

PHENOLIC PRESERVATIVE REMOVAL FROM COMMERCIAL INSULIN FORMULATIONS REDUCES TISSUE INFLAMMATION, WHILE MAINTAINING EUGLYCEMIA

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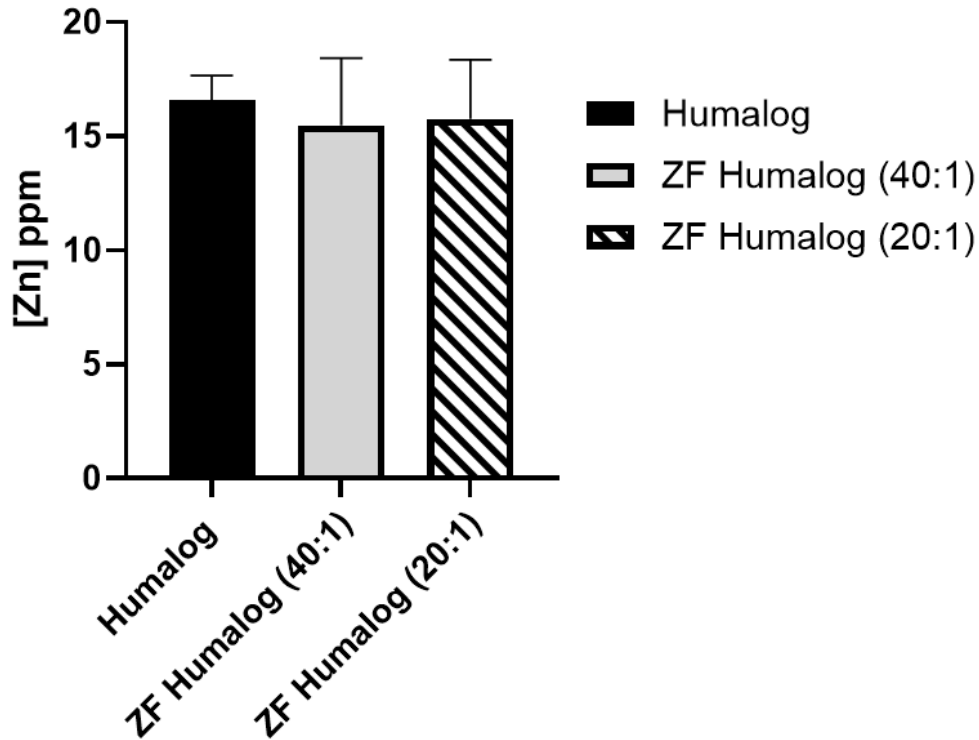


Figure S-1: ICP-MS for Zinc Filtration: Zeolite Y filtered Humalog samples were submitted to the Lumigen core facility for ICP-MS analysis. Unfiltered Humalog used as a control. This data demonstrates the selective exclusion of phenol and m-cresol by the zeolite.

