White Button and Shiitake Mushrooms Reduce the Incidence and Severity of Collagen-Induced Arthritis in Dilute Brown Non-Agouti Mice\textsuperscript{1,2}

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Abstract

Exotic mushrooms have been used in ancient Chinese medicine due to their immunomodulatory properties for the treatment and/or prevention of chronic diseases. However, only limited data exist on the health benefits of white button mushrooms (WBM), the most common in the American diet. In the current study, we investigated the effects of WBM and shiitake mushrooms (SM) on collagen-induced arthritis (CIA) using a 2 x 3 factorial design in 8-wk-old female dilute brown non-agouti mice that were fed a control diet (n = 37) or the same diet supplemented with 5% lyophilized WBM or SM (n = 27) for 6 wk. CIA was induced by immunizing mice with 100 µg bovine collagen followed by 50 µg LPS on d 20 post-collagen injection. CIA was assessed by mononuclear cell infiltration, bone erosion, plasma IL-6, TNFα, and intercellular adhesion molecule-1 (sICAM-1) concentrations. Compared with the control diet, WBM and SM tended to reduce the CIA index from 5.11 \pm 0.82 to 3.15 \pm 0.95 (P = 0.06) (median, 6–9 to 1–2) 31 d post-collagen injection. Whereas 58% of control mice had a CIA index \( \geq 7 \), only 23% of WBM and 29% of SM mice did (P = 0.1). Although both types of mushrooms reduced plasma TNFα (34%, WBM; 64%, SM), only SM increased plasma IL-6 by 1.3-fold (P < 0.05). The CIA index was positively correlated with sICAM1 (r = 0.55; P < 0.05) but negatively correlated with TNFα (r = 0.34; P < 0.05). Whether mushrooms are beneficial for arthritis management remains to be investigated. To our knowledge, this is the first report demonstrating a possible health benefit of WBM in arthritis treatment. J. Nutr. 141: 131–136, 2011.

Introduction

Rheumatoid arthritis (RA)\textsuperscript{5} is a systemic inflammatory condition of unknown etiology affecting \textsim 1% of the adult population (1). This disease affects individuals across the age spectrum, but the peak incidence usually occurs between the ages of 40 and 50 y (1). Chronic inflammation of the joints and persistent synovitis are the pathognomonic signs of RA (2). Deterioration of cartilage and bone in the affected joints may not only lead to loss of daily function due to chronic pain and fatigue but may also result in permanent disability (2). Furthermore, RA can lead to diffuse inflammation in the lungs, pericardium, pleura, sclera, and nodular lesions in subcutaneous tissue. These systemic complications increase morbidity and mortality among RA patients (3).

Although the precise pathogenesis remains unclear, the inflammation of RA is orchestrated in large part by cytokines such as IL-6 and TNFα (4). Various effector cells, including T cells, synovial fibroblasts, synovial macrophages, and chondrocytes, participate in this inflammatory process by producing cytokines locally (4). Another factor that plays a crucial role in arthritis is intercellular adhesion molecule-1 (ICAM-1), which is expressed in activated synovium by vascular endothelium, fibroblasts, lymphocytes and monocytes (5). ICAM-1, together with its ligand (leukocyte function-associated antigen-1), facilitates recruitment of mononuclear cells in the synovium, which subsequently leads to further increased secretion of inflammatory cytokines such as TNFα and IL-6. Anticytokine therapies such as infliximab have been shown to alter the disease course and improve radiographic outcomes of RA patients (6). However, the high cost of these pharmacological therapies to the health care system and the associated side effects (e.g. respiratory and urinary tract infections and hypotension) may lead to poor compliance. It is therefore important to explore cost-effective, natural, alternative approaches for the prevention of this disease or adjuvant compounds that can be used in combination with lower doses of existing drugs for treatment. In this context, several functional foods, including mushrooms, are gaining popularity due to their immunomodulatory properties (7).
Certain exotic mushrooms such as shiitake and maitake have long been used for treatment and/or prevention of chronic diseases (8). In a recent study, extracts from the *Inonotus* mushrooms showed inhibitory effects on cyclooxygenase and xanthine oxidase activity in vitro, suggesting the benefits of mushrooms as an antiinflammatory agent (9). Interestingly, supplementation of extracts from the medicinal mushroom, *Agaricus blazei Murill*, which belongs to the Basidiomycetes family significantly reduced plasma concentrations of proinflammatory cytokines, including TNFα in humans (10).

