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1. Backgraund

Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia caused by disorders of insulin secretion, insulin activity, or both [1]. According to the American Diabetes Association (ADA) and World Health Organization (WHO); it is classified as Type 1, Type 2 and other specific types of diabetes [2]. Type 1 diabetes mellitus (T1DM), formerly called insulin-dependent diabetes or youth diabetes, is an autoimmune disease characterized by a severe endogenous insulin deficiency or absence, resulting with chronic hyperglycemia [3]. There are numerous differences in insulin applications used to manage T1DM in children and adolescents [4]. Optimal glycemic control is achieved by intensive insulin therapy in T1DM patients [3]. Despite innovations in medical treatment and technology, nutritional therapy remains one of the cornerstones of diabetes care. Healthy eating and dietary recommendations suitable for all children with diabetes. The only difference compared to healthy peers is the need for insulin therapy. The International Society for Pediatric and Adolescent Diabetes (ISPAD) Consensus Guidelines underlined the importance of specific issues in nutritional management for children with diabetes. This guideline recommends a diet that provides optimal growth and development, maintains ideal weight and prevents acute and chronic complications [5].

Childhood and adolescence cover phases of growth and pubertal development with substantial changes of metabolic condition. Therefore, daily insulin doses used for the treatment of T1DM are highly variable in childhood and adolescence. Especially during puberty, physiological insulin resistance increases to some degree [6]. Each person reacts differently to insulin. For most adults, one unit of rapid acting insulin is enough for 15 grams of CHO. While a baby may require 1/2 to 1 unit of insulin for 30-45 grams of CHO, a young person may need 1 unit of insulin for every 7 to 15 grams of CHO [7]. A more flexible approach using individualized insulin to carbohydrate ratios (ICR), which enables the pre-prandial insulin dose to be matched to carbohydrate intake, should be used for children and adolescents on intensive insulin therapy. The ICR is individualized for each child according to age, sex, pubertal status, duration of diagnosis and activity [8].

To mimic normal pancreatic function in persons with diabetes, basal insulin is usually given as long acting insulin to counteract rises in blood glucose that occur independent of meal ingestion. And bolus insulins are given by either rapid acting (lispro, aspart and glulisine) or regular insulin in relation to meals, which inhibiting the increase in blood glucose after meals. Basal insulin requirement is usually constant from day to day and matching bolus insulin to carbohydrate intake using an ICR is optimal for post-meal blood glucose management. Once an ICR is established, patients can adjust their mealtime boluses based on their carbohydrate intake [7].

GI is a reference value of foods based on their acute glycemic effects compared to reference standard glucose [4]. GI is one of several tools used to assist in glycemic control. Carbohydrates with a low GI value cause a slower and more gradual increase in blood glucose levels and reduce postprandial glycemic response compared to carbohydrates with a high GI value [4]. The use of GI has been shown to provide additional benefit for glycemic control when total carbohydrate alone is evaluated. In practice GI is used as a tool to minimize post-meal glucose increases and improve dietary quality [7]. Low-GI foods can reduce post-meal hyperglycemia when selected to replace high-GI foods [9]. Low GI foods include whole grain breads, legumes, pasta, some fruits, dairy products. Not all low GI foods are good for daily choices (eg chocolate, fructose). Many factors can affect the glycemic responses of nutrients [4]. Carbohydrates are the primary macronutrient that is effective on the postprandial glycemic response [3]. However, carbohydrate is not the only stimulus for insulin secretion [10]. The amount of fat and protein in mixed meals significantly changes glycemic and insulinemic responses [11]. Insulinemic index (II), similar to GI, measures the degree a food how much raises plasma insulin levels [12]. Although it is important to measure postprandial blood glucose responses, measurement of postprandial

insulinemic responses is equally important [13]. Protein-rich foods or the addition of protein to a carbohydrate-rich meal can induce a mild increase in insulin secretion, especially in individuals with diabetes without increasing blood glucose concentrations [10]. This is due to proteins slows gastric emptying, increases incretin and protein secretion and reduces glycemic responses [11]. Similarly, adding a large amount of fat to a carbohydrate-rich meal delays gastric emptying, reduces plasma glucose responses, increases insulin secretion [11,12]. The number of studies evaluating the insulinemic responses of different foods and mixed meals is very limited.

2. Objective

The aim of this study was to develop recipes containing CHO less than ICR, prepare new snacks alternatives that could be consumed without the need for additional insulin doses, and evaluate the glycemic and insulinemic responses of these recipes.

3. Materials and methods

3.1. Study design

The study planned as a randomized crossover study with at least 2 days wash-out period. Participants will be consume 3 test foods and reference food (glucose solution, 2 times) in a random order.

