**Targeting of the AGE-RAGE axis during prediabetes can restore glucose homeostasis and induce changes to immune phenotype that reduce T1DM incidence in preclinical models**

**Aims:**  Environmental triggers such as increased consumption of ultra-processed foods contribute to increases in advanced glycation end products (AGEs). AGE and its receptor (RAGE) are associated with T1DM onset and induce inflammation, and β-cell dysfunction. In this study, we explored reducing dietary AGEs and combination therapy with oral RAGE antagonist (oRA) to understand the impact of targeting the AGE-RAGE axis during prediabetes for T1DM prevention.

**Methods:** Female NOD mice were fed with either low AGE (LAGE), or high AGE (HAGE) diet from day 50 until day 75 (prediabetes, n=10-20) where insulin tolerance by intraperitoneal insulin tolerance test (i.p.ITT), and pancreatic insulitis were assessed, or until day 200 to determine diabetes incidence (n=29-34). In a short-term immunological study, NOD mice were fed with HAGE diet from day 35 until 75, ± oRA (n=8-12). Immunophenotype was assessed in blood (BLD), spleen (SPL), and pancreatic lymph node (PLN) using a high-parameter flow cytometry panel. Additionally, splenocytes from these mice were transferred into immunodeficient NOD-SCID mice to determine diabetes incidence (n=5-11).

**Results:** Low AGE dietary intake reduced the incidence of T1D in NOD mice by 30% (p=0.002). Furthermore, LAGE fed mice showed increased plasma insulin, GLP-1 concentrations (p<0.0001), and changes in insulin tolerance (p<0.001). oRA treatment increased proportions of regulatory T cells (Treg) in SPL and PLN (p<0.05). An elevated expression of CD39+ CD73+ on Tregs in SPL and PLN, suggesting an increase in the activation status of Tregs with oRA. Additionally, NOD-SCID mice that received splenocytes from mice fed with HAGE + oRA showed delay T1D incidence (P<0.01) compared to HAGE.

**Conclusion:** oRA induces changes in T cell homeostasis while the LAGE diet improved glucose homeostasis to prevent T1DM. Therefore, disrupting the AGE-RAGE axis by reducing dietary AGE intake or delivery of oRA are potential approaches to prevent or delay T1DM onset.