

# **EARLY SUPPLEMENTATION OF NON-OBESE DIABETIC MICE WITH OLIGOSACCHARIDES ISOLATED FROM HUMAN MILK REDUCES SPONTANEOUS AUTOIMMUNE DIABETES DEVELOPMENT LATER IN LIFE**

**A.P. Vos<sup>1,2</sup>, A. Nato<sup>1,2</sup>, J. Bastiaans<sup>1</sup>, A. Leusink-Muis<sup>2,3</sup>, B. Stahl<sup>1</sup>, J. Garssen<sup>1,2</sup>, G. Folkerts<sup>2,3</sup>**

1. Nutricia Research, Utrecht, the Netherlands
2. Utrecht Institute for Pharmaceutical Sciences, Utrecht, the Netherlands
3. Curax BV, Houten, the Netherlands



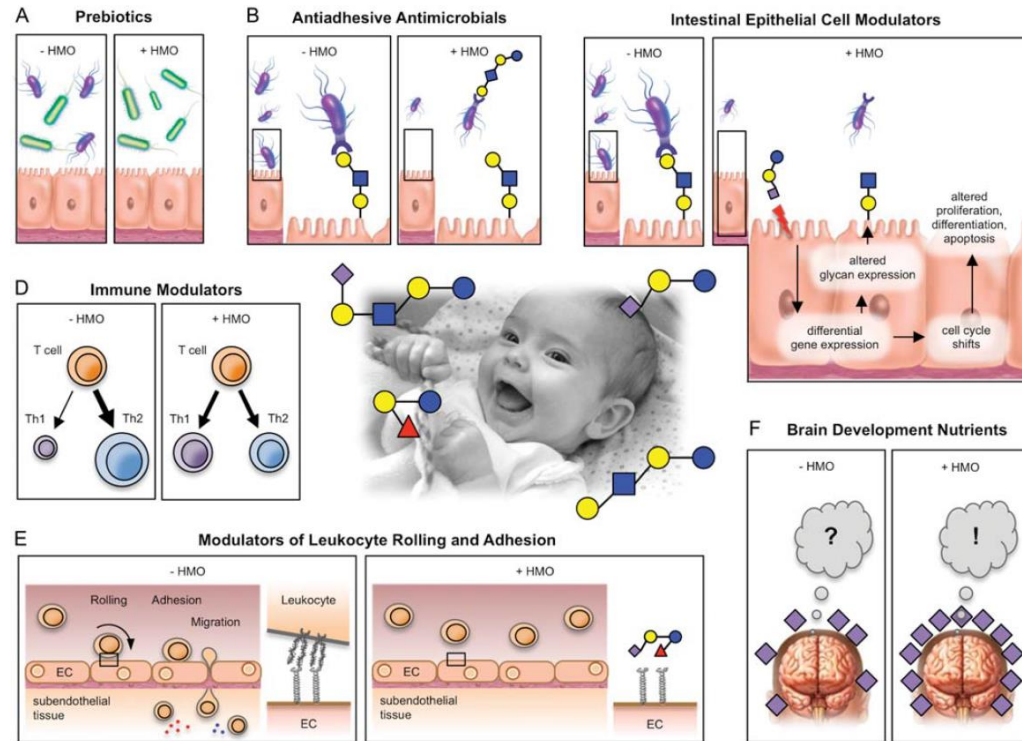
# HUMAN MILK OLIGOSACCHARIDES: EFFECTS ON THE IMMUNE SYSTEM

Breastfeeding is associated with immunological benefits (less allergies, less infections)

Human milk oligosaccharides (HMOS) can modulate the immune system directly or indirectly

→ Research questions:

- Can HMOS affect the development of autoimmune disease?
- Can early exposure to HMOS affect disease in later life (programming)?



**EVERY BABY NEEDS A SUGAR MAMA**

L. Bode, Glycobiology 22(9):1147–1162, 2012



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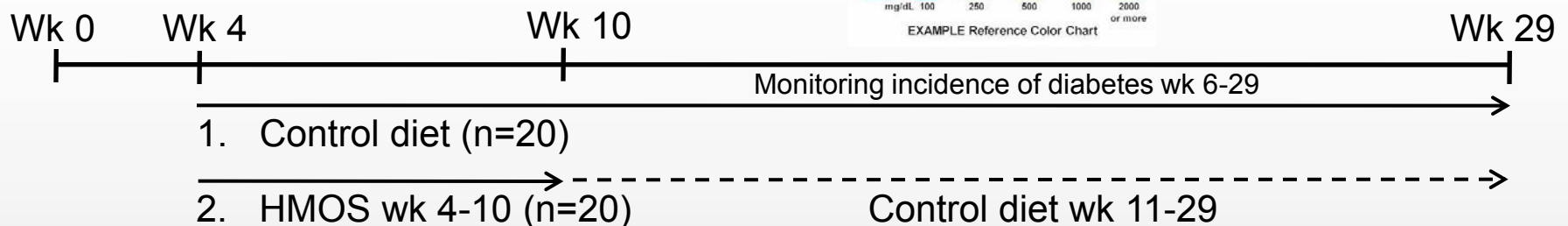
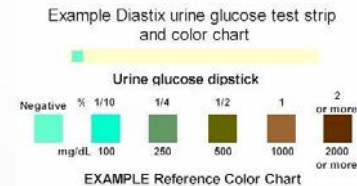