Although white button mushrooms (WBM; *Agaricus bisporus*) represent more than 90% of consumed mushrooms in the US and ~33.3% of world's production, their effects on arthritis have not been scientifically investigated. Considering that certain species of WBM have antiinflammatory properties, we hypothesized that they may reduce the incidence and severity of arthritis. Collagen-induced arthritis (CIA) is a recognized animal model for the study of human RA. Because of the many similarities to the human disease related to induction of cytokines and adhesion molecules, CIA mice have been extensively used to elucidate the pathogenesis of arthritis (11).

### Materials and Methods

#### Chemicals and reagents

Reagents were purchased from the following suppliers: *Escherichia coli* LPS, complete Freund's adjuvant from Sigma, bovine type II collagen (CII) from Sigma and Chondrex; and AIN76 diet from Teklad. IL-6, TNFα, and soluble ICAM-1 (sICAM-1) assay kits were purchased from R&D Systems. WBM and shiitake mushrooms (SM) were a gift from JM Farms and Franklin Farms.

#### Experimental design

The protocol was approved by the Institutional Animal Care and Use Committee of Oklahoma State University and all procedures were performed in a humane fashion. Eight-week-old female dilute brown non-agouti (DBA-1) mice (n = 91; body weight 15–18 g) were purchased from Jackson Laboratories. Upon arrival, all mice (5/cage) were fed the control (AIN76) diet for 7 d. The control diet contained adequate macronutrients, minerals, and vitamins to maintain optimal growth and health (12). At the end of acclimation period, mice were randomly assigned to 1 of the 3 dietary treatment groups: control diet, 5% WBM-supplemented diet, or 5% SM-supplemented diet. SM were also included in the protocol, because these mushrooms modulate the secretion of pro- and antiinflammatory cytokines and they may also affect CIA pathogenesis (13). The rationale for the 5% level of mushroom supplementation was based on the work of other investigators showing that 2.0% (which approximates normal consumption based on an energy intake of 8.4 MJ/d) provided minimal improvement in immnunity, as indicated by natural killer cell activity, whereas 10% provided the greatest immunomodulatory effect (14). Mushroom powder was added to the control diet at the expense of sucrose without altering the total energy. Mice had free access to their food and deionized water and were housed under standard 12-h-light/-dark cycle conditions. Body weight was assessed twice per month.

#### Induction of arthritis

CIA was induced as described previously (15–18). Briefly, after 42 d of receiving their assigned diets, mice that received the control (n = 19), WBM (n = 14), and SM (n = 14) supplemented diets were weighed and immunized with CII (100 μg) dissolved in 0.01 mol/L acetic acid and complete Freund's adjuvant in 1:1 ratio (in a total volume of 100 μL). Unimmunized (control) mice received 100 μL of the solvent. On d 20 post-CII injection, mice received a single i.p. injection of 50 μg of LPS dissolved in sterile 100 μL saline (0.85% NaCl), whereas the control mice received 100 μL of saline. We used LPS instead of a second dose of CII, because it has been shown to induce signs of arthritis (paw swelling) in more than 90% of mice within 5 d (15,16). Mice continued to have free access to their assigned diets after collagen and LPS injections for 10 d. They were also weighed 9 times (weekly) during this period.

#### Arthritis assessment

The severity of arthritis was assessed without knowledge of the treatment groups by coinvestigators (E.L., B.J.S., and S.L.). Clinical arthritis was assessed by using the following scoring scale (18): grade 0, no swelling; grade 1, slight swelling and redness; 2, marked edema; 3, joint rigidity, 4, severe edema of the entire paw.

