Title: Evaluating the Effect of Prebiotics on the Gut Microbiome Profile and β-cell Function in Newly Diagnosed Type 1 Diabetes

NCT: 04114357

Last Approval Date: April 11th, 2023

Evaluating the Effect of Prebiotics on the Gut Microbiome Profile and β-cell Function in Newly Diagnosed Type 1 Diabetes

Version 12.0 (April 2023)

NCT# 04114357

Protocol Physician

Heba Ismail, MB Bch
Assistant Professor of Pediatrics, Endocrinology and Diabetology,
Indiana University School of Medicine, Indianapolis
635 Barnhill Drive, Van Nunys Medical
Sciences BuildingMS2053Indianapolis, IN
46202
Tel: 317-278-7041
Fax: 317-944-3882
Email: heismail@iu.edu

This clinical study is supported by the KL2 program, the IU Health Values Funds and conducted at Indiana University, Indianapolis

TABLE OF CONTENTS: Overview and BACKGROUND 4

1.0 – OVERVIEW AND BACKGROUND

1.1 BACKGROUND

Type 1 diabetes (T1D) is a disease characterized by progressive β-cell loss with eventual dependence on exogenous insulin. The incidence of T1D is rising at a rate of nearly 3% per year, and the disease contributes over $14.4 billion annually to U.S. healthcare expenditures [1, 2]. While immunomodulatory therapy administered near the time of clinical diagnosis has shown some efficacy in attenuating T1D progression, the long-term effectiveness of these drugs has been negligible [3-7]. Given these failures, there is a critical need for a simple, tolerable, cost-effective method to mitigate the disease process and burden associated with daily diabetes management.

Compared to healthy individuals, people with both type 1 and type 2 diabetes demonstrate gut dysbiosis (microbial imbalance) with a higher gram-negative to gram-positive gut bacterial ratio (creating a pro-inflammatory milieu) and fewer bifidobacteria, an important microbial population associated with many health benefits [8-13]. Further, the gut microbiome composition of T1D children shows increased virulence factors, phage, prophage, motility genes and higher response to stress [14-18], in addition to a lower count of bacteria producing butyrate, a short chain fatty acid (SCFA) with anti-inflammatory actions [19].

The effect of gut microbiome modulation on glycemia has been assessed using both prebiotics (supplements containing substrates for the microbiota) and probiotics (supplements containing microbiota). Animal studies have shown that pre and probiotic use can change the gut microbiome profile, modulate the immune system, and improve glucose tolerance [20-22]. In persons with type 2 diabetes (T2D), prebiotics improve hemoglobin A1c (HbA1c) and postprandial glycemic excursion as well as reduce insulin resistance [23-26]. Thus, it appears that altering the gut microbiome may improve glycemia and improve insulin sensitivity.

A novel approach to mitigating T1D is perturbation of the gut microbiome using high-amylose maize starch (HAMS), a well-tolerated source of dietary fiber with selective fermentation properties. HAMS consumption shifts the gut microbiome profile towards SCFA fermenters. Further, HAMS that is acetylated and butyrylated (HAMS-AB) releases large amounts of beneficial SCFAs after colonic bacterial fermentation. Indeed, recent T1D mouse studies [22] have shown that HAMS-AB feeding shifted the gut microbiome profile towards fermenters, resulting in higher blood and fecal concentrations of the microbial metabolites acetate and butyrate (both beneficial SCFAs). These changes were associated with lower rates of progression to T1D, lower circulating inflammatory marker concentrations, and less islet autoimmunity. Data to date using HAMS in humans are more limited. When given to non-diabetic adults, HAMS lowers post-prandial glucose production and improves insulin sensitivity [27, 28].

1.2 Overview

Given these promising findings in animal models of T1D as well as adult non-diabetic studies, we hypothesize that HAMS-AB will alter the gut microbiome profile in humans with T1D and increase SCFA production and that these changes will be associated with improved β-cell function, overall glycemia, and β-cell health (as measured by markers of β-cell stress and death). We plan to perform a pilot, randomized, controlled cross-over trial of HAMS-AB in 12 individuals with recently diagnosed T1D to determine HAMS-AB effects on the gut microbiome profile, SCFA production, glycemia, markers of β-cell function, stress, and death.

This pilot study is a single center trial. Twelve newly diagnosed T1D youths with a BMI<85% and within 4-36 months of diagnosis who have measurable endogenous insulin production, assessed using serum C-peptide, and are, therefore, in the honeymoon period, will be recruited. We have chosen this cohort since the microbiome profile differs by age [29] and BMI [30], and the progression of T1D differs between children and adults [31]. Additionally, there is ample published experience studying healthy adolescents and their microbiome changes with corn fiber supplementation [32, 33], and therefore, we have chosen to pilot this intervention in a similar age group with known published data. In addition, to assess changes more accurately in β-cell function, we will include those in the honeymoon period as there is sufficient measurable C- peptide secretion during this time. The honeymoon period is the time shortly after T1D diagnosis when the β-cells undergo partial and temporary recovery and produce enough insulin to reduce insulin needs and aid with glycemic control [34, 35].

The **objectives** of this study are to assess the HAMS-AB effects on the gut microbiome profile, SCFA production, glycemia and β-cell health and function in humans with T1D. We **hypothesize** that HAMS-AB will (i) improve the gut microbiome profile in humans with T1D, (ii) increase SCFA production, and (iii) improve β-cell function, β-cell health and overall glycemia. We propose the following Specific Aims:

AIM 1: Determine the effect of HAMS-AB on the gut microbiome profile and SCFA production in children with recently diagnosed T1D. We *hypothesize* that HAMS-AB consumption will shift the gut microbiome to microbial populations that are capable of HAMS-AB fermentation and increase SCFA production. Twelve youths (11-17 years old) will consume HAMS-AB or follow the standard of care recommended diabetic diet daily for [49,50] 4 weeks and then cross over after a 4-week washout period. DNA will be extracted from fecal samples obtained followed by 16S RNA sequencing for gut microbial community analysis at baseline and at each visit during the study period [32, 33]. SCFAs will be analyzed using standard methods [36, 37].

AIM 2: Determine the effect of HAMS-AB on glycemia and β-cell function and health. We *hypothesize* that the prebiotic HAMS-AB will improve overall glycemia including reducing glycemic variability in recently diagnosed persons with T1D. We will compare glycemic/ β-cell measures pre/post intervention with HAMS-AB and between the intervention and control groups. We will measure glycemia using HbA1c, average glucose and glucose variability using continuous glucose monitoring (CGM) [38-40]. We expect to see an improvement in glycemia in response to HAMS-AB intake. We will assess β-cell function using mixed meal tolerance (MMTT)-derived C-peptide measures. We *expect* that measures of β-cell stress/death will show improvement following the use of HAMS-AB. We will determine β-cell stress using proinsulin/C-peptide (PI:C) ratios [41]. We will assess β-cell death by differential methylation of preproinsulin (INS) DNA [42].

Upon successful completion of this pilot study, we expect to assess the effect of prebiotic administration on the gut microbiome profile, SCFA production, glycemia and β-cell function in humans with T1D. The knowledge generated by the proposed research will provide critical experimental information needed to substantiate a fully powered study.

