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Effects of soy diet on inflammation-induced primary and secondary hyperalgesia in rat

Jasenka Borzan^{1,†,*}, Jill M. Tall^{2,†}, Chengshui Zhao³, Richard A. Meyer⁴, and Srinivasa N. Raja¹

¹Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins University, Baltimore, MD, USA.

²Department of Biological Sciences, Youngstown State University.

³Washington University Pain Center, Department of Anesthesiology, Washington University School of Medicine

⁴Neurosurgery, The Johns Hopkins University, Baltimore, MD, USA.

Abstract

Soy consumption is said to prevent or treat atherosclerosis, cancer, pain, and memory deficits, but experimental and clinical evidence to support these claims are lacking. We used in vivo models of inflammation to determine whether a soy diet reduces primary or secondary hyperalgesia. In all three experiments, rats were fed either a soy- or casein-based diet for at least 2 weeks before induction of inflammation and for the duration of experiments. Mechanical and heat paw withdrawal thresholds and edema were measured before and several times after induction of inflammation. Primary hyperalgesia was assessed in two models: unilateral intraplantar injection with 0.1 ml of 25% complete Freund's adjuvant (CFA) or 0.1 ml of 1% carrageenan. Unilateral injection of the intra-articular knee space with 25% CFA (0.1 ml) was used to determine the effects of soy in a model of secondary hyperalgesia. Following intraplantar injection of CFA, soy-fed animals exhibited significantly less paw edema, mechanical allodynia, and heat hyperalgesia compared to casein-fed animals. In the carrageenan model of paw inflammation, soy-fed animals were also less allodynic to mechanical stimuli, than were casein-fed animals, but showed no diet based differences in paw edema or heat hyperalgesia. Soy diet did not affect any of the outcome measures after the intra-articular injection of CFA. Our results suggest that a soy diet significantly decreases aspects of inflammation-induced primary, but not secondary, hyperalgesia in rats.

Keywords

soy; diet; arthritis; complementary and alternative medicine (CAM); carrageenan; CFA; musculoskeletal

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*Please direct correspondence to: Dr. Jasenka Borzan The Johns Hopkins Hospital 720 Rutland Ave/Ross 350 Baltimore, Maryland 21205 Office: (410) 502-5510; Fax: (410) 614-2019 jborzan1@jhmi.edu.

†These authors equally contributed to this project.

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1. Introduction

An ever-increasing number of Americans use complementary and alternative medicine (CAM) for the treatment of persistent or chronic conditions (Kessler et al., 2001; Konvicka et al., 2008; Rosenberg et al., 2008). Patients frequently turn to CAM therapies, such as herbal medicines and acupuncture, for pain control when other medical treatments are ineffective (Rao et al., 1999; Konvicka et al., 2008). In recent years, the potential influence of diet and dietary supplementation on conditions associated with pain have been the focus of considerable research (Shir et al., 2001a; Ernst, 2003; Tall and Raja, 2004; Weiner and Ernst, 2004; Perez et al., 2005; Rivat et al., 2008).

Soy and its metabolites (particularly isoflavones) have the potential to influence pain processing by inhibiting protein tyrosine kinases, pro-inflammatory cytokines, or cyclooxygenase-2 activity, as well as by acting as antioxidants, or by interacting with estrogen receptors (for review see Kurzer and Xu, 1997) (Kurzer and Xu, 1997). In the peripheral nervous system, isoflavone genistein has been shown to inhibit tyrosine kinase that can attenuate local nerve growth factor signaling (thereby attenuating further tissue inflammation). Additionally, genistein could directly inhibit excitability of nociceptive dorsal root ganglion neurons (Liu et al., 2004). Proposed antioxidant properties of soy diet phytonutrients could inhibit local recruitment of pro-inflammatory mediators to the site of inflammation (Arora et al., 1998). Additionally, it is possible that soy diet modulates pain processing via central mechanisms as well. Constituents of soy diet could have significant effects on iNOS expression, or COX activity (Corbett et al., 1996; Liang et al., 1999; Yagasaki et al., 2001; Liu et al., 2004; Ye et al., 2004; Comalada et al., 2006). Clearly, the analgesic efficacy and mechanism of action of soy are still not well understood.

