overall productivity. In addition, the interplay between force and frequency may be relevant to simple workplace modifications. This animal model is being used to determine which factor should be adjusted to have the great impact on worker health. For example, would a small decrease in joint loading force allow a large increase in frequency without risk of joint injury?

The outcomes of these studies show that biological changes can be measured and correlated to force levels and frequency levels. Potential outcomes will be used to determine the force threshold for injury and the frequency threshold for injury. These thresholds will be used to create recommendations, for example, a hand loading table incorporating force x frequency levels for hand tasks. Such a table could then be tested for effectiveness in a workplace validation study with non-invasive measured outcomes of joint pain, swelling, range of motion and so forth. Ultimately, job tasks involving repetitive hand work could be modified to appropriate force and frequency levels to avoid injury and to keep healthy workers productive and at work.

Scientific Report

Joint pain and joint disorders are associated with repetitive forceful hand activities such as those found in the workplace. Improper and/or excessive loading can be a significant factor in the development and progression of joint disease. A series of minor injuries such as repeated stereotyped activity that exceeds the injury threshold of the joint tissues can transmit excessive force and initiate damage [1]. Such stereotyped activity of the hand can be experienced in occupations with high-force repetitive gripping such as dentistry [2-5]. These biomechanical risk factors are preventable threats to worker health.

Epidemiologic studies have identified correlations between occupation and joint disorders including hand osteoarthritis (OA). A Finnish study of 291 young to middle aged female dentists (45-63 years old) has identified increased risk for finger joint OA among dentists with low task variation [2]. The study grouped dentists in clusters by the number of hours spent in low variation tasks (e.g., restoration and endodonics) and high variation tasks (e.g., surgery). The low task variation cluster had a higher risk for OA in the thumb, index and middle finger joints as measured by hand radiography which is in agreement with the hypothesis that stereotyped hand activities at work lead to hand OA.

Dentistry is emerging as one of the fastest growing professions in the United States. The number of dentists is expected to increase >9% between 2006 and 2016 and jobs for dental hygienists and dental assistants are expected to increase just over 30% [6]. The vulnerability of dental workers to occupational injury likely results from biomechanical risk factors similar to those found in manufacturing. For example, just as an assembly line requires fast-paced work to maximize productivity, the dental worker's day is scheduled to attend the maximum number of patients in order for the practice to remain profitable. Another biomechanical risk factor is the presence of forceful, repetitive hand tasks such as periodontal scaling. Scaling is a highly repetitive task performed by dentists and hygienists. High grip forces are used in scaling and range from 1.0 N to 15.7N [3]. Additional high force repetitive tasks may include probing, polishing, and flossing, although maximal grip force may occur less frequently [3]. Dentistry is just one example of a growing US labor market that would benefit from the study of biological effects of repetitive joint loading.

Additional studies find hand OA a relevant issue in additional occupations. Rossignol et al. , found that occupations with repetition of movement at machine pace are also associated with hand OA [7]. Haara et al., found that physical stress at work was associated with OA of the metacarpophalangeal (MCP) and distal interphalangeal (DIP) joints [8]. One of the first studies to compare finger joint disease between workers with hand-intensive tasks and the general population was published in 1961 [9]. Prolonged, "powerful" gripping was also identified as a high-risk occupational task for career truck drivers and farmers that results in bilateral radiographic abnormalities including joint space narrowing, prominent osteophytes, cysts, and sclerosis [10]. A more comprehensive cross-sectional study established the relationship between a specific hand task and symptoms within the specific joint used in the task [11]. In brief, the study examined the hand joints of female textile workers who performed defined, non-traumatic but repetitive manual tasks for at least 20 years and found highly significant task-related pathology affecting the structure and function of the specific joints used for the task. The outcome measures of this study were range of motion, joint degeneration, and joint malalignment. The tasks that loaded specific joints (e.g., gripping with thumb and index finger) resulted in pathological damage to only the joints used. Signs of joint damage included decreased range of motion, joint space narrowing, and increased joint malalignment (all key diagnostic features of OA) [12]. Other workers within the same mill who performed one of two different tasks loading other hand joints were affected only in those specific joints. These studies demonstrated that many occupational tasks, particularly repetitive gripping, are associated with hand OA. Thus, several occupations such as dentistry, farming, textiles, and

manufacturing would benefit from studies to determine thresholds of injurious finger joint loading.