#### Organ collection and histopathological analysis

After 10 ± 1 d post LPS injection, mice were weighed and killed by CO2 inhalation for 60 s. Blood was collected via the retro-orbital plexus in heparinized tubes and after centrifugation at 400 × g, plasma was collected and immediately frozen at −80°C for subsequent measurement of cytokines and sICAM-1.

The paws were fixed with 10% buffered formalin, decalcified in 5% formic acid, embedded in paraffin, and 5-μm sections were prepared and stained with hematoxilin/eosin. Histopathological changes (i.e., infiltration of mononuclear cells in joints, cartilage destruction, and bone erosion) were scored without knowledge of the treatment groups by the study pathologist (coauthor S.L.) as described by Joosten et al. (19). The severity of inflammation and bone erosion varied from 0 to 3, with 0, 1, 2, and 3 corresponding to none, mild, moderate, and severe and inflammation, and/or bone erosion, respectively (18,19).

#### Cytokines and sICAM-1 assay

IL-6 (Quantikine colorimetric sandwich ELISA, cat no. SM60008), TNFα (Quantikine colorimetric sandwich ELISA, cat no. SMTA00), and sICAM-1 (Quantikine colorimetric sandwich ELISA, cat no. DCDS40) were measured in plasma with commercially available kits (R&D Systems) according to the manufacturers' specifications. sICAM-1 was measured because elevated levels are usually associated with increased expression of cell membrane ICAM-1 (5,20).

#### Statistical analysis

Data were tested by 2-way ANOVA (3 diets × 2 CII treatments) with Tukey’s test as a post hoc test and Pearson correlation coefficients were calculated with SPSS version 18 and/or Microstatistical program (Microsoft). Repeated-measures ANOVA were performed for body weight to compare the mean body weight difference at age of 60, 102, 122, and 132 d and mean arthritis indices at d 0 to 32 post-CII injection. Multiple regression analysis (with diet and collagen treatment as independent variables and plasma cytokine concentrations as dependent variables) and descriptive statistics were calculated. Chi-square tests were performed to compare CIA incidence among dietary treatment groups. The proportion of mice with various severities of CIA was also compared among dietary treatment by χ² using the following definition: CIA index 0–2, 3–4, 5–6, ≥7 for none to mild, moderate, severe, and very severe. The level of significance was set at P < 0.05.

### Results

#### Body weight

At time 0 (i.e. beginning of the feeding trial, age 60 d), the overall mean body weights did not differ among the 3 dietary treatment groups. In the subgroups of unimmunized mice, the mean body weights of mice fed mushroom-supplemented diets were lower at the age of 102, 116, and 123 d than in those fed the control diet (Fig. 1; P < 0.05). By the end of study, mean body weights of these 3 dietary treatment groups were not significantly different. In the subgroup of CII-mice, the mean body weights of those fed SM-supplemented diets were ~7.8% lower than those fed the control diet at almost all ages (Fig. 1; P < 0.05). Feeding the WBM-supplemented diet resulted in a decrease (~9.4%) in body weights at 14 d (age 116 d) and 21 d (age 123 d) post CII injection (P < 0.05). Whereas injection with CII had no significant impact on weight during the first 14 d (age 116 d), after 3 wk, it decreased mean body weight in all 3 dietary treatment groups (Fig. 1, age 102 d vs. 123 d). After LPS administration to CII-mice, mean body weights further decreased in mice fed the control and SM-supplemented diets but not with the WBM-supplemented diet (Fig. 1). At the end of the study, the mean body weight of mice fed the WBM-supplemented diet was higher than the means of mice fed the control and SM-supplemented diets (P < 0.05).
Incidence and severity of arthritis. Development of edema in any of the paws of CII-treated mice is considered a positive response to immunization and induction of clinical arthritis. The incidence of clinical arthritis (i.e., visible paw swelling) was not significantly lower in mice fed SM-supplemented and WBM-supplemented diets (Table 1).