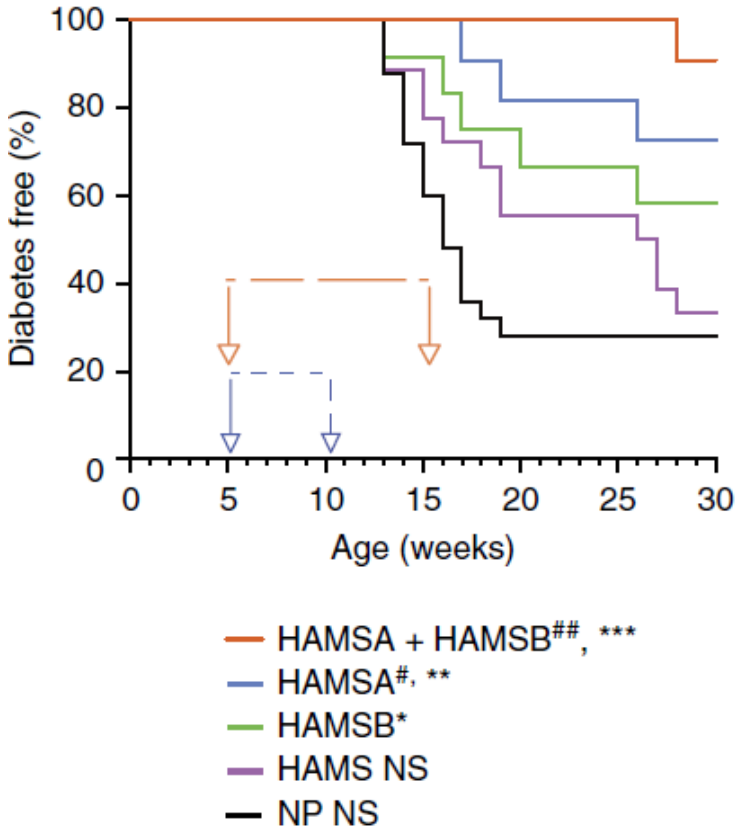
1.3 PRECLINICAL AND CLINICALEXPERIENCE

HAMS is a prebiotic that is a well-tolerated source of dietary fiber with selective fermentation properties. In T1D mouse studies [21], feeding HAMS that has been acetylated and butyrylated (HAMSA and HAMSB) shifts the gut microbiome profile towards fermenters, resulting in increased acetate and butyrate production and lower rates of progression to T1D, Figure 1.

In a human study assessing the effects of 2 levels of intake of HAMS [15 or 30 g/d (double-blind)] compared to control starch intake (0 HAMS) for 4-wk periods separated by 3-wk washouts on insulin sensitivity (SI) in participants with increased waist circumference, it appeared that the consumption of 15–30 g/d of HAMS improved insulin sensitivity significantly in men, Figure 2 [27].

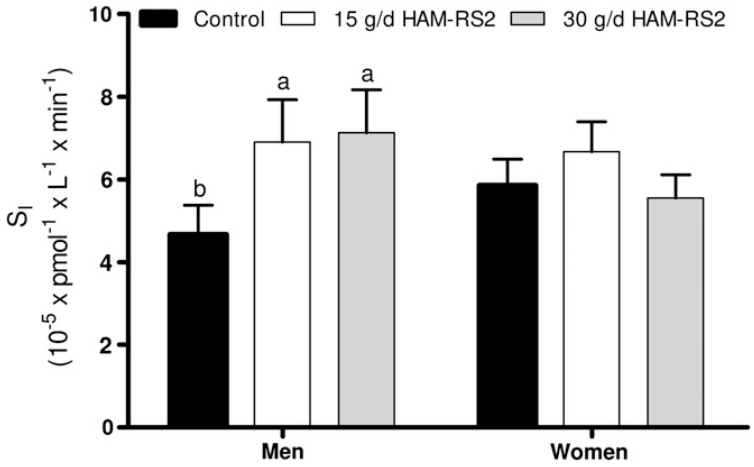
In another human randomized, double-blind, controlled study [28] in healthy adults who consumed either a high fiber scone containing a novel chemically modified high amylose maize starch or a low fiber control scone without the maize starch, the consumption of the high fiber scone significantly reduced postprandial glucose and insulin incremental areas under the curves (43–45% reduction, 35–40% reduction, respectively) and postprandial glucose and insulin maximum concentrations (8–10% and 22% reduction, respectively), Figure 3. Of note, ingestion of the high fiber scone consumption was not associated with increased gastrointestinal side effects compared with the control scone.

Figure1: Incidence of T1D in female NOD mice fed the NP diet (*n* = 25 mice), HAMS diet (*n* = 17 mice), HAMSA diet (*n* = 11 mice), HAMSB diet (*n* = 12 mice) or combined (HAMSA plus HAMSB) diet (*n* = 11 mice) for 10 weeks (HAMSA plus HAMSB; orange arrows) or 5 weeks (all other diets; blue arrows), starting at 5 weeks of age. NS (NP vs HAMS, HAMSB vs HAMS, HAMSP vs HAMS, and HAMSP vs NP); **P* = 0.0482 (HAMSB vs NP), #*P* = 0.0490 (HAMSA vs HAMS), ***P* = 0.0069 (HAMSA vs NP), ##*P* = 0.0025 (HAMSA+HAMSB vs HAMS) and ****P* = 0.0008 (HAMSA+HAMSB vs NP) (Mantel-Cox log-rank test) [22].



Conventional, non-purified (NP), High-amylose maize starch (HAMS) that has been acetylated (HAMSA) or butyrylated (HAMSB), non-significant (NS).

Figure 2: Insulin sensitivity (SI) following 4-wk feeding periods for control (0 g/d HAMS), 15 g/d HAMS, and 30 g/d HAMS in men (*n* = 11) and women (*n* = 22). Bars represent least squares geometric means and error bars extend to the value of the $\log_e$ least squares mean + 1 SEM, back transformed to the original units. Labeled means without a common letter differ, $P < 0.05$. Least squares mean and SEM values for $\log_e S_I$ were generated from repeated-measures ANOVA models containing terms for participant as a random variable, treatment condition, treatment sequence, and measures of insulin sensitivity [27].



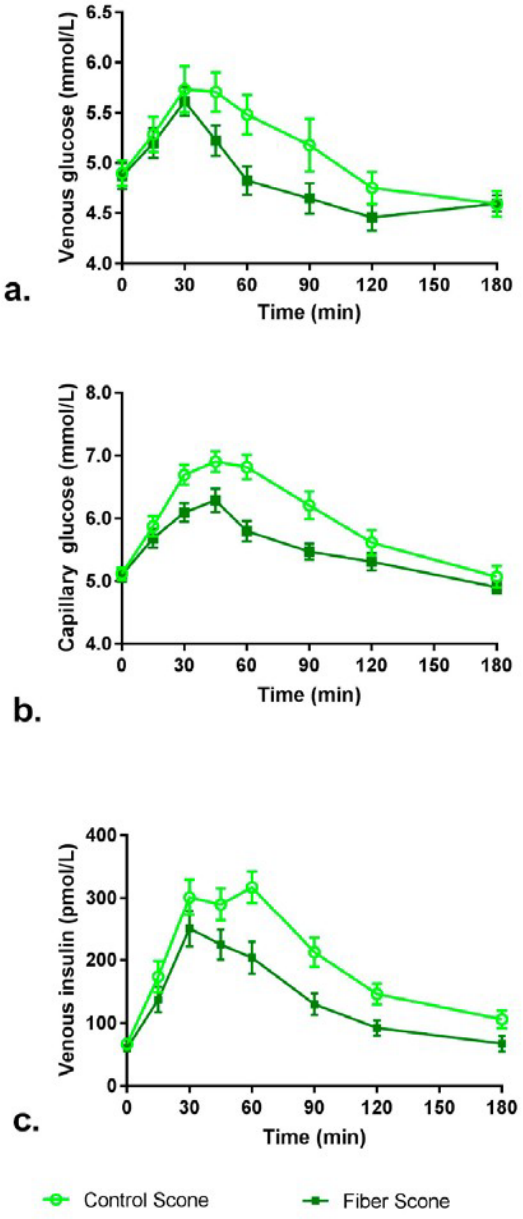


Figure 3: Mean post-prandial glucose and insulin concentrations over 180 min after ingestion of a scone containing a chemically modified maize resistant starch: a. venous glucose, b. capillary glucose, c. venous insulin. Error bars represent the standard error of the mean [28].