Musculoskeletal disorders of the joints (including joints other than in the hand) is one of the most expensive groups of disorders in this country costing about 2.5% of the Gross National Product [13]. Arthritis (all types) is the number one cause of disability reported by all adults with disabilities [14]. At this time there is no satisfactory cure for these disorders and the most commonly used treatments are analgesics for pain and prostheses to replace destroyed joints. Confounding the solution to the problem is a paucity of good early diagnostic tools and a lack of effective intervention strategies. What is clearly needed now is a better understanding of the biological mechanism that bridges the risk factors of a hand task (identified by epidemiological studies) to the painful and potentially disabling injury to the worker. The risk factors that can most easily be modified are mechanical factors of joint loading (repetition and load). Identifying how these factors affect the biological mechanism that mediates injury will lead to development of interventions that can be translated to the workplace.

Joint pain and disability are mediated by inflammation and tissue damage. The primary cause of musculoskeletal tissue damage is the loss of the key structural proteins: collagens and proteoglycans [15]. This loss occurs when the protein synthesis rate is exceeded by the degradation rate. Degradation of collagens and proteoglycans is caused by enzymes, specifically the matrix metalloproteinases (MMP) [16]. The synthesis, activation, and activity of MMP enzymes are controlled by the activity of various inflammatory and anti-inflammatory cytokines [17] and the presence of specific enzyme inhibitors known as tissue inhibitors of metalloproteinases (TIMP) [18]. The mechanical risk factors of upper musculoskeletal occupational disorders, force and repetition, have been shown by experiments using *in vitro* mechanical loading of cells or tissue explants to mediate the production of MMPs, aggrecanases, TIMPs, and cytokines [19, 20]. Although such *in vitro* studies have been performed under a variety of loading parameters and inconsistent culture conditions, it is clear that no or very low force levels of loading as well as very high force and impact load levels are damaging to joint tissues while moderate load levels are beneficial to joint tissue homeostasis. However, such *in vitro* studies are unable to recreate the physiological combination of mechanical, chemical and electrical factors that is present in the intact articulating joint. **What has not been determined by previous studies is which levels of force and repetition are damaging in physiological situations. To determine or even speculate which force levels and which frequency levels are safe for repetitive hand tasks, in vivo experiments using intact finger joints are required. Prior to this project [5 R01 0H007786], no methodology existed for in vivo testing of force and frequency at levels of cyclical joint loading that were reasonable for understanding the biological effects of occupational hand tasks.**

Thus, the goal of this R01 research project was to create an appropriate in vivo rabbit model of cyclical joint loading using force and frequency levels to simulate occupational hand tasks. A further goal was (and continues) to measure the biological effects of work-related cyclical joint loading on joint tissues to identify which loading levels are damaging to joint function (i.e., mechanobiology studies). Our studies using the *in vivo* rabbit finger flexion model moves the field of mechanobiology forward from *in vitro* studies because in the workplace setting multiple stresses and strains are acting on musculoskeletal tissues during occupational hand tasks which can be better simulated in an animal model, and thus, the *in vivo* model is more physiologically relevant than *in vitro* experiments by others. Our *in vivo* model also moves the field forward from the foundation work of epidemiologic studies because this model simulates the forces and frequencies of occupational hand tasks but also allows for the study of metabolic changes that cause occupational injury since animal tissues can be studied following cyclical loading experiments whereas collection of joint tissue of workers is impractical and unethical.

The specific aims of this study were the following: 1) The combination of force and repetition causes greater damage than repetition alone, 2) Changes in cellular response occur in joint tissues due to repetitive loading, 3) Changes in cellular response co-localize with structural damage in repetitively loaded joint tissues, and 4) There is a defined injury threshold associated with repetitive joint loading. The accomplishments of each aim are detailed in the following paragraphs. These findings were reported in the peer-reviewed literature and at international conferences as peer-reviewed poster or podium presentations.