In all 3 dietary treatment groups, mean arthritis indices increased with time post LPS injection (Fig. 2; \( P < 0.05 \)). On d 22 post collagen injection (or 48 h post LPS injection), mild but visible toe swelling was evident in mice fed the control diet but not in those fed the mushroom-supplemented diets. In fact, the mean arthritis index was higher in mice fed the control diet than fed the mushroom-supplemented diets (\( P < 0.05 \)). Between d 25 and 31 post collagen (i.e., d 5 and 11 post LPS), mice fed the control diet had elevated mean indices compared with those fed mushroom-supplemented diets. Starting on d 28, the mean arthritis indices decreased by \( \sim 31, 41, \) and 18% in the groups of mice fed the control, WBM, and SM diets, respectively. However, due to the wide variation in the CIA index within dietary treatment groups, the decreases were not significant (\( P = 0.11–0.16 \)). By the end of the study, mice fed the WBM-supplemented diet tended to have a lower mean arthritis index than those fed the control diet (\( P = 0.07 \)). Although the mean arthritis indices did not differ among the 3 dietary treatment groups at various time points (\( P = 0.45–0.92 \)), due to the wide variation, the medians were elevated in mice fed the control diet compared with those fed the mushroom-supplemented diet. For example, at d 25 post CII treatment, the median of mice fed the control diet was 6 compared with 2 in those fed the mushroom-supplemented diets. At d 28, the corresponding medians were 9.5 for the control diet and 1 for mushroom-supplemented diet. A slightly higher percent of mice fed the control diet (57.9%) than those fed the WBM (23.1%) and SM (28.6%) diets had a CIA index \( \geq 7 \) (\( \chi^2 = 10.55; df = 6; P = 0.1 \)).

Histological changes associated with arthritis, mononuclear cell infiltration in joints, and bone erosion. In unimmunized mice, there was no significant difference among study groups in the mononuclear cell distribution in joints. Histological appearance of the joints obtained from mice fed different diets was similar to that shown in Figure 3A. As expected, administration of collagen and LPS and the development of arthritis resulted in increased infiltration of leukocytes within joints (Fig. 3B–D). Histological evaluation of bone specimens from mice fed the control and mushroom-supplemented diets showed marked infiltration of leukocytes, with no significant difference in the mean infiltration scores (Fig. 3B–D; Table 1). Nonetheless, small differences in bone destruction were observed among the 3 dietary treatment groups. Whereas mice fed the control diet had massive bone erosion with arthritis development (score = 3; Fig. 3B), those fed the SM-supplemented diet (score = 1; Fig. 3C) showed nearly intact bone structure with mild infiltration of inflammatory cells. Bone histology of mice fed the WBM-supplemented diet had moderate bone loss (score = 2; Fig. 3D). The mean scores of bone erosion tended to be, although not significant, lower in mice fed the SM-supplemented diet than in those fed the control diet (\( P = 0.08 \)). When a Student’s

### TABLE 1 Incidence of CIA and severity of paw swelling in DBA mice

<table>
<thead>
<tr>
<th></th>
<th>Control-CII</th>
<th>WBM-CII</th>
<th>SM-CII</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIA incidence, ( n/\text{total} \ n (%) )</td>
<td>15/19 (79)</td>
<td>8/13 (61)</td>
<td>10/14 (71.43)</td>
</tr>
<tr>
<td>Severity of mononuclear cell infiltration score</td>
<td>2.36 ± 0.14</td>
<td>2.32 ± 0.35</td>
<td>2.00 ± 0.32</td>
</tr>
<tr>
<td>Bone erosion score</td>
<td>1.93 ± 0.26</td>
<td>2.15 ± 0.40</td>
<td>1.44 ± 0.23</td>
</tr>
</tbody>
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1 For the mononuclear cell infiltration and bone erosion scores, values are means ± SEM, \( n = 9 \) (control), 6 (WBM-supplemented diet), or 5 (SM-supplemented diet).
The Student's *t* test was used to compare the data, the mean bone erosion score tended to be lower (*P* = 0.09) in the group of mice fed the SM-supplemented diet than in those fed the WBM-supplemented diet (Table 1).