1.4 SCIENTIFIC RATIONALE

To date, there have been no studies assessing the effect of using a prebiotic, such as HAMS-AB, on the gut microbiome profile, glycemia and β-cell function in humans with T1D. In addition, there are no data linking changes in the gut microbiome and their metabolites in response to administration of HAMS-AB to metabolic changes in youths with T1D. This study will examine repurposing an existing, safe supplemental product for the treatment of T1D.

2.0 – OBJECTIVES

2.1 Primary Objective

Assess the effect of administering HAMS-AB on the gut microbiome profile in people with recently diagnosed T1D.

2.2 Secondary Objectives

Assess the effect of administering HAMS-AB on SCFA production in people with recently-diagnosed T1D.
Determine the effect of HAMS-AB on glycemia and β-cell function and health.

3.0 – STUDY DESIGN

3.1 STUDY DESCRIPTION

This study will be a single center, controlled, randomized trial in young individuals newly diagnosed with type 1 diabetes. We are enrolling youth with new onset T1D with documented continued residual C-peptide production. Within 30 days of screening during which eligibility will be determined, subjects will have a 4-week treatment period with HAMS-AB or will be provided with guidelines on the generally recommended diabetic diet for youths with T1D [49, 50]. After a 4-week wash-out period the durability of effect will be assessed in those who received the prebiotic and during this washout period, both the study and control arm will be permitted to consume a regular diet. This design has been used in other studies of prebiotic administration with a 3–4-week intervention period and a 3–4-week washout phase [21, 32, 33]. Subjects will then cross over to either HAMS-AB or diet control for another 4 weeks.

A full dosing description can be found in section 6.2.

32 STUDY DURATION

This study will screen up to 24 subjects at a single center (see Enrollment below). 12 subjects will be enrolled after meeting all eligibility criteria. If at any time during the study, a participant does not meet eligibility, participation in the study will be on hold until subject meets all eligibility criteria again. Participants may re-enter or re-enroll in the study if in the future, meet all eligibility requirements. Participants who re-enter or re-enroll will need to be reassessed for their starting point. Subjects who miss 20% of prebiotic doses (11 of 56 doses) will be replaced. The study will include the following periods:

- (1) Screening visit and eligibility determination (up to 30 days).
- (2) 4-week intervention period or diet guideline
- (3) 4-week washout period
- (4) 4-week intervention period or diet guideline.

33 ENROLLMENT

Up to 24 subjects will be screened to find 12 adequate subjects eligible for enrollment.

The plan is to recruit 24 patients with the goal of having 50% eligible and enrolled. Once 12 participants have completed the 12 week visit, we will stop recruitment. At Indiana University, as per our current recruitment strategies for other new-onset studies, patients with newly diagnosed T1D will be approached whenever possible when inpatient at diagnosis and then re-contacted after discharged home. Visits will be conducted at the Indiana Clinical Research Center (ICRC) and coordinated with diabetes clinic follow-up visits when possible.

34 STUDY ENDPOINTS

The primary outcome assessment will be to assess changes in the gut microbiome profile in response to HAMS-AB. Secondary outcome measures will be changes in SCFA production, changes in glycemia, pro-insulin to C-peptide ratios as a marker of β cell ER stress. Other β cell stress markers including unmethlyated insulin DNA will also be measured. We will also examine glycemic outcomes and β cell function as assessed by responses to MMTT, although given the variability in these measures in other reported studies of new-onset T1D [43] we do not expect to be adequately powered in this small trial to see significant differences in these outcomes.

35 STUDY RISKS

Risks include those risks related to taking HAMS-AB, having blood draws, mixed meal tolerance tests (MMTT), risk of CGM use, and the possible loss of confidentiality.

Risks of Taking HAMS-AB:
Given that most youths in the United States are consuming less than the recommended dietary intake of fiber, we anticipate the following risks:

- Likely (reported in >20% of patients): initial abdominal discomfort, increase in frequency of bowel movements, although not to the extent of causing diarrhea. These mild intestinal symptoms will likely last for only the first few days and are self-limited without intervention.

Risk of Blood Draws:
The insertion of a needle can cause some local pain and can result in bruising. Blood draws can in rare instances cause lightheadedness or fainting. There is also a risk of infection. To mitigate these risks, blood draws will be performed by trained technicians or nurses using standard aseptic techniques.

Risk of MMTT:
There are no known risks to an MMTT, but the taste of the Boost may not be well tolerated. Some persons may experience nausea after drinking Boost. Subjects with milk or soy allergies will be excluded to avoid any allergic

reactions to Boost. If the blood glucose is high at the end of the test, subjects will be instructed to give an insulin correction dose.

Risks related to CGM use:
The CGM/sensor may produce pain when it is inserted into the skin. There is a low risk for developing a local skin infection at the site of the sensor needle placement. Itchiness, redness, bleeding, and bruising at the insertion site may occur as well as local tape allergies.

Loss of Confidentiality:
There is the unlikely chance that health information is viewed by someone outside of the research team who is not authorized to see health information. All files will be kept in locked cabinets within a locked room. Any electronic data will be protected by password systems.

3.6 STUDY BENEFITS

While receiving HAMS-AB, there are potential benefits in reduction of β-cell stress for these patients. Since the gut dysbiosis may be important in the pathogenesis of T1D, accentuate β-cell stress, and have a role in the complications of the disease, these studies are of scientific importance. The benefits to be derived by mankind are expected to be substantial and may lead to advances in the prediction, prevention, and treatment of type 1 diabetes. If this study indicates that treatment can safely preserve beta cell health, then it will be a major advance in the care of diabetes.

3.7 INCLUSION OF CHILDREN

Type 1 diabetes is a disease that typically has its onset in children, adolescents, and young adults. Because we are interested in examining the role of the HAMS-AB and the gut microbiome in adolescents with T1D (and potentially eventually those at risk for developing T1D), adolescents will be studied to have data in a relevant population. As part of the consent process, in addition to consent from the parent, assent for studies and samples from all participants will be obtained.

4.0 – STUDY SUPPLEMENT

4.1 STRUCTURE AND NOMENCLATURE

CAS Registry No.:	none
Chemical Name:	high amylose maize starch
Chemical Family:	Starch
Molecular Weight:	>1000
Synonyms:	HYLON® VII

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Pure Substance or Mixture:	Pure
Appearance (physical state):	Powder.
Color:	White
Odor:	Starch
Odor threshold:	Not available
Molecular Weight:	> 10000
pH:	Not available
pH in (1%) Solution:	Approximately 5.5
Melting Point/Freezing Point (°C):	Not available
Relative Density:	Not available

Specific Gravity	1.5
Solubility(ies) in Water:	Insoluble

4.3 STABILITY AND REACTIVITY

Reactivity:	Not expected to be reactive
Chemical stability:	Material is stable under normal temperatures and pressures.
Possibility of hazardous reactions:	Hazardous polymerization will not occur.
Conditions to avoid (e.g., static discharge, shock, or vibration):	None known
Incompatible materials:	None known
Hazardous decomposition products:	This product does not undergo spontaneous decomposition. Typical combustion products are carbon monoxide, carbon dioxide, nitrogen, and water.

4.4 CLINICAL STUDIES – MECHANISM OF ACTION

HAMS appears to work by altering the gut microbiome profile and improving glycemia and insulin sensitivity [26, 27]. Additionally, the FDA has concluded that there was scientific evidence for a qualified health claim that HAMS reduced risk of type 2 diabetes development in adults.