# EXPERIMENTAL SETUP

**Non-obese diabetic (NOD/ShiLtJ) mice: spontaneous autoimmune (type I diabetes) development, sensitive to dietary influences**

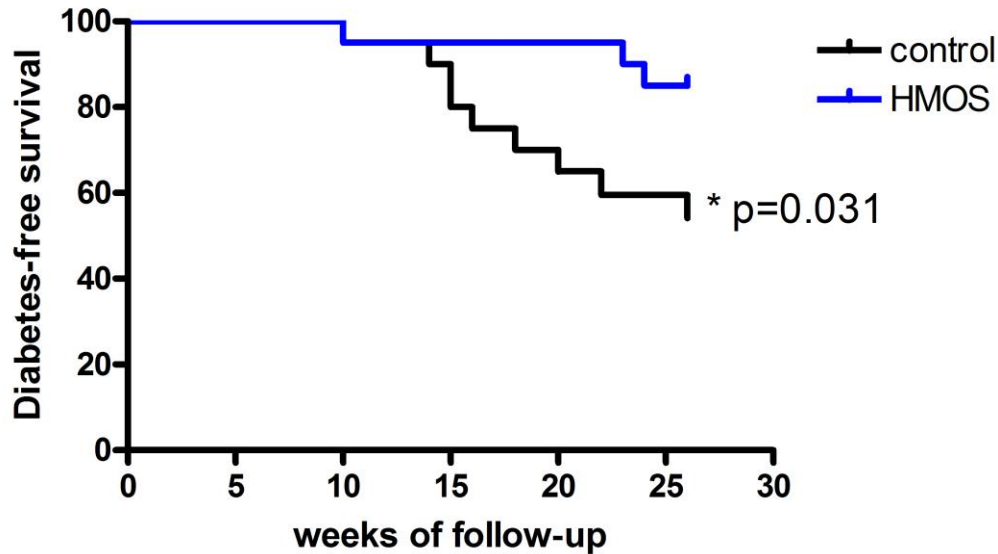
**HMOS: isolated from a pooled mature human milk sample and reduced in lactose (84% HMOS, 16% lactose; method: Geisser et al, J Chromatogr A, 2005)**

- **Comparison: AIN-93M control diet versus AIN-93M diet + 1% (w/w) HMOS**
- **Primary readout: urine glucose detection (>300 mg/dL)**
- **Secondary readouts: blood glucose, pancreas histology, flow cytometric analysis of splenocytes**