**Plasma TNFα in mice with and without CIA.** In unimmunized mice, there was no significant difference in plasma TNFα levels among dietary treatment groups (Fig. 4A). In CII-injected mice, the plasma TNFα concentration was greater in the control mice than in mice fed the SM and WBM diets, which did not differ from one another (Fig. 4A; *P* < 0.05). As expected, immunization of mice with CII fed the control diet tended to increase plasma TNFα (i.e. 38.4%) (*P* < 0.07); however, CII injection did not increase plasma TNFα in mice fed the SM and WBM diets (Fig. 4A). In fact, in mice fed the SM-supplemented diet, the mean TNFα concentration of immunized mice tended to be 53% lower than that of unimmunized mice (*P* = 0.05).

**Plasma IL-6 levels in mice with CIA.** In unimmunized mice, plasma IL-6 levels did not differ among the 3 dietary treatment groups (Fig. 4B). In CII-treated mice fed the SM diet, the mean concentration of plasma IL-6 was 2.7 times that of mice fed the control diet (*P* < 0.05). After CII injection, mice fed the SM diet had a 1.3-fold increase in plasma IL-6 levels compared with those of immunized mice fed the same diet (*P* < 0.05). No such increase was observed in mice fed the control and SM diets.

**Plasma sICAM-1.** There was no diet effect on plasma sICAM-1 in either CII-treated or unimmunized mice (data not shown). Contrary to what we expected, we observed no significant increase in plasma sICAM-1 in CII-treated mice (data not shown).

**Correlation coefficients, multiple regression analysis, and interactions between diet and collagen treatment.** In the overall subgroup of CII-treated mice, the mean CIA index was positively correlated with sICAM-1 (r = 0.55; *P* < 0.05) but was negatively correlated with TNFα (r = −0.34; *P* < 0.05). In each of the dietary treatment groups, the CIA index was positively correlated with plasma levels of sICAM-1 (r = 0.82, control diet; r = 0.96, WBM diet) and negatively correlated with levels of TNFα (r = −0.59, control diet; r = −0.85, WBM diet) (*P* < 0.05). With the SM-supplemented diet, the correlations did not reach significance. There was a strong inverse relationship between TNFα and IL-6, especially in mice with CIA fed the SM-supplemented diet (r = −0.70; *P* < 0.05). MANOVA suggested that the CIA index (P = 0.02), IL-6 (P = 0.006), and TNFα (P = 0.1) were affected by diet and collagen treatment (there was an interaction between diet and collagen treatment). When multiple regression analysis was performed with diet, CIA treatments as independent variables, and plasma cytokine concentrations as dependent variables, dietary treatment played a major role in the observed differences in CIA severity among groups (r² = 0.416; *P* < 0.05).
infliximab, have been successfully used to treat RA in humans. Anti-TNFα plays an important role in the pathophysiology of arthritis by inducing a proinflammatory cytokine cascade involving IL-1, IL-6, granulocyte monocyte stimulating factor, and IL-8, as well as several proinflammatory chemokines (21). Anti-TNFα drugs, namely infliximab, have been successfully used to treat RA in humans. Furthermore, agents that inhibit the secretion of TNFα or block their binding to TNF cell-surface receptors are being considered as potential therapeutic agents (4). Interestingly, compared with the control diet, both WBM- and SM-supplemented diets reduced plasma TNFα levels in these arthritic mice, suggesting their potential health benefits in human RA. In our study, the SM tended to protect against bone erosion to a greater extent than the WBM. This could be due to the different nutrient compositions and concentrations of β-glucans present in these mushrooms.

We measured IL-6 in this study due to its role as a B-cell differentiation factor and the fact that polyclonal B-cell activation is frequently associated with RA (22). Unlike TNFα, IL-6 is a pleiotropic cytokine that possesses biological activities that may enhance or suppress inflammation and bone destruction (23). Although IL-6 is implicated in the pathogenesis of RA by promoting osteoclastogenesis, a study conducted in IL-6 knockout mice found that IL-6 is required for controlling local and systemic acute inflammatory responses, suggesting a possible antiinflammatory role of IL-6 (24). Moreover, IL-6 deficiency has been reported to result in increased bone destruction associated with inflammation in mice (25). Furthermore, exogenous administration of IL-6 resulted in a significant reduction of cartilage destruction in zymosan-induced arthritic mice (26). These findings suggest that further research is needed to understand IL-6’s effects on connective tissue.

