

CURRICULUM VITAE

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Education

Institution and Location	Degree	Year(s)	Field of Study
University of Maryland (Baltimore, MD)	PhD	08/2012~04/2017	Nursing (Pain/Symptom Management)
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- 2010-2011 **(Part-time) Research Assistant**
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- 2010 – 2011 **Registered Nurse**
Medical Service Team, Asiana Airlines Inc., South Korea
- 2008 – 2010 **Registered Nurse**
Asan Medical Center (Pulmonary department), South Korea
- 2005 – 2008 **Undergraduate Research Assistant**
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List of Relevant Honors, Awards, and Professional Activities

Honors & Awards

- 2016 **Seoul National University Alumni Association (Greater Washington DC area) Scholarship**
Awarded by the Seoul National University Alumni Association
- 2015 **Seoul National University Alumni Association (Greater Washington DC area) Scholarship**
Awarded by the Seoul National University Alumni Association
- 2015 **Student Research Facilitation Award**
Awarded by the Biology & Behavior Across the Lifespan (BBAL) center, School of Nursing, University of Maryland
- 2015 **Mia Loizeaux Scholarship**
Awarded by the School of Nursing, University of Maryland
- 2014 **Travel Award**
Awarded by the Doctoral Student Organization (DSO), School of Nursing, University of Maryland
- 2014 **Member as a Graduate Student**
Inducted by the Sigma Theta Tau International Honor Society of Nursing (Pi Chapter)
- 2013 **Mia Loizeaux Scholarship**
Awarded by the School of Nursing, University of Maryland

- 2012-2014 **Dean Scholarship**
 Awarded by the School of Nursing, University of Maryland
- 2010 **Academic Excellence Scholarship**
 Awarded by the College of Nursing, Seoul National University,
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- 2006 **Academic Excellence Scholarship**
 Awarded by the College of Nursing, Seoul National University,
 South Korea
- 2004 **Academic Excellence Scholarship**
 Awarded by the College of Nursing, Seoul National University,
 South Korea

Grants

- 2015-2017 **American Nurses Foundation Nursing Research Grant**
 Awarded by the American Nurses Foundation/Midwest Nursing
 Research Society (\$5,000)
 Project: Genetic Mechanisms of Aromatase Inhibitor-Associated
 Arthralgia (Mentors: Dr. Cynthia L. Renn, Dr. Susan, G. Dorsey)
- 2014-2016 **Purdue Pharmaceuticals Trish Greene Pain Assessment and
 Management Grant**
 Awarded by the Oncology Nursing Society Foundation (\$25,000)
 Project: New Treatment Strategies for Aromatase Inhibitor-Associated
 Arthralgia (Mentors: Dr. Cynthia L. Renn, Dr. Susan, G. Dorsey)
- 2014-2016 **Doctoral Student Research Grant**
 Awarded by the Sigma Theta Tau International Honor Society of
 Nursing, Pi Chapter (\$1,500)
 Project: Pain Characteristics and Signaling Pathways in Aromatase
 Inhibitor-Associated Arthralgia (Mentor: Dr. Cynthia, L. Renn)

Membership in Professional Societies

- 2015 – Present American Nurses Association (#02911365)
- 2014 – Present Sigma Theta Tau International Honor Society of Nursing, Pi Chapter
 (#1177575)
- 2013 – Present Southern Nursing Research Society (#005784)
- 2013 – Present Oncology Nursing Society
- 2010 – Present Korean Society of Nursing Science

Other Professional Activities

- 2014 – 2015 **Co-leader**
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School of Nursing, University of Maryland
- 2016 – 2017 **Mentor**
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List of Publications, Presentations, and Posters

Peer Reviewed Journal Articles (Published)

1. Kim, H. J., **Yang, G. S.**, Greenspan, J. D., Downton, K. D., Griffith, K. A., Renn, C. L., ... & Dorsey, S. G. (2016). Racial and ethnic differences in experimental pain sensitivity: Systematic review and meta-analysis. *Pain, 158*(2), 194-211.
2. **Yang, G. S.**, Kim, H. J., Griffith, K. A., Zhu, S., Dorsey, S. G., & Renn, C. L. (2016). Interventions for the Treatment of Aromatase Inhibitor-Associated Arthralgia in Breast Cancer Survivors: A Systematic Review and Meta-analysis. *Cancer Nursing*.
3. Han, C.J., & **Yang, G.S.** (2015). Fatigue in irritable bowel syndrome: A systematic review and meta-analysis of pooled frequency and severity of fatigue. *Asian Nursing Research, 10*(1), 1-10.
4. Griffioen, M. A., Dernetz, V. H., **Yang, G. S.**, Griffith, K. A., Dorsey, S. G., & Renn, C. L. (2015). Evaluation of dynamic weight bearing for measurement non-evoked inflammatory hyperalgesia in mice. *Nursing Research, 64*(2).
5. **Yang, G. S.**, & Choe, M. A. (2013). Effect of anorexia and neuropathic pain induced by cisplatin on hindlimb muscles of rat. *Journal of Korean Academy Nursing, 43*(3), 361-369.

Peer Reviewed Conference Abstracts

1. **Yang, G. S.**, Yang, B.K., Renn, C.L., Dorsey, S.D., & Zhu, S. Impacts of vigorous physical exercise on pain in U.S. older adults: A longitudinal analysis of the Health and Retirement Study from 1998 to 2012. Poster discussion (podium presentation) at the 30th Annual Conference of the Southern Nursing Research Society, Williamsburg, VA (February 2016).

2. **Yang, G. S.**, Kim, H. J., Griffith, K. A., Dorsey, S. D., & Renn, C. L. (2015). Interventions for the Treatment of Aromatase Inhibitor-Associated Arthralgia in Breast Cancer Survivors: A Meta-Analysis. Poster Presentation at the Washington Regional Nursing Research Consortium 6th Annual Doctoral Student Research Conference, Washington D. C., USA (November 2015)
3. Kim, H. J., **Yang, G. S.**, & Thomas, S. A. Interventions for preventing depression in ethnic minorities in the United States: A systematic review. Poster Presentation at the Washington Regional Nursing Research Consortium 5th Annual Doctoral Student Research Conference, Washington D.C., USA (November 2014).
4. **Yang, G. S.**, Kim, H. J., & Renn, C. L. Pain management on aromatase inhibitor-associated arthralgia in breast cancer survivors: A systematic review. Poster Presentation at the Washington Regional Nursing Research Consortium 5th Annual Doctoral Student Research Conference, Washington D.C., USA (November 2014).
5. **Yang, G. S.**, Griffioen, M. A., Dernetz, V. H., Griffith, K. A., Dorsey, S. G, & Renn, C. L. Reliability of measurement tools used in the inflammatory hyperalgesia mouse model. Poster Presentation at University of Maryland 36th Annual Graduate Research Conference, Baltimore, MD, USA (March 2014).
6. Griffioen, M. A., **Yang, G. S.**, Dernetz, V. H., Griffith, K. A., Dorsey, S. G, & Renn, C. L. Evaluation of dynamic weight bearing for measuring non-evoked inflammatory hyperalgesia in mice. Poster Presentation at 28th Annual Conference of the Southern Nursing Research Society, San Antonio, TX, USA (February 2014).
7. Griffioen, M. A., **Yang, G. S.**, Dernetz, V. H., Griffith, K. A., Dorsey, S. G., & Renn, C. L. Validation of dynamic weight bearing apparatus as a measurement tool for inflammatory pain in mice. Poster Presentation at University of Maryland 35th Annual Graduate Research Conference, Baltimore, MD, USA (April 2013).
8. **Yang, G. S.**, & Choe, M. A. Effect of anorexia and neuropathic pain induced by cisplatin on hindlimb muscles of rat. Poster Presentation at the Research Institute of Nursing Science of Seoul National University the 9th International Conference, Seoul, South Korea (2013).

Newsletter

1. **Yang, G. S.** (Summer 2014). Erasing the pain of breast cancer treatment: Research seeks relief for patients experiencing AI-induced arthralgia. Nursing Forum: The Magazine of the University of Maryland School of Nursing.

Research Experience

- 2015 – 2017 Genetic mechanisms of aromatase inhibitor-associated arthralgia
The purpose of this study is to investigate genetic and biomarker correlates of aromatase inhibitor-associated arthralgia (AIA), in which the hypothesis is that the AI-treated group will show a different core set of genes that functionally relates to pain mechanisms and significantly changes compared to the control group.
School of Nursing, University of Maryland, Baltimore, MD
Role: **Principal Investigator**
- 2014 – 2016 New treatment strategies for aromatase inhibitor-associated arthralgia
The purpose of this study is to investigate the potential therapeutic effects of physical exercise and curcumin (turmeric) on AI-induced musculoskeletal pain and function, and modulation of brain-derived neurotrophic factor (BDNF) in mice treated with letrozole.
Funded by the Oncology Nursing Society Foundation, USA
School of Nursing, University of Maryland, Baltimore, MD
Role: **Principal Investigator**
- 2014 – 2016 Pain characteristics and signaling pathways in aromatase inhibitor-associated arthralgia
The purpose of this study is to examine whether up-regulation of BDNF signaling and activation of cell cycle components cause central sensitization that leads to pain behavioral response and neuromuscular dysfunction in mice treated with AIs.
Funded by the Sigma Theta Tau International Honor Society of Nursing, Pi Chapter, USA
School of Nursing, University of Maryland, Baltimore, MD
Role: **Principal Investigator**
- 2014 Development of treatment for bortezomib-induced neuropathic pain
Evaluation of the therapeutic efficacy of treatment (gabapentin, buprenorphine, CR4056) following Bortezomib injections by assessing sensory neuron function (neurometer), balance (rotarod), and wind-up in the spinal cord (electrophysiology)
School of Nursing, University of Maryland, Baltimore, MD
Role: **Research Assistant** (Advisor: Dr. Cynthia Renn)
- 2014 Examination of pain phenotype in mice with partial sciatic nerve ligation (PSNL)
Examination of pain behaviors, including mechanical allodynia, cold allodynia and weight bearing distribution, and analysis of BDNF protein expression in the spinal dorsal horn following PSNL surgery
School of Nursing, University of Maryland, Baltimore, MD
Role: **Research Assistant** (Advisor: Dr. Cynthia Renn)

-
- 2013 Evaluation of dynamic weight bearing for measuring non-evoked inflammatory hyperalgesia in mice
Validation of dynamic weight bearing apparatus by assessing thermal hyperalgesia and mechanical allodynia and verifying inflammation in the Complete Freund's Adjuvant (CFA) mouse model
School of Nursing, University of Maryland, Baltimore, MD
Role: **Research Assistant** (Advisor: Dr. Cynthia Renn)
- 2012 – 2013 Examination of pain phenotype in different genotype (knockout vs heterogeneous BDNF gene) and sex (female vs male) in cisplatin-treated mice
School of Nursing, University of Maryland, Baltimore, MD
Role: **Research Assistant** (Advisor: Dr. Cynthia Renn)
- 2012 Development of measures to recover from muscle atrophy induced cisplatin chemotherapy (writing the proposal)
Funded by Foundation for the Advancement of Science and Creativity, South Korea
College of Nursing, Seoul National University, Seoul, South Korea
Role: **Co-Investigator** (PI: Dr. Myoung-Ae Choe)
- 2010 – 2011 Development of measure to recover from hindlimb atrophy in a rat model of Parkinson's disease and determination of mechanism of the recovery measures
Funded by Korea Science and Engineering Foundation, South Korea
College of Nursing, Seoul National University, Seoul, South Korea
Role: **Research Assistant** (PI: Dr. Myoung-Ae Choe)
- 2007 – 2008 Determination of muscle atrophy induced by neuropathic pain and the development of measures to attenuate the muscle atrophy in a neuropathic pain animal model
Funded by Korea Science and Engineering Foundation, South Korea
College of Nursing, Seoul National University, Seoul, South Korea
Role: **Undergraduate Research Assistant** (PI: Dr. Myoung-Ae Choe)
- 2005 – 2007 Development of measure to prevent steroid induced muscle atrophy in a rat hindlimb muscle model
Funded by the Korea Research Foundation, South Korea
College of Nursing, Seoul National University, Seoul, South Korea
Role: **Undergraduate Research Assistant** (PI: Myoung-Ae Choe)

Teaching Experience

- Spring 2017 **Guest Lecturer, University of Maryland School of Nursing**
NURS 880: Interdisciplinary Research Center Seminar. Topic: “Non-NRSA Grant Application Process”
- Fall 2016 **Guest Lecturer, University of Maryland School of Nursing**
NURS 811: Measurement of Nursing Phenomena. Topic: “Measurement Concepts and Issues on Preclinical Research”
- Fall 2016 **Guest Lecturer, University of Maryland School of Nursing**
NURS 802: Research and Scholarship Seminar. Topic: “The Importance of Submitting NRSA/grant Applications”
- Spring 2016 **Guest Lecturer, University of Maryland School of Nursing**
NURS 880: Interdisciplinary Research Center Seminar. Topic: “Non-NRSA Grant Application Process”
- Fall 2015 **Guest Lecturer, University of Maryland School of Nursing**
PhD Student Lunch and Learn. Topic: “Bench Science: The New Window to Nursing Research”
- Spring 2015 **Guest Lecturer, University of Maryland School of Nursing**
NURS 880: Interdisciplinary Research Center Seminar. Topic: “Non-NRSA Grant Application Process”

Abstract

Title of Dissertation: Development of a New Animal Model and Treatment Strategies for Aromatase Inhibitor-Associated Arthralgia

Gee Su Yang, Doctor of Philosophy, 2017

Dissertation Directed by: Cynthia L. Renn, PhD, RN, Associate Professor, Department of Pain and Translational Symptom Science, University of Maryland School of Nursing

Background: Aromatase inhibitors (AIs) are widely used as the most effective adjuvant chemotherapy for postmenopausal women with breast cancer. Unfortunately, nearly half of women receiving AIs have reported AI-associated arthralgia (AIA), which may increase cancer-related mortality and non-adherence to the therapy. As of now, no clear biological mechanisms of AIA have been found partially due to a lack of relevant animal models, and consequently, few beneficial treatments have been developed for AIA.

Purpose: The purpose of this dissertation is to establish a clinically relevant animal model of AIA, and to examine potential treatment strategies for attenuating AIA by focusing on anti-nociceptive properties of curcumin (turmeric) and physical exercise.

Methods: To mimic the clinical condition of AIA, female immune-deficient mice underwent tumor transplantation on flanks, surgical removal of grown tumors (~300 mm³) and ovaries, and subcutaneous injections of letrozole (10µg/day for 8 weeks). Mice weekly received behavior assays of musculoskeletal pain and physical function at pre-tumor baseline, post-tumor removal, and during AI treatment. To test effects of curcumin or physical exercise on AIA, letrozole-treated mice were given either 45 mg/kg curcumin or peanut oil (vehicle) orally, or they were housed either in cages with free voluntary running wheels or cages with locked wheels for five weeks, respectively. Following the

interventions, protein expression levels of brain-derived neurotrophic factor (BDNF), a critical molecular moderator of pain behavior, were evaluated in the spinal dorsal horn.

Results: Eight weeks of letrozole injections significantly induced mechanical allodynia, thermal (cold) allodynia, joint hypersensitivity, decreased grip strength, worsened motor coordination, and anteroposterior weight shift. Curcumin administration improved almost all deteriorated nocifensive behavior and neuromuscular function except for mechanical allodynia. Physical exercise enhanced the deteriorated nocifensive behavior but did not rescue neuromuscular dysfunction. BDNF protein expression levels were down-regulated following curcumin administration and physical exercise.

Conclusion: This animal model is clinically relevant to AIA with face validity. It will provide an important platform to better understand progression and characteristics of AIA and allow examination of factors that cause AIA symptoms. Curcumin and physical exercise could be considered as effective interventions to reduce AIA symptoms and warrant studies in humans.

Development of a New Animal Model and Treatment Strategies for Aromatase Inhibitor-
Associated Arthralgia

by
Gee Su Yang

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2017

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I dedicate this thesis to
my parents, husband, lovely daughter, and those who supported me.

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Abbreviations

Abbreviation	Terms
AI	Aromatase Inhibitor
AIA	Aromatase Inhibitor-Associated Arthralgia
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
CI	Confidential Interval
CNS	Central Nervous System
DWB	Dynamic Weight Bearing
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Estrogen Receptor
HPC	HydroxyPropylCellulose
HR	Hazard Ratio
IACUC	Institutional Animal Care and Use Committee
LWT	Limb Withdrawal Threshold
NIH-SSM	National Institutes of Health Symptom Science Model
OR	Odds Ratio
OVX	Ovariectomy
PAM	Pressure Application Measurement
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QOL	Quality of Life
RCT	Randomized Controlled Trial
RM-ANOVA	Repeated Measures Analysis of Variance

SEM	Standard Error of the Mean
SERM	Selective Estrogen Receptor Modulator
SMD	Standardized Mean Difference
SNP	Single Nucleotide Polymorphism
TRPA1	Transient Receptor Potential Ankyrin 1
VAS	Visual Analog Scale

Chapter 1: Introduction, Background, and Purpose

1.1 Introduction

Breast cancer ranks second as a cause of cancer death among women, and exacts the highest national cost of cancer care (U.S. \$16.50 billion in 2010) in the U.S. (American Cancer Society, 2015; Mariotto, Yabroff, Shao, Feuer, & Brown, 2011). Third generation aromatase inhibitors (AIs) have been widely used and are the most effective standard adjuvant endocrine treatment for postmenopausal women with estrogen receptor positive breast cancer (Burstein, Griggs, Prestrud, & Temin, 2010; Cuzick et al., 2010). AIs exert a critical role in decreasing estrogen levels by 90% via covalent (e.g., exemestane) or non-covalent (e.g., letrozole, anastrozole) binding, thereby aiding in preventing breast cancer recurrence (Nabholtz, 2008). Because of an improvement in disease-survival outcomes and toxicity profiles, postmenopausal breast cancer survivors have been recommended to receive AI therapy for at least five years (Burstein et al., 2010; Goss et al., 2016).

As AI therapy has been used increasingly, a musculoskeletal adverse effect of AI therapy has been found. Women taking AIs reported significant quality of life issues, and evidence supports an increase in joint pain or stiffness and non-adherence associated with pain (Crew et al., 2007; Fontein et al., 2012; Laroche et al., 2014). Even though AI-associated arthralgia (AIA) is prevalent, and symptoms can continue years into survivorship, no clear treatment for AIA is available, due to the lack of a clear consensus on the biological mechanisms of AIA. To date, animal models have helped us explore the biological mechanisms of a certain disease and predict the analgesic efficacy of clinical applications because they control the genetic and environmental backgrounds of subjects

and allow us to obtain tissues to investigate pathogenesis from animals (Mogil, Davis, & Derbyshire, 2010). Hence, a relevant animal model of AIA is very useful as a platform to better understand the underlying mechanisms and develop treatment strategies for AIA.

1.2 Background

1.2.1 Aromatase enzyme

Aromatase enzyme plays a major role in mediating the conversion of androgens to estrogens, which is referred to as aromatization (Chumsri & Brodie, 2012). The aromatase is expressed in a wide range of tissues, such as ovaries, gonads, placenta, blood vessels, skin, bone, subcutaneous fat, liver, muscle, breast and endometrium (Smith & Dowsett, 2003). It also exists in breast cancer in both males and females (Chumsri & Brodie, 2012). Estrogen is primarily produced from peripheral sites when ovaries no longer function, such as after menopause (Chumsri & Brodie, 2012).

Aromatase is a member of the cytochrome P450 superfamily and is encoded by the CYP19A gene that contains nine coding regions, on which all are found within a 30 kilobase 3' region and many alternative untranslated exons in the 5' region (Hiscox, Davies, & Barrett-Lee, 2009). Expression of aromatase is controlled by multiple promoters driving the untranslated exons in a tissue-specific manner (Hiscox et al., 2009; Simpson et al., 1997). The aromatase gene transcribes one full-length cDNA of 3.4 kilobases, after which a protein of 503 amino acids with a molecular weight of 55kDa is produced (Simpson et al., 1994).

For aromatization, Nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase (NADPH-CPR) acts closely with aromatase as an electron donor (Simpson et al., 1994). Aromatase converts androstenedione and testosterone to estrone and

estradiol, respectively, through successive steps of oxidation upon taking electrons from reduced NADPH-CPR (Simpson et al., 1994). The conversion process requires 1 mole of NADPH and 1 mole of molecular oxygen at each step, and removes the C-19 atom to make formic acid and ring A of the steroid molecule. The reductase mediates the sequential transfer of two electrons from NADPH to the heme-iron of the cytochrome (Akhtar, Njar, & Wright, 1993).

1.2.2 Aromatase inhibitor

Since nearly 75% of breast cancer survivors have hormone receptors like estrogen receptor (ER) and/or progesterone receptor (PR), targeting those receptors has been shown to suppress breast tumor growth (Chumsri & Brodie, 2012). Estrogens are known to bind to the specific high affinity of ER, such as ER α and ER β , by interacting either indirectly through transcriptional factors (e.g., AP1, SP1, NF- κ B) or directly through estrogen response elements (EREs) (Kuiper, Enmark, Peltö-Huikko, Nilsson, & Gustafsson, 1996). Selective estrogen receptor modulators (SERMs), such as tamoxifen, hinder estrogen expression by binding to ERs, and thus breast cancer cell growth is inhibited (Hiscox et al., 2009). For this reason, SERMs had been extensively used for postmenopausal, hormone responsive breast cancer patients until 1992. Despite its beneficial effect in breast cancer patients with estrogen-dependent tumors, tamoxifen increases risk of estrogenic effects, such as endometrial cancer, thromboembolic events, and stroke in the uterus and vasculature (Cohen, 2004; Venturini & Del Mastro, 2006). In addition, the therapeutic effect of tamoxifen continues for only the first 5 years of treatment; extended tamoxifen therapy beyond 5 years does not seem to improve survival rates (Fisher, Dignam, Bryant, & Wolmark, 2001).

Brodie and colleagues proposed a more complete estrogen blockade by targeting aromatase enzyme instead of estrogen receptor (Brodie, Schwarzel, Shaikh, & Brodie, 1977). The steroidal formestane (4-hydroxyandrostenedione; 4-OHA) was developed as the first selective AI. This compound competes with androstenedione for the substrate binding site of aromatase in a time-dependent manner, and then gets metabolized to intermediates, which attach irreversibly to the active site (Ahmad & Shagufta, 2015; Brodie, Coombes, & Dowsett, 1987; Brodie et al., 1981). It leads to a reduction of estrogen synthesis in peripheral tissues and estrogenic effects in the uterus (Brodie & Longcope, 1980; Brodie et al., 1977). Due to the irreversible nature of the blockade, aromatase enzyme remains inactive even after the drug is cleared from circulation (Ahmad & Shagufta, 2015). In clinical trials, formestane had been shown to have positive outcomes in postmenopausal advanced breast cancer patients (Coombes, Goss, Dowsett, Gazet, & Brodie, 1984). However, it inhibited aromatase activity up to 90% at best, which accelerated the development of more selective and potent inhibitors of aromatase enzyme (MacNeill, Jacobs, Dowsett, Lonning, & Powles, 1995).

The current third generation AIs were developed in the early 1990s. There are two types of AIs: steroidal AIs and non-steroidal AIs, which both successfully suppress estrogen synthesis by 98% or greater (Geisler, Haynes, Anker, Dowsett, & Lonning, 2002; Johannessen et al., 1997). Exemestane (Aromasin), a steroidal androstenedione derivative, competes with endogenous androgens for the aromatase enzyme. Following binding to aromatase, it is converted to intermediates that bind irreversibly to the enzyme active site, thus inhibiting its function (Hong et al., 2007). Anastrozole (Arimadex) and letrozole (Femara) are the examples of non-steroidal AIs, which are derived from antifungal drugs,

such as ketonazole. These two compounds bind to the heme moiety of aromatase, and therefore can inhibit fungal P450 enzymes (Recanatini, Cavalli, & Valenti, 2002). The non-steroidal AIs are generally reversible, and a reduction of estrogen synthesis depends on the continuous presence of the drug (Recanatini et al., 2002).

Aromatase inhibitors are rarely used for premenopausal women with breast cancer. There are fewer estrogen receptor positive tumors in premenopausal women, and therefore the nature of premenopausal breast cancer is less estrogen dependent. Further, premenopausal women have functional ovaries producing circulating estrogens, which prevents the complete inhibition of the conversion from androgens to estrogens in the body (Dowsett & Haynes, 2003).

1.2.3 Aromatase inhibitor-associated arthralgia

Although there has been no clear definition of this chronic pain syndrome, Niravath (2013) proposed a definition of AIA by incorporating frequently reported symptoms in breast cancer survivors. Patients may be diagnosed with AIA if they meet all of the following major criteria and at least three minor criteria. Major criteria are as follows: currently taking AI therapy; joint pain that has developed or worsened since starting AI therapy; joint pain that improves or resolves within two weeks of stopping AI therapy; and joint pain that returns upon resuming AI. Minor criteria include symmetrical joint pains; pain in hands and/or wrists; carpal tunnel syndrome; decreased grip strength; morning stiffness; and improvement in joint discomfort with use or exercise (Niravath, 2013).

According to the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial, a 35% incidence of arthralgia was reported for anastrozole (Howell et al., 2005), while only

5% of patients receiving exemestane reported arthralgia (Coombes et al., 2004). Crew et al. (2007) also showed that 47% of women developed AIA, among whom half experienced new joint pain. The other half suffered from exacerbated pre-existent joint pain. Henry et al. (2008) demonstrated similar results; 45.4% of enrolled patients met criteria for rheumatologic referral based on the Health Assessment Questionnaire (HAQ) and Visual Analog Scale (VAS), suggesting that nearly half of them had joint pain. Recently, Laroche et al. (2014) reported 57% of patients with early-stage breast cancer at the start of AI treatment developed pain during one year of follow-up, and the primary characteristics of this joint pain was neuropathic, diffused, and mixed as well as persistent. Taken together, the true incidence of AIA in women receiving AI therapy seems to be approximately 50%.

Currently, joint pain is associated with a high rate of non-adherence to the AI therapy. Henry et al. (2008) did a 12-month follow-up to further characterize AIA, and found 13% of women discontinued treatment because of arthralgia. In addition, only 60% seemed to be adherent to their AI medication at 3 years, and their adherence gradually decreased over three years when analyzing records of 12,000 patients (Partridge et al., 2008).

Although the onset of AIA ranges from several weeks to more than 10 months, median onset time of AIA is 1.6 months after AI initiation. It tends to peak at ~6 months (Henry et al., 2008). Unfortunately, median time to treatment of discontinuation is 6.1 months (Henry et al., 2012), well below the recommended 5-10 years of treatment.

1.2.4 Risk factors and predictors

Several multicenter randomized controlled trials (RCTs) indicate that there may be risk factors or predictors of AI-treated joint pain and thus discontinuation of AI therapy. First, prior taxane-based chemotherapy (Hazard Ratio [HR] = 1.9, 95% CI = 1.00 - 3.6, $P = .048$) and younger age (HR = 1.4, 95% CI = 1.02 - 1.9, $P = .04$) are more associated with non-adherence to AI therapy in women with early breast cancer (Henry et al., 2012). Consistent with this study, previous chemotherapy before the initiation of AI therapy and prior hormone replacement therapy seemed to be related to a higher incidence of AIA; in particular, a history of having received a taxane was associated with a higher rate of AIA, with 62% of patients developing this problem when compared with those had never received chemotherapy (Crew et al., 2007). Second, the time since last menstrual period (LMP) is considered another significant predictor of AIA. The women who had their LMP within 5 years reported the highest probability of developing AIA (73%), while those whose LMP was ten or more years prior had the lowest probability of reporting AIA (35%) (Odds Ratio [OR] = 3.39, 95% CI = 1.21-9.44, $P < .02$) (Mao et al., 2009). Third, obesity seems to be associated with a high incidence of AIA. According to the analysis of ATAC study, obese women with a body mass index (BMI) of 30 m/kg^2 were more likely to report joint pain than normal weight women with a BMI of $< 25 \text{ m/kg}^2$ and overweight women with a BMI between 25 and 30 m/kg^2 (Sestak et al., 2008). The Intergroup Exemestane Study (IES) trial also showed a similar report that weight of $\geq 80 \text{ kg}$ could be a significant risk factor for the development of AIA (Mieog, Morden, Bliss, Coombes, & van de Velde, 2012). Fourth, genetic variation has been associated with the occurrence of AIA. In the Mao et al. (2011) study, genetic polymorphisms in

CYP19A1, which encodes the aromatase enzyme, were related to joint pain in breast cancer patients ongoing AI therapy. Subjects carrying at least one (TTTA)₈ repeat allele had a significantly lower risk of AIA compared to those carrying at least one (TTTA)₇ repeat allele (adjust OR = 0.41, 95% CI = 0.21 - 0.79, $P = .008$). This result supported the previous report that (TTTA)₇ was associated with lower estrogen levels and (TTTA)₈ was associated with higher estrogen levels in postmenopausal women (Haiman et al., 2007). CYP19A1 polymorphisms may predispose postmenopausal breast cancer patients to AIA. In another prospective cohort study (Garcia-Giralt et al., 2013), the association between single nucleotide polymorphisms (SNPs) and AIA intensity was evaluated using the VAS score. According to the analysis, SNPs in steroid 17-alpha-hydroxylase/17,20 lyase (CYP17A1) and vitamin D3 receptor (VDR) genes were shown to be significantly associated with AIA. One SNP in 25-hydroxyvitamin D1-alpha-hydroxylase (CYP27B1) gene appeared to predict therapy discontinuation, and there were interactions between CYP27B1 and both CYP17A1 and VDR SNPs. These genes are involved in the metabolism of estrogens and vitamin D, and vitamin D is known to help estrogen activated at hormone receptor sites. In the Wang et al. (2015) study, Chinese Han breast cancer survivors with receptor activator of nuclear factor-kappa B ligand (RANKL) SNP (rs7984870) and osteoprotegerin (OPG) SNP (rs2073618) risk genotypes may render a higher possibility of AIA. In these patients, rs7984870 CC and rs2073618 CC were risk genotypes.