Aim 1 (part a: validation of the model). In Aim 1 we created an animal model for repetitive finger flexion simulating dentistry, manufacturing, and other manual work. The anesthetized rabbits were laid supine with their forelimbs supported in a neutral posture. Electrical stimulation of the *flexor digitorum profundus* muscle applied to the experimental limb caused the digits of that limb to flex. A load was added to the tip of the third digit. The loading protocol was 0.42 N mean peak digit tip force, 60 repetitions per minute (1 Hz), 60 cumulative hours of exposure. Using free body analysis, we estimated that the joint contact force was 1-2 MPa. The digit joints of one limb each from a total of 12 animals were repetitively loaded. The subject number was increased to 12 at the recommendation of the Scientific Review Group in anticipation of subject drop-out due to anesthesia sensitivity. Thin sections of metacarpophalangeal (MCP) joints were prepared from digits that were repetitively flexed and loaded, or neither flexed nor loaded (i.e., experimental control joints from the contra-lateral limb). For Aim 1, photomicrographs of Safranin 0-stained thin sections of these joints were digitally analyzed for morphological features of cartilage fibrillation, uncalcified cartilage thickness, calcified cartilage thickness, mean uncalcified cartilage thickness, mean calcified cartilage thickness, osteochondral interface complexity, uncalcified cartilage cell density, and calcified cartilage cell density. We found that repetitive joint loading causes structural changes to joint tissues, namely that mean uncalcified cartilage thickness decreases with load when measured across the proximal surface of the joint. These findings were presented at an international research conference in 2005 and were published in *Osteoarthritis and Cartilage* (King, et. al, 2005).

Aim 1 (part b: flexion vs. load). To compare the relative effects of two biomechanical risk factors of injury (flexion and load) within the same set of animals, we took advantage of an anatomical difference between rabbits and humans. Due to fusion of the rabbit flexor tendons as they pass through the carpal tunnel area, all digits within the experimental limb flex upon electrical stimulation of the *flexor digitorum profundus* muscle (whereas human are able to independently move their fingers). We took advantage of this difference in rabbits by measuring the morphology of the joints that received no digit tip loading, but flexed at the same rate as the loaded digits from Aim 1, part a. Therefore, the rate of loading was 60 repetitions per minute for 60 cumulative hours, but the only force on the MCP joint was from the mass of the digit itself. Using free body analysis, we estimated that the joint contact force of these flexed-only joints was much less than those of the digit with repetitive external loading (< 0.1 MPa). We used the same histology and image analysis protocols as above to measure the joint morphology of the flexed-only joints. We found that as with added load, mean uncalcified cartilage thickness decreases with repetitive joint flexion when measured across the proximal surface of the joint. This suggested that joint flexion with a low load may cause structural tissue changes. This finding was presented at an international research conference (Marecek, et al., 2006). We continued these measurements with a larger subject number and with additional metabolic studies to determine the full extent of biological changes due to lower load (detailed below in Aim 2, part c).

Aim 1 (part c: frequency of flexion). Since the role of flexion was shown to be important in finger joint loading in Aim 1, part b, we examined the effect of flexion frequency. To accomplish this we used a new set of animals that were treated with similar muscle stimulation and digit tip loading protocols as for Aim 1 part a, with the exception that the flexion frequency

was 10 repetitions per minute (0.17 Hz). The duty cycle was lengthened so that the total work performed was equivalent to Aim 1, part a. The goal was to compare the effect of fast (part a) to slow (part c) frequency under otherwise identical conditions. We used the same histology and image analysis protocols as above to measure the joint morphology of the flexed-only joints. The morphology of fast-flexed joints was compared to those of slow-flexed joints. We found that at the slow frequency there was no difference in cartilage thickness between experimental and control joints. Therefore, while flexion alone may have caused structural joint changes, the frequency level was a key factor. We continued our study of load frequency with additional metabolic studies to determine the effect of lower frequency on joint tissue metabolism (detailed below in Aim 2, part d) .

Aim 2 (part a: cellular response with osteopontin). As a marker for cellular response, we measured the amount of osteopontin protein, which is known to increase with mechanical load. Osteopontin may also be a marker for injury since it is also found in regions of injured tissues, for example, lung fibrosis. We found in our original loading pattern (0.42 N, 60 cycles/min.) the number of cartilage cells (chondrocytes) with osteopontin immunostaining increased compared to contra-lateral control joints. This was also the first demonstration of osteopontin staining in adult chondrocytes. This finding was also published in *Osteoarthritis and Cartilage* (King, et. al, 2005).