4.5 STUDY RELATED INFORMATION

HAMS-AB will be in the form of acetylated and butyrylated HYLON® VII corn starch [a resistant corn starch type 2 (RS2) derived from high amylose maize starch and containing approximately 70% amylose that has been acetylated and butyrylated. It will be provided by Ingredion Incorporated, Bridgewater, NJ. A computer-based randomization plan will be prepared by the PI. Randomization lists will be kept by the PI.

HAMS is a resistant corn starch type 2 dietary supplement which is high in certain foods such as potatoes and green unripe bananas. RS2 is not digested in the small intestine and acts as a dietary fiber. Many short chain fatty acids (SCFAs), including acetic, propionic, and butyric acid, are formed normally during bacterial fermentation in the gut after fiber intake. Therefore, there are no expected adverse effects in general. Similarly, we do not expect any adverse effects during pregnancy. However, to be safe, female subjects who are pregnant or anticipating pregnancy will not be enrolled and pregnancy tests will be done for females of reproductive potential at all visits.

HAMS has a satisfactory safety profile based on exposure in adults and pediatrics given that it is a dietary supplement available over the counter. To date, there has been no indication of specific organ class toxicity. Additionally, there has been no indication of a particular pattern of event clustering or increased individual safety risk, and no increased vulnerabilities or major risks have been identified for the T1D and pediatric population.

5.0 –ELIGIBILITY

5.1 INCLUSION CRITERIA

Patients must meet all the following criteria:

- Be between 11-17 years of age
- Willing to consume HAMS-AB and follow a diabetic diet
- Diagnosed by ADA criteria [45] with T1D in the last 4-36 months
- Random non-fasting C-peptide of 0.17nmol/L or greater
- Willing to use an effective form of contraception if sexually active

- BMI< 85% for age and sex
- Positive for any one of the following diabetes-related autoantibodies that are tested clinically [IAA (if tested within 14 days of diagnosis), GAD, IA-2, or ZnT8].

52 EXCLUSION CRITERIA

Individuals who meet any of the exclusion criteria during the study will be replaced. However, participants may re-enroll if in the future, meet all eligibility requirements. Participants who re-enroll will need to be reassessed for their starting point. Participants must NOT meet any of the following criteria during enrollment re-entry or re-enrollment:

1. Presence of severe, active disease that interferes with dietary intake or requires the use of chronic medication, with the exception of well-controlled hypothyroidism and mild asthma not requiring oral steroids.
2. Diabetes other than T1D (Known monogenic forms of diabetes, Type 2 diabetes)
3. Chronic illness known to affect glucose metabolism (e.g., Cushing syndrome, polycystic ovarian disorder, cystic fibrosis) or taking medications that affect glucose metabolism (e.g., steroids, metformin)
4. Psychiatric impairment or current use of anti-psychotic medication
5. Any condition that, in the investigator's opinion, may compromise study participation or may confound the interpretation of the study results.
6. Female participants of child-bearing age with reproductive potential, must not be pregnant and agree to use an effective form of birth control or be abstinent during the study period (see below)
7. History of recurrent infections
8. History of on-going infections or antibiotic treatment within the past 4-6 weeks
9. History of immune compromise
10. Steroid intake (inhaled or oral)
11. Other immunosuppressant use in past 6 months
12. History of gastrointestinal disease
13. Possible or confirmed celiac disease
14. Pregnancy or possible pregnancy
15. Allergy to corn (prebiotic)
16. Allergy to milk or milk products or soy present in Boost
17. Participation in other intervention research trials within the past 3 months
18. Anticipate major changes in diabetes management during study (change from injection to pump, start of CGM usage if not already using one)
19. Consuming high fiber or vegetarian diet (consuming three or more servings of high fiber foods on 4 or more days per week) by history.
20. If taking fiber supplements or probiotics, unwilling to stop for the duration of the study.

Although generally considered to be safe, this has not been specifically studied in pregnant women and therefore, females of childbearing age will be asked to use effective Forms of Birth Control – any one of the following (additionally, participants will be counseled on the use of a second method of contraception):

a. Use of oral, injected, or implanted hormonal methods of contraception or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example, hormone vaginal ring or transdermal hormone contraception
b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
c. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository

53 EARLY TERMINATION OF A STUDY PARTICIPANT

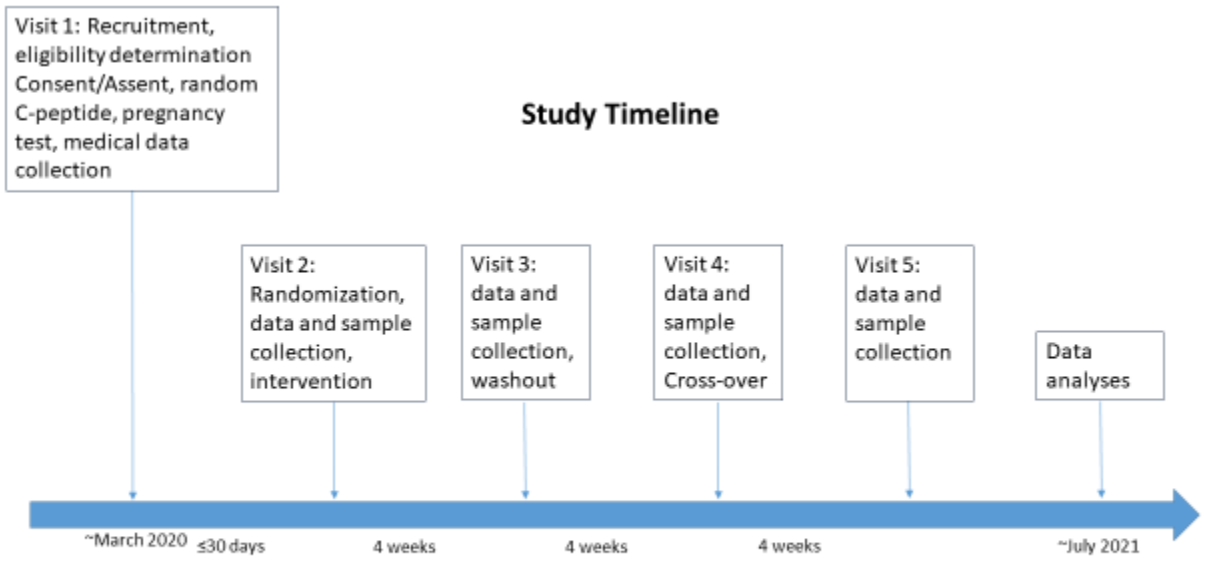
Treatment will stop and the subject will return for an exit visit if any of the following conditions occur:

- withdrawal of consent
- pregnancy (female participants)
- any medically important event such as a concurrent illness or complications

- any investigator judgment

6.0 – STUDY PROCEDURES

6.1 STUDY DESIGN



6.2 RANDOM ASSIGNMENT

Subjects will be randomly assigned (6 in each arm) to either HAMS-AB plus a recommended diabetic diet or control diabetic diet alone [49, 50] for the first 4 week of treatment period. After a suitable washout period during which participants do not need to follow the recommended diet or use the supplement, subjects will continue for 4 weeks after a cross-over. All participants will be expected to follow the guidelines for a healthy diabetic diet during the study period, except for during the 4-week washout period.

A computer-based randomization plan will be prepared by the principal investigator. The document will be kept by the study team for use. As a subject is screened and qualifies, he/she will be assigned in order of screening date on the computer-generated document. One master list will be maintained in a confidential central shared folder for use. Subjects will be replaced on an ongoing list if they drop out.