1.2.5 Biological mechanisms

Estrogen deprivation. Currently, the mechanism behind AIA is not clearly understood, though estrogen deprivation is thought to be a major cause of AIA. Estrogen

level seems to be associated with changes of neurogenic pain perception. It is known that high serum estrogen levels lead to the release of endorphins and enkephalins that can improve nocifensive perception received by the brain (Felson & Cummings, 2005). Similarly, decreased estrogen levels hinder the body's ability to improve pain perception (Zubieta et al., 2005). For example, thresholds for painful stimuli are typically elevated in pregnant women (Dawson-Basoa & Gintzler, 1996b). In addition, estrogen has direct effects on opioid pain fibers in the central nervous system (CNS) (Dawson-Basoa & Gintzler, 1996b). Especially, estrogen receptors have been found in opioid-containing neurons in the spinal cord and brain, indicating a more direct effect of estrogen on opioid production (Eckersell, Popper, & Micevych, 1998; Flores, Shughrue, Petersen, & Mokha, 2003). In some species, dorsal horn cells in the spinal cord contain aromatase that converts androstenedione and testosterone to estrogens (Evrard et al., 2000). Hence, it is speculated that decreased estrogen levels may be attributable to musculoskeletal pain in that various articular structures are innervated with nociceptive fibers from the CNS (Felson & Cummings, 2005).

Activation of transient receptor potential ankyrin 1 (TRPA1) channel. TRPA1 is a polymodal channel that may be activated by a wide range of mechanical, thermal and chemical stimuli, and is mainly found in primary sensory neurons (Bandell et al., 2004; Bautista et al., 2006; del Camino et al., 2010; Karashima et al., 2009). Fusi and colleagues (2014) demonstrated that TRPA1 is an important mediator of the pro-inflammatory and proalgesic actions of AIs, thus TRPA1 antagonists could be a promising treatment to ameliorate AI-associated pain symptoms. In the experiment, anastrozole, letrozole and exemestane seem to selectively target TRPA1 because of their

electrophilic nature; anastrozole and letrozole have nitrile moieties that possess ability of TRPA1 gating, and exemestane contains a chemical structure with a system of highly electrophilic conjugated Michael acceptor groups (Liu & Talalay, 2013). Calcium responses and currents were evoked by AIs in TRPA1-expressing HEK 293 cells; however, those responses were eliminated by a TRPA1 antagonist (e.g., HC-030031) and were not observed in neurons obtained from TRPA1-deficient mice. In addition, in *in vivo* experiment, AIs produced mechanical allodynia and decreased grip strength, which do not undergo desensitization on prolonged AI administration (Fusi et al., 2014).

Excitability of small and medium-diameter sensory neurons. Robarge et al. (2016) evaluated the effects of letrozole on mechanical, thermal (hot), and chemical sensitivity in Sprague-Dawley rats. Administration of letrozole markedly decreased mechanical paw withdrawal thresholds without changing thermal sensitivity in ovariectomized rats. Also, the excitability of neurons isolated from the dorsal root ganglion (DRG) of male rats chronically treated with letrozole was evaluated to determine whether sensitization of sensory neurons was attributable to AI-induced hypersensitivity. Consequently, both small and medium-diameter sensory neurons were more excitable, as reflected by increased action potential firing in response to a ramp of depolarizing current, a lower resting membrane potential, and a lower rheobase. However, systemic letrozole treatment did not amplify the stimulus-evoked release of neuropeptide calcitonin gene-related peptide (CGRP) from spinal cord slices, indicating that the enhanced nociceptive responses were not secondary to an increase in peptide release from sensory endings in the spinal cord. These results provided the evidence that AIs modulate

the excitability of sensory neurons, which may be a primary mechanism for the effect of the AI drugs to augment behaviors in rats (Robarge et al., 2016).

From the literature review, it is recognized that estrogen deprivation is a leading hypothesis for the cause of AIA. Most prior research has primarily focused on signaling and metabolism of estrogen and vitamin D as a key risk factor of AIA. However, this explanation has not been shown in all estrogen studies and has shifted the focus away from other promising therapies. Other possible pain-related hypotheses, such as central sensitization, could be sought as an alternative approach to reveal underlying mechanisms and develop therapeutic treatments to alleviate AIA. Recently, there emerged evidence demonstrating that CNS actions may contribute to the development of AI-induced pain. If central sensitization is associated with AIA, brain-derived neurotrophic factor (BDNF) as one of the key pain modulators also could be attributable to the occurrence of AIA. Indeed, a variety of forms of stress and/or injury to the peripheral nerves, spinal cord, and brain increase the expression of BDNF, and some of the potential deleterious effects of BDNF relate to its involvement in both inflammatory and neuropathic pain (Merighi et al., 2008; Trang, Beggs, & Salter, 2011).

1.2.6 Animal models using aromatase inhibitors

Brodie and colleagues developed the xenograft animal model of postmenopausal breast cancer conditions for aromatase inhibitor studies (Yue, Zhou, Chen, & Brodie, 1994). In this model, BALB/c athymic nude mice were inoculated with MCF-7 cells transfected with human placental aromatase gene (MCF-7Ca cells) with matrigel. Daily dosing of androstenedione (0.1 mg) was injected to provide the substrate for aromatization in ovariectomized mice bearing MCF-7Ca tumors because this

supplementation helps acceleration of tumor growth. When 1mg 4-OHA was given subcutaneously to the ovariectomized mice with MCF-7Ca tumors, tumor growth markedly slowed. The tumors of this mouse model are dependent on estrogens for their growth from an endogenous non-ovarian source. Afterwards, this research group employed this xenograft model to evaluate the effects of letrozole and anastrozole on tumor growth, as well as on the uterus (Brodie et al., 1998; Yue, Wang, Hamilton, Demers, & Santen, 1998). Arumugam et al. (2014) also employed this established xenograft mouse model to examine the role of natural steroid hormones on serum cardiovascular and bone resorption markers. Mice were ovariectomized and inoculated with MCF7-ARO cells (i.e., MCF-7 cells stably transfected with the human placental aromatase gene). During the experiment, ovariectomized mice received 0.1 mg dehydroepiandrosterone (DHEA) subcutaneously to allow the aromatization process which is responsible for much of postmenopausal hormone production and which is attacked by aromatase inhibitors. Mice were randomly administered multiple combinations of estrogen, progesterone, testosterone, and anastrozole (60 µg/day).

Recently, a few studies examined the role of AIs in inducing pain using animals. Fusi et al. (2014) investigated whether TRPA1 mediates AI-induced pain using TRPA1-deficient male mice and rats without mimicking clinical conditions. Robarge et al. (2016) used ovariectomized rats without the breast cancer surgery condition to examine a link between AI-evoked pain and excitability of sensory neurons.

From the literature review, there has been no appropriate animal model that mimics clinical conditions reflecting postmenopausal status and breast cancer removal surgery followed by long-term administration of AIs for studies of AIA. A clinically

relevant animal model of AIA is necessary for mechanistic studies of AIA and may uncover targets for future interventions.

1.3 Framework for Evaluating a Symptom

The National Institutes of Health Symptom Science Model (NIH-SSM) was recently developed to guide symptom science research using “-omics” techniques to quantify pools of biological molecules and identify potential markers (Cashion & Grady, 2015). Genomics, proteomics, and metabolomics are examples of omics. The NIH-SSM consists of sequential steps from identifying symptoms to developing therapeutic interventions to potentially reduce acute and chronic symptoms and identify biologic targets for precision medicine (Cashion & Grady, 2015). This model begins with identifying symptoms or symptom clusters, and then phenotypes of the symptom are characterized. Once phenotypes are determined based on behavioral, biological, and clinical data, potential biomarkers are explored using omics techniques to measure biological processes and pharmacologic responses, or develop therapeutic clinical applications. Diverse information collected from each step is integrated to find novel discoveries and to treat and manage symptoms (Cashion & Grady, 2015).

The arthralgia symptom fits well into the NIH-SSM. Arthralgia has been identified in postmenopausal breast cancer survivors taking AIs as a primary adverse musculoskeletal symptom. Because this model was technically developed for clinical research, it was modified by inserting one more step between biomarker discovery and clinical application to establish a revised framework guiding preclinical research in symptom science. Specifically, phenotypes of AIA were characterized by examining nocifensive behavior and neuromuscular function in an animal model. Altered protein

expression level of BDNF was measured to identify a potential biomarker that may contribute to the development of AIA. Based on the discovery of a biomarker and characterization of phenotype, we examined potential therapeutic interventions by focusing on anti-nociceptive effects of curcumin (turmeric) and physical exercise and correlated effects of the interventions to regulation of a biomarker at preclinical level. The preclinical intervention study may provide evidence for developing therapeutic clinical applications to be used for patients with AIA in the clinical setting. Below is a modified symptom science model to evaluate the AIA symptom and develop treatment strategies (Figure 1).

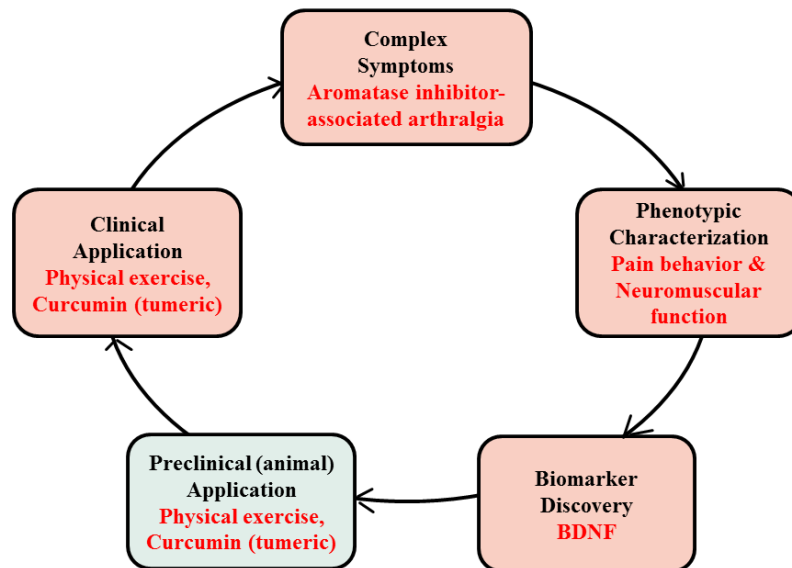


Figure 1. Application of AIA into the NIH-symptom science model. The NIH-SSM was applied for studies of AIA to characterize the symptom, discover a relevant biomarker, and develop therapeutic interventions. The preclinical (animal) application step (sky blue) was newly inserted to the existing sequential steps. BDNF=brain-derived neurotrophic factor.

1.4 Purpose and Methods

1.4.1 Purpose

The purpose of this dissertation is to (1) establish a clinically relevant animal model of AIA by phenotyping musculoskeletal pain and function present in AIA, (2) identify current pain management of AIA and evaluate the effects of interventions, and (3) examine potential treatment strategies to attenuate AIA by focusing on the anti-nociceptive properties of curcumin (turmeric) and physical exercise. The hypotheses are as follows:

1.4.2 Research hypotheses

Hypothesis 1. Phenotypes of pain sensitivity and neuromuscular function will differ among the AI-treated group and control groups (naïve control, vehicle-treated, and ovariectomy groups). To test this hypothesis, multiple characteristics of pain behavior and neuromuscular dysfunction will be examined. Assessments of pain sensitivity and joint function include mechanical pain sensitivity, cold pain sensitivity, joint pain sensitivity, grip strength, motor coordination, and weight bearing distribution.

Hypothesis 2. The AI-treated group that receives curcumin will show a decrease in pain behavior and neuromuscular dysfunction, and down-regulation of BDNF protein expression level compared to the control (vehicle) and naïve (no vehicle/treatment) groups. To test this hypothesis, changes in pain sensitivity and physical function will be evaluated before and after taking curcumin treatment in mice. Fresh lumbar SDH will be harvested to examine whether protein expression level of BDNF is down-regulated following chronic administration of curcumin.

Hypothesis 3. The AI-treated group that receives physical exercise will show a decrease in pain behavior and neuromuscular dysfunction, and down-regulation of BDNF protein expression level compared to the control (locked wheel running) and naïve (no vehicle/treatment) groups. To test this hypothesis, changes of pain sensitivity and physical function will be evaluated before and after applying voluntary running wheel exercise in mice. Whether protein expression level of BDNF in the SDH tissue is down-regulated will be evaluated following physical exercise.

Findings from this study will increase our understanding of the pathogenesis and symptom characteristics of AIA, and provide the evidence for the use of curcumin and physical exercise as potential therapeutics. If the data support our hypotheses, the new animal model will be used to perform mechanistic studies of AIA, and exercise and/or curcumin can be rapidly translated to applicable interventions because of their minimal adverse effects in the clinical setting. This study is significant to oncology nursing because the findings may ultimately help improve adherence to AI use and quality of life for breast cancer survivors taking AIs.

1.4.3 Methods

Study design and methods for this preclinical study are described in the following chapters and appendix. Aim 1 attempted to establish a new animal model of AIA by phenotyping musculoskeletal pain and function. To generate a clinically relevant animal model of AIA, mice were inoculated with breast tumor cells, and then the grown tumors and ovaries were surgically removed. Daily dosing of letrozole (10µg/day) was given subcutaneously for eight weeks until resulting nociceptive phenotype was characterized. At pre-tumor baseline, post-tumor removal, and every week during AI treatment, mice

received behavior assays, including von Frey, cold plate, algometer, grip strength, rotarod, and dynamic weight bearing.

Aim 2 provided the overview of current intervention modalities for AIA and evaluation of their effects. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guided identification of eligible studies, which were reviewed and sorted according to type of pain management. Study quality was assessed by the Quality Assessment Tool for Quantitative Studies (QATQS) tool, and effect sizes of each pain management type were evaluated by performing meta-analysis with respect to pain and subgroups.

Aim 3 pursued development of therapeutic treatment strategies based on the animal model described in Aim 1. To investigate the efficacy of curcumin, following the development of AI-induced pain, the intervention group was given 45mg/kg curcumin and the control group received peanut oil (vehicle of curcumin) daily for five weeks. For testing the effect of physical exercise, the intervention group was housed in cages with free voluntary running wheels, and the control group was housed in cages with locked running wheel cages for five weeks. Their running distance was recorded daily. During the interventions, behavior tests were evaluated weekly on pain sensitivity and neuromuscular function. Following the interventions, spinal cord lumbar enlargement was harvested to examine protein expression levels of BDNF.

1.5 Overview of Manuscripts

The following three chapters include three manuscripts completed to fulfill the requirement for the dissertation manuscript option. Data concerning the effect of physical exercise on AIA, a part of Aim 3, is briefly reported in the appendix. Chapter five

discusses the summary of the main findings, strength and limitations, and implications for research and practice based on findings of this study.

The first manuscript titled “Development of a Clinically Relevant Animal Model of Aromatase Inhibitor-Associated Arthralgia” (Chapter 2) describes how a new animal model that mimics the clinical condition of AIA was developed. The second manuscript titled “Interventions for the Treatment of Aromatase Inhibitor-Associated Arthralgia in Breast Cancer Survivors: A Systematic Review and Meta-Analysis” (Chapter 3) identifies various types of pain management for AIA and evaluates the study quality and effects of interventions on AIA in breast cancer survivors. This chapter also provides the evidence of choosing curcumin (turmeric) and physical exercise in developing therapeutic treatment strategies for improving AIA. This review paper was published in *Cancer Nursing*. The third manuscript titled “Efficacy of Curcumin on Aromatase Inhibitor-Induced Musculoskeletal Pain and Dysfunction in an Animal Model” (Chapter 4) investigates whether curcumin exerts anti-nociceptive activities in rescuing pain behavior and neuromuscular dysfunction induced by letrozole using the established animal model described in chapter 2. In the appendix, results concerning the effects of physical exercise on AI-induced pain behavior and neuromuscular dysfunction are simply presented.

Chapter 2: Development of a Clinically Relevant Animal Model of Aromatase Inhibitor-Associated Arthralgia

2.1 Introduction

Breast cancer is currently the second highest cause of cancer death among women and represents the foremost national cost of care in the U.S. (American Cancer Society, 2015; Mariotto et al., 2011). There are over 2.8 million breast cancer survivors in the U.S., and they require adjuvant chemotherapy to inhibit the recurrence of breast cancer (American Cancer Society, 2015). Aromatase inhibitors (AIs), such as anastrozole, letrozole, and exemestane, have been established as the gold standard therapy because they markedly decrease production of estrogens by targeting the aromatase enzyme that converts androgens to estrogens (Chumsri & Brodie, 2012; Nabholz, 2008). For instance, non-steroidal anastrozole and letrozole inhibit the aromatase action by binding reversibly to the aromatase active site (Miller & Dixon, 2000), and a steroidal exemestane competes with endogenous androgens and turns into intermediates that bind irreversibly to the enzyme active site (Hong et al., 2007).

With increased use of AI in more patients over longer periods of treatment, joint pain, stiffness or achiness have emerged as primary adverse effects. Approximately 50% of women taking AIs reported AI-associated arthralgia (AIA), and 20-30% evidence non-adherence to the treatment due to joint pain, which could occur in all joints (Burstein & Winer, 2007; Fontein et al., 2012; Henry et al., 2008; Laroche et al., 2014; Mao et al., 2013). AI-associated arthralgia is a very problematic issue in pain and physical function among breast cancer survivors and negatively impacts their quality of life. Although estrogen deprivation is believed to be associated with AIA, underlying mechanisms of

such a condition are poorly understood, and consequently, no satisfactory treatment has appeared that is beneficial for AIA, partially due to the lack of a relevant animal model.

To date, animal models have substantially contributed to the understanding of pain because controlled investigation is allowed for chronic pain conditions (e.g., neuropathic pain) that are not easily carried out in humans. In addition, pain-relevant tissues, such as spinal cord and brain, can only be collected from animals (Mogil et al., 2010). Furthermore, animal models offer standardization of genetic and environmental backgrounds and help explore basic physiological mechanisms of pain and predict analgesic efficacy leading to clinical drug development (Mogil et al., 2010). For this reason, animal models are useful to better understand the disease progression and characteristics of AIA. To our knowledge, there has been no appropriate clinically relevant animal model of AIA.

While a few studies used animals to examine the role of AIs in inducing pain, they were not clinically relevant. Fusi et al. (2014) investigated whether TRPA1 mediates AI-induced pain using TRPA1-deficient male mice and rats without considering clinical conditions, and Robarge et al. (2016) examined the link between AI-evoked pain and excitability of sensory neurons using ovariectomized rats without breast cancer surgery. These models did not reflect clinical conditions, such as breast cancer surgery, postmenopausal status, and long-term administration of AI. They only focused on exploring mechanisms rather than developing animal models, and therefore only one or two pain behaviors were phenotyped. In this study, we present a new animal model of AIA that mimics the clinical condition of postmenopausal status with breast cancer surgery by transplanting breast tumors and measuring a range of pain behavior and

neuromuscular function. This model system will provide important insights concerning mechanistic studies of AIA when examining factors that cause the symptoms.

2.2 Methods

2.2.1 Study design

The investigation was designed to examine the development of pain and physical dysfunction following aromatase inhibitor treatment in a mouse model. We examined the role of letrozole in inducing pain by characterizing symptoms following AIA. Mice were assigned into four groups: experimental tumor/drug group (letrozole-treated group), tumor/vehicle group (vehicle-treated group), ovariectomy group, and naïve control group (10~11 mice per group). The letrozole-treated and the vehicle-treated groups were inoculated with breast tumor cells on both flanks, and the tumors were surgically removed when the tumor size reached $\sim 300 \text{ mm}^3$; afterwards, the former group received daily subcutaneous injections of letrozole over eight weeks, and the latter group was given daily injections of vehicle, HydroxyPropylCellulose (HPC; Sigma, St. Louis, MO), during the same period. The ovariectomy group received ovariectomy surgery only, and the naïve control group did not have any treatment or procedure. At pre-tumor baseline, post-tumor removal, and every week during AI treatment, these groups were tested using behavioral assays of pain response and neuromuscular function (Figure 2).

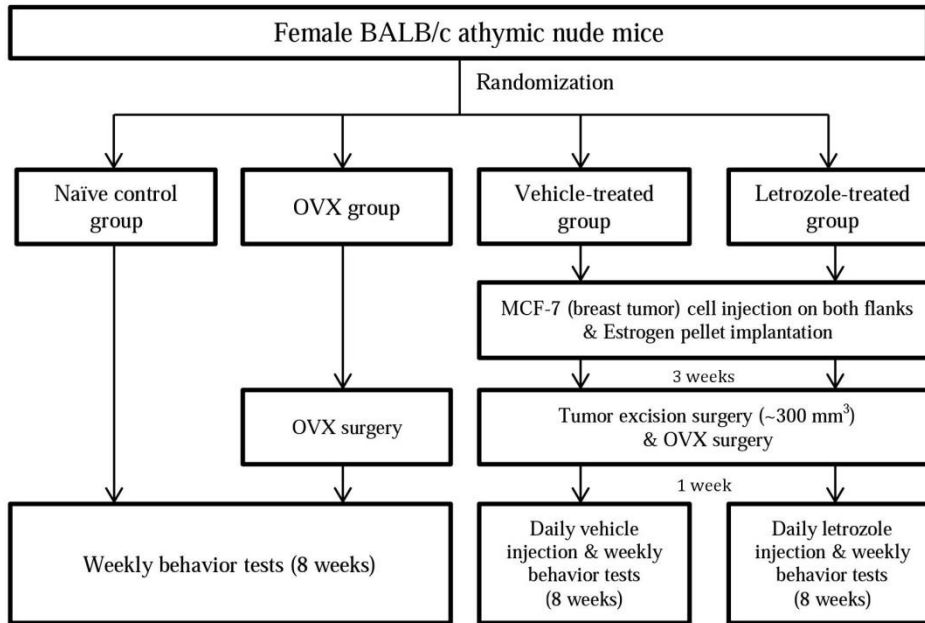


Figure 2. Experimental design for developing an animal model of aromatase inhibitor-associated arthralgia. OVX=ovariectomy.

This study was performed according to the guidelines and approval of the University of Maryland Institutional Animal Care and Use Committee (IACUC, protocol #0314008). Female BALB/c athymic nude mice at 4-7 weeks of age were purchased from the National Cancer Institute (Frederick, MD) via the Charles River Laboratories. Nude mice were used in this study because this strain has a deficient immune system, and therefore human breast cancer cells are able to be transplanted and grown into tumor lumps without an immune rejection (Brodie, Macedo, & Sabnis, 2010; Sabnis, Kazi, Golubeva, Shah, & Brodie, 2013). In addition, mice were ovariectomized to avoid side effects, such as polycystic ovarian syndrome, and interference with hormone production (Rebar, Morandini, Erickson, & Petze, 1981). Mice were housed in a pathogen-free environment under controlled conditions of a 12:12 hr light/dark cycle and humidity with sterilized food and water *ad libitum*.

2.2.2 Generation of an animal model with AIA

This animal model was made to mimic the clinical state of postmenopausal breast cancer survivors taking AIs. Female BALB/c athymic nude mice were inoculated on both flanks with MCF-7 cells and surgically excised three weeks after tumor injection. Both ovaries were removed from flanks of mice to produce the postmenopausal condition during tumor excision surgery. The ovariectomized mice were daily injected with 10 µg of letrozole to induce arthralgia over eight weeks for a total of 560 µg, and received weekly behavior assays (i.e., von Frey, cold plate, algometer, grip strength, rotarod, and dynamic weight bearing).

Tumor cell injection. MCF-7 cells (Sigma, St. Louis, MO) were cultured in DMEM plus 10% FBS, and 1% penicillin-streptomycin. Both flanks were inoculated subcutaneously with 100 µL of cell suspension containing $\sim 2.5 \times 10^7$ cells/ml in Matrigel (Sigma, St. Louis, MO). After tumor xenograft on both flanks of mice, the tumor volume was measured weekly using a caliper until the volume reached $\sim 300 \text{ mm}^3$. The formula to calculate the tumor volume is $\frac{4}{3} \times \pi \times r_1^2 \times r_2$ ($r_1 < r_2$) (Brodie et al., 2010; Sabnis et al., 2013).

Insertion of 17β-estradiol pellet. A slow-release 0.18 mg 17β-estradiol pellet (Innovative Research of America, Sarasota, FL) was implanted to the mid-scapular area of the mice using a 10-gauge trochar. Because MCF-7 cells slowly grow *in vivo*, they require estrogen to stimulate growth. The pellet helps to stimulate the tumor growth with control of estrogen release over time and minimal neurophysiological stress from excessive handling for the mice.

Excision of estrogen pellet and MCF-7 tumors. At 2~3 weeks following the tumor injection, the tumors and estrogen pellets were surgically excised from the flanks and mid-scapular area and sutured. For the pellet removal, a 2-3 mm incision was made in the skin over the estrogen pellet. The pellet was removed by cutting tissues attached to the pellet using a microscissor. The incision was closed with 5-0 proline monofilament suture and reinforced with surgical glue (Vetbond, 3M). For the tumor removal, a circumferential incision was made in the skin 2-3 mm from the border of the tumor, and the section of skin containing the tumor was removed. The skin was closed with 5-0 proline monofilament sutures along with surgical glue to reinforce the suture.

Ovariectomy surgery. Under isoflurane anesthesia, a circumferential incision was made in the skin from the para-lumbar sides approximately 1/3 of the distance between the dorsal midline and the ventral midline. A small muscle incision was made into the peritoneal cavity. The ovary and the oviduct were exteriorized through the muscle wall using sterile fine tipped forceps and the ovary and a partial oviduct were removed with a single cut through the oviduct near the ovary.

Letrozole injection. At one week following the surgery, letrozole (Sigma-Aldrich, St. Louis, MO) was prepared using 0.3% HPC in 0.9% NaCl solution, which was autoclaved. The mice were given daily subcutaneous injections of letrozole 10 μ g in the neck for eight weeks. The dose (10 μ g/mouse/day) of letrozole was chosen because it was tested and proven as an optimal dose for xenograft postmenopausal mouse model to reduce breast cancer growth (Brodie, Sabnis, & Macedo, 2007).

2.2.3 Behavior assays

The following behavior assays were used for evaluating pain behavior response and neuromuscular function in animal studies. The assays were performed weekly during the experiment in the hood to avoid pathogen environment.

Von Frey. The von Frey filament test was used to evaluate sensitivity to the mechanical stimuli (mechanical allodynia). Mice were acclimated to the testing environment in a transparent cube (2.5"×1.5"×1.5") on a wire mesh platform for approximately 60 minutes. A series of calibrated von Frey filaments with bending forces ranging from 0.04g to 1.4g (Touch test sensory evaluator kit, myNeuroLab.com, St. Louis, MO) were applied perpendicularly to the hind paws. Each filament was applied five times at intervals of 1-2 minutes. The paw withdrawal threshold (PWT) was defined as the filament with the lowest bending gram force that elicited three paw withdrawal responses out of five trials (Renn, Leitch, & Dorsey, 2009).

