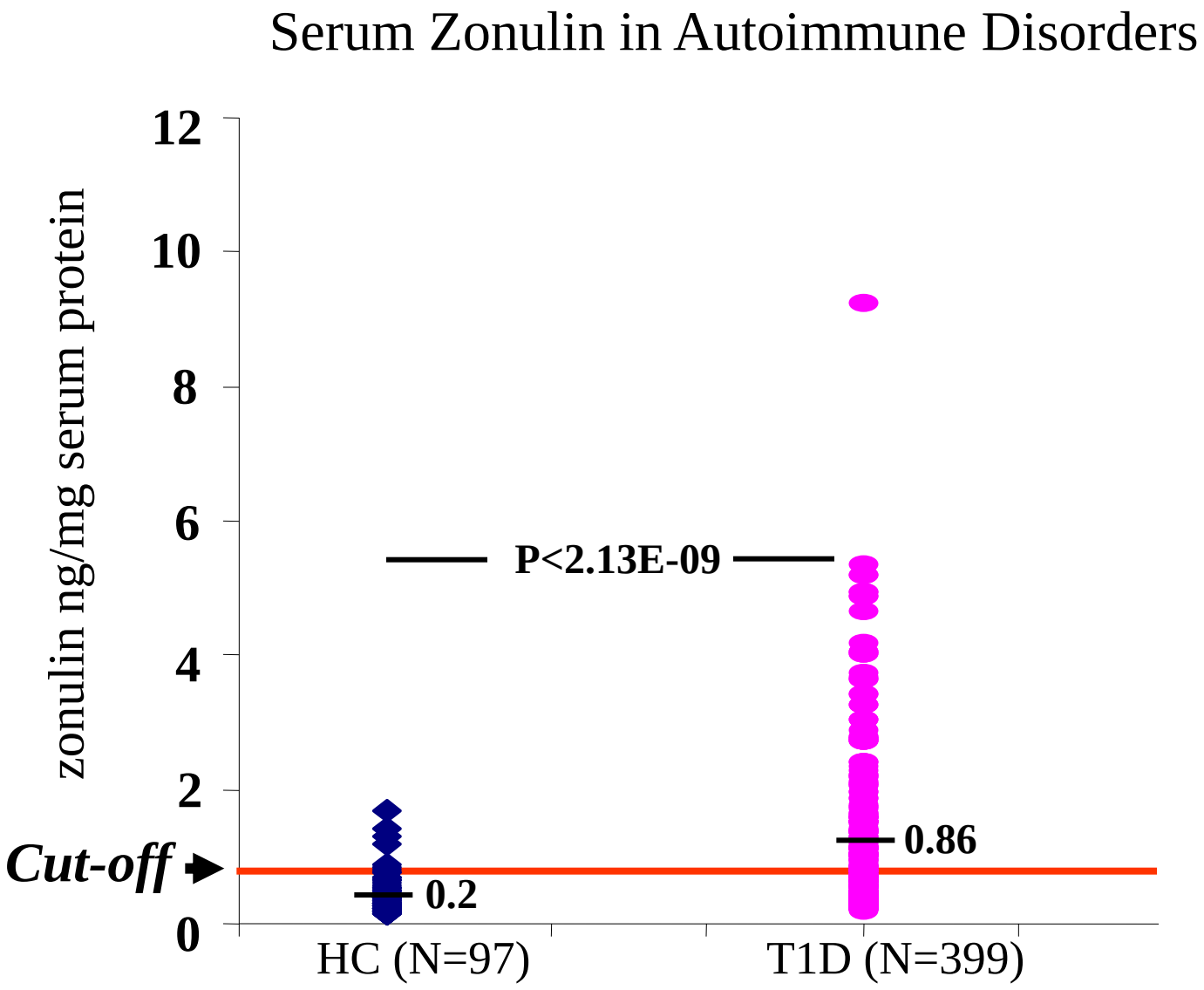
Serum zonulin and intestinal permeability before and after a gluten-containing meal in both Type 1 diabetes and in their relatives"

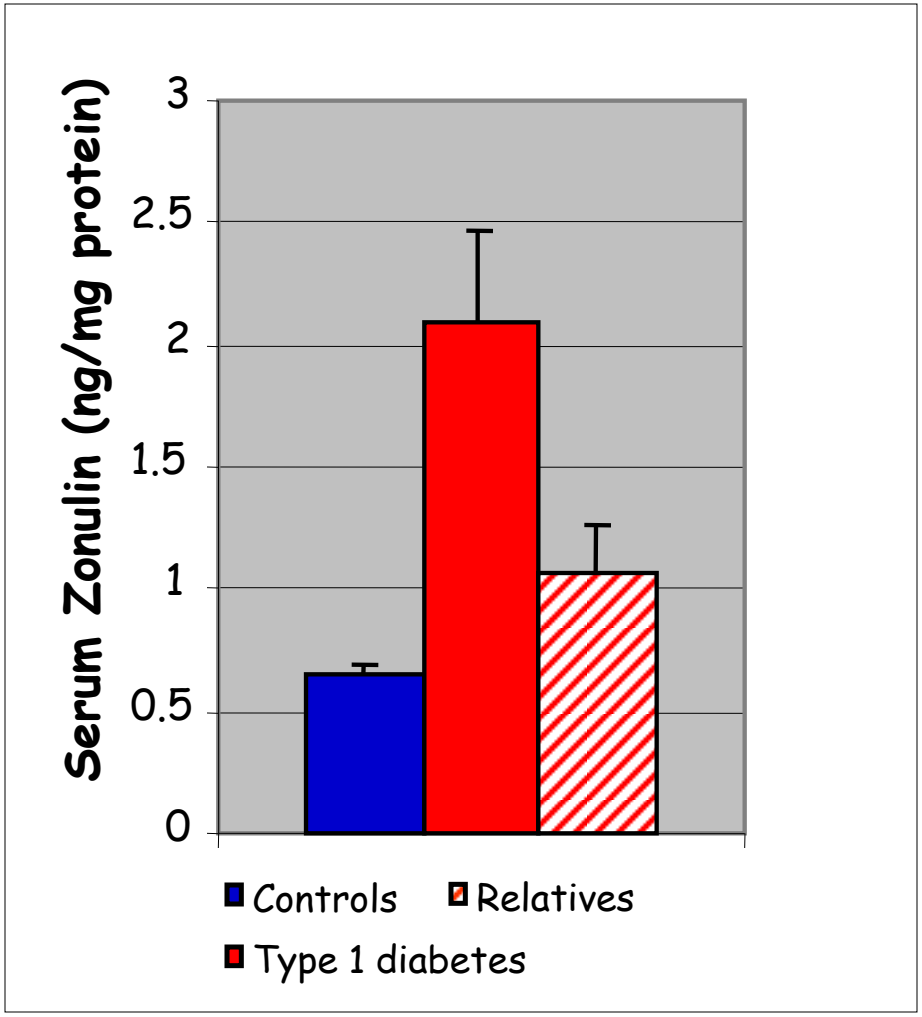
Background: The trigger of the autoimmune destruction of pancreatic beta cells in Type 1 diabetes (T1D) is unclear. One theory is that antigens absorbed through the gut may be involved. We have recently described a protein, zonulin, that opens intestinal tight junctions and allows paracellular absorption of macromolecules. We have reported that zonulin is increased in the serum of a subset of patients with T1D and first degree relatives. **Aim**: The objective of this study was to determine if dysregulation of the zonulin pathway, both at baseline and following a gluten-containing meal, is linked to increased intestinal permeability (IP) and is involved in the pathogenesis of T1D. **Methods**: After obtaining informed consent, blood was obtained from children with T1D, parents, and siblings. Zonulin was measured in the serum of children with T1D (n=11) and their first degree relatives (n=19), by sandwich ELISA. IP was determined by HPLC measurement of both serum (at baseline and every hour up to 5 hours) and urine (5 hours collection) lactulose (LA) and mannitol (MA) after subjects ingested the sugars test solution. Results were expressed as LA/MA ratio. **Results**: There was a significant increase in zonulin (53%) and IP (47 %) in subjects compared to the negative cutoff. There was a significant relationship between elevation of zonulin and increased IP ($p=0.026$) and between LA/MA ratio in post-prandial serum samples and LA/MA ratio in urine ($p=0.01$). Although there was no change in mean zonulin levels with a meal, all subjects for whom IP data is available had elevation of zonulin at baseline and post-meal, and all had an increase in IP 1-hour post-meal. The 1 h post-prandial IP increase was statistically higher in T1D subjects (5.6 folds) as compared to their relatives (2.7 folds, $p<0.01$). There was no relationship between serum glucose and zonulin levels. **Conclusions**: Zonulin is significantly correlated with increased IP in children with T1D and their first degree relatives. These data suggest that increased serum zonulin is indicative of increased gut leakiness in children with T1D. Studies of the temporal relationship of an increase in serum zonulin and the development of diabetes autoantibodies are planned to suggest a causative relationship in a subset of genetically susceptible individuals.