Participants will be instructed to consume HAMS-AB with food, such as apple sauce or oatmeal, in two divided doses at breakfast and dinner at a total daily dose to be calculated as has been previously described for children: 10g plus 1g per year of age daily [44]. The prebiotic will be dispensed by the Investigational Drug Services (IDS) at Indiana University.

HAMS-AB will be in the form of acetylated and butyrylated HYLON® VII corn starch.

6.3 SCHEDULE OF EVENTS

Study Phase	Screening	Baseline-Intervention	Washout	Crossover-Intervention	Follow-Up	Early Termination Visit
Visit Number	1	2	3	4	5	
Visit Window		≤ 30 days after Visit 1	+/- 3 days 28 days after Visit 2	+/- 3 days 28 days after Visit 3	+/- 3 days 28 days after Visit 4	
Informed consent/Assent	X					
Inclusion/Exclusion criteria	X					
Historical Data/Demographics*	X					
Pancreatic Autoantibodies †	X					
Random C-peptide concentration††	X					
Randomization		X				
Pregnancy test**	X	X	X	X	X	X
Current Medical data***		X	X	X	X	X
Pubertal Tanner staging with physical exam	X					
Ht/Wt measurement and vitals	X	X	X	X	X	X
Supervised CGM insertion (and training, if applicable on use) †††		X		X		
C-peptide by 2-hr MMTT††††			X		X	X
HbA1c		X			X	X
Blood samples for biomarkers (PI:C and *INS* DNA)		X	X	X	X	X
Provision of HAMS-AB		X		X		
CGM download			X		X	X
Stool samples****		X	X	X	X	X
Whole blood for additional T1D testing		X	X	X	X	X
Serum and plasma for storage^		X	X	X	X	X
Adverse event assessment*****		X	X	X	X	X
Dietary Assessments******		X	X	X	X	X
Verbal Diet recall	X					
Sampling of prebiotic	X					

Data Collection:

***Historical data:** Date of birth, sex, Race/Ethnicity, date of diagnosis of T1D.

**To be done in girls of reproductive potential.

*****Current Medical Data (All visits except screening):** Medication use including types of insulin, means of insulin administration, and total daily insulin dose (u/kg body weight, averaged over the 3 days prior to each study visit), use of other medications.

******Stool samples** will be collected at home using a kit (Zymo feces catcher and RNA/DNA shield fecal collection tubes) within 3 days of presentation to each visit.

*****Participants will be assessed for **safety** with questions regarding general well-being as well as specific questions to evaluate for adverse events. Participants will also be questioned about stool form and frequency, gastrointestinal symptoms, fevers, or infection.

*********Dietary Assessments** will be ideally obtained using the Automated Self-Administered 24-Hour (ASA24) dietary assessment tool [46-48], a web-based tool for administering automatically coded, self-administered recalls/food records. This will allow assessment of dietary fiber intake along with other dietary components. Participants will be asked to fill these in for the 24 hrs. diet recall prior to each stool collection in a time frame of +/- 3 days from the stool sample collection. This can also be completed during the study visit. Diet diaries will be accepted in lieu of the online questionnaire. Participants will be asked to record all meals and snacks, time of day, number of servings, major food components or description (for example chicken or protein), estimated portion sizes and whether they completed the meal or not, and any drinks including water.

†Pancreatic autoantibodies to be tested only in those with no evidence of being tested for antibodies in their medical chart to confirm T1D.

††Random, non-fasting c-peptide: C-peptide level will be analyzed at Indiana University Translation Core Lab using a two site immuno-enzymometeric assay using a Tosoh 2000 auto- analyzer (TOSOH, Biosciences, Inc., South San Francisco, CA). The C-peptide assay is calibrated against the WHO IS 84/510 standard and has a sensitivity level of
0.05 ng/mL.

†††At visit 2 (baseline/intervention) and 4 (crossover), a blinded CGM sensor will be inserted in those participants who are not currently using a CGM as part of their routine care. Training will be provided on calibration of the sensor, its use in blinded mode, and sensor insertion. Participants will be reminded to insert a sensor at home after approximately 14 days and continue using the blinded sensor for an additional 14 days. Participants will use their own home blood glucose meter or (if had been previously using one) CGM for regular blood glucose monitoring. The participant will return after 4 weeks to assess the CGM data. CGM must be used for at least 200 hours (equivalent to 8.3 days out of 14 days)

††††C-peptide by 2-h MMTT: 2-hour MMTT will be performed using the standardized protocol utilized by the TrialNet Centers [51]. The early discontinuation MMTT will only be done if it has been at least 6 weeks since the last MMTT.

^Serum and Plasma for storage – subject has option to consent or deny storage. Will be used for future type 1 diabetes research assays.

6.4 STUDY VISITS

Visit 1 – Screening

This visit will be at the hospital or research center. It will last approximately 1-2 hours and will include:

- Obtain informed consent/assent documents and determine inclusion and exclusion criteria
- Measurement of height and weight to determine BMI percentile eligibility and physical exam including pubertal staging
- Obtaining Vital signs (heart rate, temperature, blood pressure, and respiratory rate)
- Obtaining medical history
- Dietary assessment by verbal recall
- Obtaining blood sample (approximately 1.5 tablespoon) – Random non-fasting c-peptide, pancreatic autoantibodies if not previously done to confirm T1D diagnosis
- For female adolescents: urine will be collected for a pregnancy test
- Subject to sample prebiotic for palatability to ensure compliance

Visit 2 – Baseline-Intervention

This visit will be at the hospital or research center. It will last approximately 1-2 hours and will include:

- Measurement of weight and bedside nurse assessment such as general appearance, respiratory and cardiac assessment.
- Obtain vital signs (heart rate, temperature, blood pressure, and respiratory rate)
- Collecting a diet history (diary or questionnaire)
- Collecting Adverse Events (if applicable)
- Obtaining medical history and insulin use
  Obtaining blood samples (approximately 2 tablespoons) – HbA1c, Serum biomarkers of beta cell stress and death (PI:C and *INS* DNA), other T1D testing, and serum and plasma for storage.
    - Some of the blood will be stored for future T1D research use
- CGM placement and training for participants not currently using one.
- For female adolescents: urine will be collected for a pregnancy test
- Collecting stool samples prior to or the morning of the visit (a stool collection kit will be shipped to the participants once eligibility is confirmed)
- Study Randomization to either supplement or standard of care diabetes management and a diabetic diet. – Supplement (HAMS-AB) will be provided and will be taken by mouth twice daily with food such as apple sauce or oatmeal as described in Section 6.2. Instructions will be provided for recommendations on a healthy diabetic diet.
- A new stool collection kit will be provided for the next visit

Visit 3 - Washout

This visit will be at the hospital or research center. It will last approximately 2-4 hours and will include:

- Measurement of weight, assessment of CGM insertion sites, as well as bedside nurse assessment such as general appearance, respiratory and cardiac assessment.
- Obtaining Vital signs (heart rate, temperature, blood pressure, and respiratory rate)
- Collecting a diet history (diary or questionnaire)
- Collecting Adverse Events (if applicable)
- Obtaining medical history and insulin use
- CGM download
- For female adolescents: urine will be collected for a pregnancy test
- Collecting stool samples prior to or the morning of the visit
- Obtaining fasting blood sample (approximately 5 tablespoons) – Serum biomarkers of beta cell stress and death (PI:C and *INS* DNA), other T1D testing, and serum and plasma for storage and MMTT

samples (see below), and serum and plasma for storage
    - Some of the blood will be stored for future T1D research use
- Completion of a mixed meal tolerance test (MMTT). See below.
- After completion of this visit, participants will be instructed to follow a regular diet with no supplement intake for the following 4 weeks and to follow standard diabetes care as instructed by their diabetes provider.
- A new stool collection kit will be provided for the next visit

Preparing for an MMTT

- Subjects will be asked to fast except for water for at least 10 hours prior to visit.
- Subjects will be asked to limit strenuous activity for at least 10 hours prior to visit.
- Subjects will be asked to consume at least 150 g of carbohydrates each day for 3 days.
- If subject experiences hypoglycemia, morning of the visit, subjects will be instructed to treat low blood sugar and to reschedule MMTT procedure.