Incremental cold plate. Each of the mice was placed in an individual cylinder on an Incremental Hot/Cold Plate (IITC Life Sciences, Woodland hills, CA) and allowed to acclimate for several minutes. Once the instrument is activated, the temperature of surface decreases with a rate of 10°C/min from 30°C. The temperature of the plate at the time when the mouse jumped, licked a hind paw, or rotated on a tiptoe was recorded as the threshold for noxious cold. This was repeated after at least 30 minutes, and the mean of the two temperatures was determined (Renn et al., 2011).

Pressure algometer. The development of localized joint hypersensitivity was evaluated using the pressure application measurement (pressure algometer) according to the manufacturer's protocol (Ugo Basile, Comerio, Italy). Briefly, the animal was

restrained by an investigator's scruffing, and force was manually applied to the ankle joint at a rate of 50 grams per second to a maximum test duration (10 seconds). A limb withdrawal threshold (LWT), which is regarded as a sign of endpoint, was determined when mice withdrew their limbs, showed any behavioral signs of discomfort or distress, suspended hind limbs, twisted their body, or vocalized. The peak gram force was recorded immediately when mice showed LWTs. Their ankles were measured two times at one minute intervals and the mean of the LWT was calculated.

Dynamic weight bearing. The Dynamic Weight Bearing (DWB; Bioseb, Vitrolles, France) was used to measure the postural equilibrium of freely moving mice. The DWB is a floor-instrumented arena that allows an independent measurement of the weight born by each limb. The instrument is comprised of a transparent arena with a floor that contains force-transducing sensors. During data capture, the raw force data for each paw were synchronized with images that are recorded by a high-resolution video capture. The instrument software calculates the weight distribution of the mouse per limb. The mice were placed in a 4"×4" plexiglas cube with a piezo electronic sensor mat on the bottom. The sensor mat detects the amount of force being applied by each paw, which was analyzed with specialized computer software. The data were gathered on the amount of time spent on 2, 3 or 4 paws as well as the distribution of the weight of the four paws in aspects of time and segment (Griffioen et al., 2015).

Grip strength. The grip strength test was used to measure the muscle strength of rodents according to the manufacturer's protocol (Chatillon, Largo, FL). The mouse was allowed to grab a 3 mm diameter triangular bar attached to a force transducer with both fore paws, and pulled horizontally away from the bar until it released its grip. The

maximum force exerted by the pull of the mouse was recorded by a force transducer. This process was repeated six times and the mean of the top three forces exerted was normalized for gram body weight.

Rotarod. The rotarod assay has been frequently used to assess loss of motor coordination that discriminates various neurological disorders in rodents (Rustay, Wahlsten, & Crabbe, 2003). The rotarod instrument (LE 8500; Panlab, Barcelona, Spain) was used to assess the coordination or locomotion capacity of the animals after adequate training of the mice. After acclimation, the latency to fall off the rubberized rotating barrel was measured. The barrel is located 8 cm above a padded surface to minimize the risk of injury when the mouse falls. The velocity increases from 4 to 40 rpm over a 180 second interval. Each trial ended when the animal fell off the rotarod or reached 5 minute cutoff time (Zhao et al., 2014). This process was repeated three times and the mean of the three latencies was calculated.

2.2.4 Statistical analysis

A two-way repeated measures analysis of variance (RM-ANOVA) test examined changes in paw withdrawal threshold (von Frey), paw withdrawal temperature (cold plate), limb withdrawal threshold (PAM), grip strength (grip strength), and latency to fall off (rotarod), all of which were measured at each week, between groups and across time. This was followed by post hoc tests with of multiple comparisons with a Bonferroni for differences between groups. A one-way ANOVA was performed to examine differences for ratios of front paws to rear paws (DWB) at baseline and at week 8, respectively, among the four groups. A paired t-test was performed to determine differences of gait distribution between baseline and week 8 in each group, and effects of ovariectomy and

breast cancer removal surgery on musculoskeletal pain and function of mice between two measurements before and after procedures (2-tailed). All data were presented as means \pm standard error of the mean (S.E.M.), and $P < .05$ was considered significant. Statistical analysis and figure acquisition were performed using GraphPad Prism (version 7.01) software.

2.3 Results

2.3.1 General appearance and body weight change

To generate a clinically relevant animal model of AIA, the mice were subcutaneously given letrozole (10 μ g) daily for eight weeks. There were three control groups: naïve group, ovariectomy group, and vehicle-treated group. Mice having a low paw withdrawal threshold at baseline or tumor metastasis into the abdominal cavity were excluded from the study. The duration of this study was 11 weeks, during which a majority of the mice that were treated with letrozole had continuously shown allodynic signs after receiving the drug. The mice tolerated letrozole well, and showed no distress from daily needle injection. During the period of drug administration, no change of their daily behavior was observed, such as grooming, exploring, climbing on wire cage tops and making nests. The bodyweight was measured weekly to estimate their health condition and AI toxicity. The letrozole-treated group did not have a significant decrease or increase in body weight compared to the other control groups ($F(3, 38) = 2.45$, $P = .08$). The body weight of all mice started at 20-22g, and on average 5.04g was gained during the experiment (Figure 3).

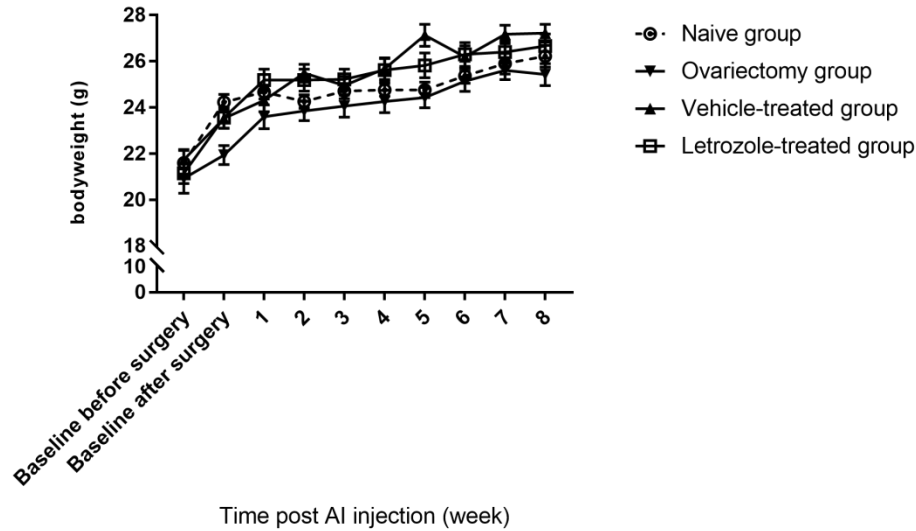


Figure 3. Effect of letrozole treatment on body weight. Body weight change was measured every week in naïve (n=11), ovariectomy (n=10), letrozole-treated (n=11) and vehicle-treated (n=10) groups during letrozole injections. No significant difference was observed among the four groups.

2.3.2 Effect of letrozole on mechanical allodynia

Recent studies showed that letrozole treatment clearly induced mechanical allodynia in rodents (Fusi et al., 2014; Robarge et al., 2016). To determine whether our mouse model of AIA also exhibited signs of pain, ovariectomized immune-deficient mice weekly underwent the von Frey filament assay. Long-term administration of letrozole decreased paw withdrawal thresholds (PWTs) to mechanical stimuli in mice. The letrozole-treated group showed lower PWTs from week 1 ($P = .01$), that persisted during the letrozole treatment compared to the naïve group ($P < .05$). In addition, the letrozole-treated group had lower PWTs at week 3, 6, 7, and 8 compared to the vehicle-treated group ($P = .04$; $P = .01$; $P = .02$; $P < .01$, respectively). The PWT values decreased from $1.27 \pm 0.06\text{g}$ to $0.61 \pm 0.13\text{g}$ in the letrozole-treated group, while other control groups maintained similar PWTs to the level of their baselines throughout the study. We also evaluated if ovariectomy and tumor removal surgery may affect sensitivity to mechanical

stimuli in the letrozole-treated group and the vehicle-treated group, both which received the procedures before initiation of drug injections. When comparing two measurements before and after procedures in each group, no significant changes were observed in the letrozole-treated group ($t = .60, P = .56$) and the vehicle-treated group ($t = 1.16, P = .28$). This result suggests that ovariectomy and tumor removal surgery were not attributable to the occurrence of mechanical allodynia before initiation of letrozole. Next, we determined if estrogen deprivation contributed to mechanical allodynia by comparing the ovariectomy group to the naive group. There was no significant difference between the two groups on mechanical allodynia throughout the experiment ($P > .05$) although there was a tendency that the ovariectomy group maintained lower PWTs than the naïve group, suggesting that low estrogen levels did not seem to evoke mechanical allodynia in this experiment. There was a significant main effect for time ($F(9, 342) = 4.82, P < .001$), treatment group ($F(3, 38) = 3.75, P = .019$), and interaction between time and treatment ($F(27, 342) = 2.518, P < .001$) for mechanical allodynia (Figure 4).

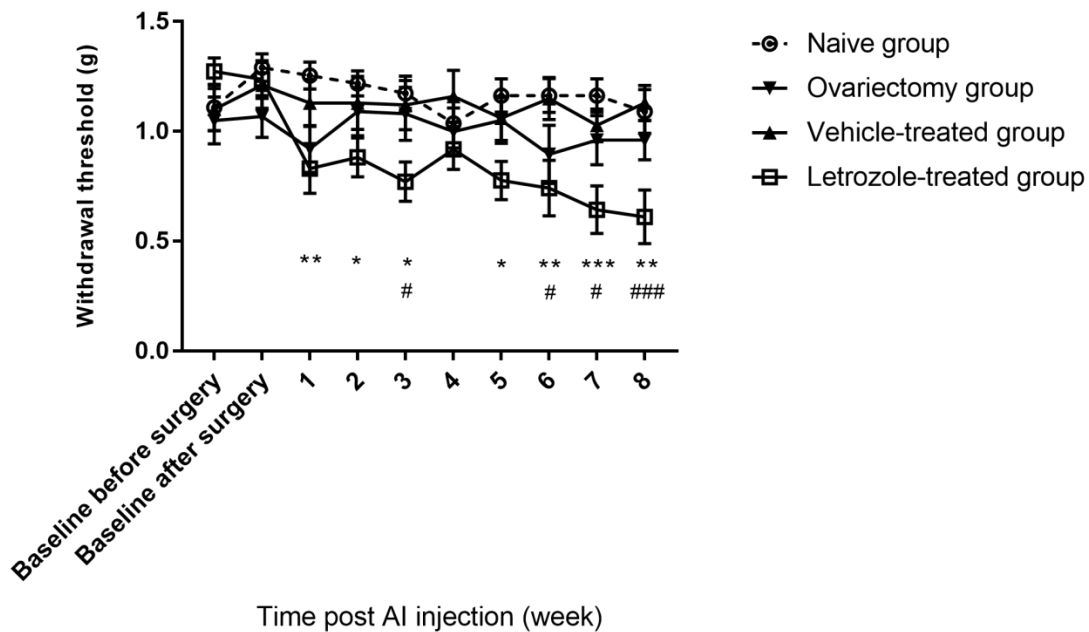


Figure 4. Effect of letrozole treatment on mechanical allodynia. Paw withdrawal thresholds (g) were assessed every week to examine mechanical allodynia in naïve (n=11), ovariectomy (n=10), letrozole-treated (n=11) and vehicle-treated (n=10) groups during letrozole treatment. Chronic administration of letrozole induced mechanical allodynia in mice. Letrozole-treated group vs naïve group: * $P < .05$, ** $P < .01$, *** $P < .001$; letrozole-treated group vs vehicle-treated group: # $P < .05$, ### $P < .001$.

2.3.3 Effect of letrozole on cold allodynia

Cold allodynia was assessed by observing agitation or jumping response by the mice. Long-term letrozole injections significantly induced cold allodynia. The letrozole-treated mice showed higher withdrawal temperatures ($^{\circ}\text{C}$) to cold thermal stimuli compared to the naïve group ($P < .001$) and the vehicle-treated group ($P < .001$) after the first week of injections. The three control groups maintained similar records to the level of baselines throughout the study. When evaluating the effects of ovariectomy and tumor removal surgery on cold allodynia, no significant changes were found between two time points of baselines before and after surgeries in the letrozole-treated group ($t = 1.17$, $P = .27$) and the vehicle-treated group ($t = 0.07$, $P = .95$). This result indicated that these

procedures had no impact on cold allodynia. In addition, the ovariectomy group exhibited higher withdrawal temperatures than the naïve group at week 2 ($P = .04$), 3 ($P < .01$), and 4 ($P = .01$), suggesting estrogen deprivation may be related to cold allodynia. There was a significant main effect for time ($F(9, 360) = 15.24, P < .001$), treatment group ($F(3, 40) = 111.1, P < .001$), and interaction between time and treatment ($F(27, 360) = 8.904, P < .001$) for cold allodynia (Figure 5).

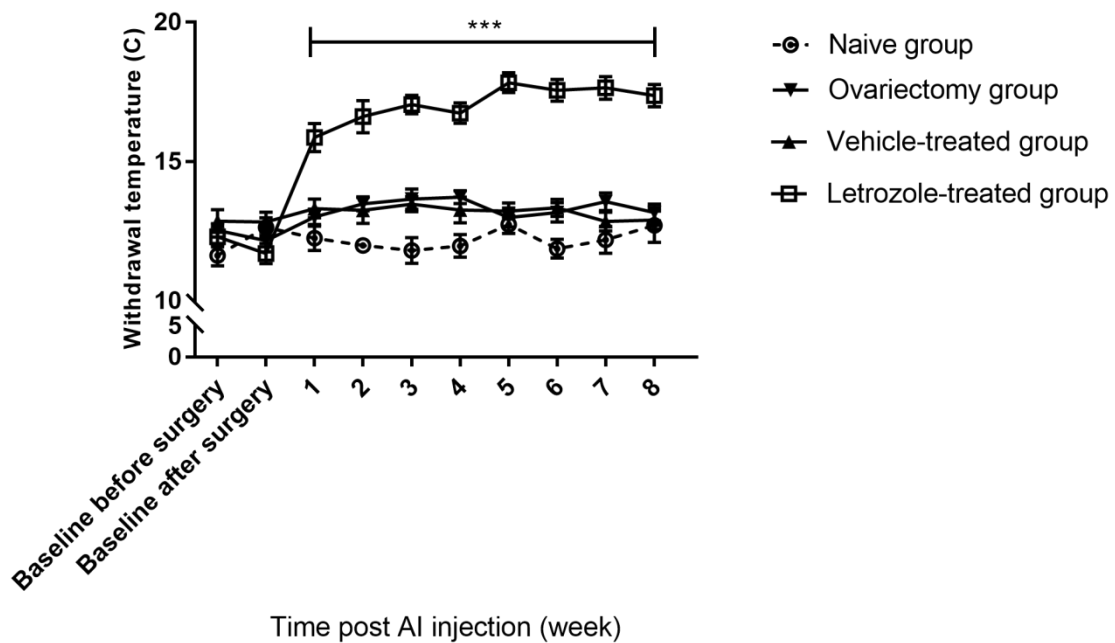


Figure 5. Effect of letrozole treatment on cold allodynia. Paw withdrawal temperature ($^{\circ}\text{C}$) was assessed every week to evaluate cold allodynia in four groups. Long-term administration of letrozole induced cold allodynia in mice. Letrozole-treated group vs naïve group and letrozole-treated group vs vehicle-treated group: $^{***} P < .001$.

2.3.4 Effect of letrozole on joint hypersensitivity

Since joint pain or discomfort is a major side effect of AI therapy in the clinical setting, joint hypersensitivity is regarded as a key indicator of determining AIA in mice. Hence, we examined whether the AI could develop local joint hypersensitivity by squeezing ankle joints with a pressure algometer device. Chronic letrozole treatment

increased joint hypersensitivity against pressure stimuli in mice. The letrozole-treated mice were less likely to endure the pressure stimuli than the naïve mice from week 3 through week 8 ($P < .05$). In addition, the letrozole-treated mice showed markedly lower limb withdrawal thresholds (LWTs) at week 5 that persisted to the last week compared to the vehicle-treated mice ($P < .05$). The LWTs decreased by nearly 25% from baseline to week 8 in the letrozole-treated group. There was no significant effect of estrogen deprivation on joint hypersensitivity for eight weeks (naïve group vs ovariectomy group: $P > .05$). There was a significant main effect for time ($F(8, 304) = 6.679, P < .001$), treatment group ($F(3, 38) = 8.425, P < .001$), and interaction between time and treatment ($F(24, 304) = 1.61, P = .037$) for the development of hypersensitivity in ankle joints (Figure 6).

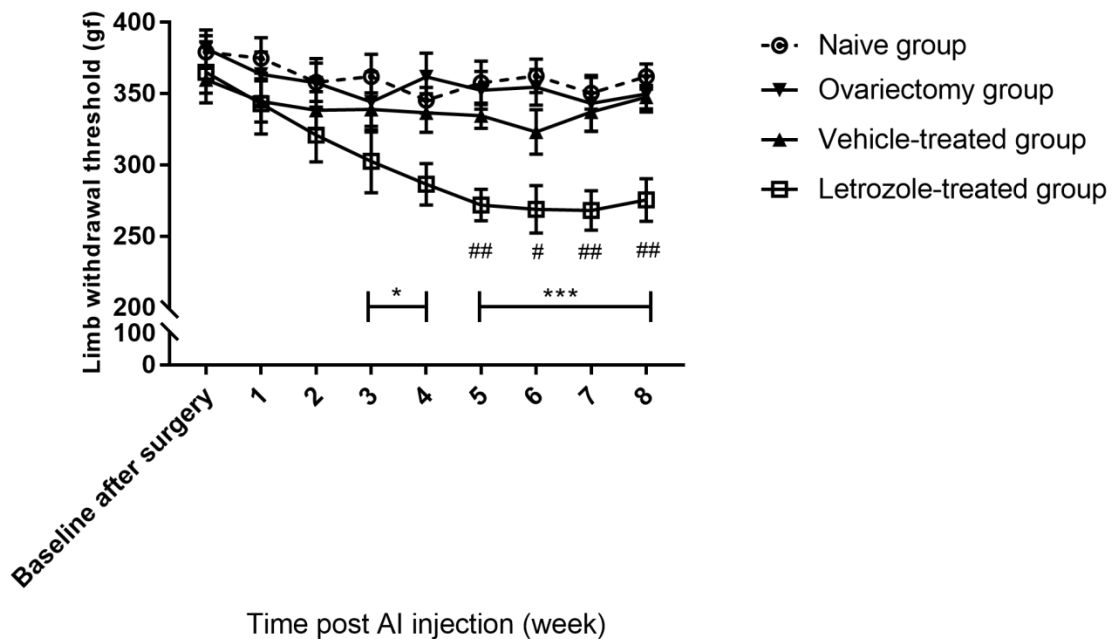


Figure 6. Effect of letrozole treatment on joint hypersensitivity. Limb withdrawal thresholds (g) were assessed every week to evaluate ankle joint hypersensitivity in four groups. Chronic administration of letrozole increased joint hypersensitivity. Letrozole-treated group vs naïve group: * $P < .05$, *** $P < .001$; letrozole-treated group vs vehicle-treated group: # $P < .05$, ## $P < .01$.

2.3.5 Effect of letrozole on weight bearing distribution

We hypothesized that mice would avoid supporting their body weight on hind limbs when their major knee joints are affected by systemic administration of letrozole. For this reason, we captured front paws and rear paws of free movement on the sensor mat for five minutes, and calculated the ratio of front paws to rear paws in regard to time and segment (area) to analyze how shifted their body weight was. As shown in Figure 7A, all groups showed similar ratios of front paws to rear paws at baseline ($F(3,36) = 0.54$, $P = .66$), and they were likely to spend a longer time shifting their weight to front paws at week 8. In particular, the letrozole-treated group showed a significantly increased time shifting their body weight forward ($t = 3.60$, $P < .01$). The ratio of front paws to rear paws in the letrozole-treated group increased from 0.17 to 0.37. The vehicle-treated group slightly increased their time shifting body weight forward ($t = 2.75$, $P = .02$); however, the change in the vehicle-treated group from baseline to week 8 was similar to other control groups. The naïve group and the ovariectomy group did not exhibit any significant changes regarding the amount of time spent on front paws (naïve group: $t = 1.93$, $P = .09$; ovariectomy group: $t = 1.63$, $P = .14$). Additionally, we examined the ratios of front paws to rear paws regarding segment, an area which mice occupied on the sensor mat, as presented in Figure 7B. All groups began with similar ratios of front paws to rear paws at baseline ($F(3,36) = 0.29$, $P = .83$), and they had a tendency to occupy more segments on their front paws at week 8. The letrozole-treated group clearly leaned forward when calculating the amount of segments ($t = 2.94$, $P = .01$), but the vehicle-treated group also increased the amount of segments on their front paws at week 8 ($t = 2.78$, $P = .02$). The ratio of front paws to rear paws in the letrozole-treated group was

increased from 0.21 to 0.38. The naïve group and the ovariectomy group also increased segments of front paws but they were not significant (naïve group: $t = 1.15$, $P = .28$; ovariectomy group: $t = 1.59$, $P = .15$).

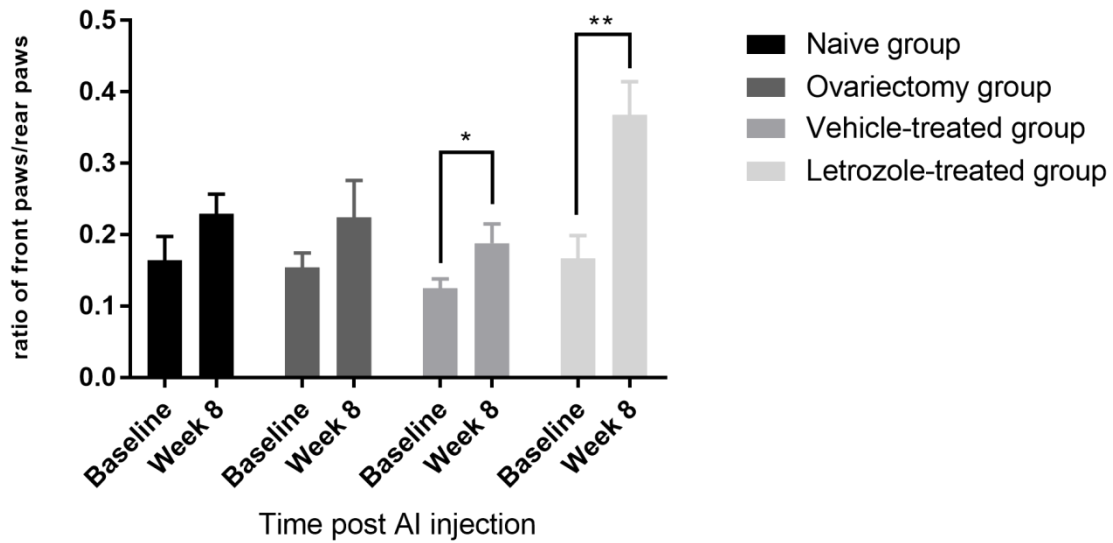


Figure 7A. Effect of letrozole treatment on weight bearing distribution (time). Weight bearing distribution was assessed by capturing free movement on sensor mat by mice at baseline and at week 8 in four groups. The amount of time spent on front paws and rear paws was recorded, and ratios of front paws to rear paws were calculated. Following chronic administration of letrozole, mice were likely to spend a longer time shifting their body weight to front paws. Baseline vs week 8: * $P < .05$, ** $P < .01$.

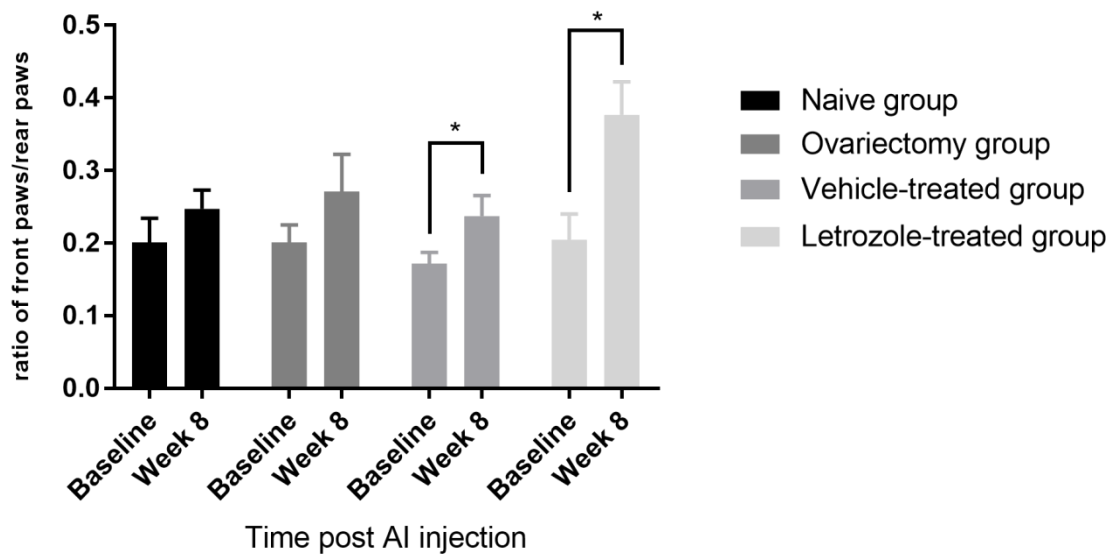


Figure 7B. Effect of letrozole treatment on weight bearing distribution (segment). The amount of segment (area) spent on front paws and rear paws was recorded at baseline and week 8, and ratios of front paws to rear paws were calculated in four groups. Following chronic administration of letrozole, mice increased the amount of segments on their front paws. Baseline vs week 8: * $P < .05$.

2.3.6 Effect of letrozole on grip strength

It was reported that breast cancer survivors taking AIs frequently experienced a decrease in grip strength along with fluid accumulation and tenosynovial changes (Lintermans et al., 2013). For this reason, grip strength of forepaws was tested to determine whether or not decreased grip strength was associated with AI administration as presented in clinical settings. Long-term administration of letrozole markedly decreased grip strength in mice. In the letrozole-treated group, a clear decrease in grip strength was observed from week 3 through 8 compared to the naïve group ($P < .01$). Interestingly, grip strength was decreased between baselines before and after ovariectomy and tumor removal surgery in the vehicle-treated group ($t = 3.80$, $P < .01$), though no significant change was observed in the letrozole-treated group ($t = 1.64$, $P = .13$). However, as seen in Figure 8B, standardized values of grip strength showed an overall

gradual decrease in all groups regardless of surgeries. In addition, the ovariectomy group had a significantly lower grip strength at week 5 and week 6 compared to the naïve group ($P = .03$, respectively), suggesting the estrogen deprivation may deteriorate grip strength in mice. There was a significant main effect for time ($F(9, 342) = 17.88, P < .001$), treatment group ($F(3, 38) = 7.55, P < .001$), and interaction between time and treatment ($F(27, 342) = 3.38, P < .001$) for grip strength (Figure 8A).

