Effect of Aging and Interleukin-1β on the Production of Adipokines by the Infrapatellar Fat Pad

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Introduction: Obesity and aging increase the risk of knee osteoarthritis (OA) by altering joint loading and increasing the production of systemic inflammatory mediators. Adipose tissue is a key source of inflammatory cytokines and chemokines (i.e., adipokines) due to the infiltration of activated macrophages following adipocyte hypertrophy that occurs with obesity. Recent studies indicate that the infrapatellar fat pad (IFP) is a significant source of adipokines. IFPs harvested from osteoarthritic joints secrete higher levels of IL-6, adiponectin, and visfatin compared to subcutaneous fat (1,2). Stimulation with interleukin-1β (IL-1β) further increases the expression of IFP-derived adipokines (3). Aging also increases the production of adipokines, although adipocytes rather than macrophages are the primary source of aging-associated inflammation due to enhanced NF-κB activation (4). NF-κB activation is redox sensitive, suggesting that an age-related increase in oxidative stress contributes to adipose tissue inflammation. We sought to determine the effect of aging on basal and IL-1β-stimulated adipokine production by the IFP. We hypothesized that aging-dependent oxidative stress and IL-1β function synergistically to increase IFP-derived inflammation.

Methods: All experiments were conducted following an approved IACUC protocol. IFPs were harvested from 10, 20, and 30-month-old male F344BN F1 rats purchased from the NIH Aging Rodent Colony. We have previously reported that knee OA increases with age in this animal model. IFPs from the left knee were snap-frozen and the ratio of reduced to oxidized glutathione (GSH: GSSG) was measured in the absence or presence of 2-vinylpyridine (Glutathione Assay Kit, Cayman; N=3 per age). Contralateral IFPs were divided in half, weighed, and cultured ex vivo in DMEM culture media for 24 hours +/- 1 ng/ml IL-1β (R&D). Paired samples were treated +/- 2.5 mM N-acetylcysteine (NAC, Sigma) for 2 hours prior to and during IL-1β treatment. Conditioned culture media was collected and stored at -80 degrees C for adipokine protein quantification (leptin, R&D; CCL2, R&D; IFN-γ, IL-1β, IL-4, IL-5, IL-13, IL-8, TNF-α, MSD 7-plex; N=3-6 per age and condition). Values below the lowest level of detection were assigned a value of one-half this value for statistical purposes. Results were normalized to IFP wet weight, log-transformed to normalize data, and analyzed by 1- or 2-factor ANOVA with Holm’s-Sidak post hoc multiple comparisons test (p<0.05; Prism v6).

Results: The IFP wet weight decreased with age (10-mo 12.2±0.6mg, 20-mo 11.1±0.3mg, 30-mo 10.1±0.5mg, p=0.016). Aging increased the production of TNFα and IL-13 by 67% and 35%, respectively, between 10 to 30-months of age (p<0.05; Fig. 1,3). Aging also altered the production of IL-8, although in a different pattern, with values being lowest in the 20-month samples (p<0.05 versus 10 and 30 months). Other cytokines were not altered with aging. While IL-1β treatment increased the production of all adipokines, except for leptin, aging did not function synergistically with IL-1β treatment to increase adipokine production. Suprisingly, leptin production was reduced over 70% with IL-1β treatment in 10-month old samples, and it was not detected in 20- and 30-month IFP samples treated with IL-1β (Fig. 2). To evaluate the effect of aging on IFP oxidative stress, we compared GSH:GSSG levels in 10- and 30-month old IFP samples. GSH:GSSG levels were not significantly altered by aging (0.92±0.11 10-month old, 1.47±0.55 30-month old; p=0.38), indicating that aging does not generally increase IFP oxidation. Rather than reducing adipokine production, treatment with NAC increased the production of IFN-γ, IL-4, IL-5, IL-8 and IL-13 (p<0.05). These results, however, were only seen in 10- and/or 20-month old IFP samples (e.g., Fig. 3). NAC did not alter adipokine production in 30-month samples (except for IL-4) or in any samples treated with IL-1β regardless of age.

Discussion: We found that basal production of select pro- and anti-inflammatory cytokines increase with age in the IFP, as indicated by increased levels of TNFα and IL-13, respectively. Contrary to our hypothesis, however, these changes were not associated with an increase in IFP oxidation as indicated by a consistent ratio of reduced:oxidized glutathione in 10- and 30-month old samples. Furthermore, TNFα and IL-13 production were not reduced by treatment with the antioxidant NAC. Rather, NAC treatment had the greatest effect on younger IFP samples, increasing the production of numerous pro- and anti-inflammatory cytokines in 10- and 20-month old IFP samples. Aging and IL-1β decreased the sensitivity of the IFP to antioxidant treatment, which is contrary to expectations and indicates a declining sensitivity to antioxidant interventions with increasing age or under pro-inflammatory conditions, such as joint injury. Our most surprising finding was that IL-1β treatment decreased the production of leptin by the IFP in an age-dependent manner. Leptin functions as a pro-inflammatory and pro-catabolic mediator in cartilage (5), and these findings suggest that increased levels of IL-1β negatively regulate the local production of leptin in the joint. Future studies are needed to understand the mechanism by which IL-1β regulates leptin secretion by adipocytes in the IFP.
In summary, we find that aging significantly alters the basal production of adipokines involved in joint tissue inflammation and the sensitivity of the tissue to antioxidant treatments. These results support age-dependent therapeutic approaches for modifying soluble mediators of joint inflammation.

**Significance:** Inhibition of joint inflammation is considered an important therapeutic approach for reducing the risk of OA due to aging, obesity, or joint injury. Here we show antioxidant treatments stimulate rather than inhibit the production of soluble pro- and anti-inflammatory mediators from infrapatellar fat in an age-dependent manner.

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**References:**

![Figure 1. Aging increases the basal production of TNF-alpha by the IFP. IL-1beta treatment further increases TNF-alpha, although the effect diminishes with increasing age. *p<0.05 versus 10-mo value (within treatment). *p<0.05 for IL-1beta treatment (within age).](image-url)
Figure 2. IL-1beta treatment decreases IFP leptin production in an age-dependent manner. *p<0.05 for IL-1beta treatment (within age).
Figure 3. Treatment with the antioxidant n-acetylcysteine (NAC) increases IL-13 production in 10- and 20-month old IFP samples. #p<0.05 versus 10-mo value (within treatment). *p<0.05 for NAC treatment (within age).