MMTT Visit procedures

Blood glucose will be tested and the MMTT started if blood glucose <200 by 10:00am. Long-acting insulin may be taken per routine, but short acting insulin must not have been given within 2 hours of MMTT.

An IV catheter will be placed in the arm by trained nurses for blood drawing purposes. Blood will be drawn 10 minutes prior to drinking Boost, just prior to drinking Boost, 15, 30-, 60-, 90-, and 120-minute post Boost consumption. High Protein Boost will be measured to a dose of 6ml/kg body weight (maximum dose 360ml). Total volume of blood will be approximately (5 tablespoons) not to exceed 5ml/kg/day.

Visit 4 Crossover-Intervention

This visit will be at the hospital or research center. It will last approximately 1-2 hours and will include:

- Measurement of weight, assessment of CGM insertion sites, as well as bedside nurse assessment such as general appearance, respiratory and cardiac assessment.
- Obtaining vital signs (heart rate, temperature, blood pressure, and respiratory rate)
- Collecting a diet history (diary or questionnaire)
- Collecting Adverse Events (if applicable)
- Obtaining medical history and insulin use
- Obtaining blood samples (approximately 2tablespoon) – Serum biomarkers of beta cell stress and death (PI:C and *INS* DNA), other T1D testing, and serum and plasma for storage
    - Some of the blood will be stored for future T1D research use
- For female adolescents: urine will be collected for a pregnancytest
- Supplement (HAMS-AB) will be provided and will be taken by mouth twice daily in the treatment arm as above in addition to insulin for diabetes management. Those in the control arm will follow standard diabetes management in addition to a recommended diabetic diet.
- New CGM supplies given, if applicable.
- Collecting stool samples 3 days prior to or the morning of the visit
- A new stool collection kit will be provided for the next visit

Visit 5 Follow-Up and/or Early Termination Visit

This visit will be at the hospital or research center, will last approximately 2-4 hours and will include:

- Measurement of height and weight and assessment of CGM insertion sites
- Obtaining vital signs (heart rate, temperature, blood pressure, and respiratory rate)
- Collecting a diet history (diary or questionnaire)
- Collecting Adverse Events (if applicable)

- Obtaining medical history and insulin use
- Obtaining blood samples (approximately 5 tablespoons) – HbA1c, Serum biomarkers of beta cell stress and death (PI:C and *INS* DNA), MMTT samples (see above), other T1D testing, and serum and plasma for storage
    - Some of the blood will be stored for future use
- For female adolescents: urine will be collected for a pregnancy test
- Completion of a mixed meal tolerance test (MMTT) See Visit 3 for details.
- CGM download
- Collecting stool samples prior to or the morning of the visit

An early discontinuation MMTT will only be done if it has been at least 6 weeks since the last MMTT.

6.5 ONGOING DIABETES MANAGEMENT

All study participants will have a treating endocrinologist who is primarily responsible for their diabetes care. In general, the expectation is that all participants will receive at least three injections of insulin daily, including short- and long-acting insulin preparations, or will utilize continuous subcutaneous insulin infusion (CSII, insulin pump). As part of diabetes monitoring during the subjects' participation, we will encourage participants to check glucose levels at least four times daily if using blood glucose monitor and if not already using a CGM. **At the time of visits the study team** will suggest changes that they believe would improve the glucose control, if necessary. Letters summarizing the study visit will also be sent to the endocrinologist after parental agreement.

In addition, insulin use, hypoglycemic events, and any episodes of ketoacidosis will be captured at each visit on the appropriate CRFs.

Participants will be requested to record the amount of insulin they have used during the 3-day period immediately preceding each study visit. Insulin use logs will be provided to participants at each study visit and collected at the next visit. These logs will serve as the source documents.

6.6 BANKED SPECIMENS

Serum and plasma will also be banked and stored at Indiana University for other type 1 diabetes related assays and for potential future use by other investigators. Participants will have the option to not have samples stored on the informed consent documents.

7.0 REPORTING OF ADVERSE EVENTS OR UNANTICIPATED PROBLEMS INVOLVING RISK TO PARTICIPANTS OR OTHERS

7.1 UNANTICIPATED PROBLEMS

In the unlikely event of an acute emergency with a subject, all procedures will be performed in a hospital setting by trained personnel.

In the event of physical injury resulting from the participation in this research, necessary medical treatment will be provided and billed as part of their medical expenses. Costs not covered by health care insurer will be the subject's responsibility. Also, it is the subject's responsibility to determine the extent of health care coverage. There is no program in place for other monetary compensation for such injuries. However, the subject will not give up any legal rights or benefits to which he/she is otherwise entitled. If any severe adverse events occur while not at a medical facility, the subject will be responsible for seeking medical care and for the expenses associated with any care received.

7.2 DATA SAFETY MONITORING

The data safety monitoring plan involves Dr. Zeina Nabhan, MD, a pediatric endocrinologist, to review this study for data for quality, rate of subject recruitment, adverse events, procedures for protecting patient privacy, and any protocol deviations every 6 months following the first subject enrolled. If the review concludes that there are significant safety concerns or quality of the data is unsatisfactory, no further enrollment will occur until these issues have been addressed and any changes to the study approved by the IUPUI IRB.

7.3 ADVERSE EVENTS

An adverse event is defined as any unintended or abnormal clinical observation that is not of benefit to the patient. Either the condition was not present prior to exposure to study supplement, or it has worsened in intensity or frequency following exposure to the study supplement. Adverse events will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria v5.0.
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf
Any suspected or confirmed toxicity should be reported immediately (within 24 hours) to the principal investigator.

Hypoglycemic and hyperglycemic events that are seen as part of daily diabetes management will not be considered adverse events unless they result in hospitalization

Principal investigators (PI) will report to the IRB as soon as possible, but in all cases within 5 working days from notification any event that appears on the List of Events that Require Prompt Reporting to the IUPUI IRB.

List of Events that Require Prompt Reporting to the IUPUI IRB: Any of the following:

- Event (including adverse events, injuries, side effects during the research study), which in the opinion of the PI
    - caused harm to one or more subjects or others, or placed one or more subjects or others at increased risk of harm; AND
    - was unexpected; AND
    - was related to the research procedures

Note: After the study is closed with the IRB, these events will only be reported if they are profound, or they demonstrate long-term risks that would necessitate notifying subjects.

- Protocol deviation/violation (as defined under this policy)
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject (e.g., purposeful and for subject safety)
- Complaint of a subject that indicates unexpected risks, or complaint that cannot be resolved by the research team
- Interim findings and safety monitoring reports that indicate an unexpected change to the risks or potential benefits of the research, in terms of severity or frequency
- Publication in the literature that indicates an unexpected change to the risks or potential benefits of the research
- Change in FDA labeling or withdrawal from marketing of the supplement used in this research study
- Noncompliance (as defined in this policy)

8.0 STATISTICAL CONSIDERATIONS

A sample size of 12 with a cross over design (i.e., 12 per arm) is considered adequate to estimate effect sizes for efficacy to aid in designing future trials for responses that are normally distributed [52]. Simple comparisons will be performed using chi-square for categorical variables and t-tests for continuous variables. Random attritions and failed screens will be replaced. An intention to treat analysis will not be appropriate given the crossover design. Adverse events will be presented as the proportion of each event seen. This is considered a minimal risk study with low risk for minor adverse events such as mild gastrointestinal symptoms that could be related to increased dietary fiber intake. Our primary outcome is changes in the gut microbiome profile. To determine the diversity and richness of the microbiome, we will calculate a diversity index for both the pre- and post-intervention time points for each recipient. Microbial sequence analysis and statistics will be analyzed using the Quantitative Insights into Microbial Ecology (QIIME) 1.8.0 software as previously described [53].