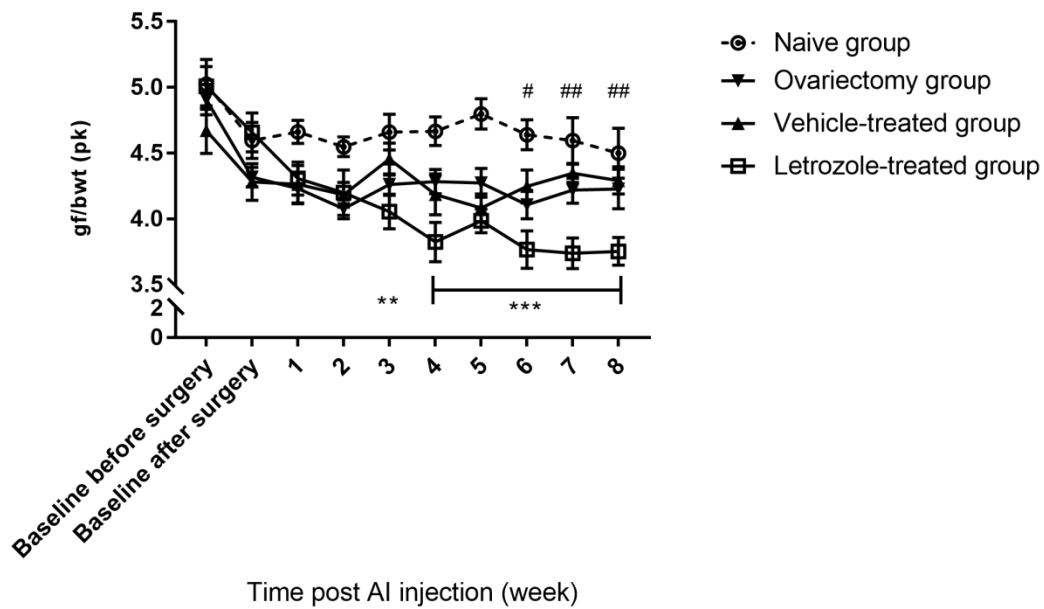


Figure 8A. Effect of letrozole treatment on grip strength. Grip strength of forepaws was assessed weekly in four groups. Long-term administration of letrozole significantly weakened grip strength. Letrozole-treated group vs naïve group: * $P < .01$, *** $P < .001$; letrozole-treated group vs vehicle-treated group: # $P < .05$, ## $P < .01$, ### $P < .001$.

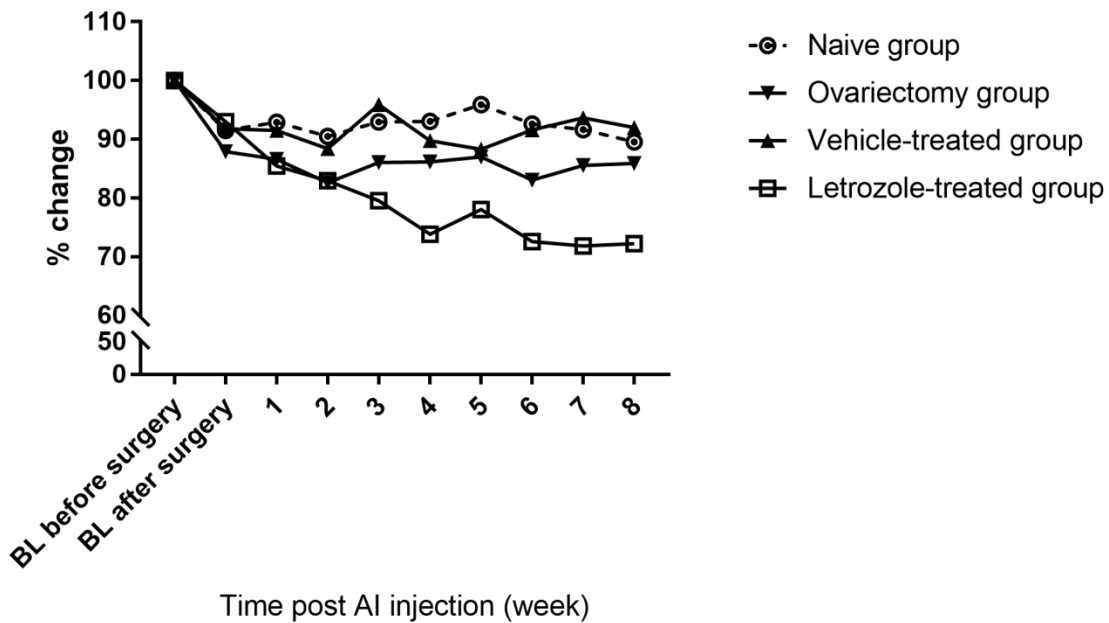


Figure 8B. Percent changes of grip strength following letrozole treatment. Grip strengths were standardized as a percent change to estimate geometrical changes in each group.

2.3.7 Effect of letrozole on motor coordination

We hypothesized that motor coordination would be impaired if systemic AI treatment affects neuromuscular function. As a result, chronic letrozole administration inhibited an improvement of motor coordination. The letrozole-treated group had a significantly lower latency at week 4 through 8 compared to the naïve group ($P < .05$), and at week 3 through 7 compared to the vehicle-treated group ($P < .05$). Regardless of ovariectomy and tumor removal surgery, all groups exhibited an increase of motor coordination until week 2. In addition, the estrogen deprivation condition did not seem to alter motor coordination when comparing the ovariectomy group to the naïve for eight weeks ($P > .05$). There was a significant main effect for time ($F(9, 342) = 12.84, P < .001$), treatment group ($F(3, 38) = 4.71, P < .001$), and interaction between time and treatment ($F(27, 342)=2.45, P < .001$) for motor coordination (Figure 9).

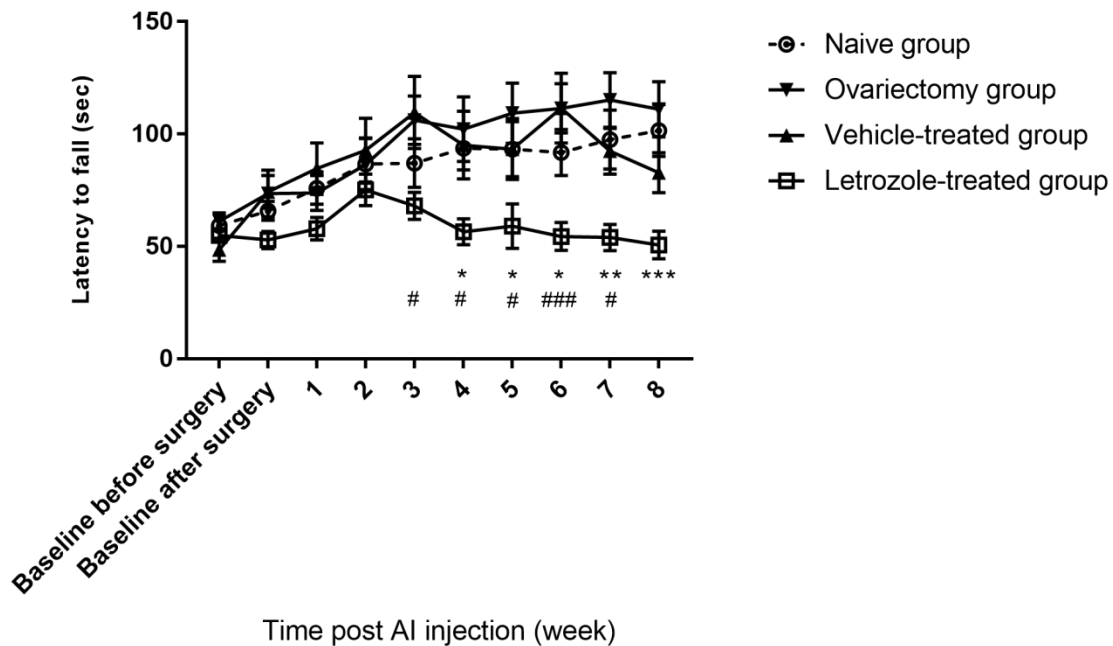


Figure 9. Effect of letrozole treatment on motor coordination. Latency to fall down (sec) was measured every week in four groups. Long-term administration of letrozole inhibited an improvement of motor coordination. Letrozole-treated group vs naïve group: * $P < .05$, ** $P < .01$, *** $P < .001$; letrozole-treated group vs vehicle-treated group: # $P < .05$, ### $P < .001$.

2.4 Discussion

One of the major reasons for the discontinuation of AI therapy is the occurrence of musculoskeletal pain in postmenopausal breast cancer survivors. Although AIA and the associated non-adherence issue increase mortality, the symptom cannot be successfully remedied at this time because no clear biological mechanisms that could inform AIA have been found. An animal model system is useful to reveal underlying mechanisms because it can provide genomic, genetic, physiological, and behavioral evidence. Herein, we have demonstrated that a clinically relevant animal model of AIA could be generated. We phenotyped multiple nocifensive behavior and neuromuscular function using ovariectomized immune-deficient mice, which allowed for low estrogen

levels and tumor xenograft. Chronic administration of letrozole significantly induced mechanical allodynia, thermal (cold) allodynia, joint hypersensitivity, anteroposterior weight shift, decreased grip strength, and worsened motor coordination. We also concluded estrogen deprivation partially contributed to cold allodynia and grip strength in this animal model.

Our animal model of AIA has face validity, in which the model accurately reflects the symptoms of the condition (Tkacs & Thompson, 2006). Face validity of an animal model can be evaluated by measuring feasible outcome variables and conducting a pairwise comparison with homologous outcome variables from human studies (Tkacs & Thompson, 2006). The proposed animal model was made by mimicking clinical conditions, such as breast tumor growth and its surgical removal, ovariectomized (postmenopausal) status, and chronic administration of AIs. In the study, animals exhibited quite similar phenotypes (e.g., joint hypersensitivity, decreased grip strength) to patients with AIA in the clinical setting. Therefore, this animal model system seems to be feasible for mechanistic studies of AIA.

Increased body weight is known as a risk factor of whether patients receiving AI therapy will discontinue treatment in several longitudinal studies (Niravath, 2013). The Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial and the Intergroup Exemestane Study (IES) study showed obese women were more likely to report joint pain than normal weight women (Mieog et al., 2012; Sestak et al., 2008). On the contrary, Crew et al. (2007) reported overweight women were less likely to experience AI-related joint pain compared to women with a normal body mass index (BMI). Therefore, we examined whether there were changes of body weight in mice treated with AIs, which

overall showed allodynic signs. When comparing body weights of four groups over eight weeks, no significant difference in body weight was found, regardless of presence of pain. This result may be due to a short duration of this study. In the Arumugam et al. (2014) study, anastrozole-treated mice and ovariectomized mice exhibited maximum body weight gain compared to mice treated with only estrogen or progesterone or both in 116 weeks of observation. It seems long-term studies are required to determine effects of the body weight on AI-induced pain in animal studies.

The use of the DWB test is suitable for validating compensatory body weight shift because the measurement allows for evaluating spontaneous and free movement of mice without the interference of an operator (Laux-Biehlmann et al., 2016). In the experiment, we observed that chronic letrozole injections significantly increased the time spent on and segment size of front paws in mice. In fact, there were few studies assessing weight distribution changes between front and rear paws in animal models of pain, except for the abdominal pain model (Laux-Biehlmann et al., 2016). In most preclinical pain research, comparisons between two rear paws have been frequently performed in aspects of asymmetrically directed behaviors of animals to assess bone cancer pain, neuropathic pain, osteoarthritis pain, and inflammatory pain (Lolignier et al., 2011; McCaffrey et al., 2014; Robinson, Sargent, & Hatcher, 2012; Ungard, Seidlitz, & Singh, 2014). This study is one of the first reports evaluating anteroposterior shifts of body weight in animal models of pain. Further research is warranted to examine how systemic injections of AIs affect joints with regard to weight bearing re-distribution by performing histological and molecular analyses of knee and ankle joints in mice.

To our knowledge, there is no literature reporting cold thermal stimuli intolerance in women receiving AIs; however, the development of cold allodynia was observed after letrozole administration in this animal model. Sometimes, there is a mismatch between the epidemiological realities of chronic pain prevalence in the human population and animal model subjects (Mogil et al., 2010). In the clinical setting, subjects experiencing AIA are female postmenopausal old-aged adults with considerable genetic variability, while this animal study is performed on young immune-deficient adult mice (at week 4~7) of a single strain. Further, in animal research, thermal and mechanical hypersensitivity is measured to evaluate pain (Mogil & Crager, 2004); which are less correlated with global ratings of pain severity in humans (Backonja & Stacey, 2004; Scholz et al., 2009). Further research may be needed to confirm the nocifensive behavior characteristics of this animal model.

Our data demonstrated that estrogen deprivation may partially contribute to a decrease in grip strength and the development of cold allodynia, as well as mechanical allodynia, though statistical significance on mechanical allodynia was not found. In alignment with this result, estrogen is known to exert anti-nociceptive and pain modulating effects on nociceptive input (Felson & Cummings, 2005). Estrogen directly affects opioid pain fibers in the central nervous system (CNS) (Dawson-Basoa & Gintzler, 1996a), and stimulates the release of endorphins and enkephalins that decrease nocifensive perception received by the brain (Felson & Cummings, 2005). Given the possible effects of estrogen on nociceptive input, the ovariectomy surgery appears to be an important procedure when employing animal models for studies of AIA, though it is unclear how the ovariectomized condition affects AI-induced pain.

Our data showed that AI administration induces nocifensive responses, such as mechanical allodynia and cold allodynia. This result may implicate that the aromatase inhibitor treatment is involved in central sensitization. It is evident that the majority of chronic musculoskeletal pain conditions are characterized by an alteration in pain processing of the CNS (Woolf, 2011). Central sensitization is commonly signified by pain which is triggered by normally innocuous stimuli (allodynia), or is augmented in response to noxious stimuli (hyperalgesia) (Latremoliere & Woolf, 2009). Central sensitization has provided a mechanistic explanation for threshold changes in acute and chronic pain and the generation and changes of abnormal pain sensitivity in the CNS (Latremoliere & Woolf, 2009). To date, most prior research has primarily focused on signaling and metabolism of estrogen and vitamin D as key risk factors in AIA, which has not been shown in all estrogen studies of AIA and shifted the focus away from promising therapies. Central sensitization could be an alternative approach to the examination of biological mechanisms of AIA and the development of new therapeutic treatments.

2.5 Conclusion

Our results indicate that a clinically relevant animal model of the AIA condition can be generated. The use of an appropriate animal model of AIA is beneficial for mechanistic studies addressing the AIA symptom. The nocifensive response and neuromuscular dysfunction elicited in this study provide evidence for better understanding the progression and characteristics of AIA. This model system will offer a platform to help find genomic, genetic, physiological, and behavioral evidence of the development of aromatase inhibitor-associated arthralgia.

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Chapter 3: Interventions for the Treatment of Aromatase Inhibitor-Associated Arthralgia in Breast Cancer Survivors: A Systematic Review and Meta-Analysis¹

3.1 Introduction

Breast cancer is the most frequently diagnosed cancer (231,841 estimated cases in 2015) and ranks second as a cause of cancer death among women in the U.S. (American Cancer Society, 2015). Third-generation aromatase inhibitors (AIs) are widely used and are the most effective, standard adjuvant endocrine treatment for postmenopausal women with breast cancer (Burstein et al., 2010; Cuzick et al., 2010). AIs play a critical role in decreasing the estrogen that primarily stimulates breast cancer progression by 90% via covalent (e.g., exemestane) or non-covalent (e.g., letrozole, anastrozole) binding, thereby aiding in preventing breast cancer recurrence (Nabholtz, 2008). Treatment with AIs improves disease free survival, lowers rates of endometrial malignancy, and has a more favorable toxicity profile compared to tamoxifen (Burstein & Winer, 2007; Crew et al., 2007). Since AI therapy has become more common, clinical experience has identified a unique musculoskeletal adverse effect of AI therapy. Nearly half of women who take AIs report AI-related joint pain or stiffness, and 20-30% discontinue the treatment because of the joint pain (Burstein & Winer, 2007; Galantino, Callens, Cardena, Piela, & Mao, 2013; Henry et al., 2008; Laroche et al., 2014). Patients report symmetrical joint pain or stiffness in hands, knees, feet, hip, and back, with a mild thickening of the soft tissues (Burstein & Winer, 2007). Neuropathic, diffused, mixed, or persistent joint pain is the most common characteristics of this condition (Laroche et al., 2014). Results from the

¹ Yang, G. S., Kim, H. J., Griffith, K. A., Zhu, S., Dorsey, S. G., & Renn, C. L. (2016). Interventions for the Treatment of Aromatase Inhibitor-Associated Arthralgia in Breast Cancer Survivors: A Systematic Review and Meta-analysis. *Cancer nursing*.

Investigation on the Duration of Extended Adjuvant Letrozole (IDEAL) trial demonstrated that a quarter of the enrolled women discontinued initial AI therapy within two years specifically because of musculoskeletal symptoms; in addition the overall non-compliance probability was 18.4% at 2.5 years (Fontein et al., 2012).

As arthralgias are quite common in breast cancer taking AIs, and such symptoms can continue years into survivorship, there is growing of a need to develop and evaluate AIA interventional strategies. Still, few forms of pain management have been established in clinical settings due to the lack of a clear consensus on the underlying biological mechanisms of development of AIA. Although it has been proposed that estrogen deprivation may contribute to the development of AIA, this has not been shown in all studies on estrogen: whether the effect results from localized estrogen deprivation or a systemic problem is also not clear (Gaillard & Stearns, 2011; Nevitt, Felson, Williams, & Grady, 2001).

If strong evidence on the effectiveness of specific interventions is provided, clinicians, policymakers, and patients can make the best decision based on the information to get the desirable treatment effect (Atkins et al., 2004). AI-associated arthralgia (AIA) is an extremely common problem among these women and negatively impacts their day-to-day well-being. Because AI therapy should be continued for a minimum of 5 years (Burstein et al., 2010), AIA may be a persistent problem for women, resulting lack of adherence to AI therapy. To our knowledge, no systematic review and meta-analysis has been conducted on all intervention types for AIA. Recently, Chien and colleagues studied the effect of acupuncture on AIA via a systematic review and meta-analysis and reported that acupuncture may alleviate AIA in breast cancer survivors;

however, they only focused on acupuncture and did not assess other types of interventions for AIA (Chien, Liu, Chang, Fang, & Hsu, 2015). Therefore, the purpose of this study was to identify current various types of pain management for AIA and evaluate the study quality and effects of interventions on AIA in breast cancer survivors.

3.2 Methods

3.2.1 Search strategy and data Sources

A systematic review and meta-analysis were guided by the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)*, which consists of a 27-item checklist and a four-phase flow diagram (Liberati et al., 2009). The PRISMA approach helps authors to formulate relevant and precise questions by using the acronym “PICOS,” which refers to five components: the information about population (P), the exposure or interventions (I), the comparator group intervention (C), the outcomes of intervention being assessed (O), and the type of study design (S).

The following databases were searched from 2000 to August 2015: PubMed, CINAHL (EBSCOHost), PsycINFO (OVID), and Web of Science (Web of Knowledge). We also identified eligible studies through a search of additional sources (e.g., Google Scholar, ProQuest for dissertations and theses, and manual search in citations of original publications). Unpublished dissertation studies were included to avoid publication bias. The keywords “Intervention,” “Management,” “Treatment,” were combined with “Arthralgia,” “Arthritis,” “Joint pain,” “Joint disease,” “Musculoskeletal disease,” “Musculoskeletal pain,” “Musculoskeletal symptom,” and “Aromatase Inhibitors,” including “Anastrozole (Arimidex),” “Letrozole (Femara),” “Exemestane (Aromasin),” as well as “Breast cancer,” and “Breast neoplasm.” The search was limited to only the

English language, publication dates from 2000 to August 2015, human subjects, and clinical experimental or quasi-experimental study design.

Each abstract and subsequent full-text article considered as qualitative synthesis was reviewed by two authors (G.S.Y. and H.J.K.) independently based on the PICOS criteria. At each step, two reviewers compared the results and discussed discrepancies to reach consensus. Eligible studies were sorted according to type of pain management, including: pharmacological approach, acupuncture, nutritional supplementation, relaxation techniques, and physical exercise. Data of each study were assessed by one reviewer (G.S.Y.) with a self-developed structured codebook, and confirmed by the other reviewer (H.J.K.) (Figure 10).

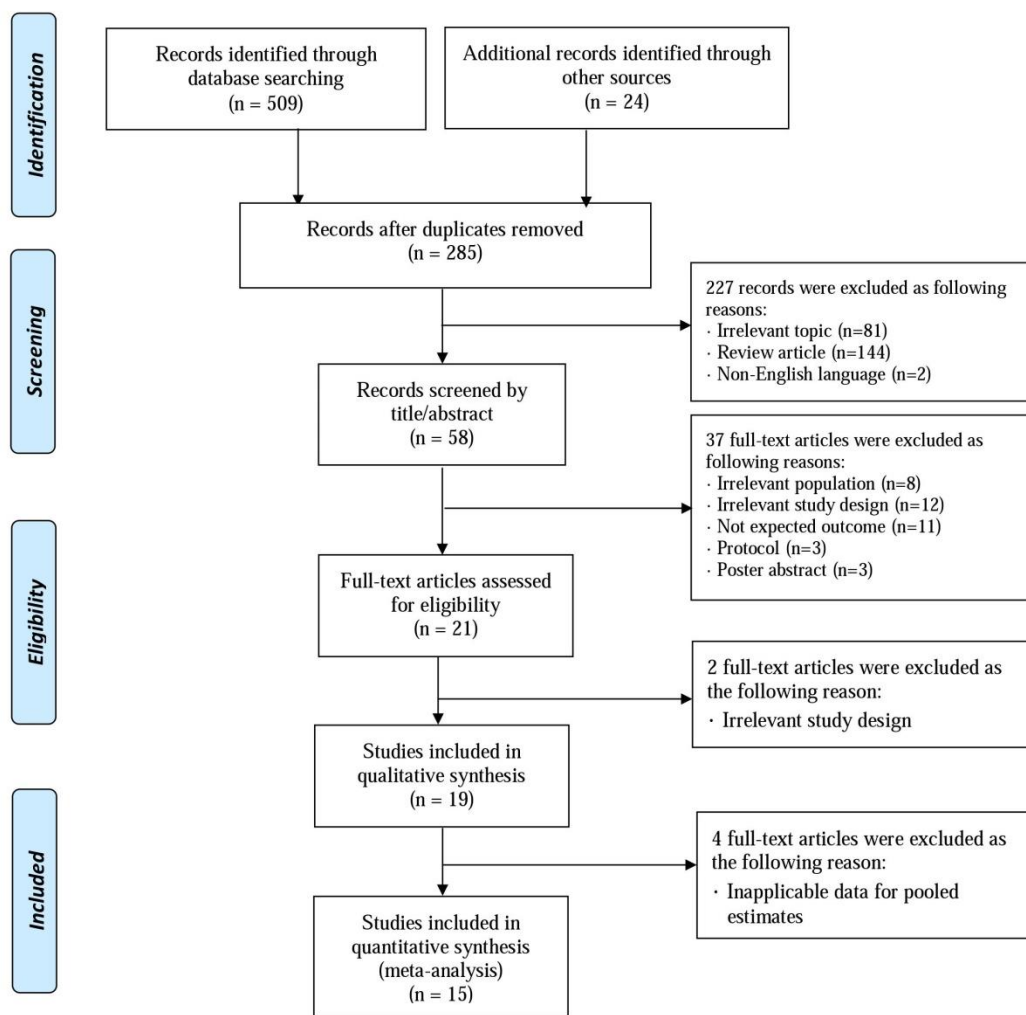


Figure 10. Flow diagram of interventions for the treatment on aromatase inhibitor-associated arthralgia in breast cancer survivors.

3.2.2 Inclusion and exclusion criteria

The inclusion criteria and exclusion criteria were assessed according to the PICOS framework. The population of interest (P) was postmenopausal women with a history of stage I, II, or III breast cancer and current use of AIs. Also, women who had joint pain associated with aromatase inhibitors and reported persistent pain or stiffness in their joints after initiation of AI therapy were also appropriate for the inclusion criteria.

Women with metastatic breast cancer (Stage IV) were excluded because of possible visceral/bone pain. For the intervention of interest (I), any intervention defined as treatment or activity strategy to decrease AIA symptoms was included. Trials with designs that did not allow for an evaluation of the effectiveness of intervention were excluded. As for comparators (C), any comparator or none was included, for example, baseline, placebo, usual care, another active treatment, or no control treatment. In terms of the outcome of interest (O), joint pain assessed by validated pain measurement tools was included. Regarding study design (S), all randomized controlled trials (RCTs) and quasi-experimental designs (e.g., one-group pre-post test studies) were included. Prospective cohort studies and longitudinal studies were also included if the study was designed to compare a treatment group and control group although no direct intervention was applied in patients. Case studies, case series, qualitative studies, systematic literature reviews, meta-analyses, and poster abstracts were excluded (Table 1).

Table 1

Study Eligibility Criteria from PICOS Framework

Category	Criteria
Population	Women aged 18 years or older, with postmenopause, a history of stage I, II, or III breast cancer; currently receiving AIs (anastrozole, letrozole, or exemestane); joint pain attributable to AI therapy; and ongoing pain or stiffness in one or multiple joints, which started or worsened after initiation of AI therapy
Intervention	Any interventions related with AIA defined as treatment or activity strategy to alleviate AIA symptoms: analgesics, other prescription medications, dietary supplements, non-pharmacological approaches, and others such as switching hormone therapy
Comparator	Any comparator or none (e.g., another active treatment, placebo, no treatment)
Outcome of interest	Musculoskeletal symptoms (i.e., joint pain or stiffness)
Study design	RCTs, quasi-RCTs, pilot study, uncontrolled trials, prospective cohort or longitudinal study

Note. AI=aromatase inhibitor; AIA=aromatase inhibitor-associated arthralgia; RCT=randomized clinical trials.

3.2.3 Quality assessment

Once all full text articles were collected, each article was assessed to evaluate quality using the *Quality Assessment Tool for Quantitative Studies* (QATQS) with a standardized guideline and dictionary for reference (Thomas, Ciliska, Dobbins, & Micucci, 2004). The QATQS tool is frequently used to assess studies by grading eight components including selection bias, study design, confounders, blinding, data collection methods, withdrawals and dropouts, intervention integrity, and analysis. The first six components were rated as strong, moderate, or weak, while the last two components were not rated; after which overall study quality was determined. The two authors compared

the six components and the overall quality of each article and then discussed discrepancies so that they could arrive at agreement (Table 2).

Table 2

Criteria for Assessing Study Quality

Components	Criteria for component ratings	Component ratings & Global rating
Selection bias	<ul style="list-style-type: none"> - Strong: Very likely to be representative of the target population and 80-100% of selected individuals who agreed to participate - Moderate: Somewhat likely to be representative of the target population and 60-79% of selected individuals who agreed to participate - Weak: All other responses 	<p>a. Each component is rated based on the criteria as one of follows:</p> <ul style="list-style-type: none"> - strong - moderate - weak <p>b. Global rating is evaluated as one of follows:</p> <ul style="list-style-type: none"> - strong: if there are four strong ratings with no weak ratings - moderate: if there are less than four strong ratings and one weak rating - weak: if there are two or more weak ratings
Study design	<ul style="list-style-type: none"> - Strong: Randomized controlled trials or clinical controlled trials - Moderate: Cohort analytic, case-control, cohort or an interrupted time series - Weak: All other study designs 	
Confounders	<ul style="list-style-type: none"> - Strong: No presence of important differences between groups prior to the intervention (control of at minimum 80% confounders) - Moderate: Control of 60-79% confounders - Weak: Uncontrolled confounders 	
Blinding	<ul style="list-style-type: none"> - Strong: Blinding of both outcome assessors and participants on study questions - Moderate: Blinding of either outcome assessors or participants on study questions - Weak: Awareness of study questions by both parts 	
Data collection methods	<ul style="list-style-type: none"> - Strong: Use of valid and reliable collection tools - Moderate: Use of valid tools but no description of reliability - Weak: Lack of reliability and validity 	
Withdrawals and drop-outs	<ul style="list-style-type: none"> - Strong: 80% or greater of follow-up rate - Moderate: 60-79% of follow-up rate - Weak: Less than 60% of follow-up rate 	

Adapted from "A process for systematically reviewing the literature: providing the research evidence for public health nursing interventions," by B.H. Thomas, D. Ciliska, M. Dobbins, & S. Micucci. *Worldviews on Evidence-Based Nursing*, 2004, 1(3), p.180. Copyright by Sigma Theta Tau International.

3.2.4 Data analysis

We primarily used the pain mean score as assessed by Brief Pain Inventory (BPI) at the end timepoint of intervention because this questionnaire was the most frequently used instrument across the studies. We calculated pooled standardized mean differences (SMDs) and 95% confidence intervals (CIs) to determine the magnitude of effect sizes of interventions on pain. If standard deviations were not provided, we obtained standard

deviations from standard errors and CIs. Publication bias was assessed with Funnel plot and Egger's test. Heterogeneity across studies was identified by I^2 statistic, which was regarded as high ($I^2 = 81.52\%$). Hence, the random-effects model was used to get pooled SMDs.(Borenstein, Hedges, Higgins, & Rothstein, 2009) Using the random effects model, subgroup analyses were performed by testing pooled SMDs for a significant difference in pain between categorized groups, such as study type, study design, study quality, adherence to intervention protocols, and the number of participants. *P*-value of less than .05 was considered statistically significant (two-tailed). Comprehensive Meta-Analysis version 3 (Englewood, NJ, USA) was used to analyze the data.