In mice fed the SM-supplemented diet, there was a significant increase in the plasma IL-6 level in arthritis mice compared with unimmunized mice fed the same diet. In parallel to increased IL-6 plasma levels, plasma TNFα significantly decreased. These data indicate that TNFα is more crucial in the development of RA than IL-6. Interestingly, SM supplementation alone significantly increased in plasma IL-6 in the arthritic mice. Hence, we speculate that elevated plasma IL-6 might be responsible for less bone erosion as evidenced by bone histology in mice fed the SM-supplemented diet; however, this would need to be confirmed by further investigation. We therefore contend that the elevated IL-6 in SM-fed arthritic mice may have a regulatory role in the bone inflammatory response and thereby prevent bone erosion. Potential mechanisms could involve the induction of acute-phase proteins with increasing IL-6 that have antiinflammatory properties (27).

ICAM-1, an important molecule involved in leukocyte recruitment during inflammation is increasingly expressed in synovial tissue during arthritis, suggesting its role in the pathogenesis of RA (28). Plasma sICAM-1 is an indirect measure of ICAM-1 in synovial tissue. In our study, we could not detect a significant increase in plasma sICAM-1 levels as expected. This may be due to insufficient shedding of sICAM-1 in synovial fluid to a level that produces a detectable change in the plasma. Because we did not measure the sICAM-1 in synovial fluid by ELISA and localize ICAM-1 expression in arthritis affected synovial tissue by immunohistochemistry, we are uncertain of the effect of mushrooms on the expression of ICAM-1. This could be considered a limitation of the present study.

Histology of bone from SM-fed arthritic mice revealed nearly intact bone structure with less infiltration of inflammatory cells compared with arthritic mice fed the control diet. Bone resorption associated with arthritis is largely mediated by the increased local production TNFα (29). TNFα plays an important role in normal remodeling but also in diseased bone metabolism (30). TNFα induces bone resorption by upregulating the production of the essential osteoclast differentiation factor, receptor activator of NFκB ligand, and/or downregulating its soluble decay receptor, osteoproengin (31).

Further, expression of TNFα is positively associated with bone destruction as evidenced by bone histology (32). A growing body of experimental and clinical evidence supports the importance of TNFα in the pathogenesis of various forms of bone loss (29). Further, infliximab, an anti-TNFα drug, improved bone histology in patients with RA (33). Hence, we speculate that the preserved intact bone structure in the histology of the SM-supplemented diet group is likely due to the reduction in TNFα.

**Discussion**

In the present study, the WBM-supplemented diet tended to reduce the incidence of clinical arthritis from 79% in the control diet to 61%. The severity of arthritis assessed by arthritis index was reduced with the WBM-supplemented diet from 5.11 to 3.16 and weight loss associated with CIA was prevented. Moreover, the WBM-supplemented diet also reduced plasma TNFα, a major proinflammatory cytokine involved in the pathogenesis of RA. Interestingly, the SM-supplemented diet tended to reduce bone erosion and severity of arthritis despite the elevated IL-6 in this animal model. The decreased bone erosion may be attributed in part to decreased TNFα levels.

