Materials and methods

Participants

Thirty patients with T1D who had been treated with basal-bolus insulin regimen were recruited at the Mustafa Eraslan and Fevzi Mercan Children's Hospital, Erciyes University in Turkey. There were 14 males and 16 females, with a median age of 16 years (range 16 to 18 years) and a median duration of diabetes 6.0 years (range 2 to 16 years). Median body mass index (BMI) was 20.5±3.01 kg/m^2 (range 15.6 to 25.0 kg/m^2) and glycated haemoglobin (HbA1c) was 7.6 (range 6.0 to 11.2%). The bolus and basal insulin regimen was consisting of lispro (n=4) or aspart (n=26) as fast-acting insulin and insulin glargine (n=23) or detemir (n=7) as long-acting insulin.

Exclusion criteria included: subjects (i) had any restrictive food intake disorders (eg celiac disease or food allergy); (ii) had any diabetes complications (eg neuropathy, nephropathy or retinopathy); (iii) diagnosed for <1 year; (iv) were using corticosteroid or any medication that could effect gastric emptying; (v) did exercise or had hypoglycemia or ketoacidosis within 24 hour before the test meals; (vi) had overweight or obese (BMI z score: ≥ 1SD and ≥ 2SD respectively); (vii) had not on follicular or peri-ovulatory phases of their menstrual cycles (for females).

Study design

This was a single center, crossover, randomized, controlled study, one standard control meal (SM) and three test meals (HPM: high protein meal using carbohydrate counting method, HPFM-a: high protein-fat meal using carbohydrate counting method and HPFM-b: high protein-fat meal using carbohydrate and fat-protein counting method) were compared on postprandial glucose response. During the first visit at the clinic, all patients and their parents were instructed about the study. The study was approved by the Ethics Committee on Clinical Research Ethics of Erciyes University, Faculty of Medicine (2013/173). A written informed consent was obtained from the patients and their parents.

Dietary intervention

Control and test meals

On four different occasions each interspersed by 1 week, patients consumed in a random order, one standard control meal (SM) and three test meals (HPM, HPFM-a and HPFM-b).

Each meal was given early in the morning at the same time (08.30 h) and prepared in the hospital kitchen. The breakfast meal was chosen as a test meal to eliminate any confounding second-meal effect. Controlled conditions were employed throughout the study and the factors that adversely affects glycemic response has been minimized: All meals were consumed within 20 min; no additional food or drink was allowed in the 4-h postprandial period unless required to treat symptomatic hypoglycemia. Activity was limited to sedentary activities in the research unit (watching DVD). Fast-acting insulin was injected on the same region on arm before the first blood sample collection.

The same amount of carbohydrate (70 g) and fiber (11.5 g) was given to each patient with control and test meals. The energy of SM (543 kcal) was calculated as 25% of subjects' age-adjusted daily energy requirement. HPM and HPHF meals contained same amount of protein (36 g), but differed in fat content (HPM= 17 g, HPHF= 30 g). For SM, HPM, HPFM-a, the insulin doses were calculated according to the carbohydrate content of the meals, for HPFM-b the insulin dose was calculated according to insulin-to-carbohydrate ratio (ICR) and additionally insulin-fat raito. The composition of standard and test meals and total insulin administration for each meal were given in Table 1.

Table 1. Composition of test meals

Parameters	Unit	SM	HMP	HPFM-a	HPFM-b
Energy	**kcal**	535	591	702	702
Carbohydrate	**g**	70	70	70	70
	%	53	49	41	41
Protein	**g**	24	36	36	36
	%	18	25	21	21
Fat	**g**	17	17	30	30
	%	29	26	38	38
Fiber	**g**	11.5	11.5	11.5	11.5
Calculated GI	**%**	60.4	60.4	60.4	60.4
Total insulin administration (mean±SD)	**IU**	7.9±1.6	7.9±1.6	7.9±1.6	11.9±1.6

Intervention

Calculation of insulin dosages

For CARB counting meals (SM, HPM, HPFM-a) all patients were subjected to intensive insulin therapy using insulin–carbohydrate ratio for dosing mealtime boluses. Current ICR which was calculated by dividing the total daily insulin dosage into 500 and basal insulin dosages were applied to the current individual therapy of the patient and did not chance

during the control and test meals. Fat and protein was not included in the carbohydrate counting algorithms.

For fat protein counting meal (HPFM-b) ICR and additionally insulin–fat ratio were used for dosing of mealtime boluses. The fat–protein unit (FPU) is defined as 100 calories of fat and protein. HPFM-b meal accounted for 4 FPU (400 kcal from fat and protein).

Assessment of HbA1c anc blood glucose concentrations

During the first visit at the clinic, fasting blood samples were collected for HbA1c. HbA1c concentration assessed using turbidimetric inhibition method (ELISA kit), in accordance with the manufacturer's instructions .Subjects arrived to the hospital in a fasting state (after 8 hours night fasting) at 8.00 am and capillary blood glucose values were collected and recorded before (0 min) and every 30 minutes thereafter for 240 minutes (30, 60, 90, 120, 150, 210, and 240 minutes) after control and test meals using glucometer (Accu-Chek; Roche, Mannheim, Germany). Subjects with fasting blood glucose (FBG)> 250 mg/dL or 200-250 mg/dL and urine ketone (+) were excluded and control and/or test meals were repeated in a different day. Test was finalized when hypoglycaemia occurred and repeated in a different day. In case of hypoglycaemia (glucose values below 70 mg/dL, 2 episodes) patients were asked to consume 2 dextrose tablets. No patient had severe hypoglycaemia during the study.

Glycemic response of the meals

Early (0-120 min), late (120-240 min) and whole (0-240 min) glycemic response of standard and test meals were evaluated by area under curve (AUC) calculation.