3.3 Results

The systematic search through PubMed, CINAHL, PsycINFO, and Web of Science yielded a total of 509 titles, and 24 were identified from additional sources (e.g., Google Scholar, ProQuest for dissertations and theses, and manual search in citations of original publications). An abstract review reduced the potential number of studies to 58, and full-text review further reduced eight RCTs and eleven cohort one-group pre-post tests, for a total of 1,076 postmenopausal women with a history of breast cancer. Average age in the studies ranged from 56 to 71 years. Participants were recruited from a variety of settings, such as university hospitals, clinics, breast cancer centers, and community health clubs. In addition, AIA intervention studies were conducted in USA, Spain, France, Japan, UK, China, and Australia. The overall adherence to intervention protocols was high (85.4%), ranging 66.7% to 100% (Table 3). Joint pain symptom outcomes of the trials were measured by validated self-reported questionnaires, including, BPI, Western Ontario and McMaster Universities Osteoarthritis Index, Visual Analogue Scale,

Fibromyalgia Impact Questionnaire, Modified Score for the Assessment and Quantification of Chronic Rheumatoid Affections of the Hands, and Health Assessment Questionnaire. The selected 19 studies were included for qualitative synthesis, among which 15 were available for meta-analysis. As for the quality assessment, they had overall moderate quality according to the QATQS tool, with a substantial agreement ($\kappa = 0.640$, $P < .001$). Studies were categorized into five intervention types: pharmacological approaches, (electro) acupuncture, nutritional supplementation, relaxation techniques, and physical exercise.

3.3.1 Characteristics and quality of the included studies

Pharmacological approaches. Four cohort one-group pre-post test studies examined the effects of pharmacological interventions in a total of 251 participants with AIA. Pharmacological approaches include taking duloxetine (Cymbalta) as a serotonin and norepinephrine reuptake inhibitor (Henry et al., 2011), prednisolone (Kubo et al., 2012), zadaxin (Thymalfasin) as immunotherapy (Zhang, Tang, & Zhao, 2010), and switching between AI drugs (Briot, Tubiana-Hulin, Bastit, Kloos, & Roux, 2010). Those studies reported no severe adverse events and demonstrated overall improvement in joint pain and stiffness. In the duloxetine trial (30 mg daily for one week and then 60 mg daily for seven weeks), 72.4% of participants experienced at least 30% reduction in the average pain level compared to baseline ($P < .001$), and approximately 80% completed the protocol (Henry et al., 2011). A short course of prednisolone (5mg of oral prednisolone once a day in the morning for one week) provided immediate relief in joint pain in nearly 70% of participants, with 63% still reporting improvement at one month (Kubo et al., 2012). Also, the participants who started letrozole after a one-month washout period of

anastrozole reported improvement in pain ($P < .001$), physical and mental quality of life (QOL) ($P < .001$ and $P = .01$, respectively), indicating that letrozole was better tolerated compared to anastrozole. However, 74% of participants still complaint of arthralgia at six months of letrozole therapy (Briot et al., 2010). Lastly, zadaxin therapy (subcutaneous injection of thymosin $\alpha 1$ 1.6mg twice a week for four weeks) had evidence of improvement in pain severity ($P = .014$), pain-related functional interference ($P = .001$), and physical well-being ($P = .001$) compared to baseline (Zhang et al., 2010). Studies using duloxetine, immunotherapy, and switching between AIs were evaluated as having moderate quality, while the prednisolone study was weak due to lack of blinding and no use of valid and reliable measurement tools.

Acupuncture. Four RCTs and two cohort one-group pre-post test studies investigated the effectiveness of traditional acupuncture and electro-acupuncture (EA) in a total of 226 women with AIA. Overall, the acupuncture interventions were well-tolerated, and infection or development of lymphedema was not observed in any participants. However, all of the studies did not demonstrate effectiveness of acupuncture. In an RCT study by Crew et al. (Crew et al., 2010), the true acupuncture (TA) group received full body and auricular acupuncture as well as joint-specific acupoints, whereas the sham acupuncture (SA) group had superficial needle insertion at nonacupoint locations. The TA group reported significant decrease in pain severity ($p = .003$), pain-related interference ($P = .002$), and stiffness ($P = .01$). Mao and colleagues also conducted an RCT of electrostimulation delivered by a transcutaneous electrical nerve stimulation (TENS) unit (Mao et al., 2014). Pain severity and pain-related interference improved more in the TA group compared to the waiting list control (WLC)

group ($P < .001$ and $P = .003$, respectively). Prior to those RCT studies, these research groups had conducted pilot studies using the same protocols to test efficacy and safety, and had found that pain was significantly decreased (Crew et al., 2007; Mao et al., 2009). On the contrary, there are another two studies reporting the opposite results.

A significant difference was not observed in decrease of pain between experimental and sham groups ($P = .31$) when comparing effects of stimulating 15 real acupoints called major *Qi* with sham acupoints that are midpoints of the line between two real acupoints (Bao et al., 2013). Oh and colleagues applied real acupuncture by connecting needles through an electrode with bilateral rotation at various acupuncture points until *de qi* sensation (e.g., tingling, numbness) occurred, while the sham group received acupuncture that did not penetrate the skin (Oh et al., 2013). There were no significant differences in joint pain, stiffness, and physical function between the sham and real acupuncture groups. In acupuncture intervention, four studies were evaluated as having strong quality (Bao et al., 2013; Crew et al., 2010; Mao et al., 2014; Oh et al., 2013), while the others were assessed as having moderate (Mao et al., 2009) and weak (Crew et al., 2007) due to issues of participants' representativeness and lack of blinding.

Nutritional supplementation. Nutritional supplementation included three studies evaluating the effects of taking a high dose of vitamin D, glucosamine plus chondroitin, and omega-3 fatty acids (O3-FAs) in 362 breast cancer survivors with AIA. Two studies were designed as RCTs (Hershman et al., 2015; Rastelli et al., 2011), and one was a cohort one-group pre-post test design (Greenlee et al., 2013). Participants taking high dose vitamin D2 (50,000 IU) in Rastelli et al.'s study showed a greater reduction in average pain ($P = .007$), pain severity ($P = .04$), and pain interference ($P = .034$)

compared to the placebo group at 2 months, though there were no differences in pain at 6 months (Rastelli et al., 2011). A daily dose of 1,500 mg glucosamine plus 1,200 mg chondroitin over 24 weeks also produced improvement in pain ($P = .05$) and stiffness ($P = .03$) as well as hand grip and pinch strength with minimal side effects at 12 weeks (Greenlee et al., 2013). In the RCT study by Hershman et al. (Hershman et al., 2015), the intervention group received daily 3.3g O3-FAs (i.e., eicosapentaenoic acid [EPA] plus dehydroepiandrosterone [DHEA] in a 40-to-20 ratio), while the control group took the placebo, a blend of soybean and corn oil, for 24 weeks. Interestingly, both placebo treatment and O3-FAs improved joint pain in participants; therefore, two groups showed no difference ($P = .52$). In addition, no changes in cholesterol ($P = .66$), C-reactive protein (CRP) ($P = .71$), and lipid profiles except for triglycerides were observed. In nutritional supplementation study, two studies had strong quality (Hershman et al., 2015; Rastelli et al., 2011), while the other one was moderate (Greenlee et al., 2013).

Relaxation techniques. There were two studies examining the effectiveness of relaxation techniques, including yoga and tai chi, in 22 women taking AIs. The studies were designed as a one group cohort with pre-post test. A feasibility study was conducted to evaluate the impact of yoga on pain, functional outcomes, and health-related QOL (Galantino et al., 2012). Iyengar yoga, comprised of precise postures (*asanas*), breathing exercise (*pranayama*), and meditation was done for two hours per week over eight weeks. The participants reported improvement in pain ($P = .016$), flexibility (functional reach: $P = .048$, sit and reach: $P = .009$), and physical function ($P = .015$) following yoga. In contrast, another study using tai chi was shown to be ineffective in decreasing pain ($P = .058$) and functional well-being ($P = .223$) (Galantino et al., 2013). This technique is

focused on body awareness, deep breathing, and weight bearing to address the symptoms for one hour twice per week over eight weeks. In this study, anxiety, depression, emotional well-being, fatigue, and sit and reach flexibility were significantly improved. Both studies were evaluated as weak quality due to selection bias and lack of blinding of outcome assessors and participants.

Physical exercise. Four studies (N = 215) were conducted to examine the effectiveness of physical exercise in participants treated with AIs. Two studies were designed as one-group pre-post test (Denysshchen et al., 2014; Nyrop et al., 2014), and two were designed as RCTs (Fields, 2015; Irwin et al., 2015). Denysshchen et al. investigated an 8-week, home based program that combined upper and lower body resistance exercise with self-selected aerobic exercise by assessing pain, functional performance, and cardiovascular endurance (Denysshchen et al., 2014). Participants reported a significantly lower number of painful joints ($P = .01$) and an improvement in physical activity ($P = .01$). Significant improvements in grip strength, biceps curl, and sit-to-stand were also observed, whereas cardiovascular endurance showed no significant changes. In the Fields' study (2015), participants carried out Nordic walking, which is a form of walking with gripping poles moved in an opposite manner to lower limb motion, to evaluate the effectiveness on pain and psychosocial components over 12 weeks. The intervention group participated in supervised Nordic walking training followed by self-managed Nordic walking session, whereas the control group received usual care. The findings suggested that both groups experienced improvement on pain, depression, and self-efficacy; interestingly, the control group showed a marginally greater decrease in pain compared to the experimental group ($P = .10$). In Irwin et al.'s study (Irwin et al.,

2015), participants were randomized into exercise (a combination of supervised resistance training program at a local health club and a home-based aerobic exercise program) or usual care group. At 12 months, the worst joint pain scores had decreased by 29% in the intervention group versus a 3% increase in the usual care group. Pain severity ($P < .001$) and pain interference ($P < .001$) were significantly lower in the experimental group. Lastly, Nyrop et al. investigated a 6-week self-directed walking program in elderly breast cancer survivors taking AIs (Nyrop et al., 2014). The women completed walking at least 5 days a week for at least 30 accumulated minutes a day. As a result, a total time of walking per week increased over a 6-week period. Joint pain, stiffness, and fatigue also decreased by 10%, 32%, and 19%, respectively, although not significantly. In the physical exercise intervention, three studies had moderate quality (Denyssen et al., 2014; Irwin et al., 2015; Nyrop et al., 2014), and one was evaluated as having weak due to selection bias, lack of blinding, and significant confounders (Fields, 2015).

Table 3

Characteristics and Quality of the Selected Studies

Author, year, location	Study design	Research question/aim	Adherence to the protocol	Age (yrs) & N (at the initiation of the treatment)	Intervention	Comparator	Duration	Outcomes	Study quality
Pharmacological Approach (n=4)									
Briot et al., 2010, France	one group pre-post test	To evaluate the effect of the switch of AIs on musculoskeletal symptoms in postmenopausal women with HR+ breast cancer, and to identify the factors associated with subsequent discontinuations from AI therapy	71.5% (128/179)	61.3±8.4 (mean), N=179	Stopping anastrozole, and then switching to letrozole (2.5 mg per day) after having 1-month washout	Baseline	6 months	Decrease in pain (BPI) and disability (HAQ), improvement in physical and mental components in relation with QOL (SF-12), no observed change in biochemical markers of inflammation	Moderate
Henry et al., 2011, USA	one group pre-post test	To determine whether duloxetine may be effective for management of AI-associated musculoskeletal symptoms	79.3% (23/29)	56 [36-70] (median), N=29	Duloxetine 30 mg daily for 7 days, then 60mg daily for 21 days; after which patients were allowed to choose whether continuing duloxetine 60 mg daily, increasing the dose to duloxetine 60 mg twice daily, or discontinuing due to adverse effects or no relieving symptoms	Baseline	8 weeks	Decrease in pain (BPI, HAQ, VAS), hot flash (HFRDI, MENQOL), depression (CESD), and sleep disturbance (PSQI)	Moderate
*Kubo et al., 2012, Japan	one group pre-post test	To determine whether low-dose prednisolone is effective against AIA	100% (27/27)	62.8 [51-81] (mean), N=27	Prednisolone 5mg daily in the morning	Baseline	1 week	Decrease in pain (VAS)	Weak
Zhang et al., 2010, China	one group pre-post test	To evaluate the feasibility of immunologic therapies for AI-related joint symptom	100% (16/16)	58 [45-72] (median), N=16	Zadaxin (thymosin α 1 ; immunologic therapy) 1.6mg twice a week s ubcutaneously	Baseline	4 weeks	Decrease in pain (BPI-SF, WOMAC), physical well-being, social well-being, emotional well-being, functional well-being (FACT-G), and interferon-gamma, increase in interleukin-4	Moderate

Table 3 (Continued)

(Electro) Acupuncture (n=6)

*Bao et al., 2013, USA	RCT	To determine the effect of acupuncture in improvements of function and pain in women with AIA	92.2% (47/51) (RA=23, SA=24)	RA: 61 [44-82], SA: 61 [45-85] (median), N=51 (RA=25; SA=26)	Real acupuncture on 15 acupoints called major Qi (vital energy) weekly	Non-penetrating retractable needles on 14 acupoints placed on the midpoint of the line between two real acupoints	8 weeks	No observed change in pain (VAS and HAQ-DI), reduction of IL-17 in both groups, no significant modulation in estradiol, β -endorphin, or other proinflammatory cytokine concentrations	Strong
Crew et al. 2007, USA	one group pre-post test	To evaluate the efficacy and safety of acupuncture in decreasing joint symptoms related to AI use	90.5% (19/21)	59 [46-73] (median), N=21	Full body acupuncture and auricular acupuncture in alternating ears for 30minutes sessions twice weekly	Baseline	6 weeks	Improvement in pain (BPI-SF), function (WOMAC) and physical well-being (FACT-G), no observed changes in emotional well-being (FACT-G) and inflammatory biomarkers (TNF- α , IL-1 β)	Weak
Crew et al. 2010, USA	RCT	To evaluate the benefits of acupuncture in the improvement of pain, stiffness, and functional ability in women with AIA	88.4% (38/43) (TA=20, SA=18)	TA: 58 [44-77], SA: 57 [37-77] (median), N=43 (TA=23; SA=20)	Full body and auricular acupuncture for 30 minutes twice weekly	Sham acupuncture at non-acupoints location via superficial needle insertion	6 weeks	Improvement in pain, stiffness and function (BPI and WOMAC), no observed change in pain, stiffness, and function (M-SACRAH and FACT-G)	Strong
Mao et al., 2009, USA	one group pre-post test	To demonstrate the feasibility of recruitment and retention to an acupuncture trial; and to explore the effects of acupuncture on fatigue, anxiety, depression, and sleep	66.7% (8/12)	59 [52-70] (median), N=12	A manualized protocol over fixed acupuncture points connected with a TENS unit twice a week for 2weeks, then weekly for 6 more weeks for a total maximum of 10 treatments	Baseline	8 weeks	Decrease in pain, stiffness (BPI), fatigue (BFI), and anxiety (HADS), no observed change in depression (HADS) and sleep disturbance (PSQI)	Moderate
Mao et al., 2014, USA	RCT	To evaluate the short-term effects and safety of electro-acupuncture for AIA	88.1% (59/67)	EA: 57.5 \pm 10.1, SA: 60.9 \pm 6.5, WLC: 60.6 \pm 8.2 (mean), N=67 (EA=22; SA=22; WLC=23)	Electro-acupuncture (EA) using a manual protocol with 2 Hz electro-stimulation delivered by a TENS unit	SA, WLC	8 weeks	Decrease in pain, (BPI, quick-DASH), stiffness, function (WOMAC), no observed change in PPT	Strong
*Oh et al., 2013, Australia	RCT	To determine the efficacy of acupuncture in aspects of feasibility and safety to treat AIA	90.6% (29/32) (RA=14; SA= 15)	RA: yr<45 (n=14), yr \geq 45 (n=1), SA: yr<45 (n=12), yr \geq 45 (n=2), N=32	RA: needles connected through an electrode to a battery-operated pulse generator at various acupuncture points until <i>de qi</i> sensation occurs twice weekly	SA: needles pressuring on contact at six real acupuncture points without causing <i>de qi</i> sensation	6 weeks	No changes in pain, stiffness, physical function (BPI, WOMAC), pain severity and interference with daily functioning (BPI-SF), QOL (FACT-G), hand strength or inflammatory markers (CRP, ESR)	Strong

Table 3 (Continued)

Nutritional Supplementation (n=3)									
Greenlee et al., 2013, USA	one group pre-post test	To test the effect of glucosamine plus chondroitin on AIA in post-menopausal women	92.5% (37/40)	61.4 [40.7-83.2] (mean), N=40	A daily dose of 1,500mg glucosamine and 1,200mg chondroitin	Baseline	2 weeks	Decrease in pain and stiffness (WOMAC, M-SACRAH, BP), increase in hand grip and pinch strength, no change in estradiol level	Moderate
Hershman et al., 2015, USA	RCT	To examine whether omega-3 fatty acids can reduce pain and stiffness in women undergoing adjuvant AI therapy for early-stage breast cancer	79.8% (209/262) (I=102, P=107)	I: 59.5, P: 59.1 (median), N=262 (I=131; P=131)	Omega-3 fatty acids (O3-FAs) (560 mg of EPA plus DHA in a 40-to-20 ratio) 3.3g per day	Placebo (a blend of soybean and corn oil)	24 weeks	Improvement in pain in both groups (BPI), no difference in symptoms (WOMAC, M-SACRAH, FACT-ES, global rating of change scales), no observed changes in cholesterol, CRP, and lipid profiles except for triglycerides	Strong
Rastelli et al., 2011, USA	RCT	To determine whether high dose vitamin D2 supplementation (HDD) in breast cancer patients who develop AIA	78.3% (47/60) (I=21, P=26)	I: 60±8.8, P: 63±7.8, (mean), N=60 (I=30; P=30)	Vit D2 50,000IU capsule for a total of eight consecutive weeks, and then once a month for the rest of the study	Placebo orally for 16 consecutive weeks and then once a month for the rest of the study	6 months	Decrease in pain and interference with daily activities (FIQ, BPI), no observed change in functional ability (HAQ-DI)	Strong
Relaxation Techniques (n=2)									
Galantino et al., 2012, USA	one group pre-post test	To evaluate the impact of yoga as a feasibility study in pain, functional outcomes, and health-related quality of life for patients with AIA	80% (8/10)	58 [50-71] (median), N=10	Iyengar yoga , combining precise postures (<i>asanas</i>), breathing exercise (<i>pranayama</i>), and meditation, twice per week for one hour	Baseline	8 weeks	Improvement in pain (BPI), functional reach, flexibility, and physical function (PSFS, FACT-B)	Weak
Galantino et al., 2013, USA	one group pre-post test	To determine the safety and feasibility of a tai chi trial and evaluate the effectiveness of tai chi in the function, pain, and quality of life for patients with AIA	75% (9/12)	59 [49-76] (median), N=12	Tai Chi focused on body awareness, deep breathing, and weight bearing to address the symptoms for one hour twice per week in a group program (16 sessions)	Baseline	8 weeks	Improvement in anxiety, depression, emotional well-being, fatigue, sit-and-stand test (HADS, FACT-B, FACIT-Fatigue), no observed change in pain, social/family well-being, functional well-being (BPI, FACT-B), functional balance (BBS), and physical function (TUG)	Weak

Table 3 (Continued)

Physical Exercise (n=4)									
Denysschen et al., 2014, USA	one group pre-post test	To determine implementation of an exercise program in reducing joint pain and improving quality of life and functional performance in breast cancer patients treated with AIs	76.5% (26/34)	63 [52-72] (mean), N=34	Home-based program combining upper and lower body resistance exercises with self-selected aerobic exercises	Baseline	8 weeks	Improvement in pain (AIMS2), depression, QOL (SF-36), grip strength, biceps curl, and sit-to-stand, no observed change in cardiovascular endurance and anthropometrics	Moderate
Fields, 2015, UK	RCT	To test whether Nordic walking improves pain and related biopsychosocial outcomes in people with AIA	90% (36/40) (I=16, C=20)	63±8 (mean), N=40 (I=20; C=20)	Nordic walking (week 1-6: supervised group Nordic walking training weekly; week 7-12: 4×30 min self-managed Nordic walking session)	Waiting list control group (usual care)	12 weeks	Improvement in pain (BPI-SF), depression (CES-D), and self-efficacy (PSEQ) in both groups; improvement in health-related QOL (SF-36) in the intervention group	Weak
Irwin et al., 2015, USA	RCT	To examine the effect of an exercise intervention on severity of AI-induced arthralgia in women receiving AIs and experiencing arthralgia	88.4% (107/121)	I:62.0±7.0 U:60.5±7.0 (mean), N=121 (I=61; U60)	A combination of a twice-per-week supervised resistance training program at a local health club and a home-based aerobic exercise program of 150 minutes per week	Usual-care group (not given any exercise instruction)	12 months	Decrease in pain (BPI, WOMAC, and DASH), no dose-response effect of exercise	Moderate
*Nyrop et al., 2014, USA	one group pre-post test	To determine whether a physical activity intervention is feasible and potentially effective in providing relief for survivors on AI therapy who report joint pain or other joint symptoms	95% (19/20)	71 [65-87] (mean), N=20	Walk With Ease (WWE): the self-directed physical activity program by walking at least 5 days a week for at least 30 accumulated minutes per day, which is based on the WWE workbook reflecting arthritis symptom management through exercise and motivation/behavioral change strategies	Baseline	6 weeks	Decrease in pain, stiffness (VAS, ASE), and fatigue (not significant), increase in total time of walking	Moderate

Abbreviations: AIMS2, Arthritis Impact Measure Scale 2; ASE, Arthritis Self-Efficacy Scale; BBS, Berg Balance Scale; BFI, Brief Fatigue Inventory; BPI, Brief Pain Inventory; CES-D, Center for Epidemiologic Studies-Depression Scale; CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; FACIT, Functional Assessment of Chronic Illness Therapy; FACT-B, Functional Assessment of Cancer Therapy-Breast; FIQ, Fibromyalgia Impact Questionnaire; HADS, Hospital of Anxiety and Depression Scale; HAQ, Health Assessment Questionnaire; HAQ-DI, Health Assessment Questionnaire Disability Index; HFRDI, Hot Flash Related Daily Interference Scale; HR-QOL, Health-Related Quality of Life; MENQOL, Menopause-Specific Quality of Life Questionnaire; M-SACRAH, Modified Score for the Assessment and Quantification of Chronic Rheumatoid Affections of the Hands; PSEQ, Pain Self-Efficacy Questionnaire; PSFS, Patient Specific Functional Scale; PSQI, Pittsburgh Sleep Quality Index; RCT, Randomized Controlled Trial; TENS, Transcutaneous Electrical Nerve Stimulation; Quick-DASH, Quick Disability of Arm, Shoulder, Hand; PPT, Physical Performance Test; TUG, Timed Up-and-Go; VAS, Visual Analog Scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

* Denoted studies were excluded in the meta-analysis.

3.3.2 Meta-analysis

Figure 11 and Table 4 show the results of the meta-analysis from 15 selected studies. Four out of 19 studies were excluded in this analysis due to the lack of sufficient raw data from the original publication. Studies included were three with pharmacological approaches (Briot et al., 2010; Henry et al., 2011; Zhang et al., 2010), four using acupuncture (Crew et al., 2007; Crew et al., 2010; Mao et al., 2009; Mao et al., 2014), three employing nutritional supplementation (Greenlee et al., 2013; Hershman et al., 2015; Rastelli et al., 2011), two with relaxation techniques (Galantino et al., 2013; Galantino et al., 2012), and three with physical exercise interventions (Denyssen et al., 2014; Fields, 2015; Irwin et al., 2015).

Publication bias. The funnel plot for measurement of pain is presented in Figure 11. In the presence of publication bias, the bottom of the plot commonly shows a high concentration of studies on one side of the mean, indicating that smaller studies are more likely to be published if they can report a large effect size (Rothstein, Sutton, & Borenstein, 2005). Although the funnel plot looked asymmetrically distributed around the pooled SMD, it did not show a high concentration on one side of the bottom. Egger's test for asymmetry was not significant ($t = 1.765$, $P = .101$). These results indicate that there was no publication bias for pain.

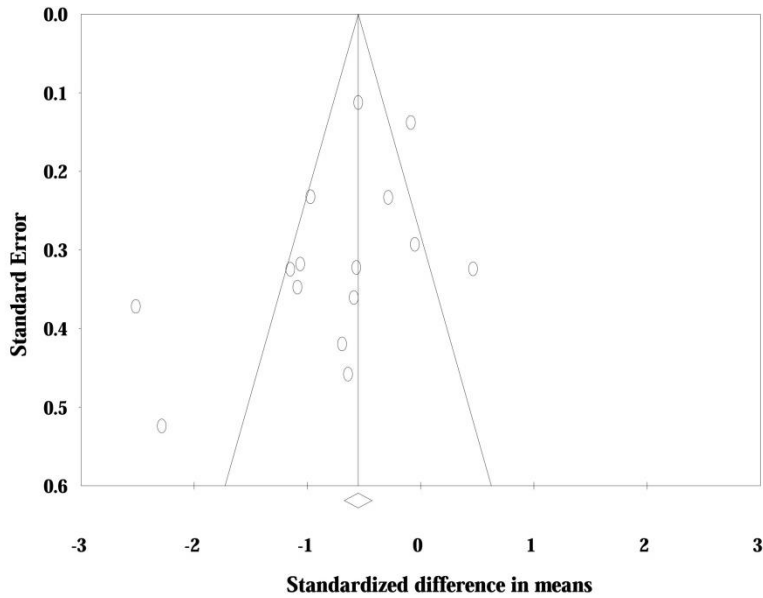


Figure 11. Funnel plot for examining publication bias on pain across studies (n=15). Evidence of negative publication bias is shown in the funnel plot (assessed with Egger's test).

Effects of interventions on pain. Statistical evidence for between-study heterogeneity in the effects of interventions on pain is shown ($I^2 = 81.52\%$, $P < .001$). Across the studies, the overall effect size of the interventions used for improving AIA was shown to be large (SMD: -0.744, 95% CI: -1.061 – -0.428). Seven of 15 studies demonstrated a significant effect in reducing AIA, among which the acupuncture method used in Henry et al.'s study (Henry et al., 2011) had the greatest effect size (SMD = -2.513, 95% CI: -3.243 – -1.783) (Figure 12).

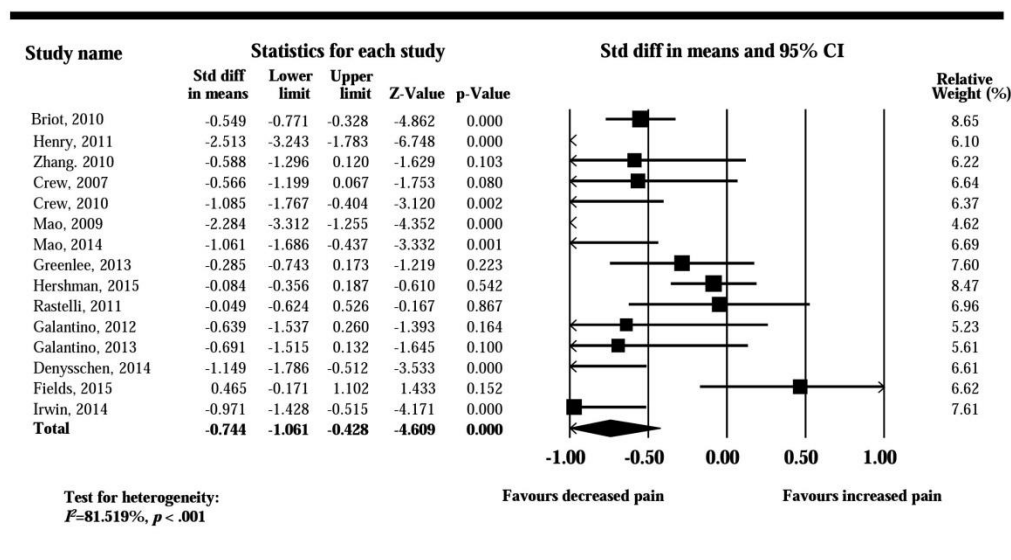


Figure 12. Forest plot for evaluating effects of interventions on pain in breast cancer survivors. Standardized mean differences and weights were obtained from a random-effects model. The square size is proportional to the weight of each study in this analysis. In the meta-analysis, there are five types of interventions: (1) pharmacological approaches (Briot et al., 2010; Henry et al., 2011; Zhang et al., 2010), (2) acupuncture (Crew et al., 2007; Crew et al., 2010; Mao et al., 2009; Mao et al., 2014), (3) nutritional supplementation (Greenlee et al., 2013; Hershman et al., 2015; Rastelli et al., 2011), (4) relaxation techniques (Galantino et al., 2012; Galantino et al., 2013), and (5) physical exercise (Denysschen et al., 2014; Fields, 2015; Irwin et al., 2014).

