Low Carbohydrate Versus Mediterranean Diet in Adolescents with Type 1 Diabetes: A Randomized Control Trial

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Study Protocol

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Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune condition caused by an absolute or relative deficiency of insulin leading to imbalanced blood glucose. Its incidence has risen worldwide by an alarming annual rate of ~4%[2]. The greatest observed increase is seen in children younger than 15 years[3]. The standard treatment to achieve balanced glycemic control and to prevent long and short-term complications, consists of daily insulin administration tailored according to lifestyle, nutrition, medical condition, and personal factors[4].

The management of T1DM in children and adolescents is a major challenge. According to the literature, the glycemic control in this group is suboptimal with an increased risk of complications[5,6]. Less than a third of youth with T1DM treated by endocrinologists meet the recommended glycated hemoglobin (HbA1c)[5]. Poor glycemic control correlates with cumulative morbidity and mortality[7].

For decades diabetes specialists focused on drug and technological development; however, the management of T1DM remains suboptimal with an average HbA1c of 8.2% [8]. It appears that current therapies are lacking the potential for optimal glycemic control. There is, therefore, a need to find an alternative path which could lead the adolescent group to achieve better glycemic control and by that avoid or delay diabetes complications.

Medical nutrition therapy (MNT) continues to be a cornerstone of diabetes care [4]. The current standard nutrition therapy consists of flexible diet combined with adjustment of daily injections of insulin. One of the greatest challenges in T1DM management is the administration of an adequate amount of insulin for each meal: too much insulin might result in a life - threatening hypoglycemic event, while too little would result in high postprandial blood glucose levels and its associated comorbidity. The standard of care guidelines published by the American Diabetes Association (ADA) advocates that patients should "match prandial insulin to carbohydrate intake, premeal blood glucose, and anticipated physical activity" [9]. In practice, most individuals with T1DM assess roughly the amount of carbohydrates in the meal, and then administer insulin according to a set insulin-to-carbohydrate ratio, with little adjustments made manually

and qualitatively for physical activity and pre-meal blood glucose levels. Overall, it was found that conventional therapy resulted in suboptimal insulin counteraction of postprandial glycemic responses. More sophisticated models exist, such as those that also adjust to the fat content of the meal, but so far, they have failed to provide a significant improvement in glycemic control, and are recommended by the ADA only for advanced patients.

Carbohydrates are the primary macronutrients that affect the postprandial glycemic response. Given the difficulty of matching carbohydrate intake with insulin dose, reducing dietary carbohydrate consumption among people with diabetes is becoming a common diet pattern. Essentially, the lower the glycemic response, the less insulin is administered, and the lower the fluctuations of blood glucose levels in absolute terms[10]. Although low carbohydrate diet (LCD) has a promising effect on glycemic control and is popularized both by social media and the patients[11], it is poorly studied and may lead to harmful dietary consequences. Carbohydrate-containing foods including grains, fruit and milk, are important sources of nutrients. Hence, low-carbohydrate diet requires attention to vitamin and energy intake to avoid micronutrient deficiencies and growth issues. Adherence to restricted diets is challenging and can have an impact on social normality. In individuals with T1DM, adverse health risks such as diabetic ketoacidosis, hypoglycemia, dyslipidemia and glycogen depletion remain clinical concerns. Parents who decide to put their child on such a regime, usually do it without proper medical guidance and may put their child at risk of nutritional depletion of essential nutrients and minerals[10]. Studies looking at glycemic outcomes from low-carbohydrate diets have largely been cross-sectional, without validated dietary data and with a lack of a control group[10].

Composition, alteration, and diversity of the gut microbiota might affect the glycemic control**.** Dysbiosis and changes in the properties of the gut barrier have been documented in T1DM subjects[12,13]. The common finding in all of these studies is the increased proportion of Bacteroides bacteria which appear at the time of the initial diagnosis and tend to decrease over time, but without reaching the levels of healthy children. According to a recent study in TIDM children, changes in diet directly influenced the intestinal microbiota composition, and therefore might correct the gut dysbiosis in patients with TID[14]. These results show the merit of studying the microbiome of already diagnosed T1D patients, with the prospect of performing microbiome interventions aimed at improving glycemic control.

We propose an integrated study examining the effect of the LCD versus the Mediterranean diet (MD) upon both the glycemic control and on the gut microbiome. In order to isolate the effect of intensive dietary intervention, we will compare LCD to MD. The MD has been widely reported as one of the healthiest models for preventing cardiovascular disease and beneficial

for HbA1c[15,16]. The SEARCH Nutrition Ancillary Study concluded that a higher MD score was associated with better glycemic control and lipid profile in youth with T1D[17]. To the best of our knowledge, this will be the first controlled study upon adolescents with T1D on a low carbohydrate diet.