In the present studies, we examined whether the consumption of a soy-based diet is effective at reducing primary hyperalgesia associated with inflammation that is induced by an intraplantar injection of complete Freund's adjuvant (CFA) or carrageenan in rats. In addition, we investigated whether a soy diet alleviates secondary hyperalgesia in the paw of rats injected with CFA in their knee joint. Our observations suggest that dietary soy suppresses aspects of inflammation and primary hyperalgesia and implicate a peripheral mechanism of action.

2. Materials and Methods

2.1 Animals

Adult male Sprague-Dawley rats were acclimated to housing for at least 1 week prior to experimentation. Rats were maintained on a 12-h light/dark cycle and were provided with food and water ad libitum. All testing was performed during the animals' day cycle. The Institutional Animal Care and Use Committee at The Johns Hopkins University approved the experimental protocol, and the studies were performed according to the Helsinki Declaration and the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983).

Each cage of rats was randomly assigned to one dietary treatment group, either the soy-based or casein-based diet. Inflammation was induced under brief isoflurane anesthesia (3%) in all experiments. Because 100% CFA induces a severe inflammatory response, we were concerned that this dose would produce a floor effect in our behavioral measures and would prevent the ability to detect an effect of soy. Hence, we first determined the lowest concentration of CFA that would produce reliable, but submaximal, levels of mechanical allodynia and heat hyperalgesia. In a pilot experiment, rats fed regular chow (Purina,

Richmond, IN) were injected with one of four concentrations of CFA diluted in sterile saline: 12, 25, 50, or 100%. Twenty-five percent CFA (0.1 ml; 1:4 v/v CFA in saline) was the lowest dose that reliably produced mechanical allodynia and heat hyperalgesia as well as paw edema (data not shown).

Our first experiment examined whether different diets would influence the development of primary mechanical allodynia, heat hyperalgesia, and paw edema after intraplantar injection of 25% CFA. In the second experiment, animals received an intraplantar injection of 0.1 ml of 1% carrageenan (diluted in saline). In the third experiment, 25% CFA (0.1 ml) was injected into the intra-articular space of the left knee. Intra-articular injection of CFA results in local inflammation of the knee but also causes secondary hyperalgesia, i.e., increased sensitivity to mechanical and heat stimuli outside of the injected area, including in the paw. Hence, we used this model to examine the effects of different diets on secondary hyperalgesia.

Rats were fed a casein (Diet #F4035, Bio-Serve, Frenchtown, NJ) or soy (Diet #F4036, Bio-Serve, Frenchtown, NJ) diet (Table 1) for at least 2 weeks before baseline testing and during the entire experimental protocol. The two week pre-treatment period was selected because it proved to be sufficient to produce a significant antihyperalgesic effect after nerve injury (Shir et al., 1998; Shir et al., 2001b; Shir et al., 2002). Each diet delivered a caloric profile of 3.6 kcal/g and possessed the same nutritional profile: 18% protein, 5% fat, 5.1 % fiber, 3.4% ash, 10% moisture, and 60.5% carbohydrate. The soy diet is produced from soy flour and contains the isoflavone compounds genistein, daidzein, and glycitein, whereas the casein diet does not. Previous studies in our laboratory have demonstrated effective pain relief with the same or similar soy diet profile in animal models of neuropathic and cancer pain (Shir et al., 1998; Shir et al., 2001b; Shir et al., 2002; Zhao et al., 2004). To assess whether the diets differentially affected animal growth, animal weight was recorded during the experimental protocol.

Animals were acclimated to laboratory environment, investigator handling, and behavioral equipment during two training sessions. For each experiment, a single investigator blinded to dietary treatment injected all animals, tested them for responses to mechanical and heat stimuli, and took measurements for paw edema. Room temperature of the behavioral testing facility was maintained at $22\pm0.5^{\circ}\text{C}$.

