



Effects of Glucose and Insulin Levels on Intervertebral Disc Metabolic Responses In an *in vitro* Rat Tail Model

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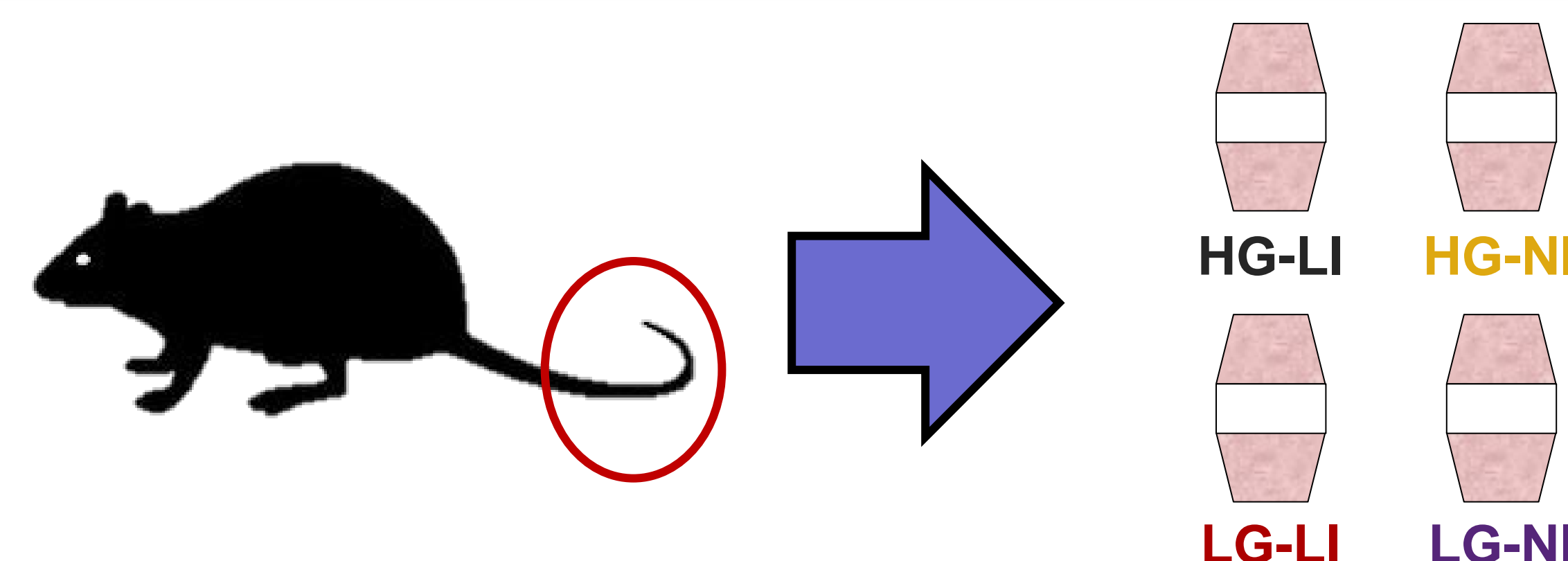
Introduction and Purpose

- Intervertebral disc (IVD) degeneration is associated with debilitating neck and low back pain, and there is evidence that there is a relationship between diabetes on IVD degeneration
- The metabolic effects of diabetes, and varying levels of glucose and insulin, on the IVD have not been established
- This study was designed to characterize the metabolic responses of IVDs varying concentrations of glucose and insulin using a rat tail whole organ IVD culture model

