Chronic Ingestion of Advanced Glycation End Products Induces Degenerative Spinal Changes and Hypertrophy in Aging Pre-Diabetic Mice

Svenja Illien-Junger, PhD\(^1\), Young Lu\(^1\), Sheeraz Qureshi\(^1\), Andrew C. Hecht, MD\(^1\), Weijing Cai, MD\(^2\), Helen Vlassara, MD\(^2\), Gary E. Striker, MD\(^3\), James C. Iatridis, PhD\(^1\).

\(^1\)Leni & Peter W. May Department of Orthopaedics/Icahn School of Medicine at Mount Sinai, New York, NY, USA, New York, NY, USA, \(^2\)Department of Geriatrics and Palliative Care, Division of Experimental Diabetes and Aging/Icahn School of Medicine at Mount Sinai, New York, NY, USA, \(^3\)Department of Geriatrics and Palliative Care, Division of Experimental Diabetes and Aging, and Division of Nephrology, Department of Medicine/Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Disclosures:

Introduction: Elderly, obese and diabetic individuals have a high incidence of back pain caused by intervertebral disc (IVD) degeneration and spinal degenerative changes [1], and these associations are likely related to increased systemic and local inflammation. The risk of developing diabetes mellitus is increased by the consumption of modern diets that are rich in calories and fat, particularly if they involve cooking at high temperatures that result in high levels of advanced glycation end products (AGEs). AGEs are well known to be associated with vascular calcification, increased oxidant stress, and inflammation [2,3]. We have previously shown that diabetic mice develop IVD degeneration which was correlated with intradiscal AGE accumulation and increased TNFα production [4]. The aim of this study was to investigate the role of a specific AGE, the carbonyl precursor methylglyoxal (MG), on IVD and vertebral pathology in aging, pre-diabetic mice.

Methods: C57BL/6 mice were pair-fed isocaloric diets with either the standard amount of AGEs, e.g. methylglyoxal-derivatives (MG+: 1.8x10^4 nmol/day; n = 9) or reduced AGEs (MG−: 0.65x10^4 nmol/day, p<0.01; n = 11) for 5 generations. Mice (F5) were sacrificed after 18 months and lumbar spines were dissected. Spines were fixed and µCT analysis was performed for trabecular bone in vertebrae and endplates (EPs), cortical bone, bone mass, vertebrae and IVD dimensions. Calcified spines were plastic embedded and vertebrae were sectioned for histology (extended FAST, von Kossa) and immunohistochemistry for the AGEs (Nε-carboxymethyl)lysine (CML) and MG, as well as COL10, TNFα and ADAMTS5). Student’s t-tests determined significance (p<0.05).

Results: MG+ mice, were insulin resistant but not hyperglycemic and had significantly higher serum CML (0.47±0.04 nmol/l vs. 0.27±0.01 nmol/l) and body weight (35.1±1.8 vs. 29±0.52g) compared to MG− mice, suggesting a type 2 pre-diabetes model. The cortical thickness and surface area (p=0.029 and p=0.047 respectively) was increased in vertebrae of MG+ mice and the superior EP had a significantly higher bone mineral density but decreased connectivity density compared to that of MG− mice (p=0.016 and p=0.0062; Figure 1, 2 A+B), suggesting inferior bone quality. Ectopic calcification deposits were detected by von Kossa stain in both, nucleus pulposus (NP) and annulus fibrosus regions of MG+ mice (Figure 2C). Further, the notochordal band in MG+ mice appeared disorganized and COL10 immunostaining was highly positive (Figure 3A+B). In MG+ mice the glycosaminoglycan (GAG) rich region of the NP was thinned and weakly stained (Figure 4A). In contrast, the GAG rich region in MG− mice was thick and intensely stained (Figure 4B), the notochordal band was more organized and COL10 staining appeared less prominent (Figure 2C+D). Immunostaining for MG suggested greater accumulation in vertebrae and EPs of MG+ mice compared to MG− mice.

