Clinical Nutrition ESPEN 31 (2019) 48–55

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Original article

Postprandial glucose response after the consumption of three mixed meals based on the carbohydrate counting method in adults with type 1 diabetes. A randomized crossover trial

Emilia Papakonstantinou [a, *], Kleopatra Papavasiliou [a], Chrystalleni Maouri [b], Emmanuella Magriplis [a], Stavros Pappas [c], Stavros Bousboulas [c], Anastasios Koutsovasilis [c], Maria Pappas [c], Alexis Sotiropoulos [c], Meropi D. Kontogianni [b]

[a] Lab of Food Chemistry and Analysis, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
[b] Department of Nutrition and Dietetics, Harokopio University, Athens, Greece
[c] Third Pathology Clinic, Diabetes Center, Nice General Hospital, Athens, Greece

ARTICLE INFO

Article history:
Received 1 May 2018
Accepted 10 March 2019

Keywords:
Carbohydrate counting method
Protein
Fat
Extra virgin olive oil
Glucose
Type 1 diabetes

SUMMARY

Background & aims: People on intensive insulin therapy usually calculate their premeal insulin dose based on the total amount of consumed carbohydrates. However, arguments have been expressed supporting that also the protein and fat content of the meals should be considered when estimating premeal insulin dose. We examined the effectiveness of the carbohydrate counting method after consumption of mixed meals, and we further explored the effects of added extra virgin olive oil in these mixed meals, in adults with type 1 diabetes.

Methods: Twenty adults (35.0 ± 8.9 years, BMI 27 ± 5 kg/m^2) with diabetes duration 17 ± 11 years, on intensive insulin therapy with multiple injections, consumed 3 mixed meals (pasticcio, chicken with vegetables and baked giant beans), with and without the addition of 11 ml extra virgin olive oil (total of 6 meals), in random order, with the insulin dose determined by using the carbohydrate counting method. Capillary blood glucose was measured at premeal (baseline) and 30, 60, 90, 120, 150 and 180 min after meal consumption. At every visit, participants were assessed for anthropometric parameters and subjective stress.

Results: Participants had mean HbA1c 7.5 ± 1.2%, mean carbohydrate to insulin ratio 9:1 IU and stable body weight, waist circumference and subjective stress throughout the study. The mean glucose concentration, for all 6 meals, 120 min postprandially was within target (<180 mg/dl) in nearly 80% of the sample. Addition of olive oil produced sustained increased postprandial glucose concentrations only to pasticcio meal, although within target, and no significant differences were noticed for the grilled chicken with vegetables or the baked giant beans (legume) meals.

Conclusions: The carbohydrate-counting method was effective for achieving postprandial glucose levels within target threshold up to 3 h postprandially. Moreover, adding small amounts of dietary fat (extra virgin olive oil) to low fat meals does not significantly alter the postprandial response within the first 3 h, whereas caused a sustained increase in postprandial blood glucose concentrations to the high energy density meal (i.e. the pasticcio meal).

© 2019 European Society for Clinical Nutrition and Metabolism. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Achieving optimal glycemic control in people with type 1 diabetes (T1D) on intensive insulin treatment with multiple insulin injections is challenging from a wide perspective for both clinicians, dietitians and patients. The need for frequent blood glucose self-monitoring, the appropriate rapid insulin dose estimation for

* Corresponding author. Lab of Food Chemistry and Analysis, Department of Food Science and Human Nutrition, 75 Iera Odos, GR-11855 Athens, Greece. Fax: +30 210 5294967.
E-mail address: emiliap@aua.gr (E. Papakonstantinou).

https://doi.org/10.1016/j.clnesp.2019.03.002
2405-4577/© 2019 European Society for Clinical Nutrition and Metabolism. Published by Elsevier Ltd. All rights reserved.

consumed meals, the different techniques used for people using multiple daily injections with pens vs continuous subcutaneous insulin infusions with pumps, the proper use of the individual correction factor, the complexity of nutrition education regarding rapid insulin matching to meals, and patients' fear of hypoglycemic episodes are some of the factors adding to the challenge of tight glycemic control for the individual with T1D. The importance of keeping blood glucose fluctuations under control has been highlighted in the past few years, as fluctuations are known to induce oxidative stress and b-cell damage in people with diabetes [1]. Indeed, increased glucose variability from peaks to nadirs has been recognized as a metabolic defect leading to CVD in people with diabetes [2].

