Introduction
Temporomandibular Joint Disorders (TMDs) are a common cause of facial pain. Although the etiology of TMDs is yet to be fully elucidated, histologic and arthroscopic studies strongly suggest that the inflammatory process is similar to that seen in osteoarthritis of other synovial joints. The Receptor for Advanced Glycation Endproducts (RAGE) is a multiligand receptor that is present on T and B lymphocytes, cells of the monocyte/macrophage lineage, vascular cells, and many tumor cells. RAGE is expressed at low levels in most normal tissues and is upregulated where its ligands are present. This receptor binds multiple ligands including a number of Advanced Glycation Endproducts (AGEs). RAGE is emerging as a key inflammatory mediator. In order to investigate the role that RAGE and its ligands play in joint inflammation, we have utilized a transgenic (tg) murine model that overexpresses human Tumor Necrosis Factor-α (tg hTNF-α), resulting in progressive inflammation and destruction of the synovial joints. To test the impact of genetic deletion of RAGE, these transgenic animals were bred into the RAGE null background. Our results demonstrate that these transgenic animals show progressive inflammation and joint destruction with increasing age, although there are significant differences in the degree of destruction between the TMJ and knee joints. Furthermore, tg hTNF-α/RAGE null mice fail to develop arthritic joint changes similar to that seen in the tg hTNF-α mice.

Materials and Methods
Mice: Transgenic hTNFα mice were bred into a C57BL/6 background. A RAGE null mouse model was cross-bred with tg hTNFα mice to yield tg hTNFα/RAGE null mice.

Weights and Lengths: To determine weights and lengths mice were sedated. The length from the base of the tail to the tip of the nose was recorded using a ruler. The mass of each mouse was measured using an electronic scale.

Joint Isolation: Mouse temporomandibular (TMJ) and knee joints were isolated aseptically, frozen and then homogenized in PBS. Total protein concentration for each sample was measured using a Lowry based protein assay kit.

Western Blot Analysis: Protein samples were loaded onto 4–12% PAGE gels and transferred to a nitrocellulose membrane. The membranes were incubated with the primary antibodies to RAGE, S100b and HMGB1. Secondary antibodies were applied for 30 minutes at RT. The membranes were then developed with Enhanced Chemiluminescence and exposed to X-ray film.

AGE ELISA: Protein samples and AGE standards were incubated in a 96 well plate overnight at 4°C. Following blocking, secondary anti-AGE antibody was added and incubated at RT for 3hrs. Secondary, HRP conjugated antibody was then added and incubated for 1hr at RT. Developing buffer was added to the wells. The reaction was stopped with dilute sulfuric acid and absorbance values were read at 490nm in a plate reader.

Microarray Analysis: Three and six month old female mouse knee and TMJs were surgically isolated and immediately frozen in liquid nitrogen. Six joints were pooled together for each sample in order to yield sufficient amounts of RNA. Joints were then homogenized in TRizol. RNA was extracted, precipitated and Affymetrix gene microarray analysis was performed.

Data Analysis: Analysis was performed by ANOVA and the Tukey-Kramer post-hoc test was performed for all pair-wise comparisons. Significance was set at p < 0.05.

Results
Arthritic mice at 4 and 5 months of age were shorter in length (Figure 1A) and lower in weight (Figure 1B) by approximately 20% and 30% respectively when the RAGE null background was introduced with tg hTNF-α mice animals, weight and lengths returned to control levels. Protein samples from knees (Figure 2A) and TMJs (Figure 2B) of tg hTNF-α mice (Lane 1) showed significant levels of RAGE and S100b at 55kDa and 24 kDa respectively compared with C57BL/6 (Lane 2) and tg hTNF-α/RAGE null (Lane 3) mice. Significant levels of HMGB1 were present in tg hTNF-α knees at 30 kDa (Lane 1) but not in C57BL/6 (Lane 2) and tg hTNF-α/RAGE null (Lane 3) mice (Figure 2C). HMGB1 in tg hTNF-α mouse TMJ samples was not detected (data not shown). Transgenic hTNF-α mice expressed significantly higher levels of AGEs than control mice (Figure 3). Interestingly, AGE levels were significantly increased in tg hTNF-α/RAGE null samples compared with tg hTNF-α mice. Microarray analysis yielded many inflammatory, TNF-associated, and joint degradation markers that were significantly upregulated in tg hTNF-α joints compared to C57BL/6 samples. Overall, knee joints had larger increases compared with TMJs. Matrix Metalloproteinase-3 showed a 5-fold increase in 6 month old tg hTNF-α TMJs while levels in the knees increased 45 fold. Prion protein, dbllet, and procollagen Type Xα1 were both significantly more upregulated in TMJ samples of arthritic mice compared to knee samples.

Discussion
The tg hTNF-α mouse appears to be a useful model of joint inflammation. Affected animals showed significant inflammatory changes with more severe disease in the knee joints when compared to the TMJs. Furthermore, RAGE upregulation was critical in arthritic progression as insertion of the RAGE null background into the tg hTNF-α mouse decreased a number of inflammatory mediators to baseline levels. Microarray analysis was consistent with other findings, where knee joints showed a more advanced arthritic disease than TMJs. Future studies will include microarray analysis of tg hTNF-α/RAGE null animals. Our studies indicate that RAGE blockade may have therapeutic value in the treatment of inflammatory arthropathies.

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