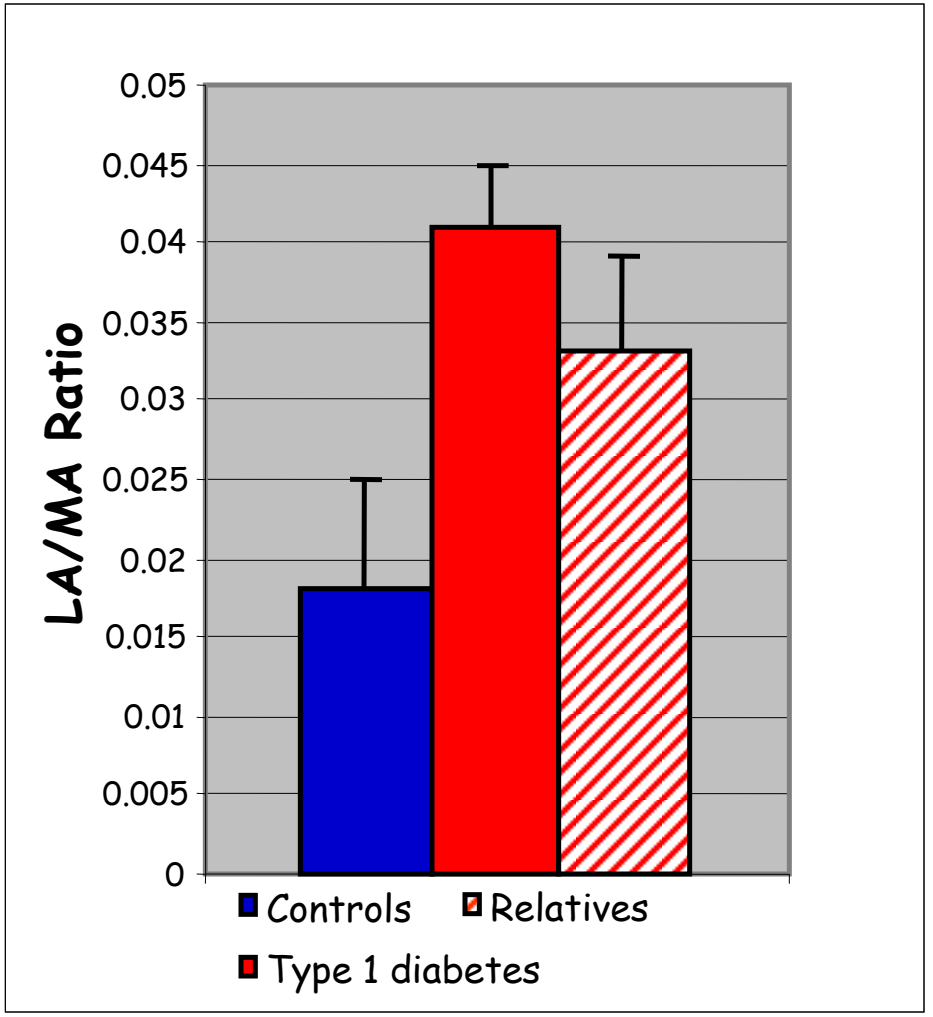
The Zonulin Pathway and Type 1 Diabetes

Zonulin Up-Regulation

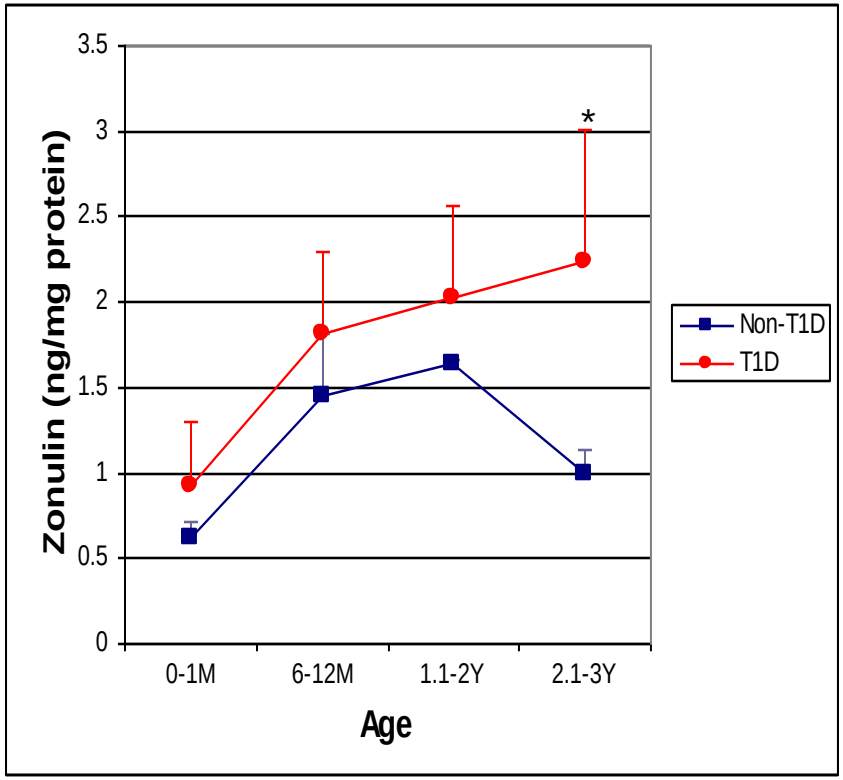


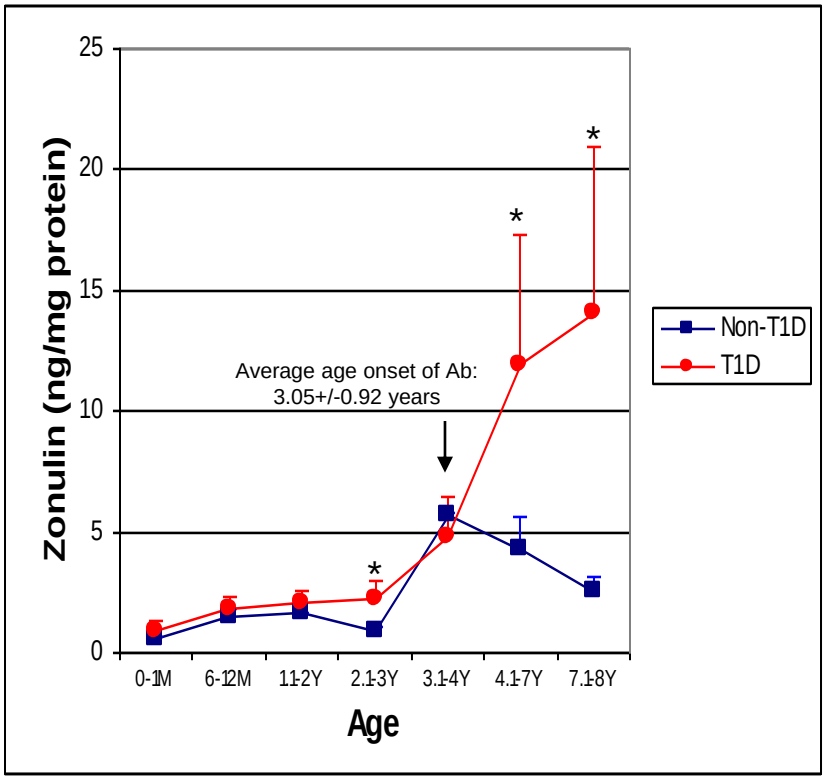
Comparison between serum zonulin and *in vivo* permeability in children with Type 1 Diabetes, their adult relatives, and healthy controls





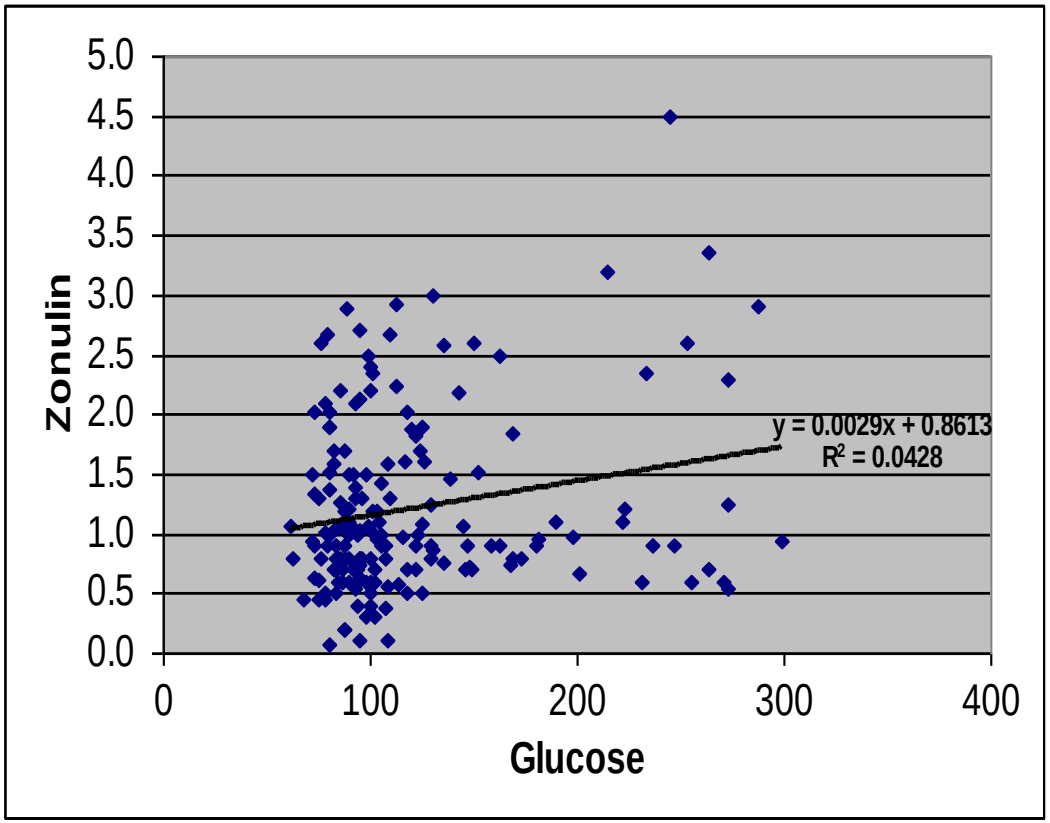
T1D and Zonulin: Prospective Studies in a Cohort of DQ2+ Neonates at Risk for T1D and Early Gluten Exposure (<6 Months)





