Comparison of the response to experimentally induced short-term inflammation in the temporomandibular and metacarpophalangeal joints of horses

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Objective—To investigate the relationship between inflammatory responses of the temporomandibular joint (TMJ) and the metacarpophalangeal (MCP) joint in clinically normal horses.

Animals—7 mature horses.

Procedures—In each horse, 1 TMJ and 1 MCP joint were injected with lipopolysaccharide (LPS; 0.0025 µg). The contralateral TMJ and MCP joint were injected with saline (0.9% NaCl) solution. Synovial fluid samples were collected from all 4 joints over 24 hours after injection. Concentrations of interleukin-6, tumor necrosis factor-α, transforming growth factor-β, and total protein were measured via immunoassay. Horses were assessed for clinical signs of joint inflammation at each time point.

Results—Concentrations of interleukin-6 were not significantly different between LPS-injected MCP joints and TMJs at any time point. Transforming growth factor-β concentrations were significantly increased in MCP joints, compared with concentrations in TMJs, at 12 and 24 hours after injection. Tumor necrosis factor-α concentrations were significantly higher in LPS-injected TMJs than in LPS-injected MCP joints at 1 and 6 hours after injection. Total protein concentration did not differ significantly between LPS-injected MCP joints and TMJs. Injection of LPS induced clinical inflammation at all time points; additionally, 2 MCP joints (but no TMJs) had an inflammatory response to injection of saline solution.

Conclusions and Clinical Relevance—The inflammatory response to LPS appeared to be attenuated more quickly in TMJs than in MCP joints of horses. The difference in response suggested that a lack of clinical osteoarthritis in the TMJ of horses could be attributable to a difference in cytokine response. (Am J Vet Res 2011;72:1586–1591)
inflammatory (TGF-β1, TGF-β2, and TGF-β3) cytokines and pathological dental conditions, although there was a slight increase in IL-8 concentrations with age.

The TMJ is a unique load-bearing synovial joint and the only one to be innervated by a cranial nerve. The surface of the joint is covered by fibrocartilage and not hyaline cartilage as is found in the appendicular joints.6,8 In a recent study12 in mice, chondrocytes from the mandibular condyle were found to be phenotypically unique from hyaline cartilage chondrocytes originating from the stifle joints. Because of these anatomic and phenotypic variations, generalizations between the TMJ and appendicular joints should be made with caution, especially with regard to responses to inflammation.9 These differences become more interesting when the frequency of osteoarthritis in the joints of the appendicular skeleton is compared with that in the TMJ in horses.

One possible explanation is that the TMJ responds to inflammation in a manner similar to that of all other synovial joints and that disease is common but clinical signs are subtle or nonexistent and thus are missed. Another possibility is that for some currently undetermined reason, the TMJ responds to inflammation in a different manner similar to that of other synovial joints but disease is rare (or that the pain and limitation to movement may be self-limiting and cease after a short period, as has been reported for TMJ disease in humans),13 despite massive repetitive motion over time. Finally, the TMJ in horses may respond to inflammation in an extremely different manner than that of other synovial joints; hence, there is a rarity of clinical disease in the TMJ. Previous studies2,4,5,6 have clearly indicated that the TMJ in horses responds to inflammatory mediators. The possibility that TMJ disease in horses is common but systematically missed is unlikely given that there is a previous study on osteoarthritis (especially if one discounts osteoarthritis secondary to synovial sepsis) of the TMJ and that other joints are commonly affected.

The purpose of the study reported here was to determine the response of the TMJ of horses to induced inflammation, compared with the response of the MCP (fetlock) joint, which is commonly affected by chronic degenerative osteoarthritis. The null hypothesis was that there would be no difference in cytokine concentrations of IL-6, TNF-α, and TGF-β between the TMJ and MCP joint of horses receiving an intra-articular injection of LPS. The alternate hypothesis was that the response of the TMJ to inflammation differs from that of the MCP joint in horses.