Subgroup analyses. Subgroup analyses were conducted on study type, study design, study quality, the number of participants, and adherence to intervention protocols as shown in Table 4. There was a significant difference in between subgroups of study type ($P = .005$). Pharmacological approaches (SMD = -1.186, 95% CI: -2.312 – -0.060) and acupuncture (SMD= -1.150, 95% CI: -1.729 – -0.571) showed a very large effect in improving pain, and relaxation technique (SMD= -0.667, 95% CI: -1.274 – -0.060) had a moderate effect on pain; nutritional supplementation (SMD= -0.124, 95% CI: -0.341 – 0.092) and physical exercise (SMD= -0.562, 95% CI: -1.499 – 0.375) showed no significant effect on pain, though they had tendency of decreasing joint pain. In addition,

study design (one group pre-post test vs RCT, $P = .124$), number of participants (less than 50 vs equal and greater than 50, $P = .238$), adherence to the intervention protocol (high adherence [80-100%] vs moderate adherence [60-79%], $P = .103$), and study quality (weak vs moderate vs strong, $P = .111$) had no differences between subgroups.

Table 4

Subgroup Analyses of the Selected Studies on Pain

Criteria	Subgroup	n	SMD (95% CI)	Test for subgroup difference
Study type	Pharmacological approaches	4	-1.186 (-2.312 – -0.060)	$Q=14.815, df=4,$ $p=.005$
	Acupuncture	3	-1.150 (-1.729 – -0.571)	
	Nutritional supplementation	3	-0.124 (-0.341 – 0.092)	
	Relaxation techniques	2	-0.667 (-1.274 – -0.060)	
	Physical exercise	3	-0.562 (-1.499 – 0.375)	
Study design	One group pre-post test	9	-0.967 (-1.405 – -0.528)	$Q=2.372, df=1,$ $p=.124$
	RCT	6	-0.454 (-0.938 – 0.031)	
Number of participants	< 50	10	-0.900 (-1.425 – -0.374)	$Q=1.390, df=1,$ $p=.238$
	≥ 50	5	-0.515 (-0.879 – -0.152)	
Adherence of the intervention protocol	High (80-100%)	9	-0.513 (-0.850 – -0.175)	$Q=2.656, df=1,$ $p=0.103$
	Moderate (60-79%)	6	-1.146 (-1.828 – -0.464)	
Study quality	Weak	4	-0.322 (-0.908 – 0.265)	$Q=4.402, df=2,$ $p=.111$
	Moderate	7	-1.102 (-1.608 – -0.595)	
	Strong	4	-0.523 (-1.078 – -0.032)	

Note. SMD=standardized mean difference; RCT=randomized controlled trial.

3.4 Discussion

This systematic review was the first to evaluate the effects of current pain management on AIA in breast cancer survivors and to identify the quality and strength of the studies. We found that the overall effect size of AIA interventions for pain improvement across the studies was large. In particular, pharmacological approaches and acupuncture showed a large effect on pain, and relaxation techniques had a moderate effect, whereas effects of nutritional supplementation and physical exercise were shown to be insignificant. This may be in part due to inconsistency of intervention outcomes on joint pain in nutritional supplementation and physical exercise studies, though their study quality was evaluated moderate to high. Further investigation into the influence of nutrition and physical exercise is needed to better discern their potential for pain management.

In this review, more than half of all studies described a procedure for an appropriate power analysis of sample size adequacy to detect 80 to 90% power (Crew et al., 2007; Crew et al., 2010; Galantino et al., 2012; Hershman et al., 2015; Irwin et al., 2015; Mao et al., 2009; Mao et al., 2014; Rastelli et al., 2011). These studies calculated the sample size based on their pilot study findings or the assumption that the standard deviation is equal to or greater than half of the mean and considering a drop-out rate. According to Turner et al. (Turner, Bird, & Higgins, 2013), when there are at least two adequately powered studies included in the meta-analysis, the under-powered studies will contribute a trivial amount to the overall power. Therefore, our results from the meta-analysis may provide sufficient power to detect the large effects of intervention on pain.

As for the effect of acupuncture, the result from this review was different from that of the previous meta-analysis of acupuncture in the treatment of AIA (Chien et al., 2015). According to that study, there was a trend for decreased pain in participants following acupuncture; however, the effect was not significant. In contrast, we concluded that acupuncture significantly reduces pain. In Chien et al.'s analysis (Chien et al., 2015), only two RCTs (Crew et al., 2010; Mao et al., 2014) were selected in meta-analysis, while we included four acupuncture studies regardless of study design, which might lead to results that are more statistically significant in terms of the intervention effect. Despite the positive impact of acupuncture on pain, its effect may be biased in our meta-analysis because the other two studies showing no observed change in pain (Bao et al., 2013; Oh et al., 2013) required exclusion because reported findings included median values or percentages only and were therefore incompatible with methods used in other studies for the meta-analysis.

Although most studies offered promising modalities because of high efficacy and safety in treating AIA, several limitations remain to be addressed in this analysis. With the exception of acupuncture, each intervention type was discussed in only two or three studies at most, which may be too small to determine their precise treatment effects on joint pain. To evaluate the individual intervention type appropriately, investigators should consider conducting more studies on pain management strategies that are underexamined or have not yet been examined but are frequently used by AIA patients in daily life. For example, pharmacological approaches may include acetaminophen, cyclooxygenase-2 (COX-2) inhibitors, or non-steroidal anti-inflammatory drugs (NSAIDs), for which

causal mechanisms have not been identified (Lintermans & Neven, 2011; Menas et al., 2012).

AIA is a complex symptom affected by the interrelation among biological, psychological, and sociocultural aspects of patients; however, we were not able to include secondary outcomes (e.g., depression, sleep disturbance, functional well-being, menopausal symptoms, etc.) in the meta-analysis. A small number of studies evaluated these aspects, and those secondary outcomes were assessed using a variety of measurement tools. Quantitatively analyzing these important secondary outcomes regarding AIA intervention effects would help researchers better understand the symptom if additional studies are conducted on secondary outcomes.

Lastly, four of the studies that used sham/placebo treatment failed to find significant differences between experimental and control groups (Bao et al., 2013; Fields, 2015; Hershman et al., 2015; Oh et al., 2013). The sham or placebo treatment may have had potential effects in the control group. Sham acupuncture may trigger physiological effects or closely correlate with real acupoints which are diffused in the body (Bao et al., 2013). Also, the placebo drug of O3-FA may have caused the natural improvement over time, contamination in the placebo group, or the possibility that the ingredients (soy/corn oil) were active in decreasing AIA (Henry & Griggs, 2015; Hershman et al., 2015). These unexpected outcomes might have had negative impacts on evaluation of study quality and the meta-analysis. To support interpretation of placebo data, objective evidence, such as musculoskeletal (e.g., hand/knee joints and tendons) sonography, may be considered in addition to self-reported questionnaires.

3.5 Implications for Practice

Oncology nurses are in an excellent position to improve AIA by directly providing patients with high-quality care. With optimal intensity of acupuncture, physical exercise or relaxation techniques, and precise dosage of medication or nutritional supplementation, nurses may be able to implement such validated interventions as pain management modalities to mitigate the symptoms. Especially, some interventions, including acupuncture and relaxation techniques, can be safely and rapidly applied to women in clinical settings as potential therapies to improve AIA symptoms according to findings from this review. Enabling oncology nurses to offer safe therapeutic pain interventions can reduce burden for the patients suffering from AIA.

In addition, nurse researchers may consider conducting larger trials to evaluate the therapeutic efficacy of AIA interventions, especially pharmacological approaches and relaxation techniques that have been proven effective in only studies with a small sample size. Large RCT studies may strengthen these bodies of evidence.

3.6 Conclusion

This review suggests the overall effect size of current interventions on AIA is large, and the evidence is based on a body of research with moderate study quality. Pharmacological approaches, acupuncture, and relaxation techniques demonstrated a significant effect on pain. Based on our findings, nutritional supplementation and physical exercise do not seem to contribute to control of joint pain. Additional studies with more methodological rigor and diversity may be required to fully evaluate precise effects of these interventional approaches.

Suboptimal adherence to AI drugs may lead to unsatisfactory therapeutic efficacy, ultimately reducing rates of disease-free survival in postmenopausal breast cancer patients. Therefore, it is important for health care providers to implement effective strategies for patients so that they remain compliant with the AIA therapy, which has considerable clinical benefit. Promising interventions to reduce AIA, including acupuncture, relaxation, and pharmacotherapy, is indicated for use based on findings reported here.

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Chapter 4: Efficacy of Curcumin on Aromatase Inhibitor-Induced Musculoskeletal Pain and Dysfunction in an Animal Model

4.1 Introduction

Currently, the standard care for treatment of hormone-sensitive postmenopausal breast cancer is an aromatase inhibitor (AI), such as anastrozole, letrozole, or exemestane. This class of medication is known to suppress circulating estrogen levels profoundly by inhibiting the conversion of androgens to estrogens. Because several trials have established AIs' superiority over tamoxifen in improvement of disease survival outcome and toxicity profile, it became a common therapy in breast cancer survivors (Burstein & Winer, 2007; Crew et al., 2007; Nabholz, 2008). Unfortunately, nearly half the patients undergoing AI therapy reported joint pain, stiffness, or achiness as a primary adverse effect, and 20-30% of patients will discontinue AI therapy because of these symptoms. Non-adherence may lead to increased cancer recurrence and mortality, as well as decreased quality of life. Despite frequent occurrence of AI-associated arthralgia (AIA), its clinical management has been a challenge because there have been quite a small number of randomized controlled trials to investigate it (Yang et al., 2016). The most widely used medication, non-steroidal anti-inflammatory drugs, does not improve the condition effectively.

Curcumin (1, 7-bis[4-hydroxy-3-methoxyphenyl]-1, 6-heptadiene-3, 5-dione, C₂₁H₂₀O₆), which is derived from the rhizome of *Curcuma longa*, has been widely used medically because it is a highly pleiotropic compound with an outstanding safety profile and documented therapeutic potency (Henrotin, Priem, & Mobasher, 2013). It has shown strong efficacy in antioxidant, anti-inflammatory, anti-cancer, and neuroprotective

activities (Gazal et al. 2014; Kant, Gopal, Pathak, Kumar, Tandan, & Kumar, 2014; Mock, Jordan, & Selvam, 2015). Furthermore, curcumin possesses anti-nociceptive properties. In clinical trials testing the efficacy of curcumin on osteoarthritis and rheumatoid arthritis, curcumin was reported to improve joint pain, stiffness and physical function (Belcaro et al., 2010; Chandran & Goel, 2012; Kulkarni, Patki, Jog, Gandage, & Patwardhan, 1991). A number of preclinical studies also have shown that curcumin treatment plays an important role in exerting anti-nociceptive effects in neuropathic, inflammatory, or visceral pain (Babu, Prasanth, & Balaji, 2015; Chen et al., 2015; Kandhare, Raygude, Ghosh, Ghule, & Bodhankar, 2012; Mendonca et al., 2013; Zhao et al., 2012).

It has not been studied whether the anti-nociceptive effects of curcumin could be extended to AIA. Its efficacy on AI-induced musculoskeletal pain and dysfunction has not been examined in either clinical or preclinical studies. For this reason, we evaluated the potential anti-nociceptive efficacy of curcumin on AIA in an animal model, which was developed by expanding on the intratumoral aromatase *in vivo* model for postmenopausal breast cancer (Brodie et al., 1998). Subsequently, multiple characteristics of nocifensive behavior and neuromuscular function were phenotyped during the AI and curcumin administration. Furthermore, we explored a potential role of brain-derived neurotrophic factor (BDNF) with regard to curcumin effects on AIA by measuring altered protein expression levels, which are known as one of key modulators for pain processing in the central nervous system. If the efficacy of curcumin on AIA is supported in this study, it could help maintain AI therapy by reducing the AIA symptoms.

4.2 Methods

4.2.1 Study design

This investigation was designed to examine the potential efficacy of curcumin on AI-induced pain and physical dysfunction in an animal model. Mice were assigned to one of three groups: intervention (curcumin-treated), control (vehicle-treated), and naïve groups. There were nine to ten mice per group. The intervention and control groups were inoculated with breast cancer cells on both flanks followed by surgical removal of grown tumors and ovaries at week 3 after the tumor transplantation; afterwards, daily injection of subcutaneous letrozole was given for eight weeks until mice showed evidence of pain. And then, the intervention group was administered curcumin, and the control group received peanut oil (vehicle of curcumin) daily for five weeks. At pre-tumor baseline, post-tumor removal, weekly during AI treatment and the period of curcumin intervention, the mice were evaluated weekly on pain sensitivity and neuromuscular function. After the intervention was complete, fresh spinal dorsal horn (SDH) was harvested to examine altered protein expression level of BDNF (Figure 13).

This study followed the guidelines and approval of the University of Maryland Institutional Animal Care and Use Committee (IACUC, protocol #0314008). Female BALB/c athymic nude mice at 4-7 weeks of age were obtained from the National Cancer Institute (Frederick, MD) via the Charles River Laboratories. A total of three mice were excluded from this study because they showed high sensitivity to mechanical stimuli at baseline or metastasis into the abdominal cavity during the course of the experiment. Mice were housed in a pathogen-free environment under controlled conditions of a 12:12 hr light/dark cycle and humidity with sterilized food and water *ad libitum*.

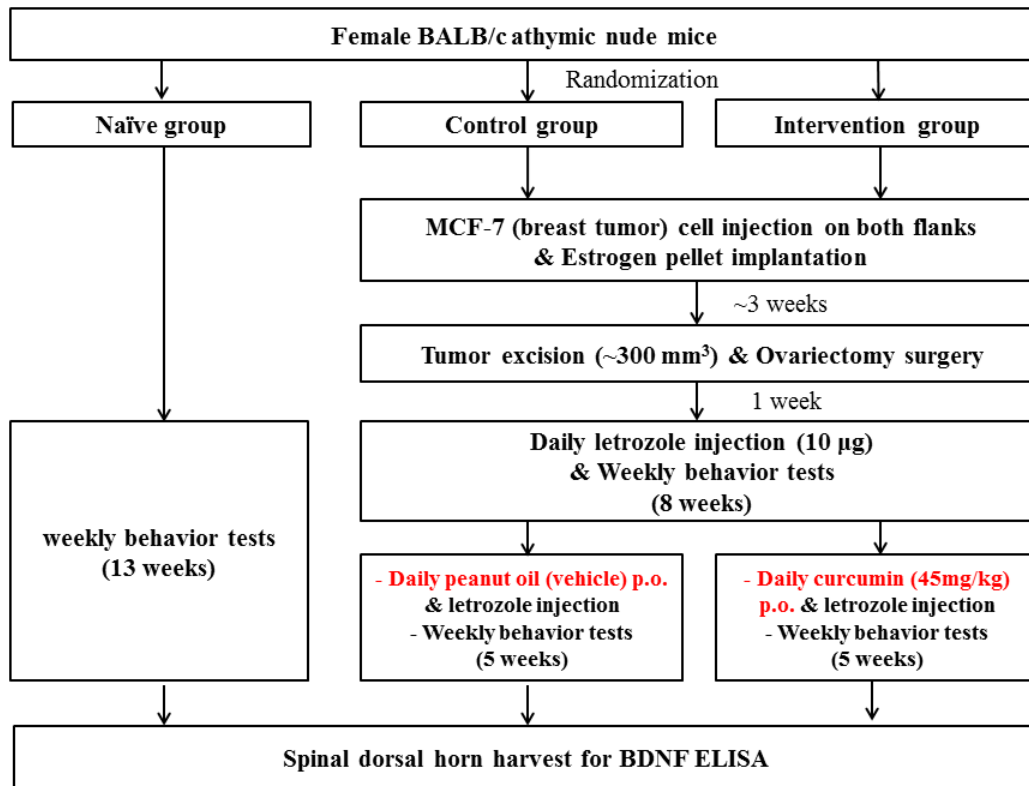


Figure 13. Experimental design for evaluating the efficacy of curcumin on letrozole-induced musculoskeletal pain and dysfunction. BDNF=Brain-derived neurotrophic factor; ELISA=enzyme-linked immunosorbent assay.

4.2.2 Generation of animal model of AIA

Procedures of the AIA animal model generation are described in Chapter 2.

4.2.3 Behavior assays

Behavior assays of nocifensive behavior and neuromuscular function are described in Chapter 2.

4.2.4 Curcumin administration

Mice received oral administration of curcumin (Sigma-Aldrich, St. Louis, MO) or peanut oil (Sigma-Aldrich, St. Louis, MO) as a vehicle per day via gavage for five weeks, with a volume of 10 ml/kg. Fresh curcumin was dissolved in peanut oil and diluted to 45

mg/kg on the day of experiment (Zhao et al., 2012). Since curcumin has poor bioavailability due to its hydrophobic nature (Shen & Ji, 2012), peanut oil was chosen as a vehicle. Dilution in peanut oil prevents modification of curcumin structure. Thus, it can bypass intestinal metabolic enzymes and be directly absorbed into chylomicrons and subsequently into the lymphatic system while bypassing the liver (Anand et al., 2010).

4.2.5 Enzyme-linked immunosorbent assay (ELISA)

The ELISA method was conducted according to the manufacturer's protocol (BDNF E_{max} ImmunoAssay System; Promega, Madison, WI). Briefly, after mice were euthanized, fresh spinal cord was rapidly removed by flushing the spinal canal in a caudal-to-rostral direction with ice-cold diethylpyrocarbonate (DEPC)-treated water. The spinal cord was then frozen on dry ice. The frozen spinal cords were dissected, and the dorsal half of the lumbar region was homogenized in 250 µl lysis buffer (TBS, 10% glycerol, 0.1% Nonidet P-40, complete mini-protease inhibitor cocktail tablet, PhosSTOP phosphatase inhibitor cocktail tablet; Roche Diagnostics) using glass and glass homogenization method and centrifuged to extract the protein at 12,000 g for 10 minutes. The plate was incubated in BDNF mAb that is diluted to 1:1000 in carbonated coating buffer overnight at 4°C, washed once with TBST, and then added 200 µl Block & Sample buffer to each well for 1 hr at room temperature, followed by one wash with TBST. Protein samples were diluted by adding 4 volumes of Dulbecco's PBS (DPBS) to approximately pH 2.6, and then acidified by adding 1 µl of 1N HCl per 50 µl of diluted sample, followed by neutralizing by adding 1µl of 1N NaOH for each 50 µl of diluted sample. The acid-treated protein samples or diluted standard (7.8-500 pg/ml; 100 µl/well) were added to the plate and shaken (400-500 rpm) for 1 hr at room temperature. A

negative control assay was also carried out on the same plate in which protein lysis buffer was used in place of protein lysate. The plate was washed three times in TBST, and 100 μ l Anti-Human BDNF pAb (1:500) was added to each well and shaken at room temperature for 2 hr, followed by five washes with TBST. Accurately 50 μ l of Anti-IgY HRP Conjugate (1:200; 100 μ l/well) was added to the plate and shaken for 1 hr at room temperature, followed by five washes with TBST. TMB One substrate (100 μ l/well) was added to the plate, which was shaken for 10 min at room temperature and neutralized with 1 N HCl. The plate was then read at 450 nm on a Perkin Elmer Wallac 1420 multi-label counter (Waltham, MA) to detect pictogram amounts of BDNF (Renn et al., 2011). As the spinal dorsal horn is a solid tissue, total protein of each sample was obtained by performing bicinchoninic acid (BCA; Pierce biotechnology, Rockford, IL) assay for normalization of BDNF concentration.

4.2.6 Statistical analyses

A two-way repeated measures analysis of variance (RM-ANOVA) test was used to examine changes in mechanical allodynia (von Frey), cold allodynia (cold plate), joint hypersensitivity (PAM), grip strength (grip strength) and motor coordination (rotarod) with the between-subjects factor (treatment group) and the within-subjects factor (time). Post-hoc multiple comparisons with Bonferroni correction were performed to test differences between groups at each time point. A Kruskal-Wallis test with Dunn's post hoc multiple comparisons was performed to examine the difference of the protein expression levels of BDNF (3~4 samples per group). All data were shown as means \pm standard error of the mean (S.E.M.), and $P < .05$ was considered significant. For statistical analysis and figures, GraphPad Prism (version 7.01) software was used.

4.3 Results

4.3.1 Effect of curcumin on letrozole-induced mechanical allodynia

To examine the efficacy of curcumin on mechanical allodynia following letrozole treatment, we first verified that the letrozole decreased low withdrawal thresholds to mechanical stimuli, after which curcumin administration was started. After one week of letrozole treatment, the mice showed significantly lower PWTs compared to the naïve group, and this allodynic condition continued until week 8 ($P < .01$). PWTs of both groups were decreased by approximately 60% from baseline to week 8. In addition, ovariectomy and tumor removal surgery did not seem to affect the occurrence of mechanical allodynia when comparing the two measurements before and after these procedures in the curcumin-treated group ($t = 1.41, P = .19$) and the vehicle-treated group ($t = 0, P > .99$). After confirming the development of mechanical allodynia, curcumin (45 mg/kg/day) was administered daily to the curcumin-treated group (experimental group), and peanut oil was administered to the vehicle-treated group (control group) for five weeks. There was no significant difference in mechanical allodynia between the curcumin-treated group and the vehicle-treated group from week 9 through the end of the experiment ($P > .99$). This result suggests that curcumin may not improve mechanical allodynia. There was a significant main effect for time ($F(14, 364) = 16.01, P < .001$), treatment group ($F(2, 26) = 14.08, P < .001$), and interaction between time and treatment ($F(28, 364) = 3.19, P < .001$) for mechanical allodynia (Figure 14).

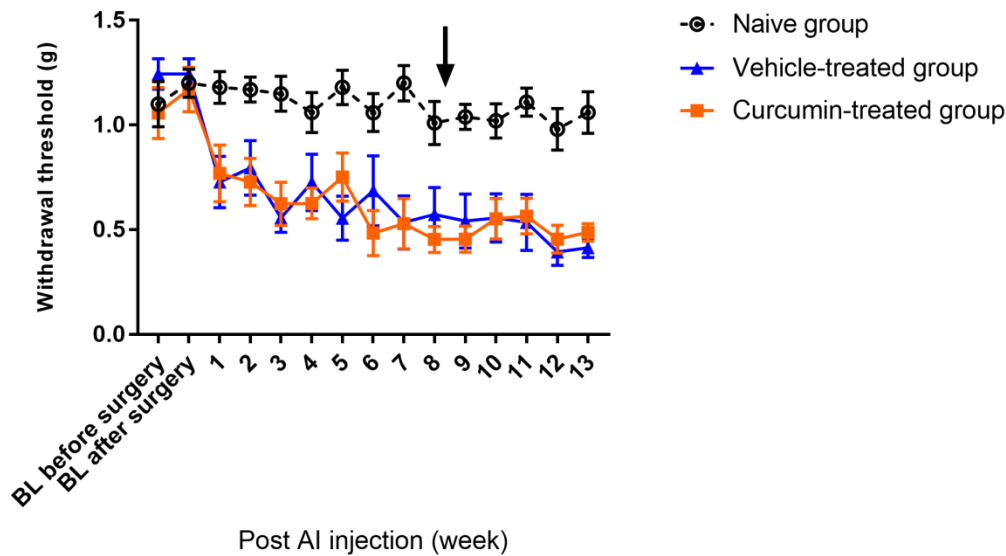


Figure 14. Effect of curcumin on letrozole-induced mechanical allodynia. Paw withdrawal threshold (g) was assessed weekly to examine mechanical allodynia during the letrozole treatment (week 1~week 13) and curcumin administration (week 9 ~ week 13) in naïve group (n=10), vehicle-treated group (n=9), and curcumin-treated group (n=10). Oral administration of curcumin was started at week 9 (black arrow), but it did not improve letrozole-induced mechanical allodynia. Curcumin-treated group vs vehicle-treated group: ns.

4.3.2 Effect of curcumin on letrozole-induced cold allodynia

The cold plate assay was performed to evaluate the nocifensive response to cold thermal stimuli following letrozole treatment and curcumin administration. In this experiment, the letrozole-treated mice showed a significantly higher response threshold temperature compared to the naïve group after one week of treatment (letrozole-treated vs naïve: $P < .001$) that persisted for eight weeks. In addition, no significant change was observed on effects of ovariectomy and tumor removal surgery on cold allodynia in the curcumin-treated group ($t = 1.99$, $P = .08$) and the vehicle-treated group ($t = 0.54$, $P = .60$). Daily curcumin administration was initiated at week 9 of letrozole treatment. Letrozole-induced cold allodynia was decreased by week 11 ($P = .05$), which persisted through week 13 ($P < .01$). There was a significant main effect for time ($F(14, 364) = 30$,

$P < .001$), treatment group ($F(2, 26) = 236, P < .001$), and interaction between time and treatment ($F(28, 364) = 9.23, P < .001$) for cold allodynia (Figure 15).

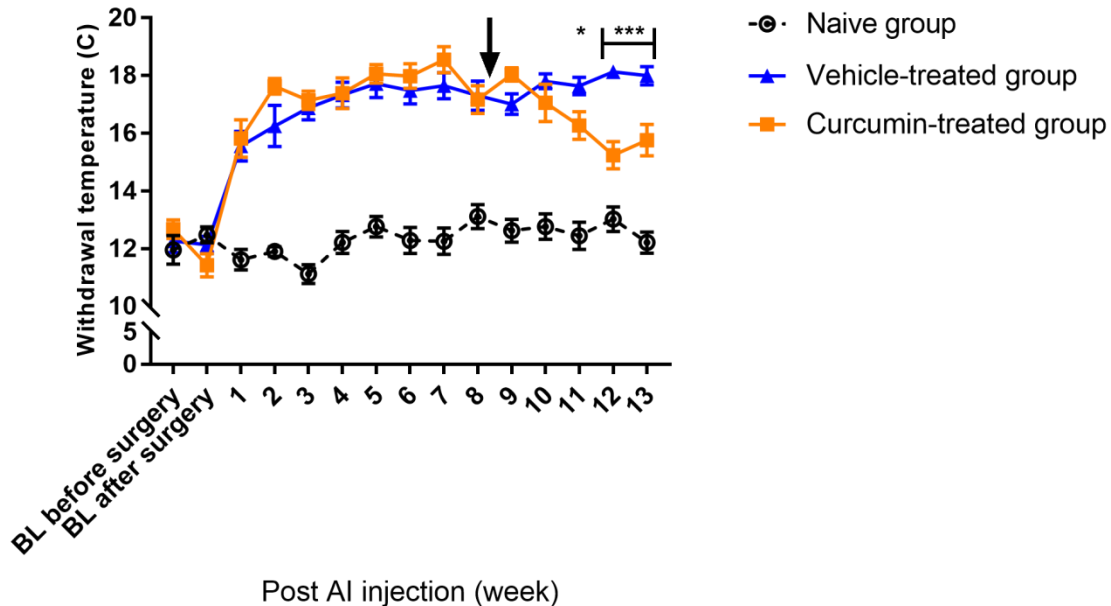


Figure 15. Effect of curcumin on letrozole-induced cold allodynia. Paw withdrawal temperature ($^{\circ}\text{C}$) was assessed weekly to examine cold allodynia during the letrozole treatment and curcumin administration in naïve group ($n=10$), vehicle-treated group ($n=9$), and curcumin-treated group ($n=10$). Oral administration of curcumin was started at week 9 (black arrow), and it improved letrozole-induced cold allodynia.

Curcumin-treated group vs vehicle-treated group: * $P < .05$, *** $P < .001$.