According to most recent data reported, only a minority of individuals meet ADA (American Diabetic Association) HbA1c goals and HbA1c levels remain particularly high in adolescents and young adults[18]. Parents and patients who may consider LCD as an option to optimize glycemic control face many concerns including hypoglycemia, ketoacidosis, nutritional insufficiency, poor growth, inadequate bone mineralization, poor metabolic profile, and psychological consideration due to the lack of data. Because more patients are choosing to follow a carbohydrate-restricted diet, there is a need to evaluate benefits and risks of LCD vs. MD among youth with T1DM after consuming LCD.

Hypothesis:

Since there are no previous studies comparing LCD vs. MD the premise is that the diets have equal effect with no superiority or inferiority.

Our Primary end point is to compare the impact of LCD and MD on the time in range (TIR) 70-180 mg/dl assessed by Continues Glucose Monitoring (CGM) among adolescents with T1DM.

Secondary end points

1. Evaluation of the influence of LCD vs. MD on HbA1c, insulin dose (average total daily dose/ kg), glycemic variability (TIR, Time bellow 54 mg/dl, CV- Coefficient Variant) and average blood glucose.
2. Assessment of the impact of dietary changes after an LCD vs. MD on gut dysbiosis.
3. Assessment of the impact of both diets on bone turnover measures, lipid profile, vitamin levels, weight and height.
4. Assessment of the impact of dietary changes after an LCD vs. MD on quality of life.

Methods:

Participants and study design

Adolescents with T1DM will be enrolled at the Pediatric Endocrinology and Diabetes unit of the Edmond and Lily Safra Children's Hospital, Sheba Medical Center (Ramat Gan, Israel). Written informed consent was obtained from participants age ≥18 years or their parents or legal guardians if <18 years. Ethic approval and monitoring is obtained from the Helsinki Committee in Sheba Hospital.

The study design is a randomized, parallel assignment clinical trial without blinding (open label) due to the impracticality of blinding diets. The criteria for eligibility are: age between 12 to 22 years , presence of T1DM (according to the American Diabetes Association criteria[19]) for at least one year, and usage of a CGMS (Dexcom, Medtronic, Libre) device. Exclusion criteria included medical history of eating disorders or any other mental illness.

Participants who will meet all inclusion criteria will be randomly assigned to either the intervention (LCD) diet group or to the MD group in a 1:1. Randomization assignments were computer generated by the study statistician prior to the start of the study using random permuted block sizes of 2 or 4.

Diet intervention

In order to maintain equal intensity of treatment, each patient will take a cooking workshop and receive a personalized diet regime at baseline, based on the randomly assigned diet. Format and quality of the materials were similar. (Appendix 1)

All diet plans will be individualized and matched for energy intake personally .

Low carbohydrate diet- The low carbohydrate diet aims to provide 50-80g of carbohydrate per a day. Planned macronutrient compositions (percentage from total calories) of the diet: 15% carbohydrate (<80 g/day), 33% protein and 58% total fat.

Mediterranean diet-The moderate-fat Mediterranean diet is rich in vegetables and low in red meat, with poultry and fish replacing beef and lamb. The main sources of added fat were 30 to 45 g of olive oil and a handful of nuts (five to seven nuts <20g) per day. The planned macronutrient compositions of the diet were 50% carbohydrate, 25% protein and 35% total fat. The diet is based on the recommendations of Willett and Skerrett[21].

Participants will meet individually with the dietitian for diet instruction and support at week 1,2,4,7,10,12 and thereafter at 24 weeks for a total of seven frontal meetings. Twice during the first 12 weeks the dietician will conduct 10-15-minute motivational telephone calls with all the participants.

Questionnaires

Demographic characteristics complete medical history (e.g diabetes duration, diabetes treatment and other medical diagnoses), and sociodemographic data will be obtained at baseline using a questionnaire and medical records.

Adherence to the diets will be evaluated by validated *Food-Frequency Questionnaire* (FFQ) [22] at baseline, 12 and 24 weeks. The FFQ that will be used in this study was developed by the nutrition department of the Israeli Ministry of Health and is the most common FFQ in Israel that was used in many studies. All participants completed a 24-hour dietary recall at each visit

with the dietician. Food diaries and registered carbohydrate intake through diabetes apps (Tidepool, Librelink, Dexcom clarity) displayed patients' responsiveness to the diet.

The Self-Efficacy for Diabetes Scales [23]a self-report measure of the self-perceptions or expectations held by persons with diabetes regarding their personal competence and confidence in their ability to successfully manage their T1D. Items are distributed across three subscales: diabetes-specific self-efficacy (24 items; total score 24-120); medical situations self-efficacy (5 items; total score 5-25); and, general situations (6 items; total score 6-30). Higher scores indicate *less* self-efficacy.