Materials and Methods

Animals—Seven horses (5 Quarter horses and 2 Paint horses; 5 mares and 2 geldings) were enrolled in the study. Age of the horses was not known; however, they were estimated to be between 5 and 10 years of age on the basis of dental examination. Results of physical examination and oral examination were unremarkable. All horses were free of lameness (before and after flexion of the MCP joints) and had no evidence (visual or during manual palpation) of effusion in the TMJs or MCP joints. Evaluation of 4 standard radiographic projections of the MCP joints obtained for all horses revealed no signs of orthopedic disease. Horses were housed as a group in an outside pen with unlimited access to hay and water. All procedures involving the use of animals received approval from the University Committee on Animal Care and Supply at the University of Saskatchewan and were conducted in accordance with the guidelines established by the Canadian Council on Animal Care.

Experimental procedures—Horses were randomly allocated by a coin flip to receive LPS or saline (0.9% NaCl) solution in the right or left MCP joint and the right or left TMJ, respectively. The contralateral TMJ and MCP joint in each horse served as a control joint. At time 0, horses were restrained in stocks and joints were injected with 0.0025 µg of LPS, and the contralateral control joints were injected with an equivalent volume of sterile saline solution. Synovial fluid samples (approx 0.5 mL) were subsequently collected at 0, 1, 6, 12, and 24 hours from all saline- and LPS-injected joints in each horse. Samples were centrifuged at 4°C for 10 minutes at 13,000 X g. Supernatant was harvested and stored at −80°C until analysis.

Horses were assessed for clinical signs of inflammation at each of the time points at which samples were collected. Assessment criteria included detection of effusion, heat or signs of pain during palpation of TMJs or MCP joints, forelimb lameness, and range of motion of the MCP joints. In the interval between samples, horses were allowed out of the stocks and given access to round-bale grass hay and water. Feeding behavior was recorded (as a marker of signs of TMJ pain). Phenylbutazone6 (4.4 mg/kg, IV) was administered to all horses at the termination of the study (24 hours after injection of LPS or saline solution).

Analysis of synovial fluid—Concentrations of IL-6 and TGF-β were quantified via ELISAs. Synovial fluid was diluted 1:2 in lysis buffer (150mM NaCl, 1% nonyl phenoxypolyethoxylethanol, 0.5% sodium deoxycholate, 0.1% SDS, 50mM tris [pH, 8.0], 5mM EDTA, and protease inhibitor cocktail [100 µL/10 mL]) and used for the assays. The protein content was quantified by use of the Bradford dye binding assay, with bovine serum albumin used as a standard. For cytokine analysis, ELISA plates were coated with antigen and bovine serum albumin diluted in carbonate buffer (pH, 7.5), and incubation for 90 minutes at 22°C. Primary antibodies (100 µL) were used at a concentration of 1 µg/mL for the detection of IL-6 and TGF-β were anti-equine IL-6 polyclonal antibody and anti-human TGF-β polyclonal antibody, respectively. Primary antibodies were allowed to bind to the respective antigens at 22°C for 2 hours. Plates were washed with PBS solution containing 0.05% Tween-20 and incubated with horseradish peroxidase–conjugated secondary antibody (1:5,000 [vol/vol]) for 2 hours at 22°C, which was followed by the addition of color detection reagents. Absorbance was measured by use of a microplate reader set at 450 nm. Tumor necrosis factor-α was quantified by use of an equine TNF-α ELISA kit as per the manufacturer’s instructions. All samples were tested in duplicate.
Table 1—Mean ± SD cytokine and total protein concentrations in synovial fluid samples collected from TMJs and MCP (fetlock) joints injected with LPS or with saline (0.9% NaCl) solution in 7 horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Joint</th>
<th>Time</th>
<th>Time of injection of LPS or saline solution was designated as time 0.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1 hour</td>
</tr>
<tr>
<td>TGF-β (µg/mL)</td>
<td>LPS-treated TMJ</td>
<td>0.524 ± 0.07</td>
<td>0.499 ± 0.05</td>
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<tr>
<td></td>
<td>Saline solution–treated TMJ</td>
<td>0.552 ± 0.07</td>
<td>0.526 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>LPS-treated MCP joint</td>
<td>0.548 ± 0.10</td>
<td>0.578 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Saline solution–treated MCP joint</td>
<td>0.617 ± 0.06</td>
<td>0.595 ± 0.06</td>
</tr>
<tr>
<td>TNF-α (µg/mL)</td>
<td>LPS-treated TMJ</td>
<td>1,017.2 ± 253.5</td>
<td>7,177.3 ± 2,227.7</td>
</tr>
<tr>
<td></td>
<td>Saline solution–treated TMJ</td>
<td>65.9 ± 44.5</td>
<td>105.8 ± 58.2</td>
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<tr>
<td></td>
<td>LPS-treated MCP joint</td>
<td>237.4 ± 54.0</td>
<td>447.3 ± 312.0</td>
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<tr>
<td></td>
<td>Saline solution–treated MCP joint</td>
<td>212.8 ± 200.5</td>
<td>174.5 ± 176.1</td>
</tr>
<tr>
<td>IL-6 (µg/mL)</td>
<td>LPS-treated TMJ</td>
<td>0.448 ± 0.054</td>
<td>0.446 ± 0.056</td>
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<tr>
<td></td>
<td>Saline solution–treated TMJ</td>
<td>1.050 ± 0.125</td>
<td>1.080 ± 0.179</td>
</tr>
<tr>
<td></td>
<td>LPS-treated MCP joint</td>
<td>0.396 ± 0.038</td>
<td>0.331 ± 0.059</td>
</tr>
<tr>
<td></td>
<td>Saline solution–treated MCP joint</td>
<td>0.488 ± 0.068</td>
<td>0.480 ± 0.078</td>
</tr>
<tr>
<td>Total protein (µg/mL)</td>
<td>LPS-treated TMJ</td>
<td>393.00 ± 143.02</td>
<td>330.81 ± 181.88</td>
</tr>
<tr>
<td></td>
<td>Saline solution–treated TMJ</td>
<td>722.76 ± 123.90</td>
<td>743.9 ± 151.52</td>
</tr>
<tr>
<td></td>
<td>LPS-treated MCP joint</td>
<td>308.48 ± 148.55</td>
<td>278.48 ± 75.28</td>
</tr>
<tr>
<td></td>
<td>Saline solution–treated MCP joint</td>
<td>1,656.31 ± 475.71</td>
<td>1,341.75 ± 203.91</td>
</tr>
</tbody>
</table>