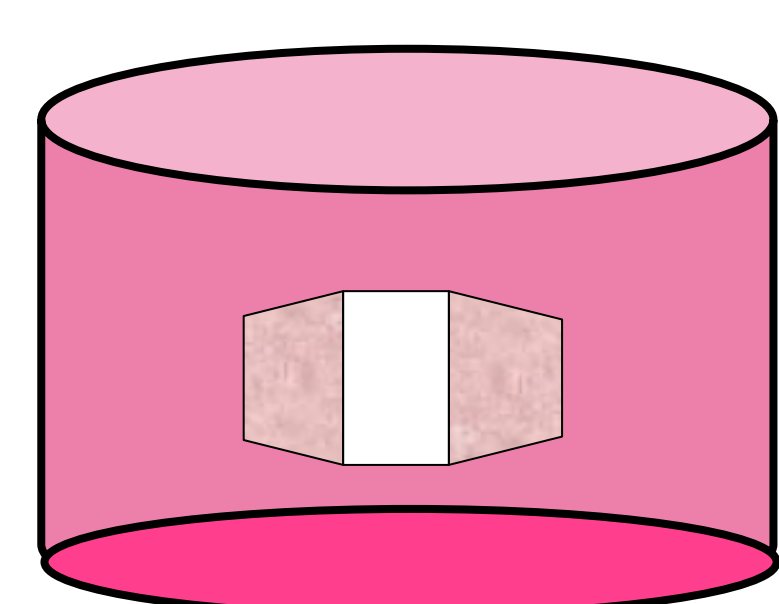
Hypothesis

- IVDs cultured in low insulin conditions will produce significantly higher levels of pro-inflammatory biomarkers compared to IVDs cultured in normal insulin conditions
- IVDs cultured in low glucose conditions will produce significantly higher levels of pro-inflammatory biomarkers compared to IVDs cultured in high glucose conditions

Methods



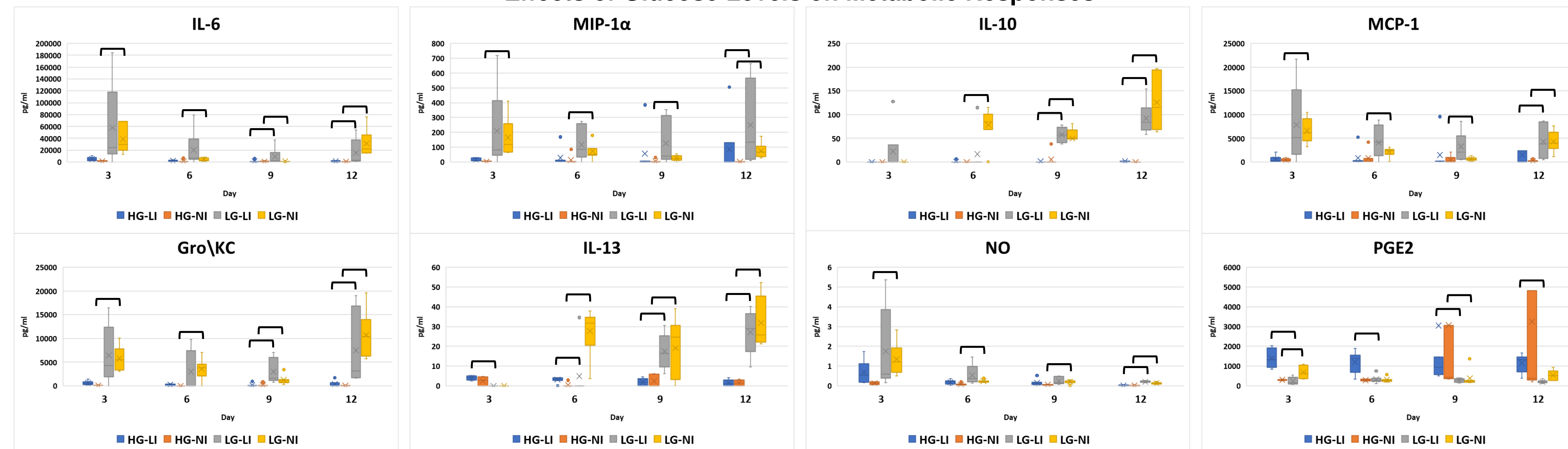
- With IACUC approval (ACUC#9435), tails were collected from skeletally mature Sprague Dawley rats (n=6) euthanatized for reasons unrelated to this study.
- IVD whole organ explants (n=24) were created and were randomly assigned to one of four culture groups based on media insulin (1 µg/ml low insulin [LI] or 10 µg/ml normal insulin [NI]) or glucose (4500 µg/ml high glucose [HG], or 1000 µg/ml low glucose [LG]) concentration.