4.3.3 Effect of curcumin on letrozole-induced joint hypersensitivity

Letrozole treatment induced significant hypersensitivity in the ankle joint by week 3 compared to naïve, that persisted through week 8 (letrozol-treated vs. naïve: $P=.01$) with LWTs gradually decreasing by nearly 30% from baseline to week 8. Starting in week 9, mice were given either curcumin or peanut oil. In the curcumin-treated group, the LWT was reversed to the level of the naïve group by week 10, which persisted through week 13; by contrast, the vehicle-treated group showed a continuous decrease in

LWTs. There was a significant main effect for time ($F(13, 338) = 11.72, P < .001$), treatment group ($F(2, 26) = 9.86, P < .001$), and interaction between time and treatment ($F(26, 338) = 2.95, P < .001$) for the development of joint hypersensitivity (Figure 16).

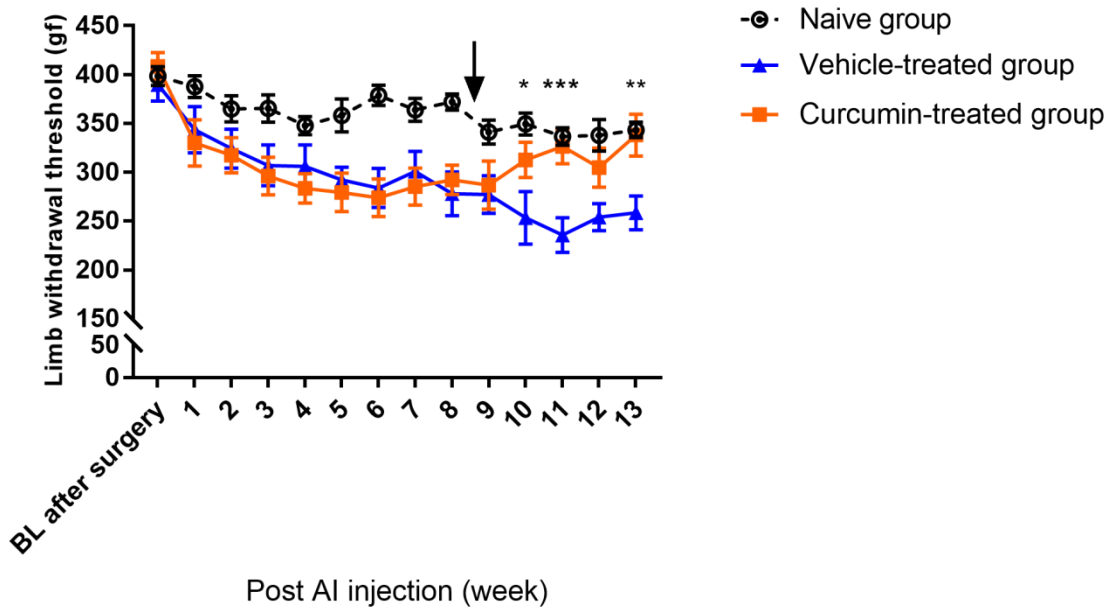


Figure 16. Effect of curcumin on letrozole-induced joint hypersensitivity. Limb withdrawal threshold (gf) was assessed weekly to evaluate joint hypersensitivity at the ankle during the letrozole treatment and curcumin administration in naïve group (n=10), vehicle-treated group (n=9), and curcumin-treated group (n=10). Oral administration of curcumin was started at week 9 (black arrow), and it improved letrozole-induced joint hypersensitivity. Curcumin-treated group vs vehicle-treated group: * $P < .05$, ** $P < .01$, *** $P < .001$.

4.3.4 Effect of curcumin on grip strength following letrozole treatment

Letrozole treatment induced a decrease in forepaw grip strength, by week 3 compared to the naïve group (letrozole-treated vs naïve: $P = .01$) that persisted through week 8. There was no change in grip strength before and after ovariectomy and breast tumor removal surgery in the curcumin-treated and vehicle-treated groups compared to the naïve group (curcumin-treated group: $t = 1.71, P = .12$; vehicle-treated group: $t = 0.96$,

$P = .36$), suggesting these procedures did not alter grip strength. At week 9, we started the daily curcumin administration in mice via gavage, and continued checking their grip strength change. Consequently, grip strength in the curcumin-treated mice significantly improved to the level of the naïve mice; however, the vehicle-treated group had a gradual decrease in grip strength until the experiment was complete. There was a significant main effect for time ($F(14, 364) = 16.42, P < .001$), treatment group ($F(2, 26) = 10.79, P < .001$), and interaction between time and treatment ($F(28, 364) = 3.51, P < .001$) for grip strength (Figure 17).

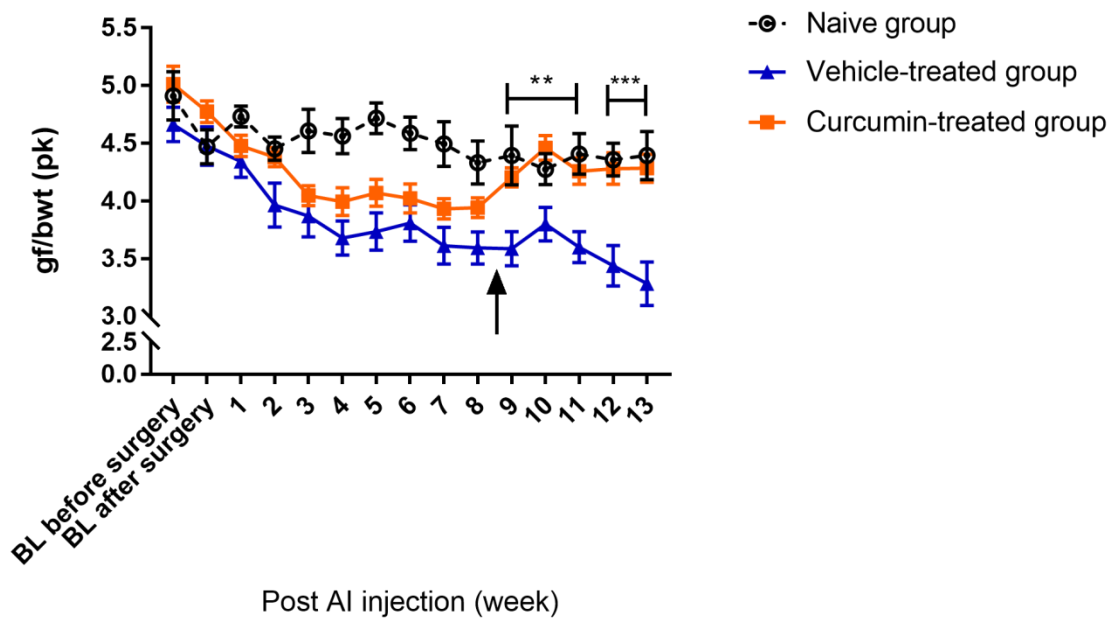


Figure 17. Effect of curcumin on grip strength following letrozole treatment. Grip strength (gf/bodyweight) of forepaws was measured weekly during the letrozole treatment and curcumin administration in naïve group (n=10), vehicle-treated group (n=9), and curcumin-treated group (n=10). Oral administration of curcumin was started at week 9 (black arrow), and it improved deteriorated grip strength. Curcumin-treated group vs vehicle-treated group: ** $P < .01$, *** $P < .001$.

4.3.5 Effect of curcumin on motor coordination following letrozole treatment

We performed the rotarod test in the mice to assess motor coordination. Latency to fall (sec) from a rubberized rotating barrel was recorded as an end point of the rotarod test. From baseline to week 8, there was no statistically significant difference in latencies when comparing the letrozole-treated groups to the naïve group ($P > .05$, respectively). Also, ovariectomy and tumor removal surgery did not affect motor coordination changes between two time points of baselines in the curcumin-treated group ($t = 0.06$, $P = .95$) and the vehicle-treated group ($t = 0.53$, $P = .61$). After initiation of curcumin administration, the curcumin-treated group's latency to fall was increased by approximately 42% from week 8 to week 13; furthermore, the mean of the curcumin-treated group was higher than that of the naïve group although it was not significant. On the contrary, the vehicle-treated group experienced a slight increase in latency, but its score was still lower than the other groups. There was a significant main effect for time ($F(14, 364) = 8.53$, $P < .001$); however, no main effect for treatment ($F(2, 26) = 0.83$, $P = .45$) and no interaction between time and treatment ($F(28, 364) = 0.61$, $P = .90$) were observed for motor coordination (Figure 18).

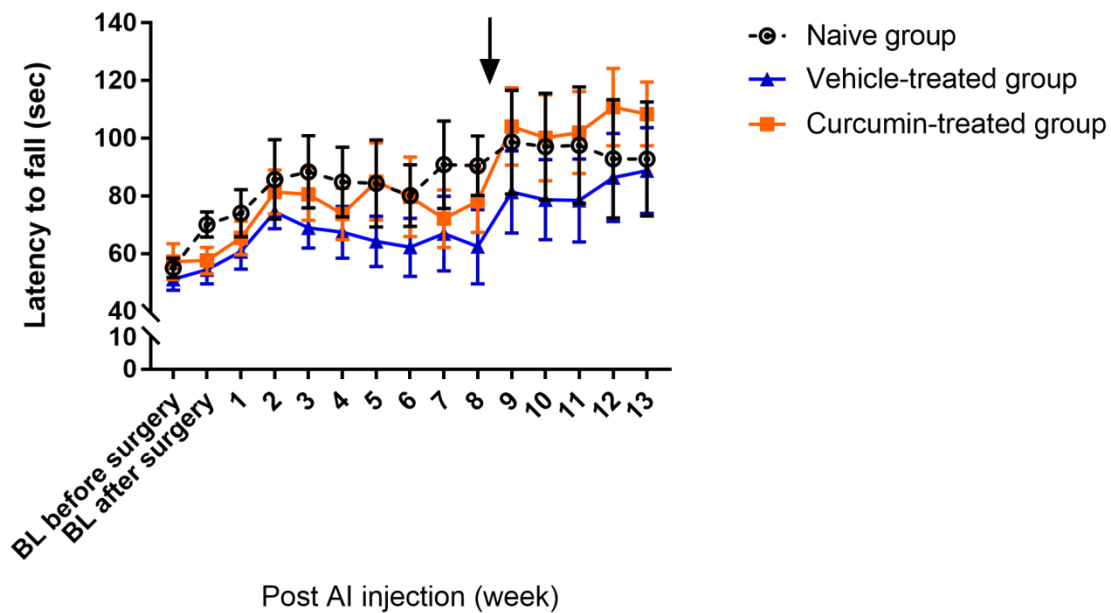


Figure 18. Effect of curcumin on motor coordination following letrozole treatment. Latency to fall down (sec) was measured weekly to evaluate motor coordination during the letrozole treatment and curcumin administration in naïve group (n=10), vehicle-treated group (n=9), and curcumin-treated group (n=10). Oral administration of curcumin was started at week 9 (black arrow). There was a tendency of enhancing motor coordination, but it was not significant. Curcumin-treated group vs vehicle-treated group: ns.

4.3.6 Protein expression level of BDNF

Following the dissection of SDH tissues from the three groups at week 13, we measured protein expression levels of BDNF in the naïve intact, vehicle-treated, and curcumin-treated groups to analyze the effect of curcumin on AI-induced pain. After the curcumin administration was complete, BDNF protein levels were evaluated by ELISA in fresh SDH tissues from each group. Mean values of BDNF protein expression levels were 268.76 ± 26.07 pg/mg in the naïve group (n=4), 403.82 ± 50.24 pg/mg in the vehicle-treated group (n=4), and 188.59 ± 18 pg/mg in the curcumin-treated group (n=3). The BDNF protein expression level of the vehicle-treated group, which had received letrozole, was higher than that of the naïve group although there was no statistical significance ($P = .14$).

The BDNF protein level was significantly lower in the SDH of the curcumin-treated group compared to the vehicle-treated group ($P = .01$), suggesting that curcumin may play a role in decreasing BDNF protein expression, which had been up-regulated due to letrozole injections. The mean value of BDNF protein level in the curcumin-treated group was also lower than that of the naïve group, although no significance was observed ($P = .50$) (Figure 19).

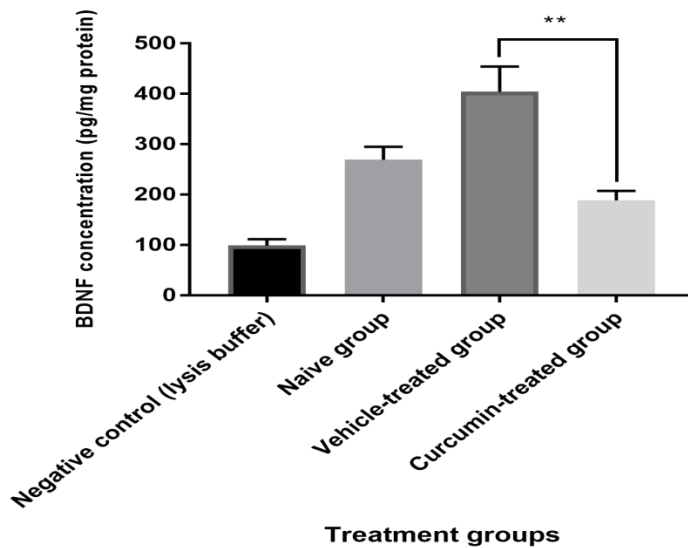


Figure 19. BDNF protein expression levels. BDNF concentrations (pg/mg protein) were measured by ELISA in spinal dorsal horn tissues from naïve group (n=4), vehicle-treated group (n=4), and curcumin-treated group (n=3). Curcumin down-regulated the BDNF protein expression level in SDH tissues from the mice treated with letrozole. Curcumin-treated group vs vehicle-treated group: $**P < .01$ (Kruskal-Wallis test with Dunn's post hoc multiple comparisons).

4.4 Discussion

We investigated the potential efficacy of curcumin treatment on AI-associated pain and physical dysfunction in a clinically relevant mouse model of AIA. Even though AIs have taken on a very important role in breast cancer therapy, the adverse effects may cause discontinuation of therapy in breast cancer survivors. To date, curcumin has been

shown to exert anti-nociceptive activities in arthritic pain, neuropathic pain, and inflammatory pain; however, its efficacy to prevent or reduce AI-associated musculoskeletal symptom has not yet been examined. Here, we demonstrate that curcumin improves cold allodynia, joint hypersensitivity, grip strength, and motor coordination, which had been induced by letrozole treatment. However, curcumin did not improve mechanical allodynia. We also found that curcumin reduced BDNF protein expression levels in SDH tissues from mice treated with letrozole.

This is the first study to evaluate the effect of curcumin on AIA. To our knowledge, neither clinical nor preclinical research has not been conducted on effects of curcumin on AIA, possibly because curcumin is thought to have low bioavailability in human and no feasible animal model exists for mechanistic studies of AIA. Therefore, we developed an animal model of AIA by performing ovariectomy, tumor transplantation, chronic letrozole treatment, and phenotyping multiple nocifensive behaviors. In particular, the dose of letrozole (10 μ g/day) used in this study seemed to achieve the greatest effect in suppressing tumor regrowth after its surgical removal and inducing allodynic signs; although two mice did develop metastasis into the abdominal cavity. In addition, this model met face validity, in which the model accurately reflects the symptoms of the condition, such as joint hypersensitivity and decreased grip strength (Tkacs & Thompson, 2006). Our animal model has homologous outcome variables from clinical settings from the standpoint of postmenopausal condition, tumor removal surgery, and pain phenotypes. Other studies using animal models of joint pain used direct injection inflammation-inducing drugs, such as complete Freund's adjuvant (CFA), into the joint space (Barton et al., 2007; Fernandes et al., 2016). In this model, the mice had joint pain induced by

systemic injections of letrozole, like patients who experience AIA. For the reason, this animal model system was regarded feasible for testing efficacy of curcumin on AIA.

We found that 45 mg/kg of curcumin treatment did not improve withdrawal thresholds in response to mechanical stimuli, which were decreased by letrozole treatment. This result indicates that curcumin may not be effective in rescuing mechanical allodynia following exposure to letrozole. However, previous *in vivo* studies found curcumin improved mechanical allodynia in rodents with neuropathic pain and inflammatory pain (Babu et al., 2015; Chen et al., 2015; Kandhare et al., 2012; Mendonca et al., 2013; Zhao et al., 2012). For instance, Chen et al. (2015) demonstrated that treatment with curcumin at 100 mg/kg, which was started 24 hours before CFA injection, attenuated articular CFA-induced mechanical allodynia; and, Babu et al. (2015) showed that curcumin 60 mg/kg significantly improved mechanical allodynia in vincristine-induced neuropathy in rats that received curcumin one hour before each vincristine injection for 14 days. Although these animal models varied regarding how they induced hypersensitivity to mechanical stimuli, curcumin demonstrated anti-nociceptive efficacy when it was co-administered with drugs (e.g., chemotherapy, inflammatory agents) or given at a high dose. In our experiment, the curcumin dose was relatively low compared to these other studies, and it was administered to animals after the AIA condition was established. If the mice would have received curcumin at a higher dose after mechanical allodynia developed or at the chosen dose simultaneously with letrozole treatment from the beginning of the study, the chance of rescuing mechanical allodynia might be increased.

Grip strength and motor coordination were improved by curcumin administration in mice subjected to letrozole treatment in this experiment. Recently, preclinical studies have shown that curcumin exerts neuroprotective activities on physical dysfunction by scavenging reactive oxygen species (ROS) or increasing antioxidant enzymes or non-enzymatic antioxidants, such as glutathione (GSH), superoxide dismutase (SOD) and catalase (Al-Obaidi, Al-Sayegh, & Nadar, 2014; Avci et al., 2012; Huang et al., 2015; Takahashi et al., 2014). Enhancement of grip strength and motor coordination may be due to the antioxidant effect of curcumin. To further examine the link between curcumin and AIA, joint histology or molecular analyses using joint and nerve tissues will be required.

The exact mechanism underlying AIA remains unclear; though, estrogen deprivation is thought to be one underlying cause of the AIA symptoms. Recently, Fusi et al. (2014) and Robarge et al. (2016) provided evidence that AI-associated pain may occur through central sensitization. Transient receptor potential ankyrin 1 (TRPA1) channels mediate calcium response and current in nociceptors of mice treated with AIs; and, AIs modulate the excitability of sensory neurons in the dorsal root ganglion, leading to the augmentation of pain behaviors in rats. Consistent with the evidence demonstrating CNS actions of AIA, we examined the potential role of BDNF as one of the key pain modulators in relation to central sensitization. In this experiment, the BDNF protein expression level of the vehicle-treated group was higher than that of the naïve group and the curcumin-treated group. This result suggested that BDNF may be one of contributing factors to AI-induced pain, and curcumin suppresses the up-regulated BDNF protein level. These results are similar to findings by Zhu et al. (2014), in which curcumin was revealed to exert an anti-nociceptive role in a dose-dependent manner in neuropathic pain by

down-regulating p300/CBP histone acetyltransferase (HAT) activity-mediated gene expression of BDNF in a rat model of chronic constriction injury. In addition, a decrease of BDNF in the spinal cord was observed after curcumin treatment. Since BDNF is a part of the causes of AIA, by using advanced “omics” discovery methodologies, such as RNA sequencing or microarray, multiple biomarkers can be found to correlate efficacy of curcumin with AIA.

In summary, this preclinical study suggests that curcumin has protective effects against cold allodynia, joint hypersensitivity and grip strength in mice treated with letrozole. Curcumin may alter BDNF protein expression levels in relation to pain and neuromuscular dysfunction. Effective anti-nociceptive agents, such as curcumin, may help maintain AI therapy by reducing adverse effects and allowing continuation for the full required period. Thus, clinical studies of curcumin on AIA should be considered to reduce joint pain in patients undergoing aromatase inhibitors.

Acknowledgements:

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Chapter 5: Summary, Strengths, Limitations, and Recommendations

5.1 Summary

Aromatase inhibitors are established for treating hormone responsive breast cancer. Unfortunately, nearly half of the women undergoing AI therapy frequently report joint pain or stiffness, which may require AI discontinuation. The symptom is very problematic and negatively impacts day-to-day well-being. AIA cannot be managed because no clear biological mechanisms have been found partially due to a lack of proper animal models of AIA, and therefore no satisfactory treatment has emerged that is beneficial for AIA. In this dissertation study, the purpose of this dissertation is to establish a clinically relevant animal model of AIA for mechanistic studies and to examine potential treatment strategies for alleviating the symptom.

Chapter two, entitled “Development of a Clinically Relevant Animal Model of Aromatase Inhibitor-Associated Arthralgia” reported on the development of a new animal model of AIA that mimics clinical conditions of postmenopausal breast cancer survivors. This study confirmed that a clinically relevant animal model could be generated. Chapter three, entitled “Interventions for the Treatment of Aromatase Inhibitor-Associated Arthralgia in Breast Cancer Survivors: A Systematic Review and Meta-Analysis” evaluated the effects of pain management on AIA in breast cancer survivors and identified the quality and strength of the studies. Chapter four, entitled “Efficacy of Curcumin on Aromatase Inhibitor-Induced Pain Behavior and Neuromuscular Dysfunction in an Animal Model” investigated anti-nociceptive effects of curcumin on AIA, and explored a potential underpinning mechanism. Lastly, results on the effect of physical exercise on AIA are briefly reported in the appendix. Whether physical exercise

exerts analgesic effects on AI-induced pain behavior and neuromuscular dysfunction is tested. Main findings are summarized below.

5.1.1 Clinically relevant animal model of AIA

For pain research, animal models are very useful because they allow controlled investigation of chronic pain conditions and help to obtain mRNAs or proteins from pain-relevant tissues (e.g., spinal cord and brain), which are not available in humans (Mogil et al., 2010). This randomized controlled experimental study examined the role of AIs in reproducing arthralgia, and characterized the symptom in a new animal model of AIA. We hypothesized that phenotypes of pain sensitivity and neuromuscular function differ among the AI-treated group and control groups (e.g., naïve group, ovariectomy group, and vehicle-treated group). We demonstrated that a clinically relevant animal model of AIA was present by phenotyping nocifensive behavior and neuromuscular function using ovariectomized immune-deficient mice, which allowed for low estrogen levels and tumor xenograft. In our experiments, eight weeks of letrozole (10µg/day) clearly induced mechanical allodynia, thermal (cold) allodynia, joint pressure hypersensitivity, anteroposterior weight shift, decreased grip strength, and worsened motor coordination. We also concluded estrogen deprivation partially contributes to the occurrence of AIA in this animal model.

The use of an appropriate animal model of AIA is beneficial for gaining mechanistic insight and for evaluating potential therapeutics of AIA. Nocifensive response and neuromuscular dysfunction elicited in this study will provide evidence for better understanding the progression and characteristics of AIA. This model system will

provide important insights concerning mechanistic studies of AIA when examining factors that cause the symptoms.

5.1.2 Current intervention modalities for the treatment of AIA

If strong evidence on the effectiveness of specific interventions is provided, clinicians, policymakers, and patients can make the best decision based on the information to get the desirable treatment effect (Atkins et al., 2004). To our knowledge, no systematic review and meta-analysis has been conducted on all intervention types for AIA. Therefore, the purpose of this analysis was to identify current pain management of AIA and to evaluate the study quality and effects of interventions.

Nineteen articles published from 2000 to August 2015 were identified using PubMed, CINAHL, PsycINFO, Web of Science, and additional records. Study quality was evaluated by the *Quality Assessment Tool for Quantitative Studies* tool. Meta-analysis was used to obtain effect sizes of interventions on pain and subgroups. Five types of interventions emerged: pharmacological approaches, acupuncture, nutritional supplementation, relaxation techniques, and physical exercise. Six studies were strong, eight were moderate, and five were weak in quality. The overall effect size of the interventions on pain was large; pharmacological approaches, acupuncture, and relaxation techniques showed moderate to large effects on pain, while nutritional supplementation and physical exercise had no significant effects on it. The evidence was based on a body of research with moderate study quality. Although the overall effect of interventions is large, further investigation into the influence of nutrition and physical exercise is needed to better discern their potential for pain management.

Researchers may consider conducting larger trials to evaluate the therapeutic efficacy of AIA interventions, especially pharmacological approaches and relaxation techniques that have been proven effective in only studies with a small sample size. Large RCT studies may strengthen further promising therapeutic modalities. Additional studies with more methodological rigor and diversity may be required to fully evaluate precise effects of these interventional approaches. Oncology nurses may be able to implement such validated interventions as pain management modalities to mitigate the symptoms so that breast cancer survivors remain compliant with AIA therapy.

5.1.3 Efficacy of curcumin on AIA

Curcumin has been widely used because of its highly pleiotropic compound with an outstanding safety profile and therapeutic potency (Henrotin et al., 2013). It has been shown to exert anti-nociceptive properties in various types of pain; however, its potential efficacy to prevent or reduce AIA has not yet been well examined. We focused on its analgesic actions for treating AI-induced pain and neuromuscular dysfunction, and explored a potential underpinning mechanism by measuring BDNF protein expression levels.

We hypothesized that the AI-treated group receiving curcumin would show a decrease in pain behavior and neuromuscular dysfunction, and down-regulation of BDNF protein expression level compared to the control (vehicle) and naïve (no vehicle/treatment) groups. Mice were assigned into three groups: intervention (curcumin-treated), control (vehicle-treated), and naïve groups. The intervention group and the control group underwent the xenograft and ovariectomy procedures followed by eight weeks of letrozole injection to evoke pain. After which, the intervention group received

curcumin, and the control group had peanut oil (vehicle of curcumin) daily for five weeks. After the intervention was complete, fresh spinal dorsal horn (lumbar enlargement) was harvested to examine altered protein expression levels of BDNF. We demonstrated that 45mg/kg of curcumin improved cold allodynia, joint hypersensitivity, grip strength, and motor coordination, which had been deteriorated by letrozole. However, curcumin did not improve mechanical allodynia induced by long-term administration of letrozole. We also found that BDNF protein expression level was down-regulated following curcumin administration.

Effective anti-nociceptive agents could help AI therapy by reducing adverse effects and enabling patients to continue the therapy for full required period. Thus, curcumin as a therapeutic treatment should be considered for patients undergoing aromatase inhibitors in clinical settings.

5.1.4 Effect of physical exercise on AIA

Exercise is known to exert elastic and compressive forces on the joint cartilage, and decrease the risk of osteoarthritis at low and medium magnitudes (Juhl, Christensen, Roos, Zhang, & Lund, 2014; Park, Hung, & Ateshian, 2004). It is still controversial whether physical exercise may improve AI-induced joint pain as described in chapter 3. We hypothesized that the AI-treated group that engages in physical exercise shows a decrease in pain behavior and neuromuscular dysfunction, and down-regulates BDNF protein expression levels compared to the control (locked wheel running) and naïve (no vehicle/treatment) groups.

This study was conducted using the clinically relevant animal model described in the chapter two. Mice were assigned to one of three groups: intervention, control, and

naïve groups. After mice received the xenograft and ovariectomy procedures, and eight weeks of letrozole injections, the intervention group was habituated in individual activity cages, and they ran every day for five weeks, while the control group stayed in cages with locked wheel. We showed that physical exercise improves mechanical allodynia and cold allodynia, while this intervention does not rescue joint hypersensitivity, and deteriorated grip strength and motor coordination induced by long-term administration of letrozole. Evidence presented here suggests that exercise may improve pain behaviors while having little impact on overall neuromuscular functioning.

Further studies are warranted to find biological mechanisms between physical exercise and AI-induced pain. In addition, whether running exercise is appropriate for alleviating AIA needs to be sought.

5.2 Strengths and Limitations

In order to improve symptom management and intervention science in oncology nursing, animal models are useful in examining preclinical testing of biologic-based interventions to reduce symptom mechanisms and severity experienced by patients with cancer (Beckett, 2008). Translational research attempts to transfer animal findings to the clinical condition (Tkacs & Thompson, 2006). Findings from this dissertation offered a new animal model for allowing mechanistic studies and testing analgesic effects of treatment. Using animal models has several strengths in study quality and design.

First, this preclinical study did not require a large sample size such as with clinical trials. In each aim, the number of animals needed was approximately ten mice per group based on literature and unpublished experiment data to detect statistical significance with power of 0.90. The sample size was satisfactory to overcome

considerable variability associated with *in vivo* studies, provided statistically meaningful results, and avoided unnecessary repetition of experiments.

Second, the use of animal model allows study procedures to be more rigorously controlled, including maintenance of timetables and standardization of manipulations and treatments (Page, 2004). Data acquisition and timing of measurements also can be determined and carried out by the investigator (Page, 2004). In this study, mice underwent complicated but uniform surgery procedures (e.g., breast tumor xenograft, ovariectomy), daily injections of drugs, and multiple behavior assays to mimic the clinical condition of postmenopausal breast cancer. These procedures required strict and efficient timelines. By restricting a wide range of environmental and extraneous variables, the study had been completed according to the scheduled timeline.