Statistical analysis—Data were entered into a commercial spreadsheet program.1 Descriptive statistics were used to summarize the reported outcomes of each treatment group for both the TMJs and MCP joints at each time period.

The absolute change in the measured concentrations of all cytokines and total protein after the injections of LPS or saline solution were determined for samples obtained 1, 6, 12, and 24 hours after the start of the study by subtracting the concentration measured in each joint at time 0. The resulting differences from time 0 were examined for each outcome in a mixed model that included fixed effects for time (1, 6, 12, and 24 hours), treatment (LPS or saline solution), joint type (TMJ or MCP joint), and all interactions between time, treatment, and joint type.1 The model accounted for both the similarity of observations within each horse by use of a random intercept for horse and repeated measures within individual joints over time in an autoregressive correlation structure. The model results were examined to identify changes in the differences after time 0 within each joint and the differences between LPS- and saline solution–treated joints at each time point. Only values with significant (P < 0.05) differences were reported. Least squares mean and SE values generated by use of the model were plotted to illustrate significant differences between LPS-treated TMJs and MCP joints at each time point during the study.

Results

Results of the cytokine and total protein analyses were summarized for each joint on the basis of time of sample collection and treatment group (Table 1). There were no significant (P > 0.15) differences in the adjusted IL-6 concentrations within individual joints over time, between LPS-treated and saline solution–treated joints of 1 type at any time point, or between LPS-treated TMJs and MCP joints at any time point (Figure 1).