Carbohydrates is the macronutrient that mainly affects the glycemic response and thus, is the major nutritional consideration for T1D regarding achievement of long-term glycemic control [3–5]. Carbohydrates consumption increases postprandial glucose concentrations in a dose-dependent response manner [5,6]. Matching insulin to the amount of consumed carbohydrates, known as the carbohydrate counting method, is an effective and efficacious strategy for improving glycemic control [3,4]. The advantages of this method include a more precise adjustment of dietary carbohydrates consumed to the insulin needs and flexibility of the dietary choices [7]. Several meta-analyses of randomized controlled clinical trials (RCT) and current RCTs in adults with T1D have shown that the carbohydrate counting method can improve glycemic control by a reduction of hemoglobin A1c (HbA1c) up to 1.2%, quality of life, overall well-being and fewer hypoglycemic episodes [8–12]. In particular, the systematic review and meta-analysis by Bell et al. [9] showed that in studies confined to adults, the carbohydrate counting method leads to a clinically and statistically significant reduction of about 0.6% in HbA1c concentration.

However, arguments have been expressed regarding the effects of dietary protein and fat content of a meal on postprandial blood glucose concentrations and concerns have been raised for the effectiveness of the carbohydrate counting method. Some small intervention studies suggest that high intakes of dietary protein may increase postprandial glucose concentrations in a dose-dependent response manner [13–15]. Other studies have also suggested that high intakes of fat cause a delayed increase of postprandial glucose concentrations and a sustained late postprandial hyperglycemia [13,16,17]. Some studies suggest that the combination of high amounts of dietary protein and fat in energy dense foods increase significantly postprandial blood glucose concentrations leading to increased rapid insulin meal needs by 1–3 units [18–21]; whereas one study showed that high intakes of dietary protein and fat did not significantly increase postprandial glucose excursions, but increased the duration of postprandial hyperglycemia [22]. However, severe hypoglycemic events have been recorded when protein and/or fat have been included in the estimation of rapid insulin meal needs [18,19].

Although delayed postprandial hyperglycemia is an important problem for glucose control, the clinical focus this far, the diabetes nutrition education systems and the instruction given to patients in real life situations still target 2 h post-meal blood glucose levels. Thus, for people with T1D using pens, the critical issue is how to estimate their individualized rapid insulin needs for meal consumption to achieve acute (2 up to 3 h) within target postprandial blood glucose concentrations.

Given the aforementioned data on the effects of macronutrients on the glycemic response in people with T1D, the aims of the present study were: a) to explore the effectiveness of the carbohydrate counting method on achieving acute (up to 3 h) postprandial blood glucose concentrations within the proposed targets, in people with T1D, after the consumption of 3 typical Greek mixed meals with varied macronutrient profiles, mimicking real life conditions, and b) to further explore the effect of a small amount of added extra virgin olive oil, typical fat used in food preparation in Greece, to the aforementioned 3 meals on the participants' glycemic response.

2. Subjects and methods

2.1. Subjects

Adults (18–55 years) with T1DM (duration >2yrs), non-smoking, men and women were eligible for enrollment in this study. Subjects were chosen via notices at the outpatient Diabetes Center of the General Hospital of Nice, Piraeus, Greece and the Agricultural University of Athens. Inclusion criteria were treatment with multiple insulin injections (rapid acting analogs: insulin lispro, glulisine and aspart) and long acting insulin analogs (insulin glargine or insulin detemir) for at least 6 months. Exclusion criteria included insulin pump therapy, pregnancy, lactation, celiac disease, serious microvascular and macrovascular diabetes complications including autonomic neuropathy and any other chronic disease apart from diabetes (e.g. coronary heart disease, liver or renal disease), gastrointestinal disorders, attending competitive sports and high alcohol consumption. Patients meeting the inclusion criteria and showing an adequate level of compliance to the purpose of the study were asked to participate. The study design requirements were discussed for participation feasibility, particularly work schedules and lifestyle habits. The protocol was approved by the Bioethics Committee of the General Hospital of Nice, Peiraeus, Greece, was carried out in accordance with the Declaration of Helsinki (1997) and registered at ClinicalTrials.gov NCT02928016. All patients provided their written consent.