This study will be conducted according to the guidelines laid down in the Declaration of Helsinki. The Clinical Research Ethics Committee of the Erciyes University approved the protocol (2019/767) on 13 November 2019, and all participants will give written informed consent.

3.2. Participants

Fifteen subjects will be selected from the individuals who see the announcements from internet and who would like to participate to the study. Healthy subjects with normal body weight (18.5-25 kg / m^2 BMI) and between the ages of 19-30 will be included to the study. Exclusion criteria includes use of tobacco, weight change of >5 kg in the last 3 months, the presence of any chronic disease or lactose intolerance, regular medicine use, pregnancy or lactation, performing competitive sports or endurance sports, fasting plasma glucose concentration >100 mg / dL, the presence of eating disorder, sensitivity or allergies to any food that used in the study. Dutch Eating Behavior Questionnaire (DEBQ) will be applied by participants to detect and exclude people with eating disorders from the study.

3.2.1. Eating Behavior Questionnaire

The Dutch Eating Behavior Questionnaire (DEBQ) will be applied by participants to evaluate the eating disorder. This questionnaire was developed by Van Strein et al. in 1986 [14]. Validity and reliability study for Turkey was conducted by dietician Nuray Bozan [15]. The 33-item survey consists of 3 sub-scales that evaluate emotional eating behaviors, external eating behaviors, and restricted eating behaviors. The items in the questionnaire are evaluated with a 5-point Likert scale. Participants with scores >3.5 will be excluded from the study [16].

3.2.2. Anthropometric measurement

3.2.2.1. Body weight (kg): Weight measurement will be measured with sensitivity of ±0.1 kg. During the measurement, individuals will be advised to keep a minimum level of clothing on their bodies. The measurement will be done while the individual are in an upright and motionless position [17].

3.2.2.2. *Height (cm):* The height of the participants will be measured using a stadiometer. During the measurement, the person's feet will be bare and heels touching the wall, standing and upright, looking straight ahead and taking care that the gaze will be on the Frankfort plane [17].

3.2.2.3. *Body Mass Index (BMI):* BMI will be calculated as weight (kg) divided by the square of height (m^2) [17] and converted age- and sex-specific z-score according to WHO criteria [18].

3.3. Test Foods

The recipes of test foods were developed for this study. The recipes will be prepared by the researcher using the ingredients and preparation method specified in Annex-1. The nutrient composition calculated from the labels. The nutrient composition of one portion test foods is given in Table-1. Each food contains less than 5 g of available carbohydrate per serving. Each test food will be analyzed at the beginning of the study. According to these results, the amount of test foods containing 25 g available CHO will be determined and presented to the participants. Glucose solution will be used as a reference food and tested twice. GI studies usually determine the amount of available carbohydrates as 50 g. However, to achieve this amount with low-carb test foods, participants will be need to consume unrealistic portions (>1500 kcal) of food within 15 minutes. For this reason, similar to previous studies, each test food and reference food will be given in portions containing 25 g available carbohydrates. The glucose solution will be prepared by dissolving 25 g of glucose in 250 ml of water. 250 ml of water will be given alongside each test food [13].

Table-1: The Nutritional Composition of a Portion of the Test Foods

Food	g/portion	Calorie	CHO	Protein	Fat	Fiber
Blueberry Cake	20 g (1 muffin)	68,2 kcal	4,2 g	2,5 g	4,5 g	1,4 g
Snack with Cheese	20 g (2 small 10g snacks)	68,7 kcal	4,8 g	2,3 g	3,8 g	0,9 g
Spicy Crackers	28 g (4 small 7 g crackers)	114,3 kcal	4,5 g	3,4 g	8,2 g	4,5 g

3.4. Study protocol

3.4.1. Preparation of Participants:

Participants will be asked to avoid unusual physical activity and not to change their normal eating patterns, avoid consuming legumes and alcohol the day before the test, and have not consumed any food for at least 10 hours before test morning [11]. They will be also asked to consume at least 150g of carbohydrates for 3 days before the test and all food and beverages they consume within these 3 days will be written daily [19]. Diet composition will analyzed by the BeBiS Nutrition Information System software version 8.2 [20]. This database contains Turkish food composition tables for all foods. In addition, in order to control the possible effects of the menstrual cycle on hormonal changes in female participants, test foods will be consumed during the follicular phase of the menstrual cycle [10].