2.2 Mechanical stimuli

The 50% paw withdrawal threshold (PWT) to a static mechanical stimulus was assessed with the up-down method (Dixon, 1980). Animals were placed under a plexiglass chamber on a wire mesh floor and allowed to acclimate for 20 min. A series of eight calibrated von Frey filaments, ranging from 0.41–15.8 g in log increments, was used. Testing was initiated with the 2.0-g von Frey filament, the middle of the filament series. The filament was applied to the ventral surface of each hind paw for 4–6 seconds. A positive paw withdrawal response was recorded if the animal briskly lifted the hind paw. Questionable responses, such as the animal shifting body weight or lifting following the removal of the stimulus, were not recorded as a positive response, and the trial was repeated. In the absence of a paw withdrawal response to the initial filament, the next stronger stimulus in the series was presented; if the stimulus evoked a positive withdrawal response, the next weaker stimulus in the series was used during the next trial. The test continued until a) the responses of five more stimuli after the first change in response had been obtained or b) the upper/lower end of the von Frey set was reached. In cases where continuous positive or negative responses were observed to the exhaustion of the filament set, the paw withdrawal threshold was assigned to be 26.5 or 0.3 g, respectively. The resulting pattern of positive and negative responses was used to calculate the 50% PWT with the following equation: $50\% \text{ PWT} =$

$10^{[F+k\delta]}$, where F = force of the final von Frey filament used in log units; k = tabular value for the pattern of positive and negative responses (Dixon, 1980); δ = mean difference between stimuli in log units. Because the Dixon threshold procedure produces data that are not normally distributed, the data were analyzed by non-parametric tests.

2.3 Thermal stimuli

Paw withdrawal latencies (PWL) to radiant heat stimuli were recorded from the left and right hind paw of each rat (Hargreaves et al., 1988). Rats were placed into Plexiglas chambers on a glass surface that was heated to 28–30 °C. Following a 20-min acclimation period, a radiant heat stimulus was alternately applied to each hind paw, and the time to paw withdrawal was measured. Each hind paw was tested five times with an inter-trial interval of 5 min. A 20-s exposure limit was imposed to prevent tissue damage. The PWL of each rat was calculated as the median of five trials.

2.4 Edema measurement

Paw thickness was used as a measurement of inflammation-induced edema (Buritova et al., 1995; Wei et al., 1999). Briefly, the dorso-ventral thickness of each hind paw was measured with a caliper placed at the metatarso-phalangeal border, touching, but not squeezing the hind paw. Similar measures were taken to evaluate knee edema before and after the intra-articular injection of 25% CFA, except that the caliper was placed medio-laterally over the middle of the shaved knee.

2.5 Statistical Analysis

All data were analyzed with Statistica version 6.0 from StatSoft, Inc, Tulsa, OK. Paw or knee thickness, PWL, and animal weight measurements were analyzed by a repeated-measures analysis of variance (ANOVA) followed by Fisher's post hoc tests or paired t-tests (including Bonferroni's correction for multiple comparisons). Data are presented as the mean $\pm$ S.E.M in the figures and mean $\pm$ S.D. in the text. Mann-Whitney U test was used to compare PWT data between soy-fed and casein-fed animals. A probability level <0.05 was considered to be statistically significant.

3. Results

Animals' weight gain over time was similar in rats fed the two different diets in all of our experiments (Fig. 1).

3.1 Intraplantar injection of 25% CFA or 1% carrageenan

Paw thickness was comparable between soy- and casein-fed animals at baseline. All CFA-treated rats ($n = 8/\text{group}$) displayed a significant increase in ipsilateral paw thickness as compared to baseline at every time point after injection. Importantly, dietary soy significantly suppressed the inflammation-induced paw swelling (Fig. 2A; main effect of diet $F(1,14) = 26.8$, $P < 0.0001$) compared to casein-fed controls. The peak effect was observed 1 d after inflammation with soy fed animals having an ipsilateral paw thickness of 6.59 ± 1.38 mm, and casein fed animals 8.33 ± 0.53 mm. Two-way ANOVA also revealed a significant main effect of time $F(4,56) = 113.5$, $P < 0.0001$, and a significant interaction $F(4,56) = 6.6$, $P < 0.001$. Fisher LSD post hoc test indicated a significant difference in paw edema between soy- and casein-fed animals at 5 h ($P < 0.02$), 1 day ($P < 0.001$), and 4 days ($P < 0.02$) after the CFA injection. These data indicate that soy consumption reduces the severity of CFA-induced edema. Carrageenan effectively induced ipsilateral paw swelling at every time point (main effect of time $F(4,56) = 75.4$, $P < 0.0001$), but different diets did not affect carrageenan-induced paw edema (Fig. 2D; $n = 8/\text{group}$). The peak effect was observed