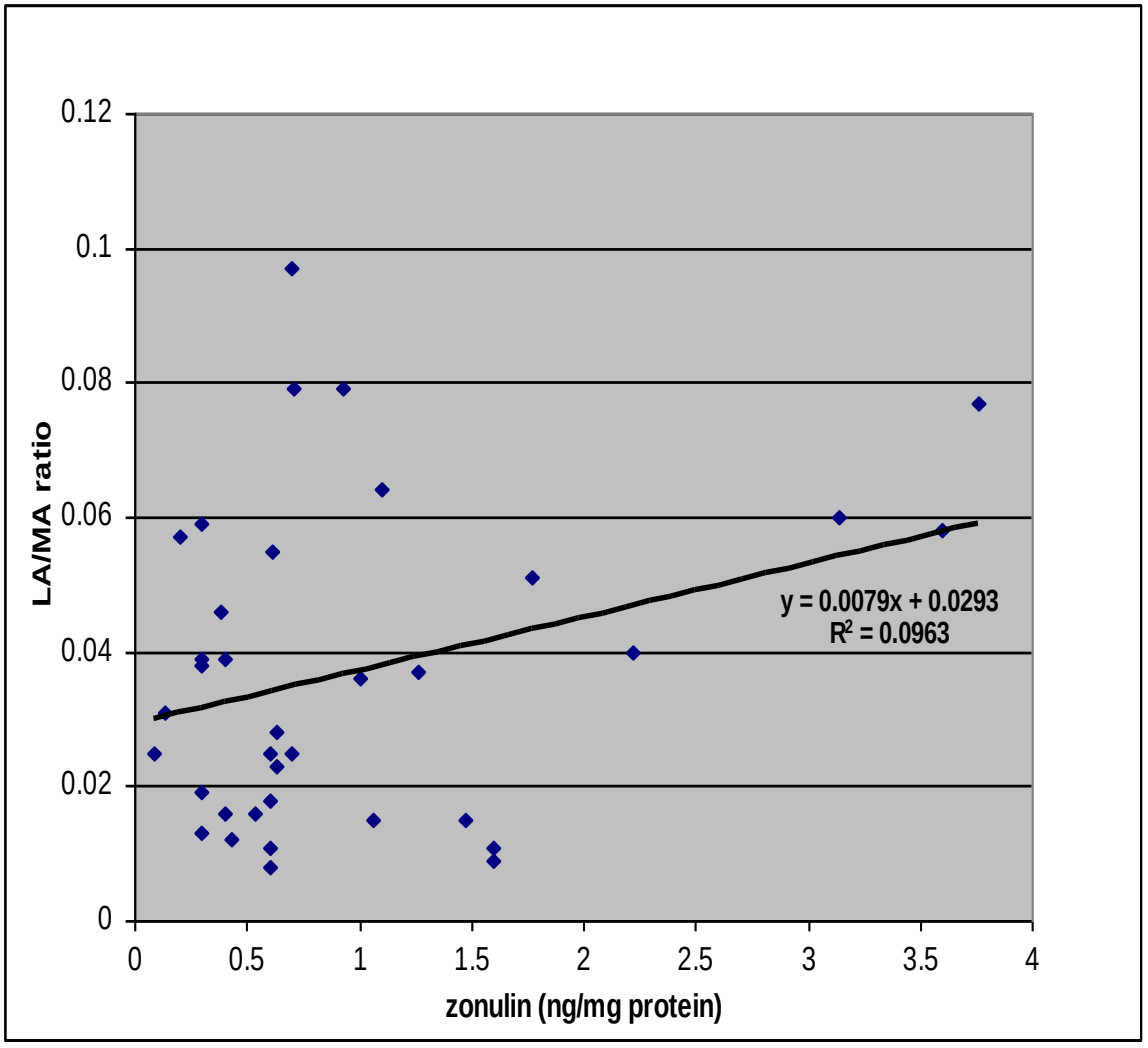
*p<0.02

Zonulin-Serum Glucose Relationship

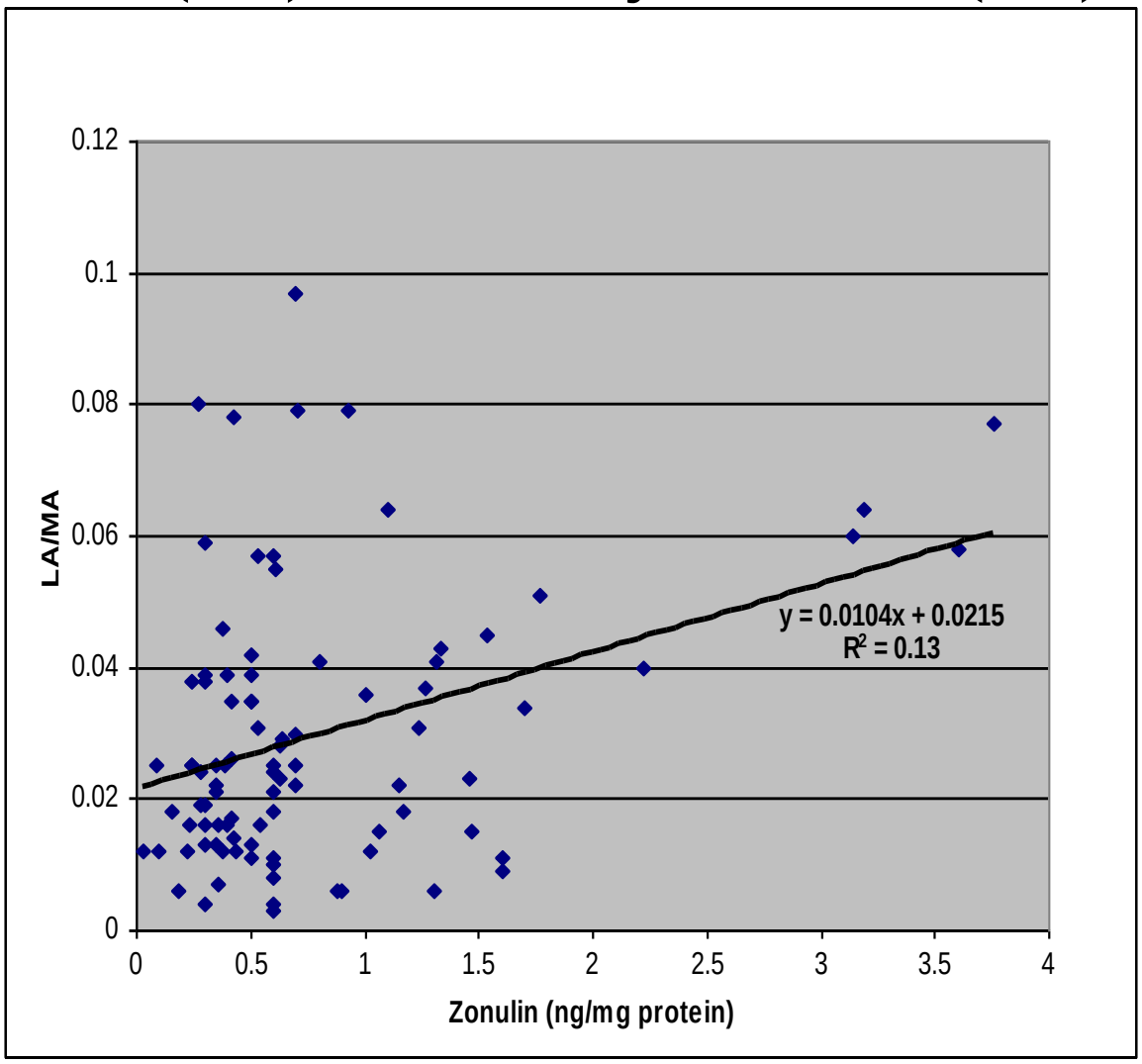


Zonulin-Permeability Relationship