Mean adjusted TGF-β concentrations were significantly higher in both the saline solution–treated (0.073 µg/mL; P = 0.029) and LPS-treated (0.088 µg/mL; P = 0.009) TMJs and also in the LPS-treated MCP joints (0.066 µg/mL; P = 0.033) at 1 hour, compared with concentrations at 24 hours. Similarly, the mean adjusted concentration of TGF-β was significantly (P = 0.013) higher at 12 hours (0.083 µg/mL) than at 24 hours in the LPS-treated TMJs. The adjusted concentration of TGF-β at 24 hours was significantly (P = 0.013) higher in the saline solution–treated MCP joints than in the saline solution–treated TMJs (0.083 µg/mL). Similarly, adjusted concentrations of TGF-β at both 12 (0.066 µg/mL; P = 0.048) and 24 (0.087 µg/mL; P = 0.010) hours were significantly higher in the LPS-treated MCP joints than in the LPS-treated TMJs (Figure 2).
The adjusted concentrations of TNF-α were significantly higher in the LPS-treated TMJs at 1 (5.466 µg/mL; \( P = 0.007 \)) and 6 (5.491 µg/mL; \( P = 0.005 \)) hours than at 24 hours after injection. The adjusted TNF-α concentrations were also significantly higher in LPS-treated TMJs, compared with concentrations in saline solution–treated TMJs, at 1 (3.770 µg/mL; \( P = 0.002 \)) and 6 (3.676 µg/mL; \( P = 0.001 \)) hours after LPS treatment. Finally, the adjusted TNF-α concentrations were significantly higher in the LPS-treated TMJs, compared with concentrations in LPS-treated MCP joints, at 1 (4.798 µg/mL; \( P = 0.03 \)) and 6 (4.787 µg/mL; \( P = 0.02 \)) hours after treatment (Figure 3).

The mean adjusted total protein concentration was significantly higher in the saline solution–treated MCP joints than in the TMJs at 6 (691 µg/mL; \( P = 0.001 \)), 12 (426 µg/mL; \( P = 0.008 \)), and 24 (352 µg/mL; \( P = 0.002 \)) hours after injection. The adjusted total protein concentration was also significantly (\( P = 0.004 \)) higher in the saline solution–treated MCP joints than in the LPS-treated MCP joints (667 µg/mL) at 6 hours after injection. The LPS-treated TMJs had lower adjusted total protein concentrations than did LPS–treated MCP joints at 1, 12, and 24 hours after injection, but these differences were not significant (Figure 4).

All LPS-treated joints had clinical signs of inflammation at 1, 6, 12, and 24 hours. This was determined by detection of effusion, heat, and signs of pain during palpation of the TMJs and MCP joints, lameness while walking, and decreased range of motion in the MCP joints. In 2 saline solution–injected MCP joints, we detected heat and signs of pain during palpation of the joint capsule; none of the saline solution–injected TMJs had evidence of inflammation.

All horses were observed eating throughout the 24 hours of the experiment. Subjectively, there was no apparent alteration of masticatory movements.

**Discussion**

In extensively managed horses (ie, those that spend most [if not all] of their time at pasture, rather than being in a box stall or housed in a barn), grazing occupies approximately 14 h/d.\(^{13} \) This constitutes a high number of repetitive movements of the TMJ during the lifetime of a horse; however, the incidence of reported disease (not withstanding septic arthritis or degenerative joint disease secondary to septic arthritis) of the TMJ is low.

The hypothesis that there would be no difference in concentrations of IL-6, TNF-α, and TGF-β between the TMJs and MCP joints of horses receiving intraarticular injections of LPS was rejected. Analysis of the data supported our alternate hypothesis, namely that the TMJ in horses responds differently to LPS-induced inflammation than does the MCP joint in that the TMJ is better able to control acute inflammation.

Acute inflammation, such as that induced in response to LPS, is characterized by activation of cells such as macrophages, elaboration of cytokines, and recruitment of cells such as neutrophils and monocytes.\(^ {14-17} \) The role of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β in the early phase of LPS-induced inflammation has been established. In the present study, no differences were observed in IL-6 concentrations within and between saline solution– or LPS–treated MCP joints and TMJs. We focused our attention on the expression of TNF-α in the LPS–treated MCP joints and TMJs and detected an increase in TNF-α concentrations in the treated TMJs. An increased concentration of TNF-α has also been established in the induction of LPS–induced inflammation.\(^ {18-20} \) Increased concentrations of TNF-α have been reported in osteoarthritis and following rupture of the cranial cruciate ligament in dogs, and TNF-α is most likely produced by the synovial membranes or new inflammatory cells recruited into the joints.\(^ {21,22} \) Interestingly, the LPS-
treated TMJs had higher concentrations of TNF-α, compared with concentrations in the LPS-treated MCP joints. Similarly, the total protein concentrations were higher in TMJs, compared with concentrations in the MCP joints, at 6 hours after LPS treatment, although the saline solution–treated MCP joints had higher protein concentrations than did the TMJs. Protein concentration in the inflammatory effusion is an indication of altered microvascular permeability, which, in turn, is regulated by various inflammatory cytokines. The present study does not provide any explanation for the differences in TNF-α and protein concentrations between the 2 joints, although 1 difference may be in the nature of the synovial membrane, especially in its vascularity. Nevertheless, the LPS-induced inflammation in TMJs suggests the presence of toll-like receptor-4 in TMJs and the ability of TMJs to respond in an inflammatory manner in the face of agonists.