5 h after injection in soy fed (9.35 ± 0.36 mm) and casein fed (9.63 ± 1.48 mm) animals. In both CFA and carrageenan models the size of the ipsilateral paw was significantly greater than the contralateral paw at all time points except at baseline. There was no observable edema on the contralateral paw.

We examined the effects of soy consumption on CFA- and carrageenan-induced primary mechanical allodynia. PWT to mechanical stimuli were similar for soy-fed animals (20.9 ± 8.6 g for CFA and 24.6 ± 3.7 g for carrageenan) and casein-fed animals (23.0 ± 6.5 g for CFA and 26.5 ± 0 g for carrageenan) prior to inflammation ($P > 0.05$). Intraplantar injection of 25% CFA or 1% carrageenan produced significant ipsilateral mechanical allodynia in both groups of animals beginning 1 h after injection. However, 4 days after CFA or carrageenan injection, the mechanical threshold in soy-fed animals was no longer different from baseline in either model (Figs. 2B and 2E, respectively; 18.4 ± 8.9 g for CFA and 17.3 ± 10.2 g for carrageenan). Casein-fed animals still had significant mechanical allodynia 4 days after CFA, but not after carrageenan (8.5 ± 6.7 g for CFA and 17.1 ± 8.9 g for carrageenan). In contrast, in both models, there were no significant changes in the PWT on the contralateral side at any time point compared to its respective baseline value. In both models, Mann-Whitney U tests indicated a significant difference between soy- and casein-fed animals on certain test sessions. In the CFA model, soy diet attenuated mechanical allodynia 4 days after the CFA injection ($P < 0.02$). Soy-fed animals had significantly higher mechanical thresholds than casein-fed animals 4 h ($P < 0.05$) and 1 day ($P < 0.001$) after carrageenan-induced inflammation. These data indicate that consumption of a soy diet may promote faster recovery after inflammatory insult and may help reduce primary mechanical allodynia.

We also examined whether the consumption of soy affected CFA- or carrageenan-induced primary heat hyperalgesia. Diet did not influence baseline paw withdrawal latencies in either model. In the CFA model, two-way ANOVA revealed a significant effect of diet ($F(1,14) = 4.9$, $P < 0.05$) and a significant effect of time ($F(4,56) = 17.2$, $P < 0.0001$), but no significant interaction between factors (Fig. 2C). By 1.5 h after intraplantar injection, 25% CFA had induced significant heat hyperalgesia in all animals (6.6 ± 3.0 s for soy and 4.3 ± 0.8 s for casein groups). Casein-fed animals showed significant hyperalgesia at all time points tested ($P < 0.001$), but soyfed animals exhibited signs of recovery 4 days after induction of inflammation ($P > 0.05$ compared to baseline). These results indicate that the consumption of soy attenuates CFA-induced primary heat hyperalgesia. Two-way ANOVA for the carrageenan-induced inflammation model indicated no main effect of diet, a significant main effect of time ($F(4,56) = 27.6$, $P < 0.0001$), and no significant interaction. Primary heat hyperalgesia peaked at 4.5 h after injection (3.4 ± 0.7 s for soy and 3.0 ± 1.1 s for casein groups). However, diet did not affect the severity of carrageenan-induced heat hyperalgesia (Fig. 2F). These results indicate that consumption of soy can reduce the severity and duration of primary heat hyperalgesia induced by CFA but did not affect carrageenan-induced heat hyperalgesia. It is also noteworthy that in both models, there was no significant heat hyperalgesia on the side contralateral to the inflammation.