2.2. Study design

The intervention was preceded by a mean of 14 days run-in period during which patients underwent nutrition education sessions with the study dietitians and the medical team. During this period participants had three face to face meetings and daily telephone contacts with the study dietitians and doctors to achieve optimal pre- and postprandial glucose concentrations before entering the study. Participants filled two 7-day food weighed dietary records along with capillary blood glucose measurements before and 2 h post meal or snack consumption, to optimize basal insulin needs and calculate patients' insulin to carbohydrate ratio and insulin correction factor. Individuals were instructed to consume their regular meals and snacks during the run-in period, containing a variety of carbohydrate grams, which ranged between 15 and 75 g. The insulin to carbohydrate ratio and insulin correction factor calculated for each patient were then used throughout the study. After the run-in period and according to a randomized crossover design, participants were assigned to the interventions using a sequence of random numbers extracted from computer software. A researcher not involved with the collection and the analysis of the scientific data, was responsible for the randomization of the volunteers to the intervention days examining the test meals.

The last rapid insulin adjustment (i.e. correction doses) had to be 12–15 h before the study day and the last long-acting insulin dose adjustment 48 h before the study day. The study dietitians contacted the participants the evening before and the morning of each study day to ensure that participants avoided hypo- or hyper glycemic events. In case of reported hypoglycemia (blood glucose <65 mg/dL) or hyperglycemia (premeal blood glucose >130 mg/dL

or post-meal blood glucose >180 mg/dL) the test procedure was scheduled on another day. In the mornings preceding the test meals, patients consumed the same light breakfast to avoid second-meal effect bias. They were also asked to avoid strenuous physical activity on the day before and on the morning of the test meal. Premeal insulin doses, injected just before eating, were based on the individual insulin to carbohydrates ratio determined during the study's run-in period. Thus, for each patient, insulin doses were the same for each mixed meal without and with the addition of extra virgin olive oil.

Participants came at the outpatient Diabetes Center of the General Hospital of Nice, Peiraeus, Greece between 1200 and 1300 h, 6 times in total, with 1-week wash-out period. Participants received 3 ready-made standardized typical Greek mixed meals [pasticcio (a dish made with thick pasta, minced beef and béchamel sauce), grilled chicken with boiled vegetables and baked giant beans (gigantes)] (Sklavenitis, SA, Peristeri, Greece) without and with the addition of 11 ml of extra virgin olive oil, in different weeks, with random sequence. The carbohydrate content (in grams) used for premeal insulin dose calculation was derived from the meals' food labels made available by Sklavenitis SA. Patients came in at the same time and day of the week, ruling out the possibility of confounding effects of different days of the week. The test meals were freshly prepared on the day of testing and served by the study's dietitians who were also present during their consumption by volunteers.

2.3. Test meal composition

The typical Greek mixed meals differed in macronutrient composition, mimicking real life conditions (Table 1). Chicken with vegetables was chosen as a high protein, low carbohydrate (non-starchy nature), low fat meal. Baked giant beans was chosen as a legume dish, high in complex carbohydrates and dietary fiber, low in fat, meal. Pasticcio was chosen as a high energy density meal (with non-carbohydrate calories coming mainly from lipids). Extra virgin olive oil was chosen as the typical source of mono-unsaturated fat used in food preparation in Greece. The whole content of olive oil (11 ml) was added to the meals just before consumption and the study's dietitians ensured that all the quantity was consumed.