We have employed a CIA murine model for our experiment, because it shares a number of clinical, histologic, and immunologic features of clinical RA (20). Similar to previous reports by other investigators who studied clinical and experimental arthritis (4), we found that plasma TNFα concentrations were also elevated in mice with arthritis and fed the control diet. TNFα, primarily secreted by monocytes/macrophages, has a central role in the pathophysiology of arthritis by inducing a proinflammatory cytokine cascade involving IL-1, IL-6, granulocyte monocyte stimulating factor, and IL-8, as well as several proinflammatory chemokines (21). Anti-TNFα drugs, namely infliximab, have been successfully used to treat RA in humans. Furthermore, agents that inhibit the secretion of TNFα or block their binding to TNF cell-surface receptors are being considered as potential therapeutic agents (4). Interestingly, compared with the control diet, both WBM- and SM-supplemented diets reduced plasma TNFα levels in these arthritic mice, suggesting their potential health benefits in human RA. In our study, the SM tended to protect against bone erosion to a greater extent than the WBM. This could be due to the different nutrient compositions and concentrations of β-glucans present in these mushrooms.
In summary, the novel and most salient findings of the current study are: 1) WBM reduced arthritis incidence, plasma TNFα levels, and severity of visible paw edema (CIA index) and prevented weight loss associated with CIA; and 2) SM reduced TNFα plasma levels but increased IL-6 levels and attenuated bone erosion associated with CIA. To the best of our knowledge, this is the first scientific report documenting the health benefits of WBM, a widely consumed mushroom, in experimental arthritis. Based on the results of our study, we conclude that WBM and SM may be beneficial in arthritis prevention.

The major limitation of our study is that we did not directly measure inflammatory cytokines in the synovium. However, considering that increased cytokine expression, especially TNFα, in the synovium usually parallels increased plasma TNFα in clinical and experimental arthritis, the observed changes in cytokine levels are still valid in regard to the possible health benefits of WBM (and SM) in RA. Although our observations provide a rationale for the use of WBM as a functional food for the prevention and/or adjuvant treatment of RA, whether or not the health benefits of WBM translate to clinical RA remains to be determined.

Acknowledgements

We thank Cheley Adami for editing the grammar of this manuscript. We also thank the department of Pathology at Oklahoma State University for processing tissues and preparing slides for histopathology. S.K. designed the study, participated in all phases of the study (feeding mice, assessment of CIA, killing mice, statistical analysis, and writing the manuscript); L.C., E.A.L., S.L.C., H.A., D.T., and B.J.S. participated in the research at the time the mice were killed; E.A.L. and B.J.S. also assessed edema in collagen-treated and untreated mice; D.T. assisted in statistical analysis; S.L. and L.C. performed histopathology; S.K. and L.C. wrote the manuscript; and S.K. and C.L. had primary responsibility for full content. All authors read and approved the final manuscript.

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ERRATUM


All symbols for micrograms were inadvertently removed from the PDF of this article before online publication. The online PDF has been corrected by the publisher to reflect this change.

In addition, in the third paragraph on page 134, under the heading “Plasma IL-6 levels in mice with CIA,” the following change should be made:

In unimmunized mice, plasma IL-6 levels did not differ among the 3 dietary treatment groups (Fig. 4B). In CII-treated mice fed the WBM diet, the mean concentration of plasma IL-6 was 2.7 times that of mice fed the control diet ($P < 0.05$). After CII injection, mice fed the SM diet had a 1.3-fold increase in plasma IL-6 levels compared with those of immunized mice fed the same diet ($P < 0.05$). No such increase was observed in mice fed the control and WBM diets.

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ERRATUM


Please note the following corrections:

An error occurred on page 1392, in the last sentence of the first paragraph of the statistical analyses. The sample value for IDA pups is incorrect and indicated that “analyses were based on 9 dams (4 IDA and 5 IS) and 25 pups (9 IDA and 16 IS, i.e., 4 and 5 siblings, respectively).” The corrected sentence should read: “analyses were based on 9 dams (4 IDA and 5 IS) and 27 pups (11 IDA and 16 IS, i.e., 5 and 5 siblings, respectively).”

In the sixth sentence of the second paragraph of the statistical analyses, “IDA and IS siblings were reduced to 3 and 5 ...” should read “IDA and IS siblings were reduced to 4 and 5...”

Corrections should be also applied to the abstract on page 1390, next to the last sentence: “IDA siblings ($n = 3$), compared with the IS siblings ($n = 5$), had significantly elevated ABR thresholds” should read “IDA siblings ($n = 4$), compared with the IS siblings ($n = 5$), had significantly elevated ABR thresholds.”

In the Figure 3 legend on page 1393, the sample value for IDA pups should read as $n = 4$, instead of $n = 3$.

This error does not affect the outcomes and findings.

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