3.2 Intra-articular injection of CFA

Baseline PWTs, PWLs, and knee thickness did not differ between soy-fed ($n=11$) and casein-fed ($n=10$) rats. Soy- and casein-treated animals developed equally significant CFA-induced knee edema ipsilateral to the injection (main effect of time, $F(4,76) = 79.1$, $P < 0.0001$) that lasted for the duration of the experiment (Fig. 3A). Dietary treatment did not affect behavioral measures of secondary hyperalgesia in the paw or knee edema. There was also no change in knee swelling on the contralateral side. Intra-articular injection of 25% CFA resulted in a significant decrease in the ipsilateral PWT from baseline in both soy- and casein-treated animals (all time points tested $P < 0.05$; Fig. 3B) whereas there was no

significant change in the contralateral PWT. Dietary treatment did not influence the development or maintenance of secondary mechanical allodynia following CFA-induced inflammation (Fig. 3B). Furthermore, secondary heat hyperalgesia (main effect of time $F(4,76) = 5.2$, $P < 0.001$) was present ipsilaterally (but not contralaterally) in both groups without a significant effect of diet (Fig. 3C).

4. Discussion

In the current studies we sought to investigate the effects of dietary soy on inflammation-induced edema, primary and secondary mechanical allodynia, and heat hyperalgesia. The soy-based diet reduced aspects of mechanical allodynia, heat hyperalgesia and edema induced by intraplantar injection of 25% CFA. However, in the carrageenan model, consumption of soy attenuated only primary mechanical allodynia. Diet did not affect intra-articular CFA-induced knee swelling or the behavioral responses in the secondary hyperalgesic region. The results of these experiments suggest that soy consumption may be beneficial in reducing primary, but not secondary, hyperalgesia in inflammatory pain conditions.

Taken together with our previously published reports, our current results suggest that beneficial effects of soy are not species or strain specific (Sprague-Dawley or Wistar (Shir et al., 1998; Shir et al., 2001a; Shir et al., 2001b; Shir et al., 2002; Zhao et al., 2004)), not dependent on the source of soy protein (Bio Source or PMI Feeds), or on the type of thermal device (CO2 laser, radiant heat (Shir et al., 1998; Shir et al., 2001a; Shir et al., 2001b; Shir et al., 2002)) used.

Our observations suggest that the soy diet has mild, but significant, anti-inflammatory properties. Because we used a mild inflammatory dose of CFA (25%), we were able to observe a significant effect of diet on primary mechanical and heat hyperalgesia and paw edema. However, the dose of carrageenan injected was a standard dose typically used by other investigators and may cause a more intense inflammatory response that makes the effect of soy less apparent. Additionally, the differences in results can be attributed in part to the fact that CFA and carrageenan have somewhat different mechanisms of action, and a soy diet may not be equally effective at reducing both types of inflammation. For example, behavioral hyperalgesia that develops following carrageenan injection is similar to that produced by CFA in that local proinflammatory mediators, such as bradykinin, and reactive oxygen species directly activate and sensitize nociceptors (Morris, 2003). However, although carrageenan causes an immune response demonstrable by an increase in leukocyte count, it is generally considered a non-immune inflammatory stimulus, whereas CFA is specifically used as an immunostimulatory inflammatory agent (Stein et al., 1988; Woolf et al., 1997; Holmdahl et al., 2001; Morris, 2003; Ferreira et al., 2007).

It is also important to note that the onset of the beneficial effect of soy varied in two different inflammatory models. In the carrageenan model, inflammation-induced hyperalgesia peaks earlier (4-6 hrs after injection) than after a CFA injection (typically 24 hrs after injection). We observed that soy diet reduced mechanical allodynia earlier in the carrageenan model (4 hrs and 1 day after inflammation), than in the intraplantar CFA model (day 4). However, soy diet attenuated CFA inflammation induced edema and heat hyperalgesia overall, whereas it had no effect on those variables in the carrageenan model. Future studies need to investigate the specific influence of soy diet on various stages of the inflammatory cascade.