8.0 PRIVACY/CONFIDENTIALITY ISSUES

Data sources will include paper and electronic medical records and test results. Data will be recorded on paper, desktop computers, and laptop computers. The Principal Investigator, Research Coordinators, Co-Investigators, Biostatistics, and Governmental Agencies will have access to the individually identifiable data. This data will be safeguarded by locking storage cabinets and office doors, keeping information in areas with limited public access, password protecting computers and files, and regular back-ups of electronic data. Data will be retained indefinitely. When confidential data need to be discarded, paper records will be shredded, CD's and diskettes will be destroyed, and data will be permanently deleted from computers. When possible, only de-identified data will be shared. If identifiable data will be shared, it will be done by fax in a secured area, on a shared drive with password protection, or by personal delivery by authorized research personnel.

10.0 FOLLOW-UP AND RECORD RETENTION

10.1 RECORD RETENTION

The duration of this study is expected to be 2 years. All specimens and records will be held as required by law and until no further questions arise from the data.

11.0 REFERENCES CITED

1 DIAMOND Project Group. Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. Diabet Med. 2006;23(8):857-66.

2 Tao B, Pietropaolo M, Atkinson M, et al. Estimating the cost of type 1 diabetes in the U.S.: a propensity score matching method. PloS one. 2010;5(7):e11501.

3 Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J M. 2002;346(22):1692-8.

4 Herold KC, Gitelman S, Greenbaum C, et al. Treatment of patients with new onset Type 1 diabetes with a single course of anti-CD3 mAb Teplizumab preserves insulin production for up to 5 years. Clin Immunol. 2009;132(2):166-73.

5 Orban T, Bundy B, Becker DJ, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. Lancet. 2011;378(9789):412-9.

6 Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al. Rituximab, B-Lymphocyte Depletion, and Preservation of Beta-Cell Function. N Engl J M. 2009;361(22):2143-52.

7 Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, Gitelman SE, Gottlieb PA, Krischer JP, Linsley PS, Marks JB, Moore W, Moran A, Rodriguez H, Russell WE, Schatz D, Skyler JS, Tsalikian E, Wherrett DK, Ziegler AG, Greenbaum CJ; Type 1 Diabetes TrialNet Study Group. An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. N Engl J Med. 2019 Jun 9. doi: 10.1056/NEJMoa1902226. [Epub ahead of print] PubMed PMID: 31180194.

8 Kostic AD, Gevers D,Siljander H, Vatanen T, Hyotylainen T, Hamalainen AM, Peet A, Tillmann V, Poho P, Mattila I, et al.: The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell Host Microbe 2015,17:260-273

9 Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C. Review article: bifidobacteria as probiotic agents —physiological effects and clinical benefits. Aliment Pharmacol Ther. 2005;22(6):495–512.

10 Brugman S, Klatter FA, Visser JT, Wildeboer-Veloo AC, Harmsen HJ, Rozing J, Bos NA: Antibiotic treatment partiallyprotects against type 1 diabetes in the bio-breeding diabetes-prone rat. Is the gut flora involved in the development of type 1 diabetes? Diabetologia 2006, 49:2105–2108.

11 de Kort S, Keszthelyi D, Masclee AA. Leaky gut and diabetes mellitus: what is the link? Obes Rev. 2011;12(6):449–58. 17. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7): 1761–72.

12 Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, Drew JC, Ilonen J, Knip M, Hyöty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW: Toward defining the autoimmune microbiome for type 1 diabetes. ISME J 2011, 5:82–91.

13 Vaarala, O. Is the origin of type 1 diabetes in the gut. Immunol. Cell Biol. 90, 271–278 (2012).

14 Mejía-León, M. E., Petrosino, J. F., Ajami, N. J., Domínguez-Bello, M. G. & de la Barca, A. M. Fecal microbiota imbalance in Mexican children with type 1 diabetes. Sci. Rep. 4, 3814 (2014).

15 Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC Med. 2013;11:46

16 de Goffau M, Luopajärvi K, Knip M, Ilonen J, Ruohtula T, Härkönen T, Orivuori L, Hakala S, Welling GW, Harmsen HJ, Vaarala O: Fecal microbiota composition differs between children with beta-cell autoimmunity and those without. Diabetes 2013, 62:1238–1244.

17 de Goffau MC, Fuentes S, van den Bogert B, Honkanen H, de Vos WM, Welling GW, et al. Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. Diabetologia. 2014;57(8):1569–77. Doi: 10.1007/s00125-014-3274-0. Epub 2014 Jun 15.

18 Brown CT, Davis-Richardson AG, Giongo A, Mukherjee N, Novelo LL, Casella G, Drew JC, Ilonen J, Knip M, Hyöty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW: Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. Plos One 2011, 6:e25792.

19 Louis P, Flint HJ: Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. Fems Microbiology Letters 2009, 294:1–8.

20 Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, et al. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes. 2011;60(11):2775–86.

21 Parnell JA, Reimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. Br J Nutr. 2012;107(4):601–13.

22 Mariño E, Richards JL, McLeod KH, Stanley D, Yap YA, Knight J, McKenzie C, Kranich J, Oliveira AC, Rossello FJ, Krishnamurthy B, Nefzger CM, Macia L, Thorburn A, Baxter AG, Morahan G, Wong LH, Polo JM, Moore RJ, Lockett TJ, Clarke JM, Topping DL, Harrison LC, Mackay CR. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. Nat Immunol. 2017 May;18(5):552-562. doi: 10.1038/ni.3713. Epub 2017 Mar 27. Erratum in: Nat Immunol. 2017 Oct 18;18(11):1271. Nat Immunol. 2017 Jul 19;18(8):951. PMID: 28346408

23 Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. Br J Nutr. 2014;111(7):1147–61.

24 Dehghan P, Gargari BP, Jafar-Abadi MA, Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. Int J Food Sci Nutr. 2014; 65(1):117–23.

25 Cani PD, Daubioul CA, Reusens B, Remacle C, Catillon G, Delzenne NM: Involvement of endogenous glucagon-like peptide-1 amide on glycaemia-lowering effect of oligofructose in streptozotocin-treated rats. J Endocrinol 2005, 185:457–465.

26 Chen J, Wang R, Li XF, Wang RL: Bifidobacterium adolescentis supplementation ameliorates visceral fat accumulation and insulin sensitivity in an experimental model of the metabolic syndrome. Br J Nutr 2012, 10:1429–1434.