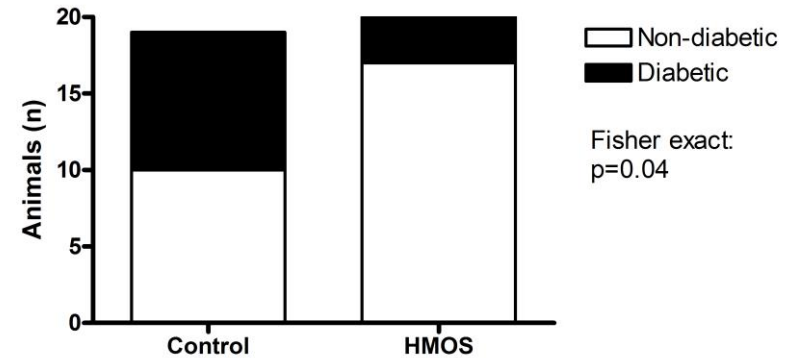
# EARLY HMOS DIETARY EXPOSURE REDUCES DIABETES DEVELOPMENT

## Diabetes development

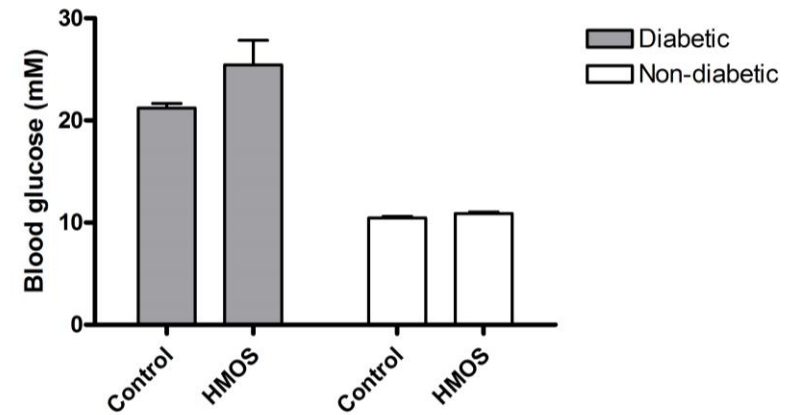


## Endpoint measurements

### Urine glucose score results



### Blood glucose levels

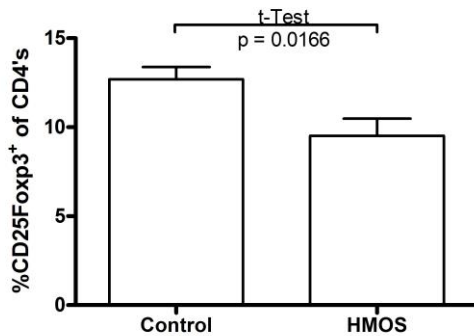


food intake & body weight were similar between experimental groups

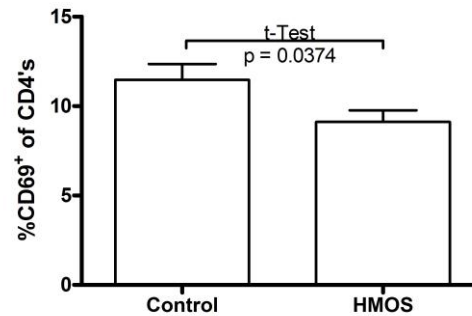


# REDUCED ACTIVATION OF SPLEEN CD4 T-CELLS AND REGULATORY T-CELLS IN HMOS GROUP

## Reduced T-regulatory cells

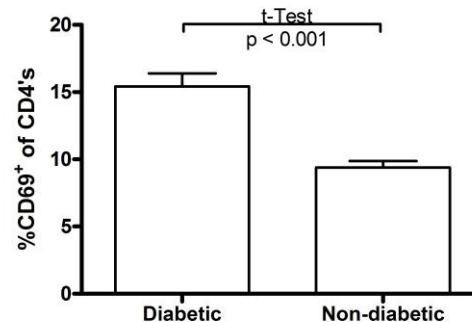
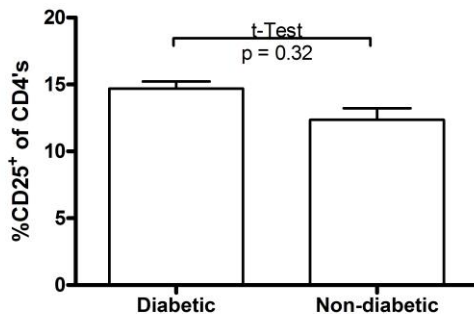


## Reduced activated T-helper cells



No increase in Tregs was observed, in contrast to effects of breastfeeding on T1D in BB rats (Brugman et al. 2009, Diabetes Metab Res Rev, 25(4):380-7)

## Related to decreased overall immune activation marker expression?



No differences were observed in % of Th1, Th2 or Th17 cells between dietary groups

# SCORING METHOD TO QUANTIFY PANCREAS INFLAMMATORY ISLET INFILTRATION

Each islet of each section was scored by this system:

0 = No Insulitis

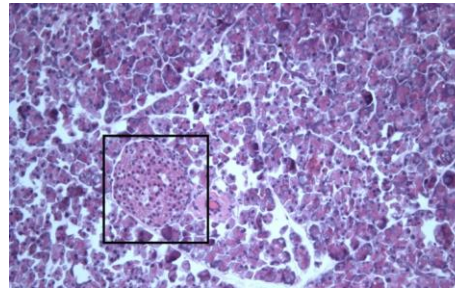
1 = Peri-Insulitis

2 = Insulitis affecting less than 50% of the islet area

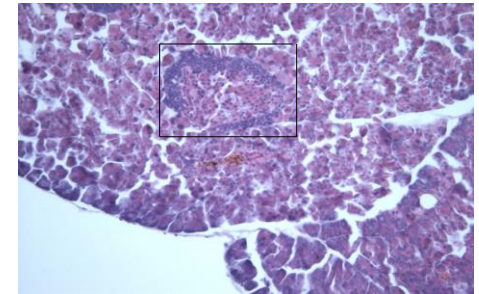
3 = Insulitis affecting more than 50% of the islet area