- Explants were cultured for 12 days with media changed and collected on day 3, 6, 9, and 12 for biomarker analysis
- Media were analyzed for concentrations of NO, PGE2, MIP-1α, IL-6, IL-10, MCP-1, GRO-KC, and IL-13.
- A Kruskal Wallce with post-hoc analysis and Bonferroni correction were used to determine significant differences between groups at each time point with significance set at p<0.05.

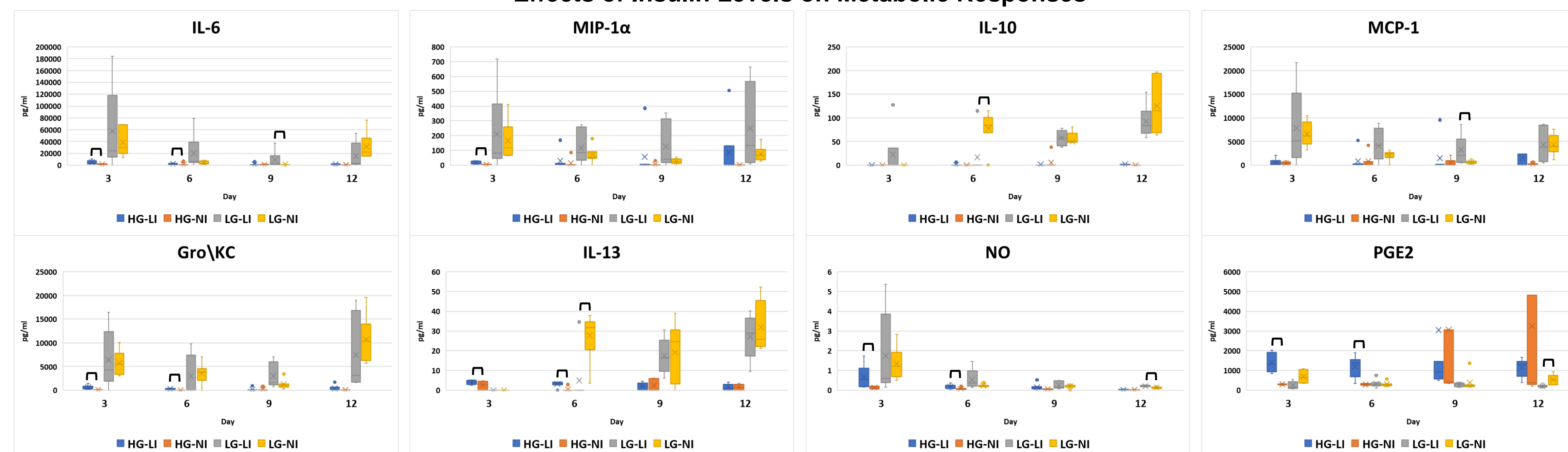
Results

Effects of Glucose Levels on Metabolic Responses



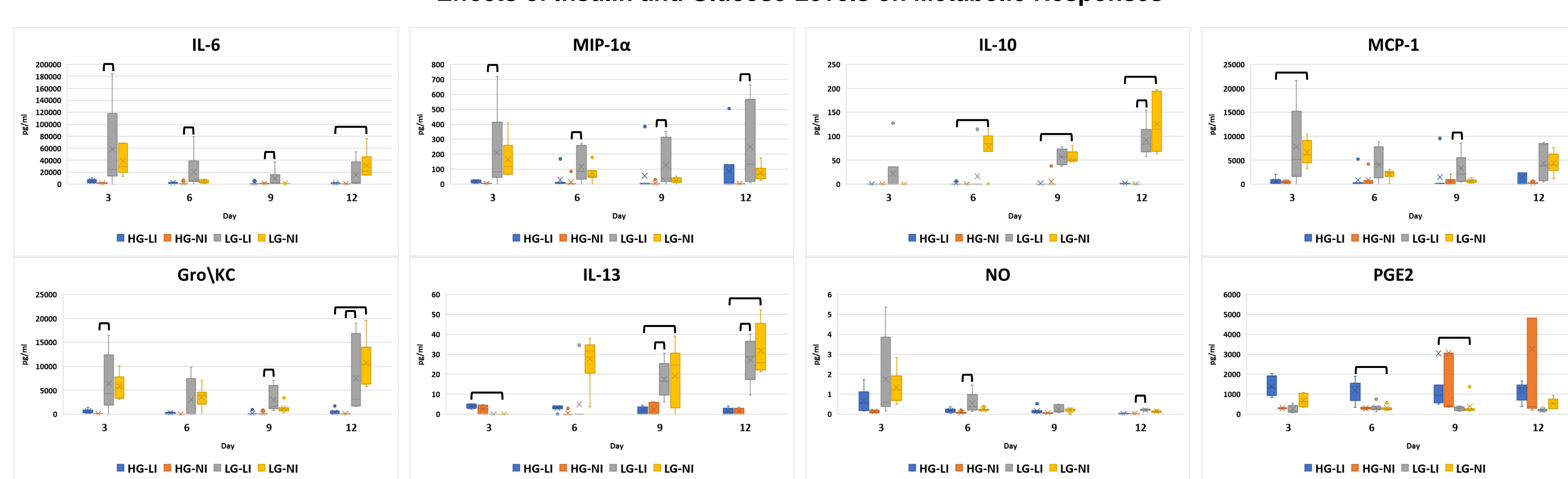
- The HG-LI group produced significantly higher levels of IL-13 (days 3-6), PGE2 (days 3-12), and IL-6 (days 9-12), and significantly lower levels of IL-13 (days 9-12), MIP-1α (day 12), IL-10 (days 9-12), GRO/KC (days 9-12), and NO (Day 12), compared to the LG-LI during culture.
- The HG-NI group produced significantly higher levels of PGE2 (day 9), and significantly lower levels of IL-13 (days 6-12), PGE2 (day 3), MIP-1a (day 3-12), IL-6 (days 3-12), IL-10 (days 6-12), MCP-1 (day 3-12), GRO/KC (days 3-12) and NO (days 3-12) compared to the LG-NI group during culture.

Effects of Insulin Levels on Metabolic Responses



- The HG-LI group produced significantly higher levels of IL-13 (Days 3-6), PGE2 (days 3-6), MIP-1a (day 3), IL-6 (days 3-6), GRO/KC (days 3-6), and significantly lower levels of NO (days 3-6), compared to the HG-NI group during culture.