Discussion: Chronic ingestion of oral AGES promoted early degenerative changes in vertebrae, vertebral EPs, and IVDs in aging mice. We observed a significant increase of bone mass in the insulin resistant pre-diabetic MG+ mice compared to MG− mice whose diet contained reduced amounts of AGES. Diabetic human patients have similarly increased bone mass compared to their age matched controls, although the increased bone mass also involved reduced bone quality and greater fracture risks [5]. The decrease in bone quality in insulin resistant MG+ mice becomes apparent with analyzing the vertebral EPs. Here, the superior but not the inferior EPs were higher in bone mass but had a significantly decreased connectivity density suggesting inferior bone quality and increased fracture risk. Such regional specific variations in bone density and quality have been previously reported and are explained by altered anatomy and loading patterns [6]. MG+ induced spinal degeneration may also be due to systemic or local inflammation known to occur in diabetic models [4] as well as pathological calcification of the EP. MG accumulation was mainly observed within EPs of MG+ mice and indicates that degenerative effects are largely attributable to exogenous AGES which have accumulated in vertebrae and EPs. EP calcification can block the capillary pores and lead to impairment of nutrient supply and IVD metabolic processes. Increased COL10 staining within the NP together with the von Kossa calcification staining within the IVD supports impaired metabolic processes and hypertrophy of IVD cells. Moreover, the NP of MG− mice was rich in GAG, suggesting it was healthy, an important factor for its ability to retain water and to resist compression due to loading. Decreased GAG and calcifications in IVDs of MG+ mice suggests that GAGs in hypertrophic IVDs may have undergone more extensive degradation, consistent with the relationship between pathological calcification and proteoglycan degradation.
previously reported in an ovine model [7]. MG+ mice had notochordal cells which appeared more disorganized and resembled a more degenerative phenotype compared to MG- mice, as reported in diabetic mice [4], suggesting accelerated aging in MG+ mice. Results indicate that quality of food consumed (and not just quantity) is an important factor in accelerated aging of spinal structures since all mice were fed an isocaloric diet but MG+ mice had greater body weight, abdominal obesity, and insulin resistance than MG- mice. These data reinforce our previous studies showing that diabetes induces spinal pathologies and IVD degeneration [4], highlights a clear role for AGE accumulation and hypertrophy in that degenerative process, and is among the first studies to show how a poor diet, particularly those high in MG, and pre-diabetes or frank diabetes may be factors in musculoskeletal diseases.

**Significance:** This study identifies a relationship between spinal pathology and AGE accumulation in aging pre-diabetic mice and indicates that diet rich in AGEs can induce heterotopic calcification and IVD degeneration. The pathophysiology of diet and diabetes induced spinal pathology may provide a mechanism for age-accelerated IVD degeneration and lead to early and minimally invasive interventions to treat painful musculoskeletal conditions in the general public.

**Acknowledgments:** Funded by NIH/NIAMS (RO1 AR051146 & RO1 AG023188). The authors thank Damien Lauder and Yury Borisov for their technical contribution.


Figure 3: Representative images of COL10 immunohistochemistry for MG+ (A) and MG- (B) mice. Brown stain indicates COL10 presence. COL 10 is highly expressed in NP of MG+ mice (C) compared to MG- mice (D).
Figure 1. \(\mu\)CT analyses of MG+ and MG- vertebral endplates. *\(p<0.05\) +\(p=0.09\)
Figure 4: Histology and Immunohistochemistry of intervertebral discs. A+B: Extended FAST of MG+ (A) and MG- (B) Red-orange = proteoglycan content. C+D: Immunohistochemistry for MG (brown) of MG+ (C) and MG- (D). High dietary AGEs promoted loss of proteoglycan in the intervertebral disc (white arrow) and vertebral endplate accumulation of MG (black arrow). Scale bar=200 μm