Aim 2 (part b: cellular response with extracellular matrix structural proteins, proteoglycan and collagen). We used Fourier Transform InfraRed (FTIR) microspectroscopy to simultaneously localize and measure the amounts of proteoglycan and collagen, two molecules that are vital to the structure and function of joint cartilage. FTIR is a sensitive and quantitative technique. Fewer rabbits (n = 6) were required to detect statistically significant changes. We found that proteoglycan increases in the deep zone of cartilage after *in vivo* cyclical joint loading. This was the first study to apply the technique of FTIR microspectroscopy to occupational medicine. The findings of this study were presented at an international research conference in 2006 and were published in *Arthritis Research and Therapy* (Saadat, et al., 2006).

Aim 2 (part c: cellular response with extracellular matrix structural proteins, proteoglycan and collagen in low force *in vivo* cyclical joint loading). We compared joints that were flexed and loaded with joints that were only flexed using FTIR as above. Unlike the flexed and loaded joints above (Aim 2, part b) we measured no change in proteoglycan in the flexed-only joints. However, it should be noted that a trend of increased proteoglycan was observed in the deep zone of the flexed-only joints (N = 7 rabbits, P= 0.11). These findings were presented at an international research conference in 2007 and are included in a manuscript currently in peer-review for publication.

Aim 2 (part d: cellular response with extracellular matrix structural proteins, proteoglycan and collagen in low frequency *in vivo* cyclical joint loading). Similarly, we compared joints that were flexed and loaded at the slow frequency to the joints flexed and loaded at the fast frequency (Aim 2, part b), again using FTIR. We measured no change in proteoglycan at low frequency. No significant change (nor trend) was found in any of the cartilage zones in the low frequency joints.

Aim 3 (localized cellular response). The goal of Aim 3 was to measure localized cellular response at the site of structural changes. In human cartilage degeneration, the first signs of injury appear in the region of highest mechanical force. In the rabbit MCP joint there also is such a high load region which is where we measured cellular response to loading in all experiments. In addition, cartilage can be anatomically divided from the articulating joint surface to the bone into regions or "zones" based on morphological, metabolic, and mechanical aspects: the superficial zone, the mid zone, and the deep zone. The responses measured in this study were

the amounts of proteins that are markers and/or effectors of tissue injury or damage, and these proteins were measured in each of the three cartilage zones.

Aim 3 (part a: proliferation). Cellular proliferation is a common response to injury. More cells would be available to produce structural extracellular matrix proteins (for example, proteoglycan) in order to restore tissue properties and thus restore joint mechanical function. In addition, in late osteoarthritis, osteophytes are formed as a failed attempt to restore joint stability. An early step in osteophyte formation is cell proliferation. Ki67, a nuclear antigen that is present in proliferating cells. We measured Ki67 in the flexed and loaded joints. We found a slight but non-statistically significant decrease with loading in the number of Ki67-positive cells per area. This suggested that in our original loading conditions (Aim 1, part a) cellular proliferation is not a significant response. We continued our study of cell proliferation and osteophyte formation with additional studies at higher force levels as below in Aim 4, part a, to determine the effect of high force on joint tissue metabolism.

Aim 3 (part b: matrix degradation). As reviewed above, many members of the matrix metalloproteinsase family are involved in injury and repair. MMP-13 cleaves native cartilage collagen and is known to be involved in osteoarthritis. MMP-2 degrades denatured collagen after an initial MMP-13-type cleavage. Together these two types of MMPs can remove collagen from the extracellular matrix. These proteinases can also degrade other matrix proteins such as proteoglycan. We found no change in MMP-13 between loaded joints and their controls. We did, however, find a slight decrease in MMP-2 with loading (N = 10, P = 0.03). This suggests that either our original loading pattern (Aim 1, part a) is anti-degradative or that at the time of our examination the cells were in a "repair" stage.

Aim 3 (part c: matrix degradation in low force in vivo cyclical ioint loading). To identify if a decrease in MMPs also occurred whether or not an external load was added, we measured MMP-2 and MMP-1 3 in the flexed-only joints. In flexed-only joints there was no change in the number of cells that were positive for MMP-2 staining in any of the three cartilage zones. However, the percent of total cells that were positive for MMP-1 3 was 6% higher than controls in the superficial zone. It should be noted that the subject number for this experiment was low (N 5 rabbits) and a single high value may have influenced the result. Applying the non-parametric Wilcoxon signed-rank test to these data indicated this trend was not significant. We are continuing this experiment with a larger subject number.

Aim 3 (part d: matrix degradation in slow frequency in vivo cyclical ioint loading). To identify if the effect on MMPs was similar in low frequency loading we measured MMP-2 in the slow frequency joints. Unlike the fast frequency loaded joints detailed above, we measure no change in the deep zone at the slow frequency. However, there was a decrease in the number of MMP-2 positive cells in the superficial zone (N = 14 rabbits, P = 0.04).