- The LG-LI group produced significantly higher levels of IL-6 (day 9), MCP-1 (day 9), and NO (day 12), and significantly lower levels of IL-13 (day 6), PGE2 (day 12), and IL-10 (day 6), compared to the LG-NI group during culture.

Effects of Insulin and Glucose Levels on Metabolic Responses



- The LG-LI group had significantly higher production of MIP-1a (days 3-12), IL-13 (days 9 and 12), IL-6 (days 3-9), IL-10 (day 12), GROKC (days 3, 9, 12), MCP-1 (day 9), and NO (days 6 and 12) compared to the HG-NI group.
- The LG-NI group had significantly higher production of MCP-1 (day 3), IL-10 (days 6-12), IL-13 (days 9 and 12), IL-6 (day 12), and GRO\KC (day 12), and significantly lower production of IL-13 (day 3) and PGE2 (days 6 and 9), compared to the HG-LI group.

Conclusions

- The data from this study indicate that inflammatory metabolic responses of IVDs are sensitive to changes in glucose and insulin levels
- A low-glucose environment appears to be more pro-inflammatory than a high-glucose environment, and low insulin conditions appears to exacerbate the effects of glucose concentration
- These results suggest that insulin and glucose levels may play direct roles in exacerbating the inflammation-mediated effect on IVDs in diabetic patients