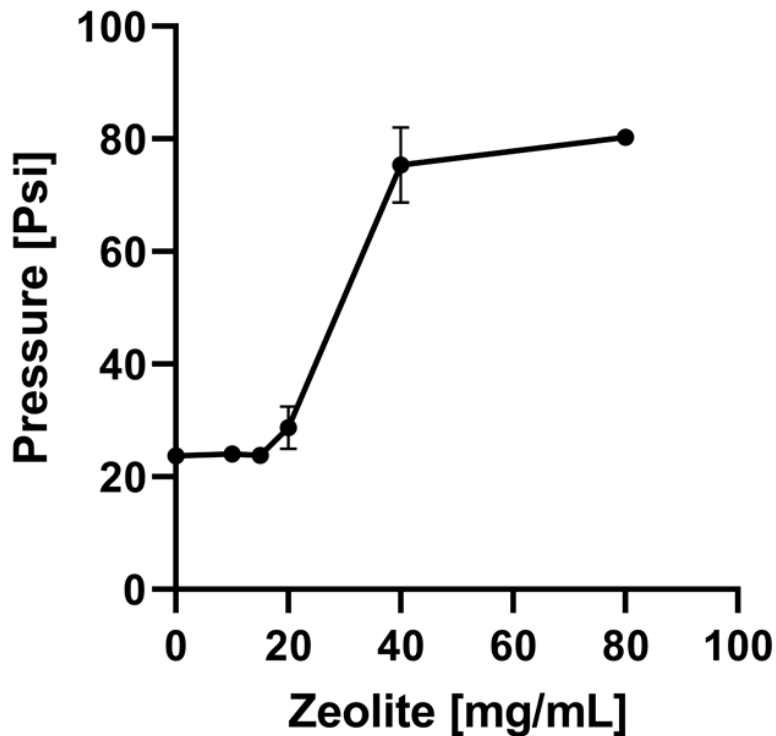


Figure S-2: In-line Pressure Monitoring: As the amount of Zeolite Y in the in-line filter system increased, in-line backpressure increased proportionally. This data demonstrates the need to minimize zeolite/ alleviate pressure in the proposed system.

| Cell Type | | RAW Macrophage | | BMMC | | 3T3-L1 Fibroblast | | 3T3-L1 Fat Cell | |
|----------------|-----------|----------------|---------|---------|---------|-------------------|---------|-----------------|---------|
| Dilution Ratio | Treatment | 1-dav | 3-dav | 1-dav | 3-dav | 1-dav | 3-dav | 1-dav | 3-dav |
| | | 1/3 | I vs. D | NS | NS | NS | NS | <0.04 | NS |
| I vs. S | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| I vs. ZI | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| D vs. S | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| D vs. ZD | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 1/6 | I vs. D | <0.0001 | <0.0001 | <0.0001 | 0.0002 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | I vs. S | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | I vs. ZI | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | D vs. S | <0.0001 | <0.0001 | <0.0001 | <0.0001 | NS | <0.0001 | <0.0001 | NS |
| | D vs. ZD | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | NS |
| 1/12 | I vs. D | <0.0001 | <0.0001 | <0.01 | <0.01 | NS | <0.0001 | 0.0136 | NS |
| | I vs. S | <0.0001 | <0.0001 | <0.0001 | <0.0001 | NS | <0.0001 | <0.0001 | <0.0001 |
| | I vs. ZI | <0.0001 | <0.0001 | <0.0001 | <0.0001 | NS | <0.0001 | NS | <0.0001 |
| | D vs. S | <0.0001 | <0.0001 | <0.0001 | <0.0001 | NS | <0.0001 | <0.0001 | <0.001 |
| | D vs. ZD | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.02 | <0.0001 | NS | 0.03 |
| 1/24 | I vs. D | <0.0001 | <0.0001 | <0.01 | <0.01 | NS | <0.01 | <0.001 | NS |
| | I vs. S | <0.0001 | <0.0001 | <0.0001 | NS | NS | <0.0001 | <0.0001 | <0.0001 |
| | I vs. ZI | <0.0001 | <0.0001 | <0.0001 | NS | NS | NS | 0.0029 | <0.0001 |
| | D vs. S | NS | <0.0001 | <0.0001 | 0.01 | NS | <0.0001 | NS | <0.0001 |
| | D vs. ZD | NS | <0.0001 | NS | NS | NS | NS | NS | NS |
| 1/48 | I vs. D | NS | <0.01 | 0.001 | <0.0001 | 0.01 | 0.01 | NS | NS |
| | I vs. S | NS | <0.0001 | NS | <0.0001 | NS | <0.0001 | <0.0001 | NS |
| | I vs. ZI | NS | <0.0001 | <0.0001 | <0.0001 | NS | <0.0001 | NS | <0.02 |
| | D vs. S | NS | 0.0021 | <0.0001 | NS | NS | <0.0001 | NS | <0.01 |
| | D vs. ZD | NS | NS | NS | NS | NS | <0.0001 | NS | NS |
| 1/96 | I vs. D | NS | NS | <0.001 | NS | NS | NS | NS | NS |
| | I vs. S | NS | NS | <0.0001 | NS | <0.0001 | NS | NS | 0.02 |
| | I vs. ZI | NS | NS | <0.0001 | 0.02 | 0.02 | <0.0001 | <0.01 | <0.0001 |
| | D vs. S | NS | NS | NS | NS | <0.0001 | NS | NS | 0.003 |
| | D vs. ZD | <0.001 | NS | 0.001 | <0.0001 | <0.0001 | NS | NS | NS |
| 1/192 | I vs. D | <0.01 | NS | NS | NS | 0.0014 | NS | <0.01 | <0.0001 |
| | I vs. S | NS | <0.0001 | <0.01 | 0.02 | <0.0001 | NS | <0.01 | <0.0001 |
| | I vs. ZI | <0.001 | NS | NS | <0.0001 | NS | NS | NS | <0.0001 |
| | D vs. S | <0.01 | <0.0001 | <0.0001 | 0.04 | <0.01 | NS | NS | NS |
| | D vs. ZD | <0.001 | NS | <0.0001 | <0.0001 | NS | NS | <0.001 | NS |