Aim 3 (part e: preliminary data on cytokine level after in vivo cyclical ioint loading). To identify if cytokines are involved in the structural and metabolic effects of in vivo cyclical loading, we measure the number of cells positive for cytokines. We began with TGFR. This cytokine has traditionally been considered an anti-inflammatory and extracellular matrix-building element. In a pilot study we found that TGF(3 expression (number of positive cells) corresponded with the proteoglycan amount but inversely corresponded to MMP-2 expression. It may be that in vivo cyclical joint loading at our original load pattern (Aim 1, part a) increases TGFR which inhibits MMP-2 action of degrading proteoglycan resulting in a net increase in proteoglycan. Current experiments are investigating this mechanism as well as alternative mechanisms and additional cytokines.

Aim 4 (injury threshold). The goal of Aim 4 was to determine the injury threshold of repetitive finger joint loading. Three force levels, two exposure duration levels, and two frequency levels were measured. Morphological and cellular data were measured as described above. The cellular responses measured were the amounts of proteins that are markers and/or

effectors of tissue injury or damage. Cell proliferation was determined by measuring immunohistological staining for Ki67 antigen. Proteoglycan and collagen contents were measured using FTIR microspectroscopy. The presence of matrix degrading enzymes was detected with immunohistological staining for MMPs -13 and -2. The presence of cytokines that control enzyme expression was detected with immunohistological staining for TGFR. Detection of bone remodeling was done with immunohistological staining for alkaline phosphatase.

Aim 4 (part a: force dose-response). We used the rabbit model of cyclical joint loading to compare the effect of three different force levels, -0.08 MPa (flexed only), -1.8 MPa (flexed and loaded), -2.2 MPa (flexed and loaded). These force levels are the joint contact forces as estimated using free-body analysis, a biomechanical method for calculating the transmission of force from a starting point (e.g., finger tip) to a specific point of interest (e.g., the finger joint). The levels of joint contact force correspond to the load applied at the rabbit's digit tip of 0, 0.42, and 0.5 N, respectively. The frequency of all three groups was 60 cycles per minute. In summary of data reported above, we found no significant changes at the lowest force level. We found some significant changes, possibly extracellular matrix promoting, at the intermediate force level. We are currently collecting data from the high force level. We have noted osteophyte formation in one of the 0.5 N loaded joints which would indicate that the high force level leads to OA-like damage to the joint. We are now measuring the characteristics of osteophyte formation across the entire group of high force loaded joints and their controls.

Aim 4 (part b: duration dose-response). We used the rabbit model of cyclical joint loading to compare the effect of three different durations of exposure. We compared our original duration (60 cumulative hours) to a longer duration (80 cumulative hours), to determine if more extensive damage occurs with longer exposure. For both groups, the frequency was 60 cycles per min. and the load added to the digit tip was 0.42N. Morphological data as well as cellular response data were collected. For the most part, data at this load level were similar, the only exception being a decrease in MMP-2 in the superficial zone that was significant in the 80 hour joints but not in the 60 hour joints.

Aim 4 (part c: frequency dose-response). We used the rabbit model of cyclical joint loading to compare the effect of two frequency levels, fast (60 cycles/min.) and slow (10 cycles/min.). Both morphology and cellular response data were measured. In summary of data reported above, the slow frequency produced none of the changes observed with the fast frequency.

Additional work completed: joint function relative to dose. All previous assessment of joint tissue health or injury following repetitive loading has been at the microscopic scale using either histological staining for general morphology, immunohistological staining or infrared spectroscopy for specific protein quantification. These are excellent and specific methods to determine if the necessary joint tissue structural proteins (or their modulators) are present and their relative amounts after loading. From this we can infer that if the structural proteins are lost due to loading, then joint function, which is dependent on these proteins, has been compromised. For future studies, it would be valuable to be able to directly measure joint function. Since the function of articulating joints is to resist compressive loads, tissue properties related to compression are important. To this end, we measured changes in joint cartilage bio mechanical properties due to repetitive loading. These tests allow us to better characterize loss of joint function due to loading. The preliminary development of tools for nano-scale measurements of tissue material properties of stiffness, resistance to deformation, and tissue modulus has been completed. The results of this study have been published in the *Journal of Biomaterials Research (Li,* et al., 2006).