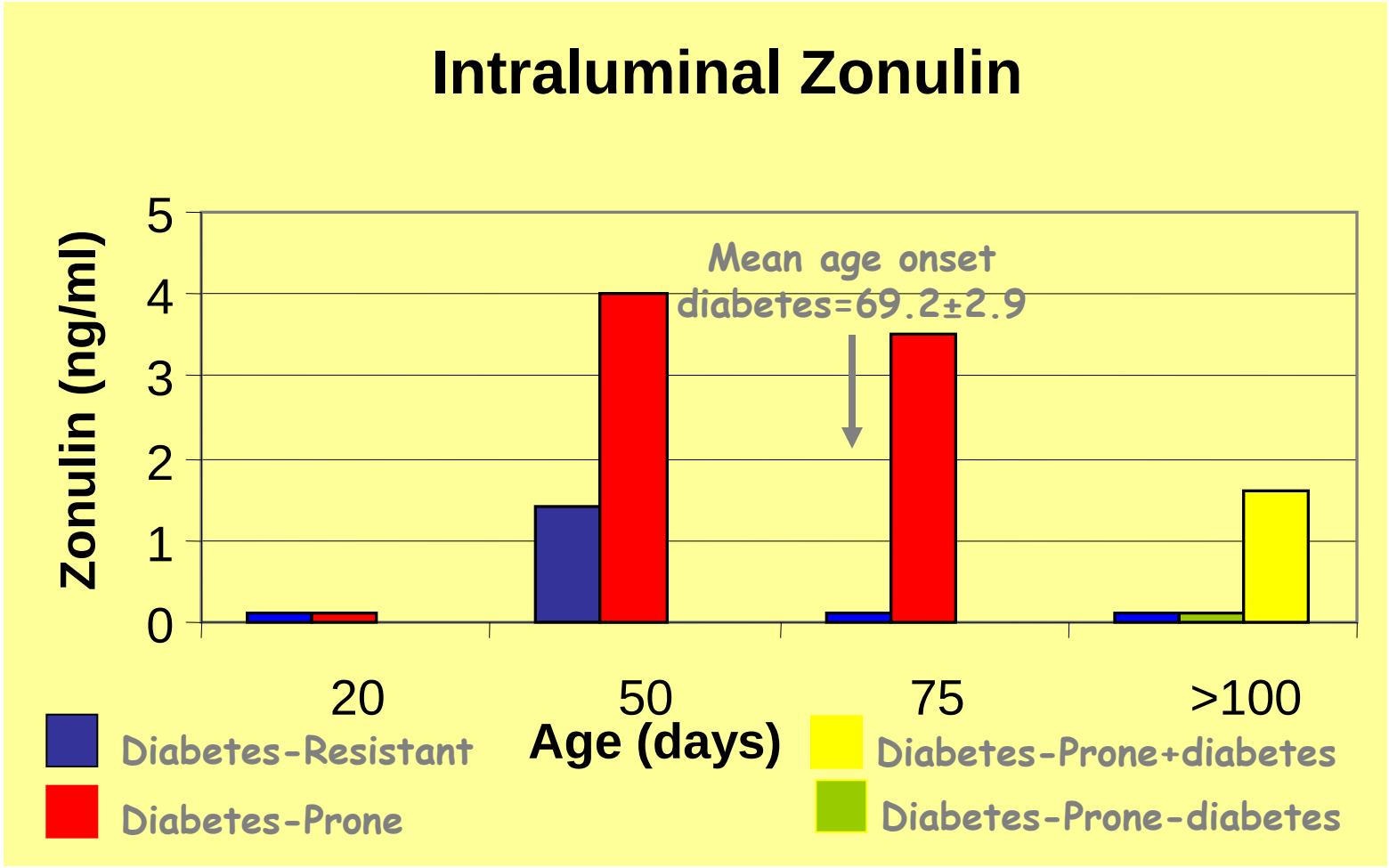
T1D Subjects



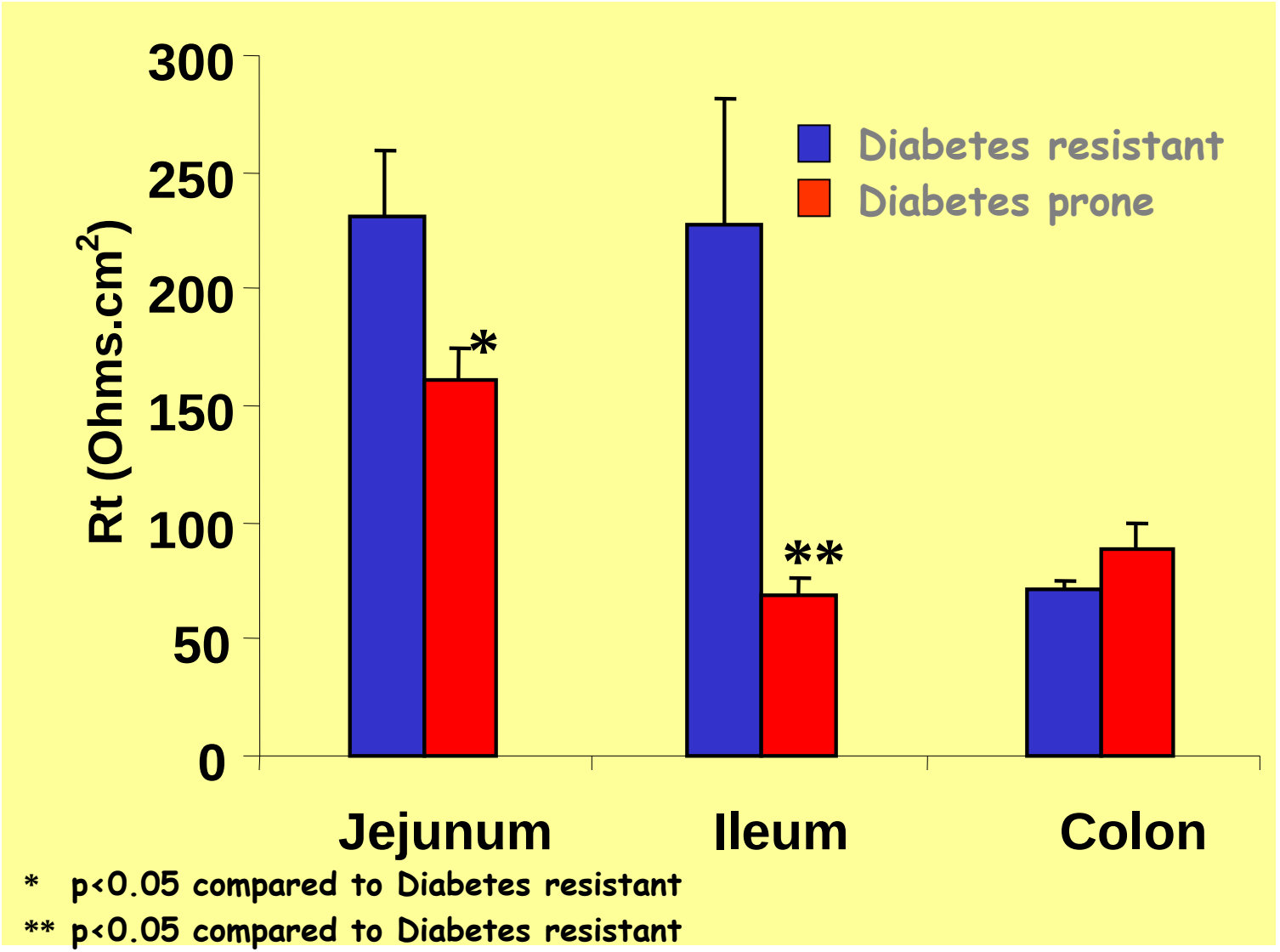
Zonulin-Intestinal Permeability Relationship: Combined Data
T1D (N=) and Family Members(N=)