The Diabetes Quality of Life Scale for Youth (DQOL) [24] is a modification of the Diabetes Quality of Life Scale[25] used to assess children's and adolescents' perceptions of the impact of intensified regimens on their general satisfaction with life, and on diabetes-related concerns over social, school and peer relationships. Three subscales measure Disease Impact (21 items; total score 21-84), Disease-related Worries (8 items; total score 8-32) and Diabetes Life Satisfaction (18 items; total score 18-72). Higher scores indicate greater negative impact and worry (poor quality of life), but better life satisfaction. Both the Self-Efficacy for Diabetes Scales and DQOL were given at baseline and 12and 24 weeks.

Glycemic control

HbA1c was collected from blood samples at baseline, 12 and 24 weeks. Time spent in range (TIR) 70-180 mg/dl assessed by CGM was downloaded at each visit with the dietician. Severe hypoglycemia was defined as an episode requiring assistance and was confirmed by documentation of a blood glucose value less than 54 mg per deciliter.

Glycemic variability

Glycemic variability from continuous blood glucose monitoring (Libre, Medtronic, Dexcom) and included SD Time spent in range 70-180 mg/dl, Time between 70-54mg/dl, time under 54 mg/dl and CV (coefficient variant) assessed by CGM was downloaded at each visit (baseline, and weeks 1,2,4,7,10 and 12) with the dietician.

Anthropometric Measurement

At the beginning of each meeting with the dietician the following data were collected: height, weight, waist circumference, blood pressure and pulse rate. All were measured according to

standardized protocol by trained and certified staff. Body mass index (BMI), is calculated as weight (kg)/height squared (m^2).

Biochemical Parameters

Fingertip blood samples will be collected and processed for measurements of ketones (Beta-hydroxybutyrate) with Abbott Freestyle Optium Neo- Blood Glucose & Ketone test meter at each visit held with the dietician.

Blood samples including; HbA1c, zinc (Atomic absorption with flame technique) , vitamin C, lipid profile, creatinine, folic acid, thiamin, magnesium, vitamin C, transferrin, ferritin, zinc, carnitine, copper, CRP, TSH, FT4, were collected under metabolic stability conditions, defined as no episode of diabetic ketoacidosis within 1 month before the visit, and after ≥12 hours of fasting. All fasting blood samples will be taken at baseline, 12- and 24-weeks from a forearm vein and processed at the Sheba Medical Center laboratories.

Assessment of bone turnover markers

Blood samples were obtained from the participants after a 12-hr fast at baseline,12 and 24 weeks. Bone markers: procollagen type 1 amino-terminal propeptide (P1NP) and bone resorption C-terminal telopeptide of type 1 collagen (CTx1) were measured using ELISA method.

DNA extraction, PCR amplification, and sequencing

Fresh fecal samples were collected during weeks 1,4 and the last week of the experiment for the gut microbial 78 characterization. Bacterial genomic DNA was extracted from frozen fecal samples stored at −80°C. 79 DNA extraction and PCR amplification of the variable region 4 (V4) of the 16S rRNA gene using 80 Illumina adapted universal primers 515F/806R was conducted using the direct PCR protocol 81 [Extract-N-Amp Plant PCR kit (Sigma-Aldrich, Inc.)] as previously described [26]. Briefly, PCRs were 82 conducted in triplicate in a 96 well plate [denaturation for 3 min at 94 °C; 35 cycles (98 °C, 60 s; 55 °C, 83 60 s; 72 °C, 60 s) followed by elongation for 10 min at 72 °C]. Positive amplicons were pooled in 84 equimolar concentrations into a composite sample that was size selected (300–500 bp) using agarose 85 gel to reduce non-specific products from host DNA. Sequencing was performed on the Illumina 86 MiSeq platform with addition of 20% PhiX, and generating paired-end reads of 175b in length in 87 each direction.

Statistical Analysis

The sample size was based on the primary endpoint, ie delta in the time spent in range (TIR 70-180 mg/dl). To be able to detect a difference of 20% with 80% power at a 5% significance level with a two-sided paired t-test. Thirty-four participants should complete the study. To account a 40% attrition rate, we included a total of 40 participants in the study. The sample size was calculated using PASS 2020 software.

Data will be analyzed with the IBM SPSS software (IBM SPSS Statistics for Windows, Version 24;IBM Crop., Armonk, NY, USA, 2016). Categorial variables were assessed for frequency and percent. Continuous variables described as frequency and percent. Continuous variables were assessed for normal distribution using histogram and Q-Q plots. Normally distributed continuous variables were described as means and standard deviations (SD), and non-normally distributed parameters were described as median and interquartile rang (IQR). Categorical variables in the same group were compared using a dependent sample t test. Differences between groups in continuous data were compared using independent-sample t tests (normally distributed data) or Mann-Whitney test (skewed data). For variables that were non-normally distributed, the median interquartile range (IQR) were presented and Kruskal-Wallis test was applied to evaluate he differences.