In primates and humans, secondary hyperalgesia following a cutaneous injury is typically characterized by increased sensitivity to mechanical but not heat stimuli in a test site away

from the injury (Raja et al., 1984; Ali et al., 1996). Secondary hyperalgesia induced by CFA (or carrageenan) injection into the intra-articular space of the knee is atypical in this regard because animals develop increased sensitivity to both mechanical and heat stimulation of the paw following inflammatory insult. The underlying mechanisms of secondary hyperalgesia following intra-articular CFA injection have been described as being driven by central sensitization of dorsal horn neurons (Sluka and Westlund, 1993; Wu et al., 1998). Joint inflammation leads to increased immunoreactivity for glutamate, substance P, calcitonin gene-related peptide, and both neuronal and inducible nitric oxide synthase (nNOS and iNOS, respectively) in the spinal cord (Sluka et al., 1992; Sluka and Westlund, 1993; Wu et al., 1998). The absence of effect of soy in the intra-articular CFA model may suggest that soy does not alter the central processing of pain signals and that its effects are predominantly peripheral. Another line of evidence for this argument is the fact that there were no contralateral differences between soy or casein fed animals after any of the inflammatory models.

A soy diet is rich with a variety of bioactive compounds that can attenuate the peripheral inflammatory response observed after intraplantar injection of CFA. Several mechanisms have been implicated as being relevant to pain processing, such as inhibition of tyrosine kinase, direct antioxidant properties, cytokine and cyclooxygenase inhibition, and interaction with estrogen receptors (Corbett et al., 1996; Arora et al., 1998; Liu et al., 2004; Mueller et al., 2004; Ye et al., 2004; Comalada et al., 2006). The isoflavone from soy, genistein, has tyrosine kinase-inhibiting properties that can attenuate local nerve growth factor signaling (thereby attenuating further tissue inflammation); alternatively it can directly inhibit excitability of nociceptive dorsal root ganglion neurons (Liu et al., 2004). However, genistein could also be expected to reduce central sensitization because tyrosine kinase inhibitors (including genistein) have been shown to reduce the expression of iNOS in the spinal cord dorsal horn. Perhaps the potency of bioactive compounds in soy at the dose ingested in these experiments was not high enough to exert both peripheral and central anti-inflammatory effects via tyrosine kinase inhibition.

Reactive oxygen species participate in cell injury and promote the inflammatory process. Phytonutrients found in soy have been shown to have excellent antioxidant properties that potentially slow the recruitment of pro-inflammatory mediators to the site of inflammation (Arora et al., 1998). In vitro studies indicate that soy proteins inhibit cytokine release and/or cyclooxygenase activity in various cell types under inflammatory conditions (Corbett et al., 1996; Liang et al., 1999; Yagasaki et al., 2001; Ye et al., 2004; Comalada et al., 2006). A recent report indicates that genistein relieves neuropathic pain by multiple mechanisms, including inhibition of nuclear factor κ B, iNOS, nNOS, and cytokines (Valsecchi et al., 2008).

Understanding the role of estrogen-like properties of isoflavones in pain processing is complicated by the fact that the role of estrogen hormones in pain is not clear (Craft et al., 2004). In the central nervous system, pro-estrogenic effects of soy could have pro-nociceptive effects, as central administration of estradiol produces an enhanced licking response after intraplantar formalin injection (Aloisi and Ceccarelli, 2000). On the other hand, systemic administration of estradiol can have anti-nociceptive and anti-inflammatory effects. Therefore, it is possible that in response to inflammation, soy reduces CFA-induced pain behavior by acting on peripheral estrogen receptors (Tada et al., 2004) but this beneficial effect is attenuated by the central pronociceptive effects of phytoestrogen in soy. Future studies will help us to determine if one or more of these mechanisms are involved in reducing primary hyperalgesia associated with CFA injection.

Our observations in models of peripheral inflammation may appear discrepant to our previously published results that soy diet reduces secondary, but not primary hyperalgesia in animal models of bone cancer (Zhao et al., 2004). Injection of sarcoma cells into the medullary cavity of the femur bone establishes a bone cancer model of secondary hyperalgesia because the affected bone is away from the test site (i.e. the paw). Behaviorally, mechanical and heat hyperalgesia following CFA-induced knee inflammation or femur bone cancer growth may appear similar, but the underlying pain mechanisms are not the same (Luger et al., 2002). Osteoclast mediated bone-destruction is a major mechanism suggested in bone cancer pain (Honore et al., 2000a;Honore et al., 2000b). Although some bone destruction may be a long term result of intraplantar CFA administration (Chan et al., 1999), this is clearly not the primary source of CFA-induced pain behavior. Furthermore, CFA-induced primary hyperalgesia and cancer-induced primary hyperalgesia (calcaneus bone cancer model) also seem to be governed by different underlying mechanisms. The lack of lymphocyte and neutrophil infiltration and the absence of obvious neuropathology at and around the tumor site suggest that cancer-induced primary hyperalgesia is not purely inflammatory or neuropathic in origin (Wacnik et al., 2001;Cain et al., 2001). Rather, it has been argued that the release of endothelin evokes pain in calcaneus bone cancer model (Wacnik et al., 2001).