3.4.2. Consumption Of Test Food

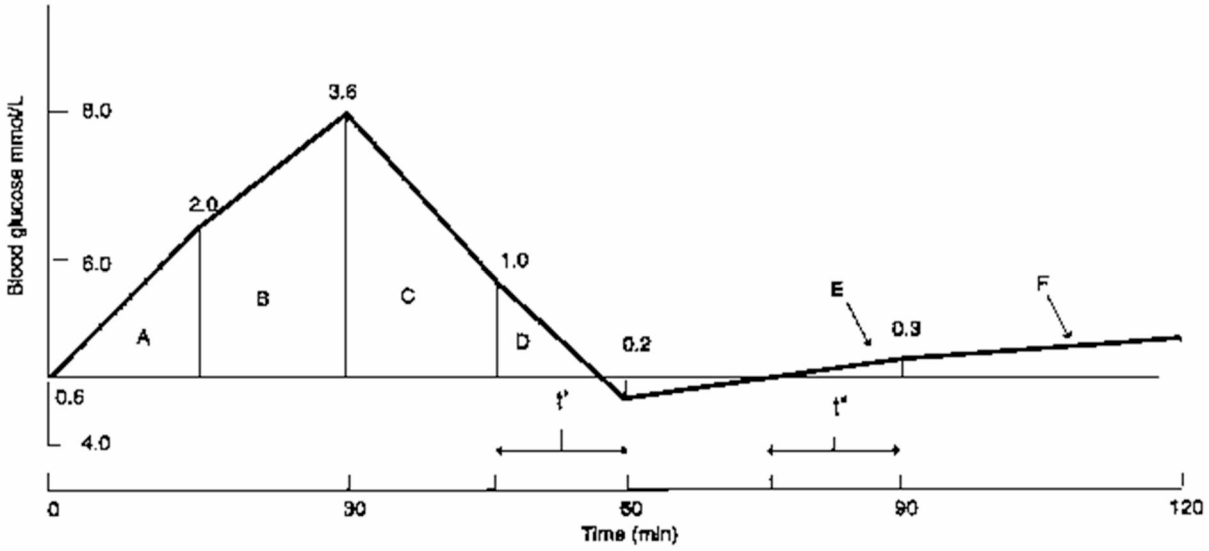
The test will start before 10:00 a.m. Participants who arrived at the hospital will rest for 10 minutes and have a catheter inserted into the arterial vein. The participants will consume the test food in 15 minutes, along with 250 ml of water. Taking the first bite from test food is considered as 0 min. Blood samples will be collected at time points 0,15, 30, 45, 60, 90 and 120 min. Glucose and insulin analyzes will be performed from blood samples [21].

3.4.3. Sensory Evaluation

Each participant, after consuming the test nutrients, will fill the 100 mm Visual Analog Scale (VAS) to indicate their sensory evaluation [22]. Participants will be asked to make a single vertical mark at the appropriate point between the 2 anchors on each scale corresponding to their feelings.VAS includes 5 questions to evaluate the visual appeal, smell, taste, aftertaste, and palatability [11].

3.5. Calculation of glycemic index and insulinemic index

Blood glucose and insulin values of 0, 15, 30, 45, 60, 90, 120 minutes of reference food and test foods containing 25 g of available carbohydrates consumed by each participant will be marked on charts. Calculations will be made using the Incremental Area Under Curve (IAUC) method, which is recommended by the FAO / WHO Committee of Experts as the most appropriate method for calculating the glycemic index value. A horizontal straight line will be drawn from the blood glucose and insulin value at the starting point (0 minutes), only the area remaining above the line will be calculated [23]. The calculation example is shown on the FAO website dated 06.09.2019 as follows [24].



- The area of triangle A = 2.0 × 15/2 = 15.0
- The area of trapezoid B = (2.0 + 3.6) × 15/2 = 42.0
- The area of trapezoid C = (3.6 + 1.0) × 15/2 = 34.5
- The area of triangle D = 1.0 × t72 since: t'/15=1.0/(1.0+0.2)
  therefore: t' = 15 × 1.0/1.2 = 12.5
  therefore the area of triangle D = 1.0 × 12.5/2 = 6.25
- The area of triangle E = 0.3 × t''/2 since: t''/30 = 0.3/(0.3 + 0.2)
  therefore: t'' = 30 × 0.3/0.5 = 18
  therefore the area of triangle D = 0.3 × 18/2 = 2.7
- The area of trapezoid F = (0.3 + 0.6) × 30/2 = 13.5
  Therefore, IAUC = 15.0 + 42.0 + 34.5 + 6.25 + 2.7 +13.5 = 114 mmol.min/L

GI= Blood glucose level after test food / Blood glucose level after reference food X 100

II= Blood insulin level after test food / Blood insulin level after reference food X 100

GI and II = IAUC (test food) / IAUC (reference food) X 100

4. Statistical analysis Plan

4.1. Sample Size

According to glycemic index methodology, the inclusion of 10 participants often provides the necessary power and precision to measure the glycemic index [21]. Considering some possibilities (eg. some participants may drop out the study, small differences may not be detected, the study may not reach the required power), 1.5 fold of this number was chosen and the study was planned for 15 people. After 10 participants have completed the study, a power analysis will be performed and the study will be terminated if the required power and precision is reached.