Third, various physiologic outcomes can be sought by obtaining specimens of tissue, blood, and organs from the euthanized animals, or directly measuring nerve, organ, and hemodynamic activity in the anesthetized organism (Page, 2004). In particular, for pain research, animal models are needed to analyze mRNAs or proteins from pain-relevant tissues (e.g., spinal cord and brain), which is not available in humans (Mogil et al., 2010). In this experiment, BDNF protein expression levels were correlated with curcumin effects by dissecting spinal cord lumbar enlargement from mice, which helped to reveal a potential biological mechanism of curcumin on AIA.

Lastly, our animal model of AIA has face validity, in which the model accurately reflects the symptoms of the condition (Tkacs & Thompson, 2006). Face validity of an animal model can be evaluated by measuring feasible outcome variables and conducting a pairwise comparison with homologous outcome variables from human studies (Tkacs &

Thompson, 2006). The proposed animal model in the dissertation was generated by mimicking quite similar procedures of the postmenopausal breast cancer condition and long-term administration of AIs, and characterizing musculoskeletal pain and function. Indeed, characteristics of the animal model exhibited similar phenotypes to symptoms in the clinical setting.

Despite that the use of animal models helps increase the efficiency of the study, there were limitations encountered during the experiment. First, investigators may have been subconsciously influenced when assessing outcomes, supplying additional care, or withdrawing animals from the experiment (van der Worp et al., 2010). In this study, maintaining the blind condition on group assignments was difficult because treatment groups had surgical scars on both flanks after receiving tumor removal and ovariectomy surgery, which distinguishes them from other control groups. Awareness of treatment group assignment may have led to selective exclusion of animals based on predictive factors (Altman et al., 2001; van der Worp et al., 2010).

Second, AIA is a complex symptom affected by the interrelation among biological, psychological, and sociocultural comorbidities of patients. In clinical settings, patients experience depression, sleep disturbance, and menopausal symptoms. However, this study only focused on pain symptoms, and other comorbidities were not considered in phenotyping the animal model of AIA. When testing the role of letrozole in inducing pain, experiments were placed on measuring pain behavior and neuromuscular function, while other psychological factors were not assessed to decrease distress from multiple behavior tests.

Third, in animal research, thermal and mechanical hypersensitivity is frequently measured (Mogil & Crager, 2004). However, these nocifensive behaviors are less correlated with global ratings of pain severity in humans (Backonja & Stacey, 2004; Scholz et al., 2009). This issue may result in a mismatch between the epidemiological realities of chronic pain prevalence in the human population and animal model subjects (Mogil et al., 2010). In this study, cold allodynia was observed after letrozole administration; however, no information has been found on responses to cold thermal stimuli in women receiving AIs.

5.3 Recommendations

5.3.1 Research implications

This study is timely because the NINR has specifically requested symptom science research to provide a better understanding of the symptoms of chronic illness and improve quality of life across diverse populations. In alignment with the request, this study attempted to elucidate pain characteristics and progression of aromatase inhibitor-associated arthralgia. This is the first study to establish an animal model that mimics the clinical condition of AIA, and satisfies face validity. The animal model system may offer an important platform for mechanistic studies to help find genomic, genetic, physiological, or behavioral evidence of AIA development. Also, it may contribute to the development of novel therapeutic targeted therapies to prevent or decrease AIA if clear biological mechanisms are found.

In this dissertation, the animal model was the basis for intervention studies by focusing on the anti-nociceptive properties of physical exercise and curcumin, which seemed to improve AI-induced musculoskeletal pain and function in mice. The result is a

prologue to later clinical trials by providing a preliminary data. Therefore, researchers may consider conducting larger trials of physical curcumin and physical exercise that have been proven effective in this animal study. Large RCT studies may strengthen these bodies of evidence.

5.3.2 Practice implications

As adherence to AI therapy is tied to the lower risk of recurrence and mortality, nurses must aggressively manage the adverse effects of AI treatment. Oncology nurses are in an excellent position to improve AIA by directly providing patients with evidence supported interventions. The blending of the study findings with clinical management will enhance their understanding of the etiology of AIA and its impact on the patient, and enable them to identify abnormal symptoms promptly and expect progression. With optimal intensity of physical exercise or precise dosage of curcumin, nurses may be able to implement effective pain management to mitigate the symptoms. Enabling oncology nurses to offer safe therapeutic pain interventions can reduce burden for the patients suffering from AIA.

5.4 Conclusion

This preclinical study demonstrated that a clinically relevant animal model of aromatase inhibitor-associated arthralgia could be developed. For this, musculoskeletal pain and physical function were phenotyped using ovariectomized immune-deficient mice. Chronic administration of letrozole significantly induced mechanical allodynia, thermal (cold) allodynia, joint hypersensitivity, anteroposterior weight shift, decreased grip strength, and worsened motor coordination. Subsequently, this animal model was utilized for interventional studies by focusing on the anti-nociceptive properties of

curcumin (turmeric) and physical exercise. Curcumin administration improved almost all deteriorated nocifensive behavior and neuromuscular function except for mechanical allodynia. Physical exercise enhanced the deteriorated nocifensive behavior, but did not rescue neuromuscular dysfunction. BDNF protein expression levels were down-regulated following curcumin administration and physical exercise. BDNF could be one of biomarkers in relation to effects of curcumin or physical exercise on AIA.

The animal model system, which has face validity, may provide an important platform for mechanistic studies to obtain genomic, genetic, physiological, or behavioral evidences of AIA development. Effective treatments, such as curcumin and physical exercise, could be considered for large clinical trials to help maintain AI therapy by reducing AIA symptoms. Further studies are warranted to find biological mechanisms of those treatments with AIA.

Appendix I: Effect of Physical Exercise on Aromatase Inhibitor-Induced Musculoskeletal Pain and Dysfunction in a Mouse Model (Brief Data Report)

This study tested the effect of physical exercise on pain response and physical function following AI treatment in mice. The hypothesis was that the AI-treated group that engages in physical exercise will show a decrease in pain behavior and neuromuscular dysfunction and down-regulation of BDNF protein expression level compared to the control (vehicle) and naïve (no vehicle/treatment) groups. To test this hypothesis, changes in pain sensitivity and joint function were examined before and after voluntary running wheel exercise. Behavior assays and statistical analysis are the same as described above in Chapter 4 except for interventions.

Briefly, the voluntary wheel running occurred in polycarbonate cages with free access to stainless steel activity wheels (diameter: 23cm; width: 5cm) with a ball-bearing axle (Actimetrics, Wilmette, IL). The wheel could be turned in either direction. It was connected to a computer that automatically recorded the distance travelled by each animal in the wheel (Cobos et al., 2012). The intervention group was habituated in individual activity cages, and ran every day except during the behavior tests for five weeks. The control group stayed in cages with locked wheel during the same period. One mouse was excluded from the data analysis because it was not cooperative with running wheel exercise. Exercise group ran on average 2.69 km per day. Figures are presented below.

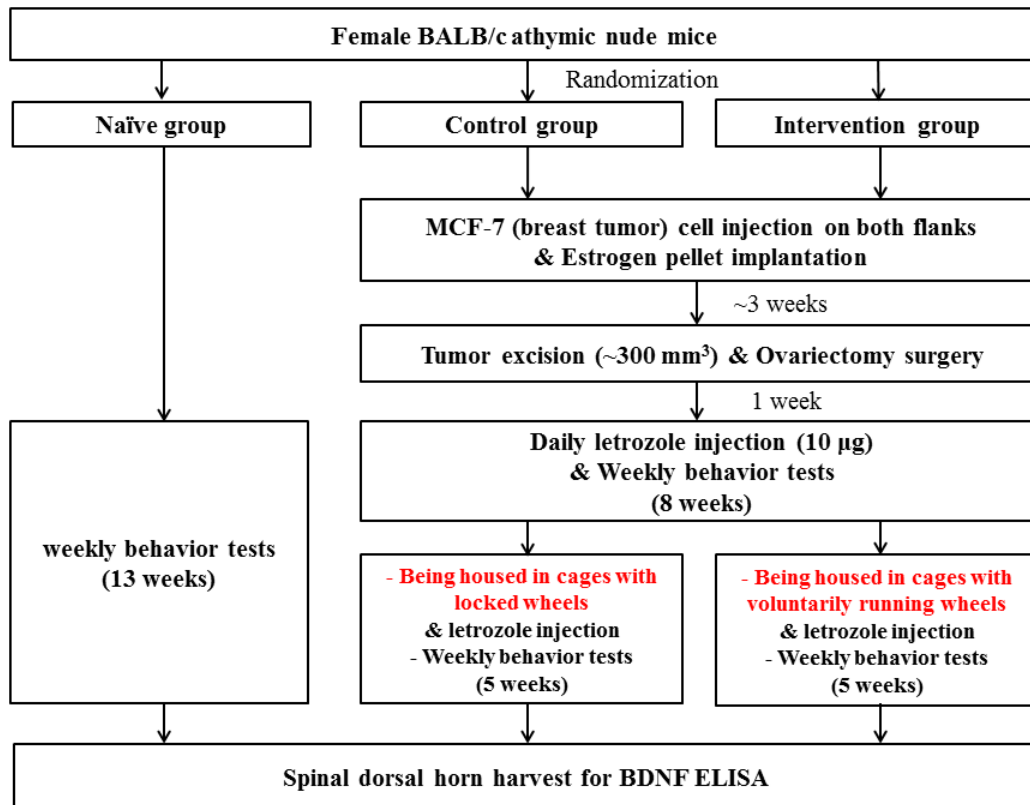


Figure A. Experimental design for evaluating the effect of physical exercise (voluntary wheel running) on letrozole-induced musculoskeletal pain and dysfunction. BDNF=Brain-derived neurotrophic factor; ELISA= Enzyme-linked immunosorbent assay.

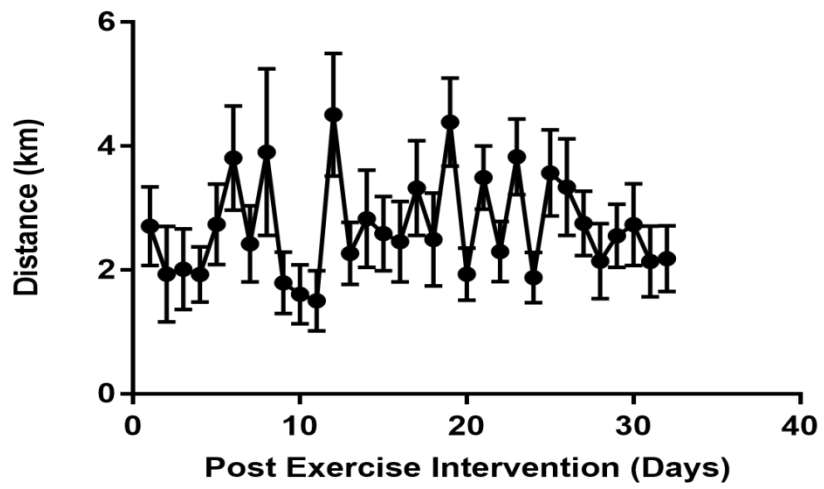


Figure B. Distance record. The distance was daily recorded for five weeks in the exercise group (n=9). Overall, mice ran 2.69 km on the wheel per day.

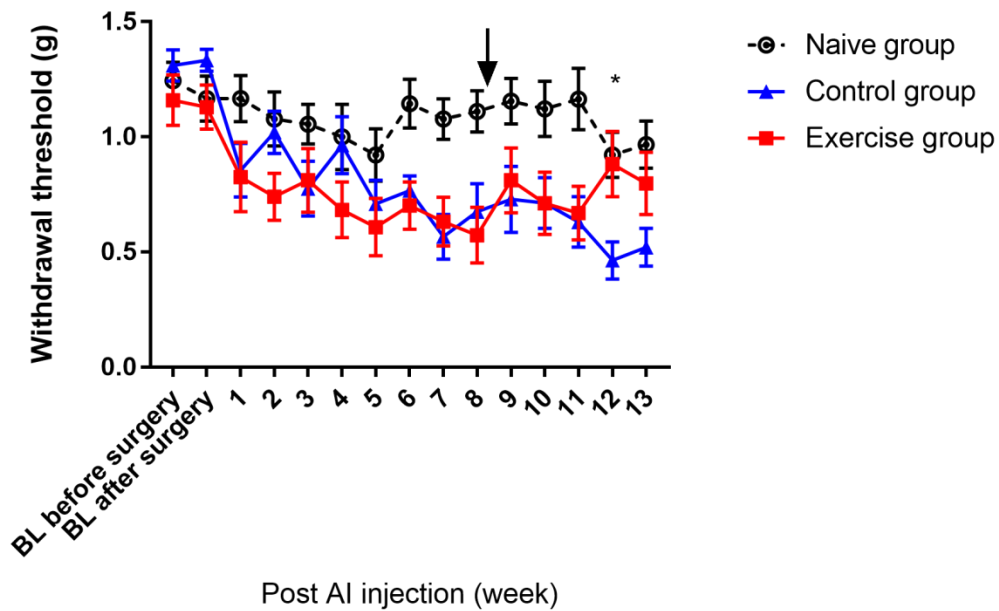


Figure C. Effect of physical exercise on letrozole-induced mechanical allodynia. Paw withdrawal threshold (g) was assessed weekly to examine mechanical allodynia during the letrozole treatment (week 1~week 13) and physical exercise (week 9 ~ week 13) in naïve group (n=9), control group (n=9), and exercise group (n=9). Physical exercise was started at week 9 (black arrow), and it improved letrozole-induced mechanical allodynia at week 12. There was a significant main effect for time ($F(14, 336) = 10.21, P < .001$), treatment group ($F(2, 24) = 3.96, P = .03$), and interaction between time and treatment ($F(28, 336) = 2.76, P < .001$) for mechanical allodynia. Exercise group vs control group: $*P < .05$.

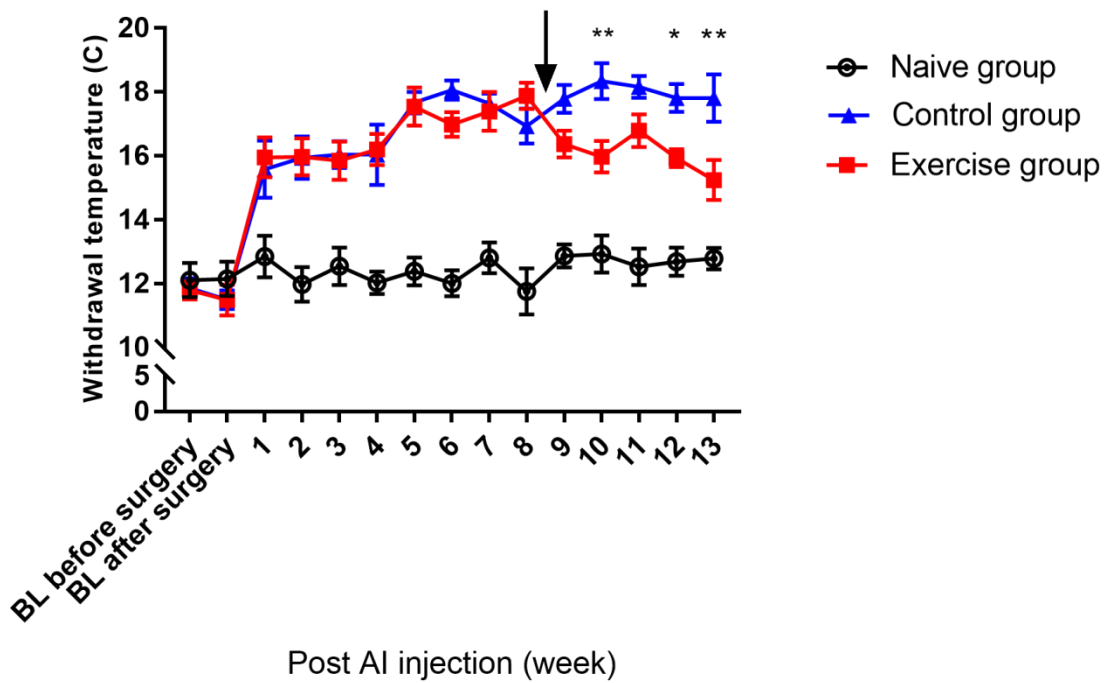


Figure D. Effect of physical exercise on letrozole-induced cold allodynia. Paw withdrawal temperature (°C) was assessed weekly to examine cold allodynia during the letrozole treatment and physical exercise in naïve group (n=9), control group (n=9), and exercise group (n=9). Physical exercise was started at week 9 (black arrow), and it improved letrozole-induced cold allodynia. There was a significant main effect for time ($F(14, 336) = 20.11, P < .001$), treatment group ($F(2, 24) = 111.2, P < .001$), and interaction between time and treatment ($F(28, 336) = 5.47, P < .001$) for cold allodynia. Exercise group vs control group: * $P < .05$, ** $P < .01$.

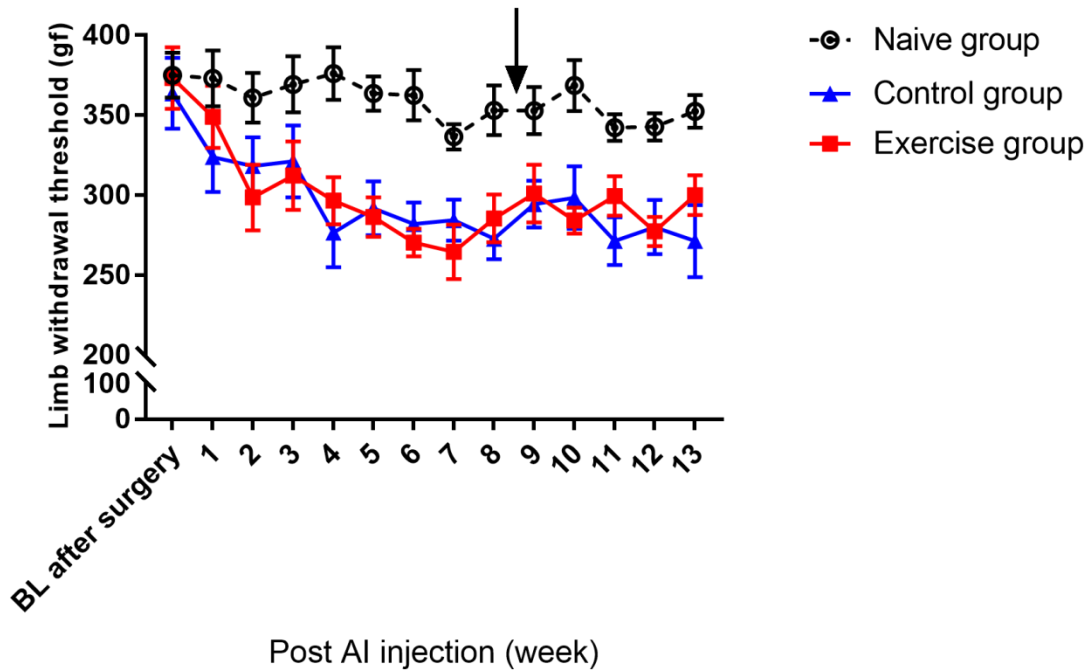


Figure E. Effect of physical exercise on letrozole-induced joint hypersensitivity. Limb withdrawal threshold (gf) was assessed weekly to evaluate joint hypersensitivity at the ankle during the letrozole treatment and physical exercise in naïve group (n=9), control group (n=9), and exercise group (n=9). Physical exercise was started at week 9 (black arrow), and it did not improve letrozole-induced joint hypersensitivity. There was a significant main effect for time ($F(13, 325) = 6.79, P < .001$) and treatment group ($F(2, 24) = 15.4, P < .001$), but no significant effect was found in interaction between time and treatment ($F(26, 325) = 1.02, P = .23$) for joint hypersensitivity. Exercise group vs control group: ns.

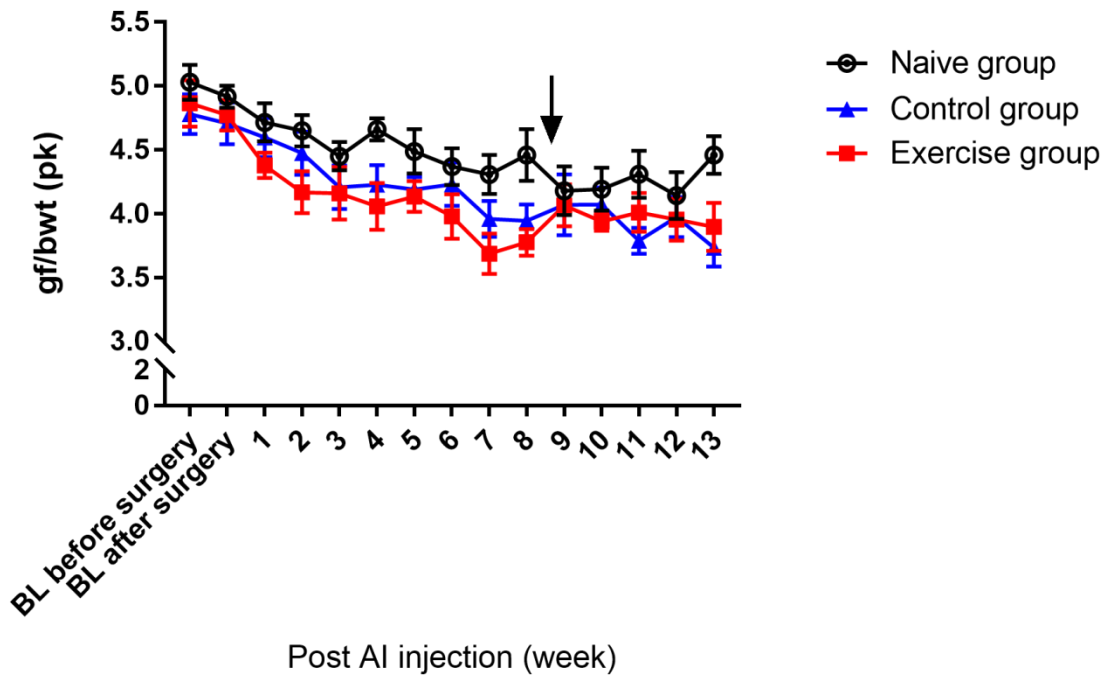


Figure F. Effect of physical exercise on grip strength following letrozole treatment. Grip strength (gf/bodyweight) of forepaws was measured weekly during the letrozole injections and physical exercise in naïve group (n=9), control group (n=9), and exercise group (n=9). Physical exercise was started at week 9 (black arrow), and it did not improve deteriorated grip strength. There was a significant main effect for time ($F(14, 336) = 19.94, P < .001$), but no significant effect was found in treatment group ($F(2, 24) = 3.04, P = .07$) and interaction between time and treatment ($F(28, 336) = 1.23, P = .20$) for grip strength. Exercise group vs control group: ns.

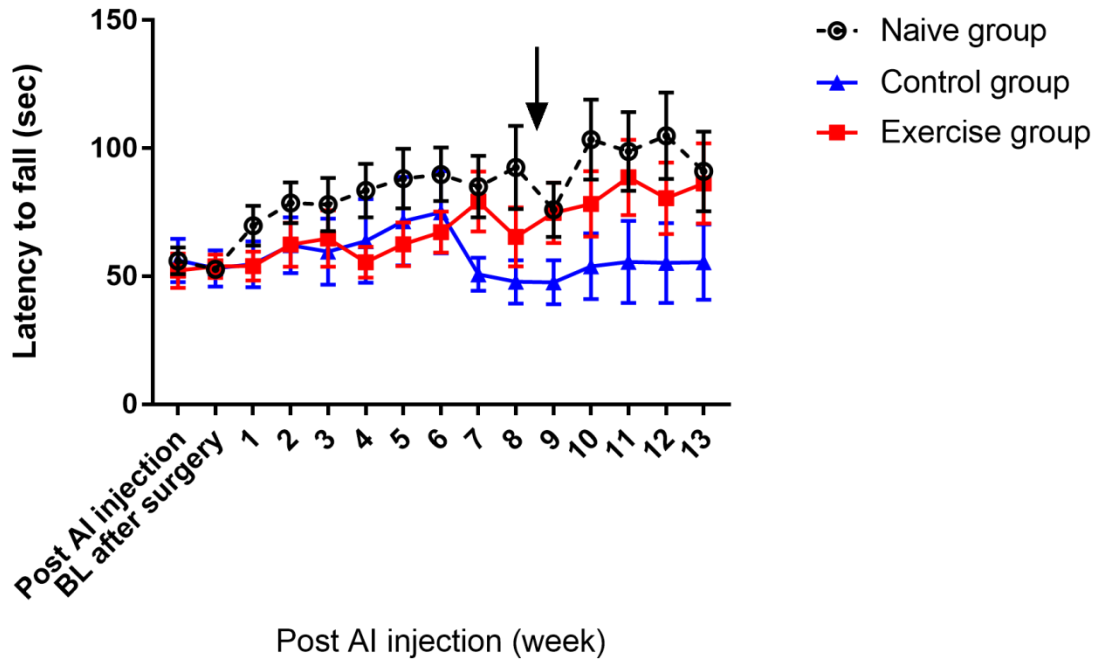


Figure G. Effect of physical exercise on motor coordination following letrozole treatment. Latency to fall down (sec) was measured weekly to evaluate motor coordination during the letrozole injections and physical exercise in naïve group (n=9), control group (n=9), and exercise group (n=9). Physical exercise was started at week 9 (black arrow). There was a tendency of enhancing motor coordination, but it was not significant. There was a significant main effect for time ($F(14, 336) = 3.46, P < .001$) and interaction between time and treatment ($F(28, 336) = 1.55, P = .04$) but no significant effect was found in treatment group ($F(2, 24) = 2.18, P = .014$) for motor coordination. Exercise group vs control group: ns.

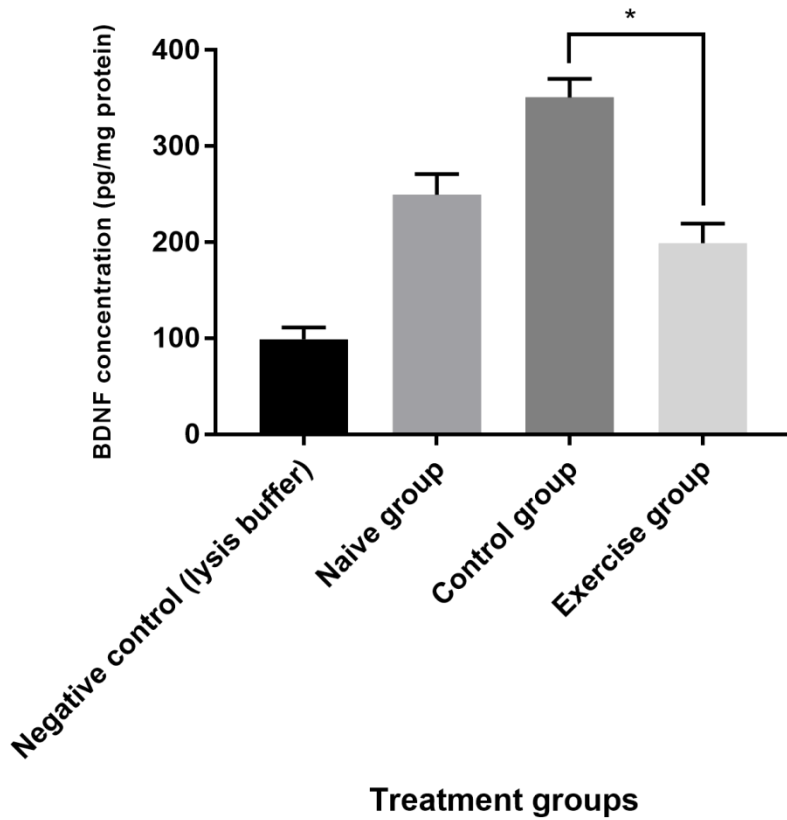


Figure H. BDNF protein expression levels. BDNF concentrations (pg/mg protein) were measured by ELISA in spinal dorsal horn tissues from naïve group (249.63 ± 21.40 pg/mg, $n=3$), control (350.79 ± 19.31 pg/mg, $n=3$), and exercise group (199.04 ± 20.49 pg/mg, $n=3$). Physical exercise down-regulated the BDNF protein expression level in SDH tissues from the mice treated with letrozole. Exercise group vs control group: $P < .05$ (Kruskal-Wallis test with Dunn's post hoc multiple comparisons).

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