Table S-1: *In vitro* Mouse cell Statistics: Statistics calculated using two-way Anova ($p < 0.05$) for 1- and 3-days of exposure in mouse RAW Macrophages, 3T3-L1 Fibroblast, 3T3-L1 derived Fat cells, and BMMC. Tukey multiple comparisons made between saline (S), diluent (D), insulin (I), Zeolite filtered insulin (ZI), and Zeolite filtered diluent (ZD).

| Cell Type | | PBMC | | HMC-1 | | THP-1 | |
|----------------|-----------|------------|---------|---------|---------|---------|---------|
| Dilution Ratio | Treatment | 1-day | 3-day | 1-day | 3-day | 1-day | 3-day |
| | | 1/3 | I vs. D | NS | NS | NS | NS |
| I vs. S | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| I vs. ZI | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| D vs. S | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| D vs. ZD | <0.0001 | | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 1/6 | I vs. D | <0.0001 | <0.0001 | 0.02 | <0.01 | <0.01 | NS |
| | I vs. S | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | I vs. ZI | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | D vs. S | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | D vs. ZD | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 1/12 | I vs. D | NS | <0.0001 | NS | 0.03 | <0.01 | <0.0001 |
| | I vs. S | NS | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | I vs. ZI | <0.001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | D vs. S | NS | <0.0001 | <0.0001 | <0.0001 | <0.0001 | NS |
| | D vs. ZD | 0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 1/24 | I vs. D | NS | <0.0001 | NS | NS | <0.0001 | <0.0001 |
| | I vs. S | NS | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| | I vs. ZI | NS | 0.0002 | <0.0001 | NS | <0.0001 | <0.0001 |
| | D vs. S | NS | NS | <0.0001 | <0.01 | <0.0001 | 0.0304 |
| | D vs. ZD | <0.0001 | NS | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 1/48 | I vs. D | NS | <0.0001 | NS | NS | <0.0001 | <0.0001 |
| | I vs. S | NS | <0.0001 | <0.0001 | <0.01 | <0.0001 | <0.0001 |
| | I vs. ZI | <0.0001 | <0.001 | <0.0001 | NS | <0.0001 | <0.0001 |
| | D vs. S | NS | 0.04 | <0.0001 | 0.03 | 0.02 | NS |
| | D vs. ZD | NS | <0.0001 | 0.0015 | 0.02 | NS | NS |
| 1/96 | I vs. D | <0.0001 | <0.0001 | 0.0126 | NS | <0.001 | <0.0001 |
| | I vs. S | NS | <0.0001 | <0.0001 | NS | <0.0001 | NS |
| | I vs. ZI | NS | NS | 0.0051 | NS | <0.0001 | <0.0001 |
| | D vs. S | NS | NS | <0.0001 | NS | <0.01 | <0.0001 |
| | D vs. ZD | <0.01 | <0.01 | NS | 0.02 | <0.01 | NS |
| 1/192 | I vs. D | NS | <0.0001 | 0.001 | <0.0001 | NS | NS |
| | I vs. S | NS | <0.0001 | <0.0001 | NS | <0.0001 | 0.0008 |
| | I vs. ZI | NS | <0.0001 | 0.003 | NS | <0.0001 | <0.0001 |
| | D vs. S | <0.03 | >0.9999 | <0.0001 | NS | <0.0001 | 0.0408 |
| | D vs. ZD | <0.0001 | <0.0001 | 0.0001 | <0.0001 | <0.0001 | <0.0001 |

Table S-2: *In vitro* Human cell Statistics: Statistics calculated using two-way Anova ($p < 0.05$) for 1- and 3-days of exposure in human PBMC, HMC-1, and THP-1 cells. Tukey multiple comparisons made between saline (S), diluent (D), insulin (I), Zeolite filtered insulin (ZI), and Zeolite filtered diluent (ZD).