Experiments in Diabetic BB/Wor rats



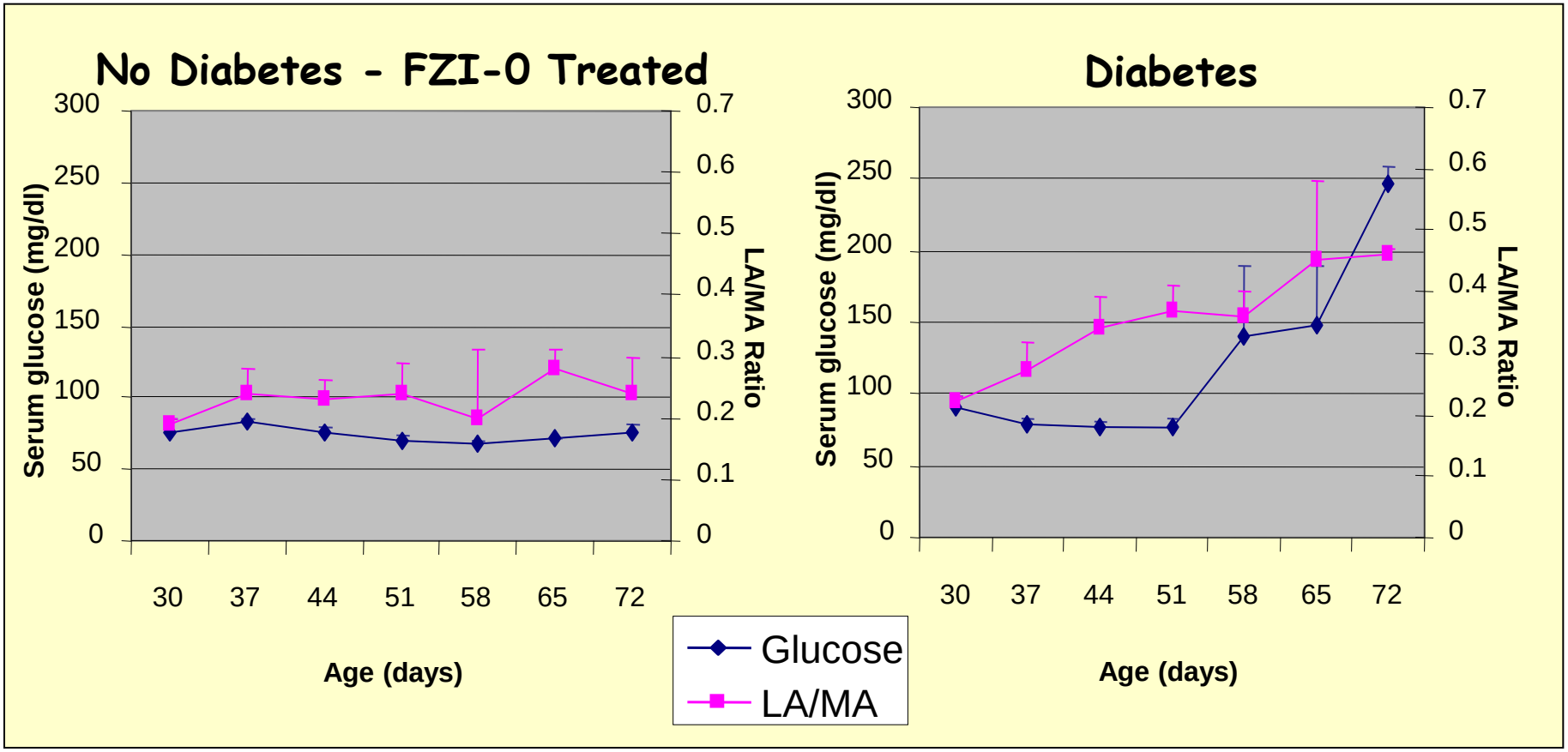
EX-VIVO INTESTINAL PERMEABILITY



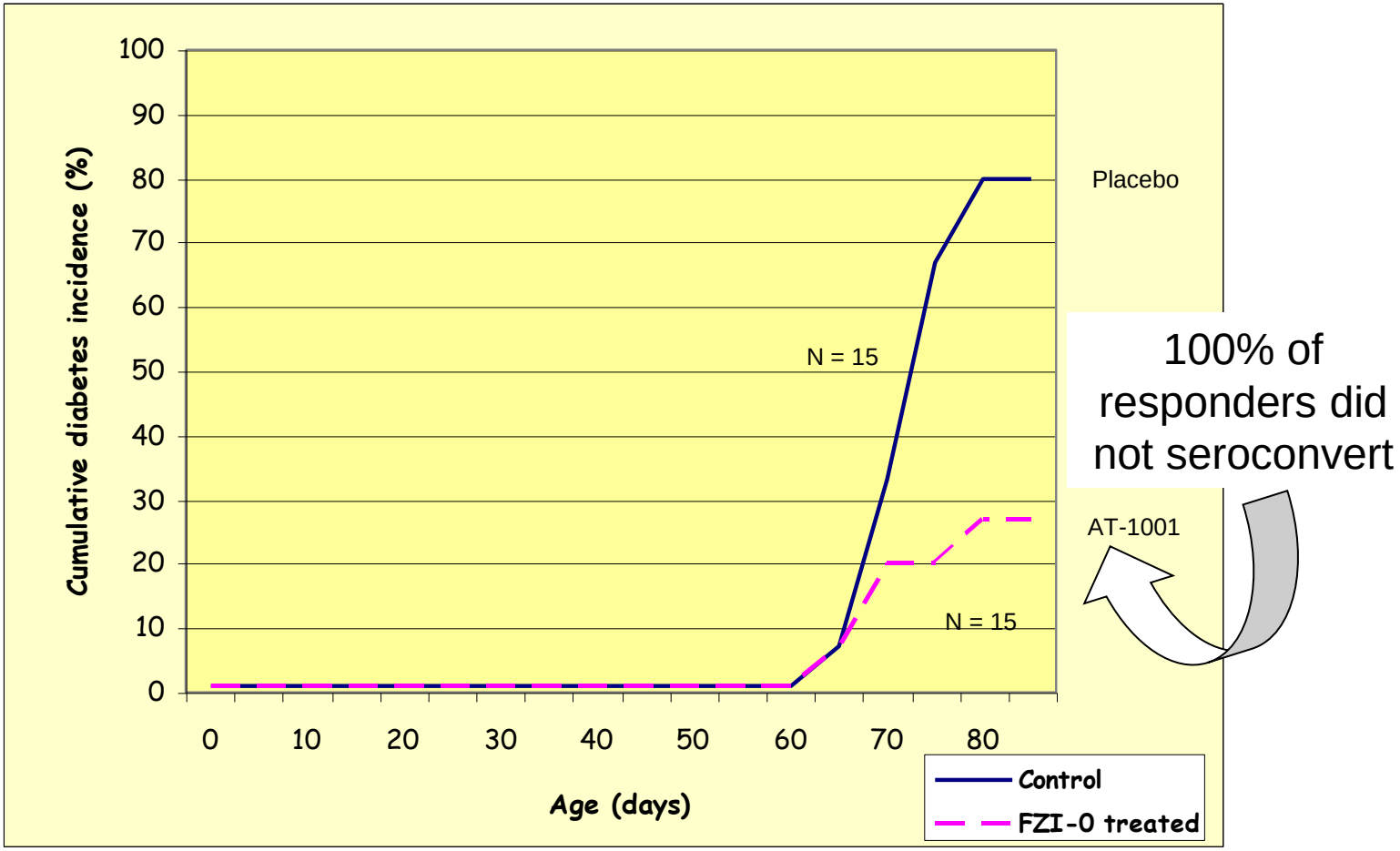
Treatment of DP rats with zonulin inhibitor AT-1001 (“FZI/0”)

- DP animals age 20 days divided in two groups
- Group 1 (n=15): AT-1001 daily in drinking water + HCO3
- Group 2 (n=15): drinking water + HCO3

In Vivo permeability and serum glucose levels in DP rats either untreated or treated with AT-1001



Zonulin Antagonism and Type 1 Diabetes



Cumulative diabetes incidence, BB/Wor rats treated w/ Pbo or AT-1001

Source: Watts et al, personal communication

Serum Zonu

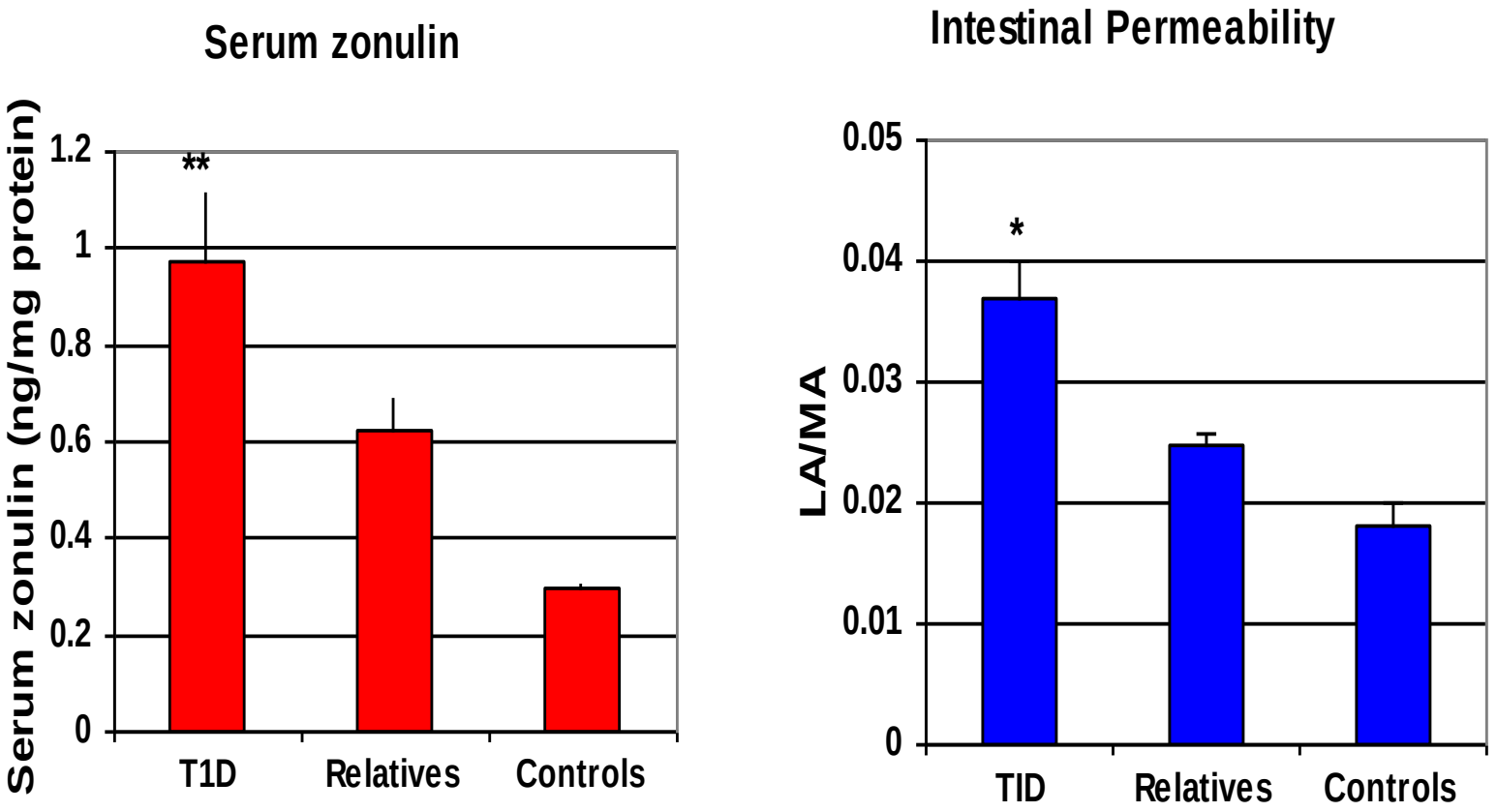


Fig. 11. Intestinal permeability and serum zonulin levels in T1D patients and their relatives. Both parameters were significantly higher in T1D subjects as compared to their relatives. *p=0.04; **p=0.02

XXXx

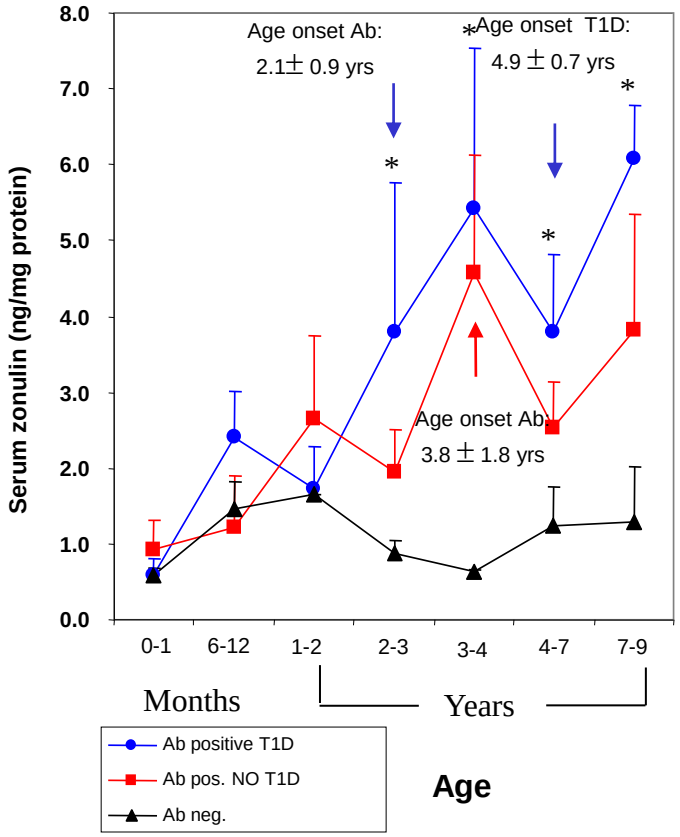


Fig. 12. Serum zonulin levels in subjects at risk for T1D that developed auto-Ab and T1D (circles N=4), auto-Ab but not T1D (squares N=6) and those that did not develop autoimmunity (triangles, N=6). *p<0.05.