4.2. Data analysis

Statistical analysis will be performed using the Statistical Package for the Social Sciences (version 22.0; IBM SPSS Statistics) software. Data will be expressed as the number (n) and percentage (%) for categorical variables, and means ± SDs, medians (25^{th}-75^{th} percentiles) for continuous variables. Normality will be assessed using the histogram and normal Q-Q plots, and also Shapiro-Wilk test. Furthermore, continuous variables will be examined for skewness and kurtosis, and log-transformed before analysis and reported back-transformed geometric means (G) ± standard error (S.E) when required [25]. One-way (1-factor) analysis of variance (ANOVA) for repeated measures will be applied to determine statistical differences between groups and for continuous variables with normal distributions will be analysed. The data will be analysed by using 2-factor (time x meal) repeated-measures ANOVA, and Bonferroni post hoc tests will be applied to significant time x meal interactions. Friedman test will be used for continuous variables without normal distributions. For all statistical analyses, p values less than 0.05 will be considered to have statistical significance [26].

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ANNEX-1

RECİPES

Blueberries Cake

Ingredients	Size	Amount	Definition
Coconut Flour	3 tbsp	30 g	Packaged product Coconut Flour (200 g)
Almond Flour	2 tbsp	20 g	Homemade
Egg	3	150 g	Middle size
Oil	3 tbsp	30 ml	Sunflower oil
Raisins	3 tbsp	50 g	Packaged product Yellow raisins (140 g)
Blueberries	1 tbsp	15 g	Dried Blueberries
Baking Powder	1 tsp	3 g	Packaged product Baking Powder (10 g)

Preparation:

- Raisins are kept in warm water overnight (8-10 hours).

- It is made homogeneous, semi-fluid by passing through blender.

- Eggs are taken into mixing bowl and whisked for 5 minutes.

- Other ingredients (coconut flour, almond flour, egg, oil, blueberries, baking powder, prepared semi-fluid raisins) are added.

- Ingredients are mixed, water can be added according to consistency.

- The mixture is poured into cake molds and cooked in preheated 170^0C oven for 15-25 minutes.

Preparation of Almond Flour:

- Almonds are kept in warm water overnight (10-12 hours).

- Filter the water, peel the shells.

- Spread on a clean surface to dry, wait until dry (about 1 day) or dry in oven (60^0C) for 5-6 hours.

- Dried almonds are made as thin as flour.

Snack with Cheese

Ingredients	Size	Amount	Definition
Zucchini	1 little size	110 g	Green Zucchini
Oat Flour	5 tbsp	50 g	Packaged product. Whole Oat Flour (1 kg)
Almond Flour	2 tbsp	20 g	Homemade
Egg	1/2	25 g	Middle size
Oil	2 tsp	5 ml	Sunflower oil
Triangle Cheese	5 big triangle	90 g	Packaged product. Large size triangle cheese (18 g x 5)
Dill (dry)	2 tsp	5 g	Homemade
Baking Powder	1 tsp	2 g	Packaged product Baking Powder (10 g)

Preparation:

- Zucchinis are washed, the ends are cut. Finely grate the whole.

- With the help of a thin hole strainer, the water is well drained and then added to the mixing bowl.

- Oat flour is added, mixed, rested for 30 minutes.

- After resting, add other ingredients (almond flour, egg, oil, triangle cheese, dill, baking powder) and mix well.

- Give shape, optionally decorate with sesame/black seed.

- Bake in preheated oven at 170^0C in 20-25 minutes until starting to brown.

- Leave to warm. Ready to serve after 10 minutes.

Spicy Crackers

Ingredients	Size	Amount	Definition
Harricot Bean Flour	5 tbsp	50 g	Packaged product Pre-cooked Harricot Bean Flour (250 g)
Egg	1/2	25 g	Middle size
Oil	3 tbsp	30 ml	Sunflower oil
Baking powder	1 tsp	2 g	Packaged product. Baking Powder (10 g)
Vinegar	1 tsp	2 ml	Grape vinegar
Salt	½ tsp	1 g	Iodized salt
Mint (dry)	1 tsp	1 g	Homemade
Ginger	½ tsp	0,5 g	Powdered ginger
Turmeric	½ tsp	0,5 g	Powdered Turmeric
Sumac	½ tsp	0,5 g	Spice
Chili Peppers	½ tsp	0,5 g	Spice

Preparation:

- Mix all ingredients into mixing bowl.

- The amounts of salt and other spices can be adjusted according to taste.

- Pastry molds to be used for shaping are coated with oil.

- Leave in the freezer for 10-15 minutes to take shape.

- After taking shape it is removed from the moulds, cooked in a 180° C oven for 15-20 minutes until it begins to brown.