The fact that a soy diet reduced edema in the paw but not in the knee may potentially be attributable to differences in tissue type at the site of injection. Paws are composed mostly of soft tissue that expands much more upon inflammation than the restricted space of the knee synovium. Similarly, knee diameter might not be a sensitive measure of changes in knee edema because it is based on two points as an indication of swelling. Perhaps measuring knee volume or biochemical markers of cartilage degradation would be more accurate and would reveal a difference between soy- and casein-fed animals. For example, Arjmandi et al found a significant improvement in serum markers of cartilage degradation (glycoprotein 39 and insulin-like growth factor-I) in osteoarthritic men who consumed soy protein for 90 days compared to men who consumed milk protein (Arjmandi et al., 2004). The results of our studies suggest that a soy rich diet may reduce primary, but not secondary, hyperalgesia in inflammatory pain conditions.

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Figure 1.

Animal weight was not affected by either diet or inflammation in any of the experiments: intraplantar CFA (25% CFA), intraplantar carrageenan (1% CARR), or intra-articular CFA (25% ia CFA). No statistically significant difference in body weight was observed between the soy- and casein-consuming rats during the 2-week pretreatment or following inflammation ($P>0.05$).

Figure 2.

Soy diet attenuated inflammation-induced edema, mechanical allodynia, and heat hyperalgesia, and edema following intraplantar injection of 25% CFA ($n=8/\text{group}$). Soy diet also reduced primary mechanical allodynia following intraplantar injection of 1% carrageenan, but did not affect paw edema or heat hyperalgesia (1% CARR, $n=8/\text{group}$). (A) In animals fed a soy diet, paw swelling was significantly reduced 5 h, 1 day, and 4 days after CFA injection compared to animals fed a casein diet. (B) Soy-fed animals recovered more quickly from CFA-induced allodynia to mechanical stimulation than did casein-fed animals (4 days, $P<0.05$). (C) Soy diet significantly attenuated primary heat hyperalgesia induced by 25% CFA. (D) Carrageenan injection induced peak paw edema 5 h after injection. However,

the level of edema did not differ between the two treatment groups at any time point. (E) Carrageenan evoked peak mechanical allodynia 4 h after injection. Soy-fed animals had less intense allodynia to mechanical stimuli than did casein-fed animals 4 h and 1 day after induction of inflammation. (F) Soy-fed animals did not differ from casein-fed controls in behavioral responses to heat after carrageenan injection. CARR = carrageenan. * $P < 0.05$, *** $P < 0.001$ soy vs. casein groups.

Figure 3.

Soy diet did not influence behavioral signs of secondary hyperalgesia or edema following 25% CFA injection into the intra-articular cavity of the left knee (Soy diet, n=11; casein diet, n=10). No significant differences were observed in knee diameter (A), paw withdrawal threshold (B), or paw withdrawal latency (C) between soy-fed and casein-fed animals.

Table 1

Composition of soy and casein diets (Bio-Serv Co., Frenchtown, NJ)

Ingredient	Casein Diet (g/kg) Diet #F4035	Soy Diet (g/kg) Diet #F4036
Casein protein	200	0
Soy protein (toasted soy flour)	0	338.46
Sucrose	500	361.54
Corn starch	150	150
Fiber	50	50
Corn oil	50	50
Mineral mix, American Institute of Nutrition, AIN-93GMX	35	35
Vitamin mix, American Institute of Nutrition, AIN-93G	10	10
DL-methionine	3	3
Choline bitartrate	2	2