EX-VIVO INTESTINAL PERMEABILITY

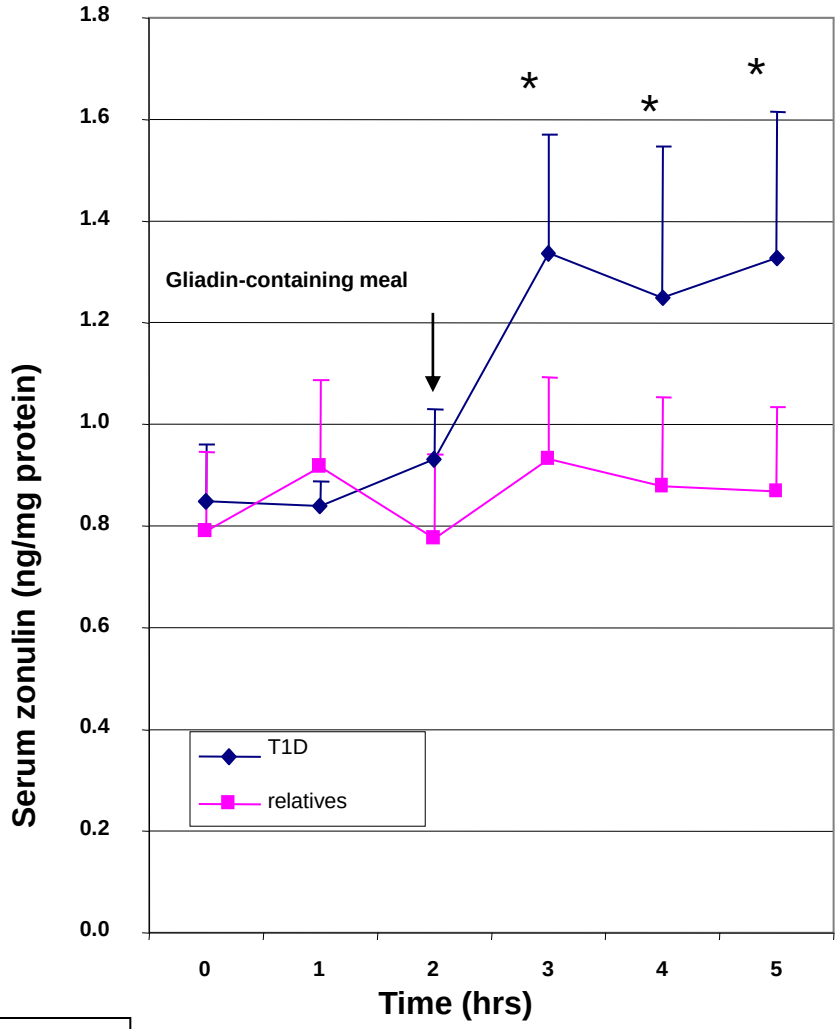


Fig. 13. Serum zonulin levels in T1D patients (n=16) and their relatives (N=35) before and after a gluten-containing meal. *<0.05 compared to baseline.

EX-VIVO INTESTINAL PERMEABILITY

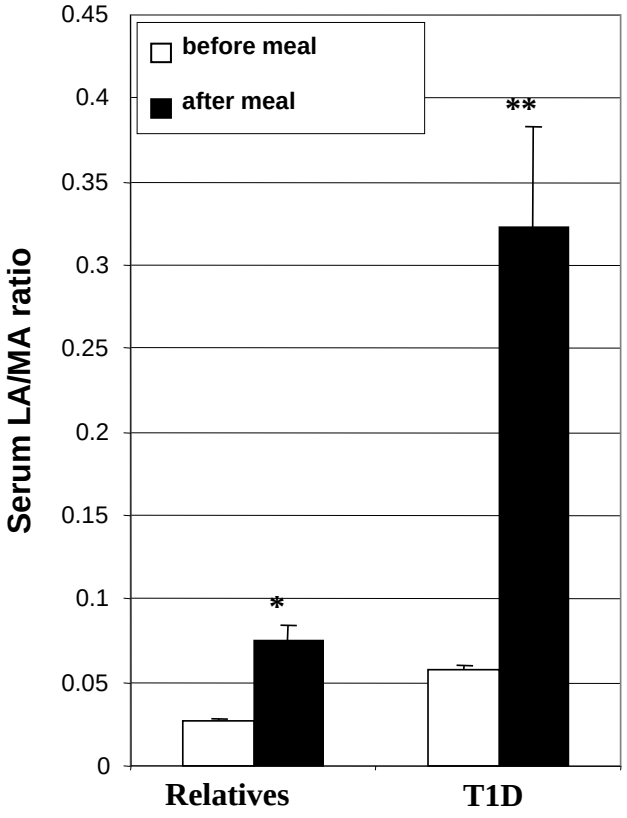


Fig. 14. Serum LA/MAin T1D patients (n=16) and their relatives (N=35) 1 hour before and 1 hour after a gluten-containing meal. *<0.0002; **<0.0005 compared to before meal LA/MA.