4 = Complete Insulitis

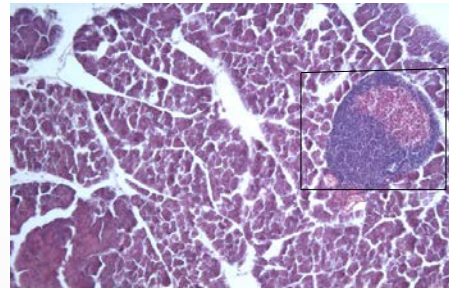
Average of 46 islets per animal were analyzed



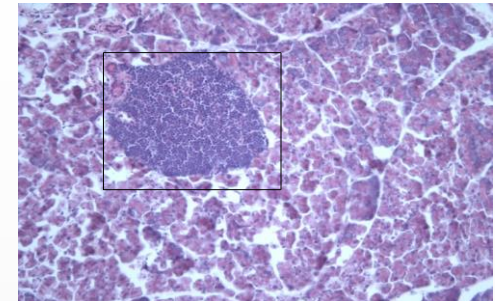
No insulitis



Peri-insulitis



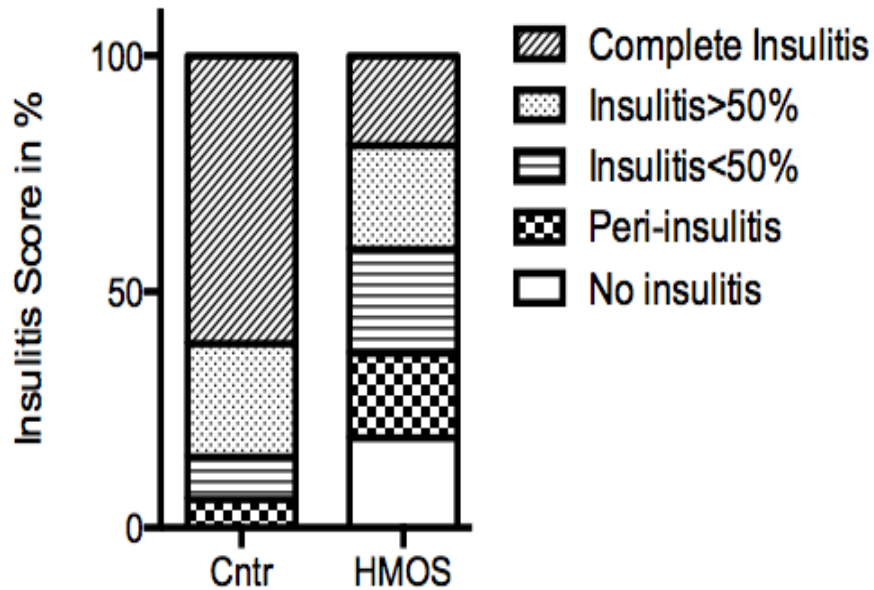
Insulitis >50%



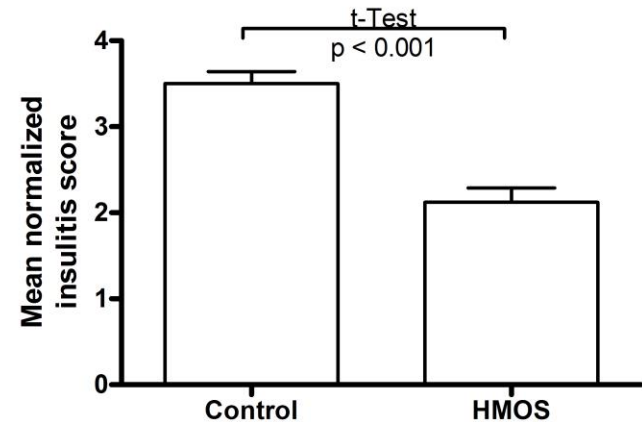
Complete insulitis

# DECREASED INSULITIS IN HMOS GROUP

## Most prevalent insulinitis score per animal



## Normalized score (range 0-4)



Insulinitis scores showed a partial correlation with urine glucose values, but many normoglycemic animals showed variable levels of insulinitis

# CONCLUSIONS

**Low level supplementation with the complex mixture<sup>1</sup> of HMOS in early life reduces autoimmune diabetes development in NOD mice later in life**

**Urine & blood glucose levels**

**Pancreas inflammation**

**Analysis of systemic immune cell populations revealed lower CD4 T-cell activation levels and lower percentages of Tregs**

**Lower Treg levels may be related to lower immune activation**

**HMOS in early life modulate immune responses in later life: an example of immunological programming**

B. Stahl et al, Anal Biochem 223(2):218-26, 1994



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