In contrast, the LPS-treated MCP joints in the present study had an increased concentration of TGF-β, compared with concentrations in the LPS-treated TMJs. It is generally accepted that TGF-β is an anti-inflammatory cytokine that is believed to block or inhibit activation of lymphocytes and macrophages. However, there are data that indicate TGF-β increases vascular permeability and its inhibition protects mice against endotoxin-induced edema and death. The later phase of inflammation provoked by a single challenge with endotoxin generally has resulted in increased TGF-β concentrations in concert with an influx of monocytes and apoptotic death of migrated neutrophils. The role of TGF-β may be important because it inhibits TNF-α–induced expression of matrix metalloproteinases in inflamed tissues, which may be evaluated in further experiments. Most evidence indicates an anti-inflammatory role for TGF-β, which also fits with the higher concentration of TNF-α in synovial fluid of TMJs along with a lower concentration of TGF-β.

Results of the present study should be interpreted cautiously, especially with regard to the differences in cytokine concentrations between MCP joints and TMJs. The cytokine concentrations in controlled anatomic spaces such as joint cavities are likely to be determined by the baseline presence of responder cells, vascularity of the synovial membrane, presence of receptors such as toll-like receptor-4 that bind LPS, and volume of the synovial fluid in the joint cavity. Additionally, inflammation is a highly complex response that involves many cytokines and their interactions with each other. Therefore, cytokines other than those evaluated in the present study may play important roles. Although we had planned to evaluate the expression of IL-8, IL-1, IL-10, and interferon-γ, insufficient volume of joint fluid precluded their analysis. There was an LPS-dilution effect in the joints, which favored the MCP joint. Because the MCP joint is larger than the TMJ, the standard LPS concentration and volume that was injected into the MCP joints and TMJs would have resulted in a greater dilution of the LPS and dilution of the cytokines in the MCP joints and, potentially, in a cytokine-concentrating effect in the TMJs. This may have confounded our analysis. There was also a volume-depletion effect in each joint, which again favored the MCP joint. The standard volume removed at each time point resulted in more rapid depletion of the small joint (ie, TMJ) than the large MCP joint. This could also have confounded our analysis. The LPS-induced inflammation in TMJs did not cause an apparent change in feeding behavior during the study, which is in contrast to the experience of one of the authors (JLC) that horses with unilateral TMJ inflammation (sepsis or periarticular abscesses with reactive synovitis) have clinically obvious dysmastication with a marked reluctance or inability to open their mouths. One of the reasons for this difference in clinical behavior may be the complex nature of agonists involved with processes such as sepsis or abscessation in clinical cases, compared with the use of LPS in the present study. Therefore, further carefully controlled studies with volume adjustments are required to address the physiologic process of the inflammatory response in TMJs.

Analysis of the data for the study reported here indicated that the acute inflammation in TMJs differs from that in MCP joints as evident by higher concentrations of TNF-α and protein in conjunction with lower concentrations of TGF-β. Analysis of the data suggested that within the time frame of the study, TMJs have a greater inflammatory response, compared with that of MCP joints, and the response of TMJs is also better able to dampen acute joint inflammation, compared with that of MCP joints. However, the complete mechanism has not been elucidated and further studies are needed to evaluate the complex set of pathways involved in the immune response of joints. These include repeating the study reported here by use of a joint volume–adjusted LPS concentration, measurement of substance P concentrations, evaluation of cartilage oligomeric matrix protein II as an inflammatory mediator of interest, and performing complete and differential WBC counts.

References

6. Weller R, Cauvin ER, Bowen IM, et al. Comparison of radiol-