References cited in this Scientific Report:

1. Felson DT, Zhang Y: **An update on the epidemiology of knee and hip osteoarthritis with a view to prevention.** *Arthritis and Rheumatism* 1998, 41:1343-1355.
2. Solovieva S, Vehmas T, Riihimaki H, Takala EP, Murtomaa H, Luoma K, Leino-Arjas P: **Finger osteoarthritis and differences in dental work tasks.** *J Dent Res* 2006, 85(4):344-348.
3. Zappa U, Cadosch J, Simona C, Graf H, Case D: **In vivo scaling and root planing forces.** *J Periodontol 1991,* 62(5):335-340.
4. Dong H, Loomer P, Villanueva A, Rempel D: **Pinch forces and instrument tip forces during periodontal scaling.** *J Periodontol* 2007, 78(1):97-103.
5. Bramson JB, Smith S, Romagnoli G: **Evaluating dental office ergonomic. Risk factors and hazards.** *Journal of the American Dental Association* 1998, 129(2):174183.
6. Dohm A, Shniper L: **Employment outlook: 2006-2016. Occupational employment projections to 2016.** *Monthly Labor Review* 2007:86-125.
7. Rossignol M, Leclerc A, Allaert FA, Rozenberg S, Valat JP, Avouac B, Coste P, Litvak E, Hilliquin P: **Primary osteoarthritis of hip, knee, and hand in relation to occupational exposure.** *Occup Environ Med* 2005, 62(11):772-777.
8. Haara MM, Manninen P, Kroger H, Arokoski JP, Karkkainen A, Knekt P, Aromaa A, **Heliovaara M: Osteoarthritis of finger joints in Finns aged 30 or over: prevalence, determinants, and association with mortality.** *Ann Rheum Dis* 2003, 62(2):151-158.
9. Lawrence JS: **Rheumatism in cotton operatives.** *British Journal of Industrial Medicine* 1961, 18:270-276.
10. Williams WV, Cope R, Gaunt WD, Adelstein EH, Hoyt TS, Singh A, Pressly TA, English R, Schumacher HR, Jr., Walker SE: **Metacarpophalangeal arthropathy associated with manual labor (Missouri metacarpal syndrome).** *Arthritis and Rheumatism* 1987, 30: 1362-1371.
11. Hadler NM, Gillings DB, Imbus HR, Levitin PM, Markuc D, Utsinger PD, Yount WJ, Slusser D, Moskovitz N: **Hand structure and function in an industrial setting, influence of three patterns of stereotyped repetitive usage.** *Arthritis and Rheumatism* 1978, 21:210-220.
12. Altman RD, Hochberg M, Murhpy WJ, Wolfe F, Lequesne M: **Atlas of individual radiographic features in osteoarthritis.** *Osteoarthritis and Cartilage* 1995, 3:3-70.
13. Yelin E, Callahan LF: **The economic cost and social and psychological impact of musculoskeletal conditions.** *Arthritis and Rheumatism* 1995, 38:1351-1362.
14. Centers for Disease Control and Prevention: **Prevalence of disabilities and associated health conditions among adults - United States, 1999.** *Morbidity and Mortalilty Weekly Report* 2001, 50:120-125.
15. Murphy G, Knauper V, Atkinson S, Butler G, English W, Hutton M, Stracke J, Clark I: **Matrix metalloproteinases** in arthritic **disease.** *Arthritis Research & Therapy* 2002, 4 **Suppi** 3:S39-49.
16. Kevorkian L, Young DA, Darrah C, Donell ST, Shepstone L, Porter S, Brockbank SM, Edwards DR, Parker AE, Clark IM: **Expression profiling of metalloproteinases and their inhibitors in cartilage.** *Arthritis and Rheumatism* 2004, 50(1):131-141.
17. Tetlow LC, Adlam DJ, Woolley DE: **Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: Associations with degenerative changes.** *Arthritis & Rheumatism* 2001, 44(3):585594.
18. Nagase H, Woessner JF, Jr.: **Matrix metalloproteinases.** *Journal of Biological Chemistry* 1999, 274(31):21491-21494.

19. Wong M, Siegrist M, Goodwin K: **Cyclic tensile strain and cyclic hydrostatic pressure differentially regulate expression of hypertrophic markers in primary chondrocytes.** *Bone 2003,* 33(4):685-693.