27 Maki KC, Pelkman CL, Finocchiaro ET, et al. Resistant starch from high-amylose maize increases insulin sensitivity in overweight and obese men. *J Nutr.* 2012;142(4):717–723. doi:10.3945/jn.111.152975

28 Stewart ML, Zimmer JP. Postprandial glucose and insulin response to a high-fiber muffin top containing resistant starch type 4 in healthy adults: a double-blind, randomized, controlled trial. Nutrition. 2018 Sep;53:59-63. doi: 10.1016/j.nut.2018.01.002. Epub 2018 Feb 13. PMID: 29655779

29 Odamaki T, Kato K, Sugahara H, Hashikura N, Takahashi S, Xiao JZ, Abe F, Osawa R. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. BMC Microbiol. 2016 May 25;16:90. doi: 10.1186/s12866-016-0708-5. PMID: 27220822

30 Peters BA, Shapiro JA, Church TR, Miller G, Trinh-Shevrin C, Yuen E, Friedlander C, Hayes RB, Ahn J. A taxonomic signature of obesity in a large study of American adults. Sci Rep. 2018 Jun 27;8(1):9749. doi: 10.1038/s41598-018-28126-1. PMID: 29950689

31 Wherrett DK, Chiang JL, Delamater AM, DiMeglio LA, Gitelman SE, Gottlieb PA, Herold KC, Lovell DJ, Orchard TJ, Ryan CM, Schatz DA, Wendler DS, Greenbaum CJ. Defining Pathways for Development of Disease Modifying Therapies in Children with Type 1 Diabetes: A Consensus Report. Diabetes Care. 2015 Oct; 38(10):1975-85.

32 Whisner CM, Martin BR, Nakatsu CH, McCabe GP, McCabe LD, Peacock M, Weaver CM: Soluble maize fibre affects short-term calcium absorption in adolescent boys and girls: a randomized controlled trial using dual stable isotopic tracers. Br J Nutr 2014, 112:446-456.

33 Whisner CM, Martin BR, Nakatsu CH, Story JA, MacDonald-Clarke CJ, McCabe LD, McCabe GP, Weaver CM. Soluble corn fiber increases calcium absorption associated with shifts in the gut microbiome. A randomized dose-response trial in free-living pubertal girls. J Nutr., 2016 Jul; 146 (7): 1298-306. Doi: 10.3945/jn. 115.227256. Epub 2016 Jun 8.

34 Abdul-Rasoul M, Habib H, Al-Khouly M. 'The honeymoon phase' in children with type 1 diabetes mellitus: frequency, duration, and influential factors. Pediatr Diabetes. 2006; 7:101–7. [PubMed: 16629716]

35 Davis AK, DuBose SN, Haller MJ, Miller KM, DiMeglio LA, Bethin KE, Goland RS, Greenberg EM, Liljenquist DR, Ahmann AJ, Marcovina SM, Peters AL, Beck RW, Greenbaum CJ; T1D Exchange Clinic Network. Prevalence of detectable C-Peptide according to age at diagnosis and duration of type 1 diabetes. Diabetes Care. 2015 Mar;38(3):476-81. doi: 10.2337/dc14-1952. Epub 2014 Dec 17. PMID: 25519448

36 Rose DJ, Patterson JA, Hamaker BR: Structural differences among alkali-soluble arabinoxylans from maize (Zeamays), rice (Oryzasativa), and wheat (Triticumaestivum) brans influence human fecal fermentation profiles. J Agric Food Chem 2010, 58:493-499.

37 Kendall CW, Esfahani A, Hoffman AJ, et al.: Effect of novel maize-based dietary fibers on postprandial glycemia and insulinemia. *J Am Coll Nutr.* 2008;27(6):711–718. 10.1080/07315724.2008.10719748

38 Bergenstal RM, Beck RW, Close KL, Grunberger G, Sacks DB, Kowalski A, Brown AS, Heinemann L, Aleppo G, Ryan DB, Riddlesworth TD, Cefalu WT. Glucose Management Indicator (GMI): A New Term for Estimating A1C From Continuous Glucose Monitoring. Diabetes Care. 2018 Nov;41(11):2275-2280. doi: 10.2337/dc18-1581. Epub 2018 Sep 17. PMID: 30224348

39 Bergenstal RM, Gal RL, Connor CG, Gubitosi-Klug R, Kruger D, Olson B, Willi S, Aleppo G, Weinstock RS, Wood J, Rickels M, DiMeglio LA, Bethin KE, Marcovia S, Tassopoulos A, Lee S, Massaro E, Bzdick S, Ichihara B, Markman E, McGuigan P, Woerner S, Ecker M, Beck RW for the T1D Exchange Racial Differences Study Group. Racial Differences in Hemoglobin Glycation Affecting the Relationship of Glucose Concentrations and HbA1c Levels. 2017. Ann Internal Med. July 18; 167(2):95-102.

40 Baghurst PA. Calculating the mean amplitude of glycemic excursion from continuous glucose monitoring data. *Diabetes Technol Ther.* 2011;13(3):296-302.

41 Watkins RA, Evans-Molina C, Terrell JK, Day KH, Guindon L, Restrepo IA, Mirmira RG, Blum JS, DiMeglio LA. Proinsulin and heat shock protein 90 as biomarkers of beta-cell stress in the early period after onset of type 1 diabetes. Transl Res. 2016 Feb;168:96-106.e1. doi: 10.1016/j.trsl.2015.08.010. Epub 2015 Sep 4. PMID: 26397425

42 Fisher MM, Watkins RA, Blum J, Evans-Molina C, Chalasani N, DiMeglio LA, Mather KJ, Tersey SA, Mirmira RG. Elevations in circulation methylated and unmethylated preproinsulin DNA in new-onset type 1 diabetes. Diabetes. 2015 Nov;64(11):3867-72. doi: 10.2337/db15-0430. Epub 2015 Jul 27. PMID: 26216854

43 Greenbaum CJ, Beam CA, Boulware D et al. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. Diabetes 2012;61(8):2066-2073.

44 Aryana K, Greenway F, Dhurandhar N, Tulley R, Finley J, Keenan M, Martin R, Pelkman C, Olson D, Zheng J. A resistant-starch enriched yogurt: fermentability, sensory characteristics, and a pilot study in children. F1000Res. 2015 Jun 1;4:139. doi: 10.12688/f1000research.6451.1. eCollection 2015. PMID: 26925221

45 American Diabetes Association. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes—2018*. Diabetes Care Jan 2018, 41 (Supplement 1) S13-S27; DOI: 10.2337/dc18-S002

46 National Cancer Institute. Automated self-Administered 24-hour recall (ASA24) Dietary Assessment Tool National Cancer Institute; 2018 (https://epi.grants.cancer.gov/asa24/). Last accessed January 7th,2019.

47 Blanton CA, Moshfegh AJ, Baer DJ, Kretsch MJ. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. The Journal of nutrition. 2006;136(10):2594-9.

48 Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, et al. The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes. The American journal of clinical nutrition. 2008;88(2):324-32.

49 The American Diabetes Association, Create Your Plate! http://www.diabetes.org/food-and-fitness/food/planning-meals/create-your-plate/#seven last accessed 03/15/2019.

50 Smart CE, Annan F, Higgins LA, Jelleryd E, Lopez M, Acerini CL. ISPAD Clinical Practice Consensus Guidelines 2018: Nutritional management in children and adolescents with diabetes. Pediatr Diabetes. 2018 Oct; 19 Suppl 27:136-154. doi: 10.1111/pedi.12738. DOI: 10.1111/pedi.12738 PMID: 30062718

51 Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Burkhard Haastert, Ludvigsson J, Pozzilli P, Lachin JM, Kolb H. Mixed-Meal Tolerance Test Versus Glucagon Stimulation Test for the Assessment of β-Cell Function in Therapeutic Trials in Type 1 Diabetes. Diabetes Care Oct 2008, 31 (10) 1966-1971; **DOI:** 10.2337/dc07-2451

52 Julious S. Sample size of 12 per group rule of thumb for a pilot study. Pharmaceutical Statistics 2005;4(4):287-291

53 Sanchez-Alcoholado L, Castellano-Castillo D, Jordan-Martınez L, et al. Role of gut microbiota on cardio-metabolic parameters and immunity in coronary artery disease patients with and without type-2 diabetes mellitus. Front Microbiol

2017; 8: 1936.