BB/wor DP Rat
Beta Cell Rescue Study

BB/wor DP Treatment Study: Study Design

Age: 52-54 days

Steps

Baseline AT1001 Treatment T_1

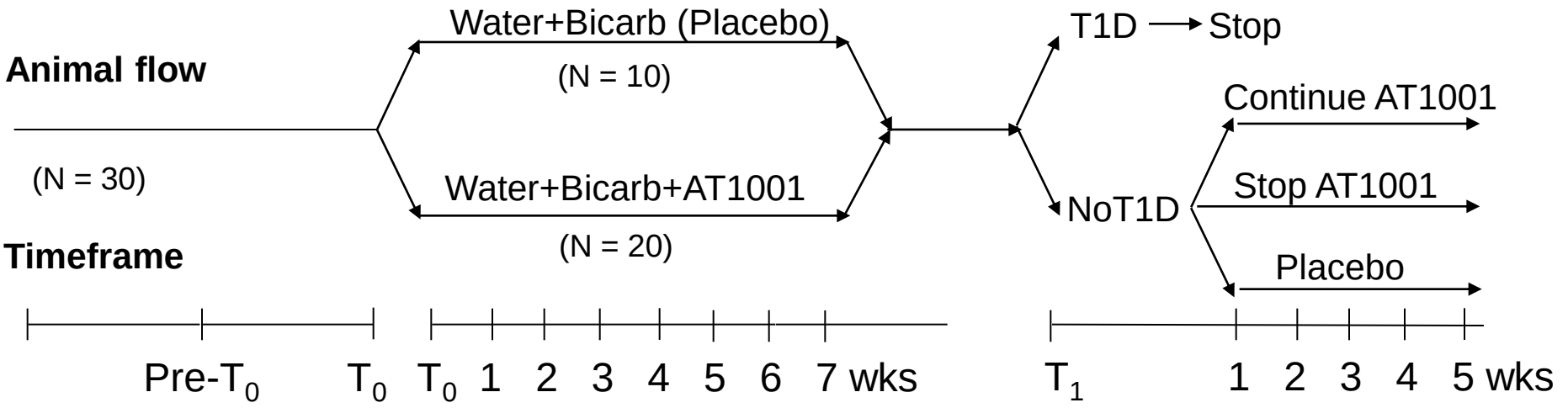
Intervention

Weight
Serum Glucose
ICA
Daily water intake

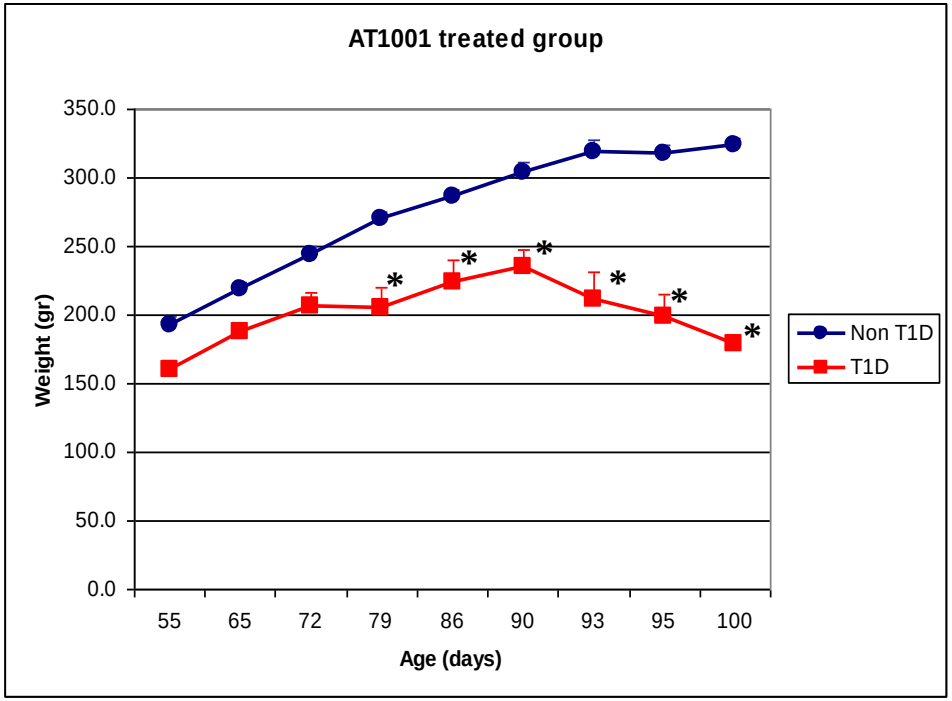
Randomization

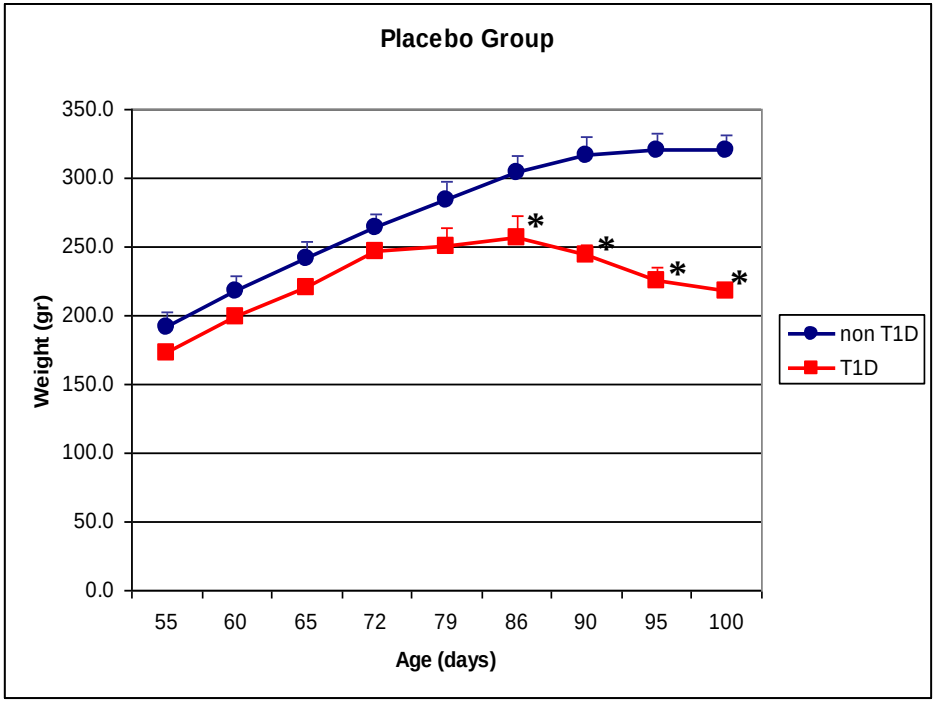
Water intake (daily)
Weight (weekly)
Serum glucose (weekly)

Serum glucose
ICA



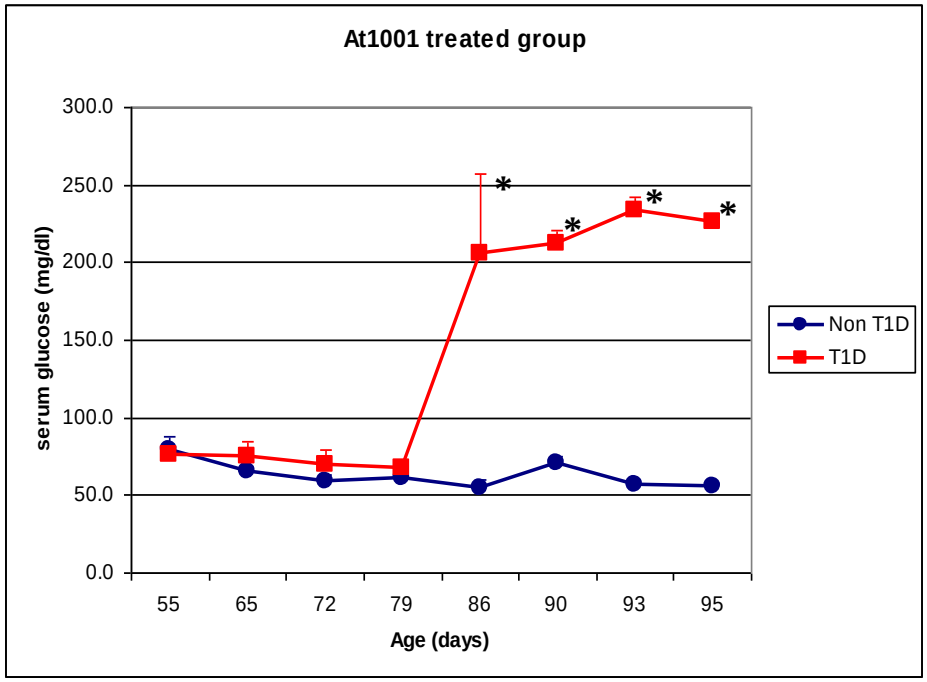
BB/wor DP Treatment Study: Weight Gain

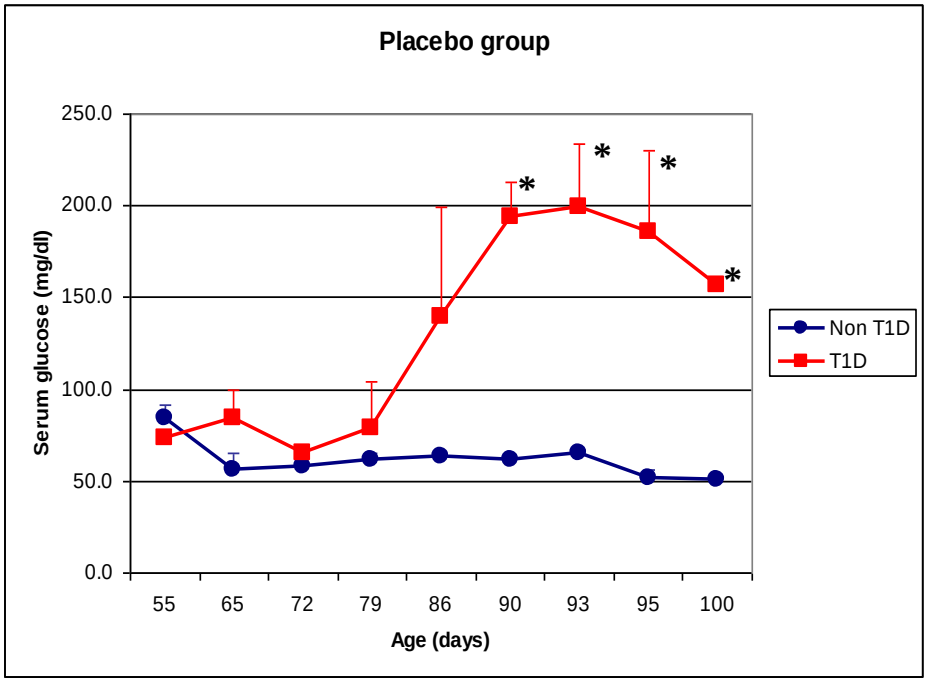




*p<0.01 compared to non-T1D

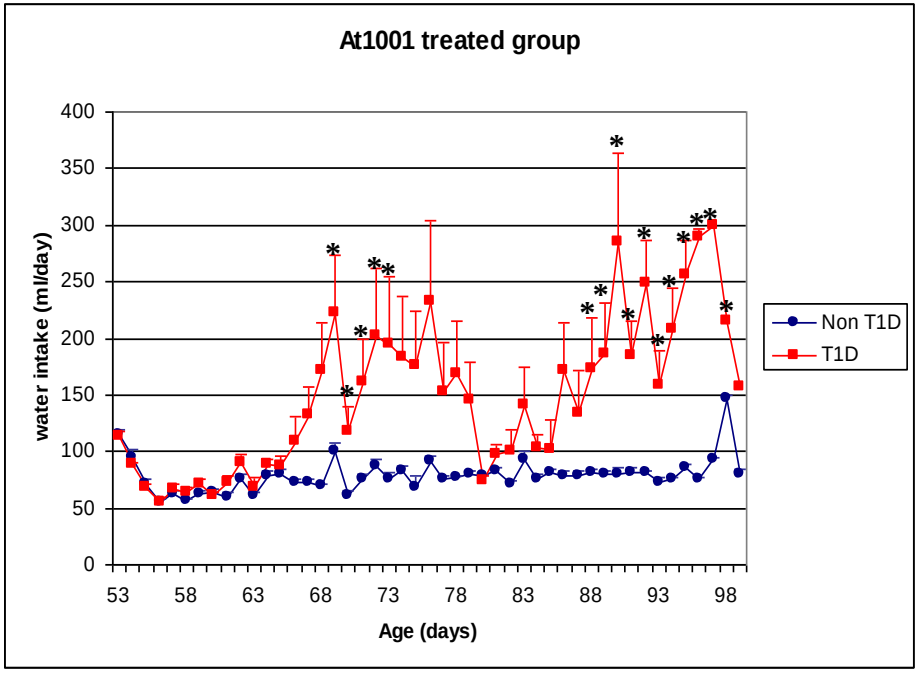
BB/wor DP Treatment Study: Serum Glucose Levels