20. Archambault JM, Jelinsky SA, Lake SP, Hill AA, Glaser DL, Soslowsky LJ: **Rat supraspinatus tendon expresses cartilage markers with overuse.** *Journal of Orthopaedic Research* 2007, 25(5):617-624.

Publications Resulting from this Study

1. KB King, C Opel, and D Rempel. Cyclical articular joint loading leads to cartilage thinning and osteopontin production in a novel *in vivo* rabbit model of repetitive finger flexion with loading. Osteoarthritis and Cartilage. 13:971-978, 2005.

2. C Li, LA Pruitt, and KB King. Nanoindentation differentiates tissue-scale functional properties of native articular cartilage. Journal of Biomaterials Research. 17A:729-738, 2006.

3. E Saadat, H Lan, S Majumdar, DM Rempel and KB King. Long-term cyclical *in vivo* loading increases cartilage proteoglycan content in a spatially specific manner: .an infrared microspectroscopic imaging and polarized light microscopy study. Arthritis Research and Therapy. 8:R147, 2006.

4. C Li, KB King, and LA Pruitt. Zone-specific changes in micromechanical, biochemical, and structural properties in articular cartilage from a rabbit joint flexion model. Materials Research Society Symposium Proceedings. 1097E:1-6, 2008.

5. E Saadat, S Majumdar, DM Rempel, and KB King. Effect of cyclical load frequency on cartilage proteoglycan in rabbits. *In review.*

Published, peer-reviewed abstracts

1. DM Ebenstein, K King, and L Pruitt. A nanoindentation technique for measuring mechanical properties of finger joint cartilage. Transactions of the 29th Annual Meeting, Society for Biomaterials, Reno, NV, April 30 – May 3, p. 718, 2003.

   LA Pruitt, C Li, and KB King. Nanoindentation assessment of rabbit finger joint cartilage. 7th World Biomaterials Congress. 2004. Sydney, Australia

3. C Li, DM Ebenstein, K King, and LA Pruitt. Nanoindentation of finger joint cartilage in a fluid cell. Transactions Orthopaedic Research Society. 50:539, 2004.

4. A Medellin, C Opel, and KB King. *In vivo* repetitive joint loading leads to uncalcified cartilage loss: Possible tidemark advancement. 8th International Conference on the Chemistry and Biology of Mineralized Tissues, October 17-22, 2004, Banff Centre, Alberta, Canada.

5. C Opel, D Rempel, and KB King. In vivo cyclical joint loading decreases unmineralized cartilage thickness in the rabbit metacarpophalangeal. Transactions Orthopaedic Research Society. 51:1638, 2005.

6. E Saadat, S. Majumdar, A. Burghardt, and KB King. Cyclical *in vivo* loading increases cartilage proteoglycan content in the rabbit metacarpophalangeal joint. Transactions Orthopaedic Research Society. 52:1352, 2006.

7. GS Marecek, CF Opel, DM Rempel, and KB King. Repetitive finger joint flexion without external load leads to articular cartilage thinning in an *in vivo* rabbit model. Transactions Orthopaedic Research Society. 52:1898, 2006.

8. C Li, K King, and L Pruitt. Depth-dependent nanomechanical response of soft biomaterials. Materials Research Society. San Francisco, CA.

9. C Li, K King, and L Pruitt. Changes in biomechanical properties in articular cartilage due to cyclic loading. Annual Fall Meeting of the Biomedical Engineering Society. October 11-14, 2006. Chicago, IL. 714.

10. C Li, KB King, DM Ebenstein, J. Lin, and L Pruitt. Analysis of force-displacement curves of cartilage and biomaterials. Transactions Orthopaedic Research Society. 53:1565, 2007.

11. E Saadat, S. Majumdar, D. Rempel, and KB King. The effect of frequency and force of in *vivo* loading on proteoglycan content of rabbit articular cartilage. Transactions Orthopaedic Research Society. 54:50, 2008.

12. C Li, KB King, and L Pruitt. Nanoindentation measurement in articular cartilage from an in *vivo* loading model. Transactions Orthopaedic Research Society. 54:649, 2008.

13. C Li, KB King, and L Pruitt. Zone-specific changes in micromechanical, biochemical, and structural properties in articular cartilage from a rabbit joint flexion model. Materials Research Society Spring Meeting. San Francisco, March 25-28, 2008, GG1.10