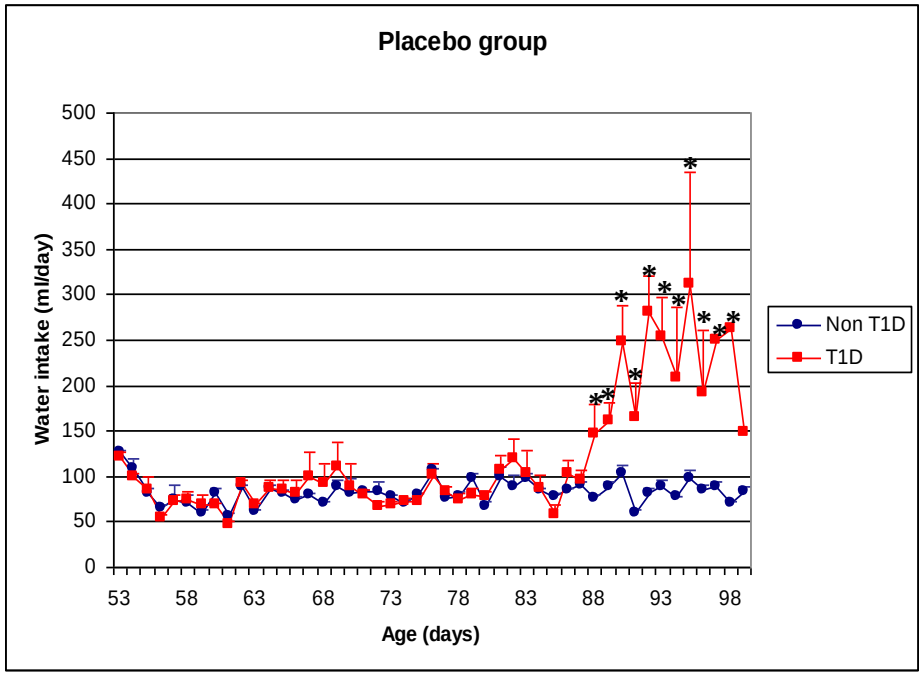




*p<0.01 compared to non-T1D

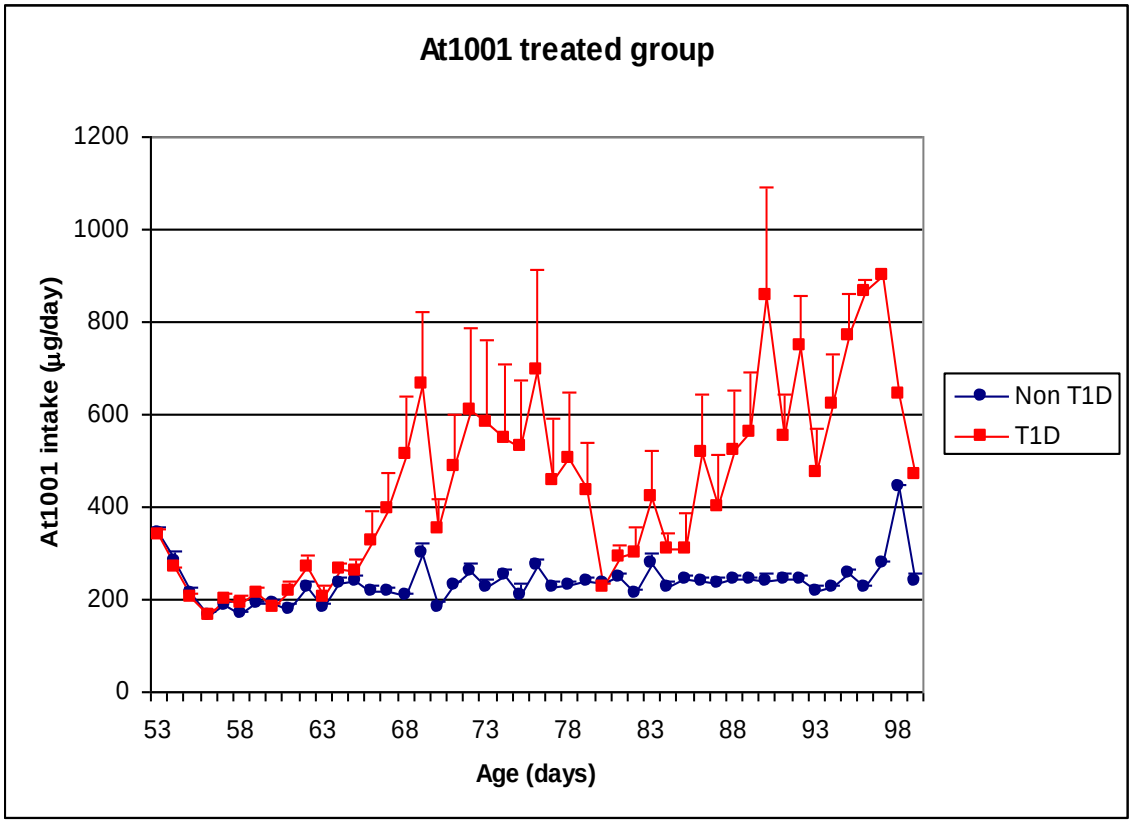
BB/wor DP Treatment Study: Water Intake



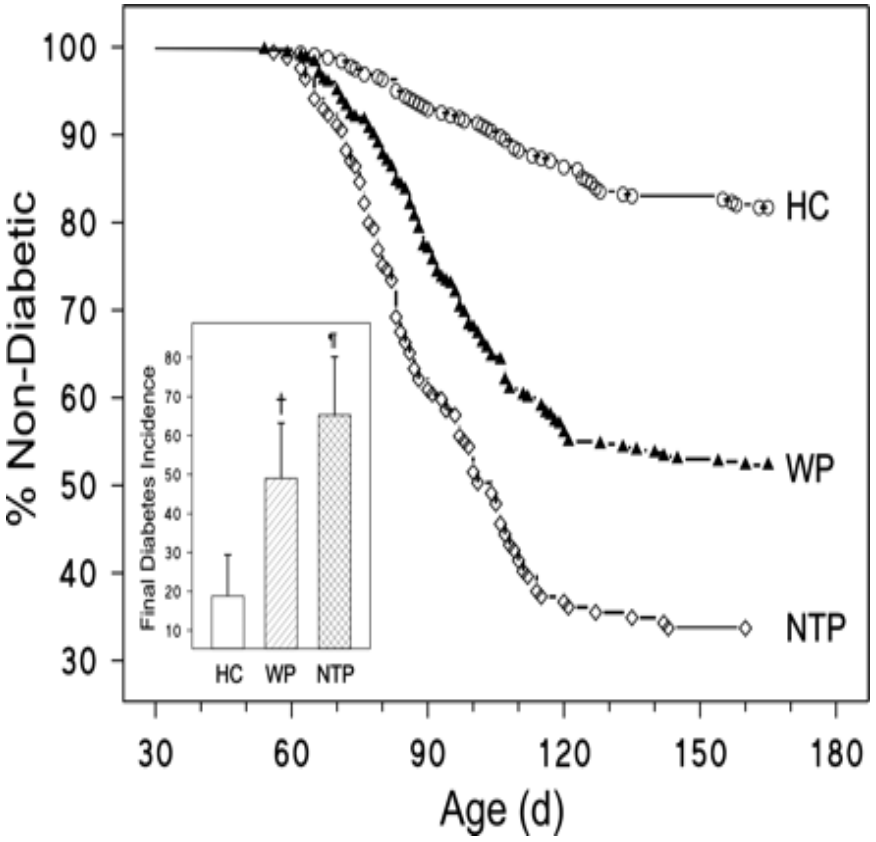


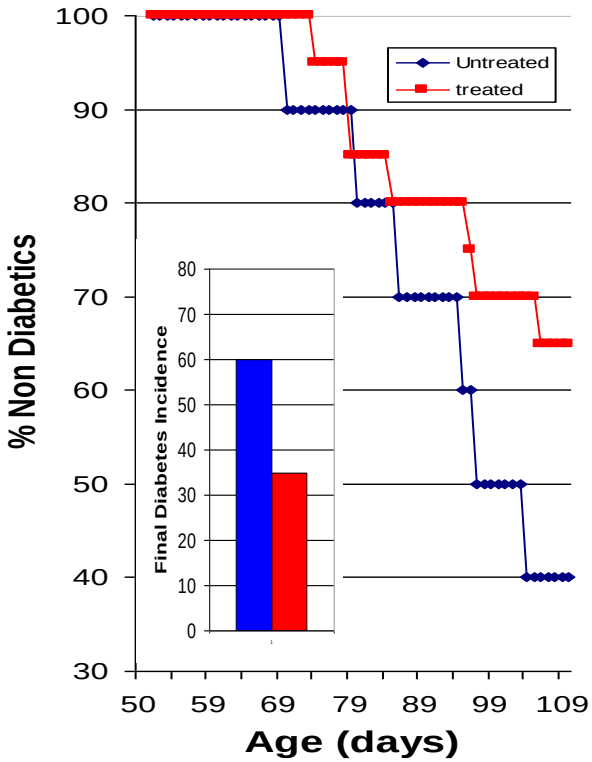
*p<0.05 compared to non-T1D

BB/wor DP Treatment Study: AT1001 Intake



BB/wor DP Treatment Study: T1D Incidence





MacFarlane J.A. et al J Biol Chem 2003, 278:54-63