2.4. Measurements

To determine blood glucose concentrations, trained individuals from our research team, performed the capillary blood glucose monitoring procedure by skin pricking according to the scheduled time. To standardize all data collection procedures, capillary blood glucose monitoring was performed at the fingertip (distal phalange of the third finger). The participants could choose the hand for capillary blood glucose monitoring. Capillary blood samples were collected at baseline and at 30, 60, 90, 120, 150 and 180 min after meal consumption. All blood glucose measurements were conducted by using the FORA Comfort lux GD50 blood glucose meter (ATCARE ltd, Greece). Blood glucose was measured with glucose dehydrogenase FAD dependent (GDH − FAD) test strips which show no reactivity to any sugars other than glucose and has better heat-resistance and oxygen-resistance. The allowed deviation limits of glucose meters for glucose results ≥100 mg/dL were within 15% of the reference method. The coefficient of variation (CV, %) was less than 5% both in intermediate precision and repeatability. The blood glucose value recorded was the mean of three measurements.

The within target 2 h postprandial blood glucose levels were set between 70 and 180 mg/dl [23]. 0–3 h postprandial blood glucose incremental areas (iAUC) for blood glucose were calculated by the trapezoidal method as the area under the curve above the baseline value. Blood glucose peak was calculated as the maximal blood glucose excursion from the fasting value over the 3 h period. Time to blood glucose peak was calculated as the time at which the maximal blood glucose excursion was observed. To account for the few hypoglycemic events (blood glucose value < 65 mg/dL) observed, we used the last recorded blood glucose value before treating hypoglycemia for the calculation of iAUC blood glucose.

During the run-in period, fasting blood samples were collected for biochemical analyses. Hemoglobin A1c (HbA1c) was measured with RXDaytona analyzer (Randox Laboratories, MA, USA). Total cholesterol (mmol/L), triglycerides (mmol/L), and high-density lipoprotein cholesterol (HDL-c) (mmol/L) were measured by the AEROSET/ARCHITECT c8000 System (Abbott, Chicago, IL, USA). Low density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula [24].

2.5. Dietary, physical activity and psychological assessment

Before entry to the study, dietary habits were assessed through a semi-quantitative food frequency questionnaire and the quality of the background diet was assessed through the MedDietScore that assesses adherence to the Mediterranean dietary pattern [25]. The range of the diet score is 0–55, with higher values indicating greater adherence to the Mediterranean diet. At every visit, a 24 h recall was completed. Both the 24 h recalls and the 7-day weighed food records, administered for estimating the carbohydrate to insulin ratio, were analyzed using Diet Analysis Plus software (version 6.1, ESHA Research, OR, USA). The database was extensively modified to include new foods and recipes. Physical activity of the participants was assessed through a validated questionnaire [the Athens Physical Activity Questionnaire (APAQ)] [26]. This questionnaire collects the previous week's self-reported physical activity and examines the time spent in light, moderate, high

Table 1
Energy and nutrient content of the test meals.

	Grilled chicken with boiled vegetables	Baked giant beans	Pasticcio	Grilled chicken with boiled vegetables+11 ml extra virgin olive oil	Baked giant beans+11 ml extra virgin olive oil	Pasticcio+ 11 ml extra virgin olive oil
Per serving						
Energy (kcal)	399	502	794	498	601	893
Energy from lipids and protein (kcal)	286	296	510	305	395	605
Carbohydrates (g)	25	34	68	25	34	68
Sugars (g)	4	9	5	4	9	5
Lipids (g)	10	24	38	21	35	49
Proteins (g)	49	20	42	49	20	42
Fibers (g)	6	31	8	6	31	8

intensity activities and sleep. Based on the metabolic equivalents of all activities, the mean daily energy expenditure and the physical activity level (PAL) were estimated. The reason behind estimating habitual diet and physical activity was to ensure that participants would not change their typical dietary and physical activity patterns, which could possibly have an impact on the glycemic response. The study dietitians monitored these data and counseled participants if needed.

At every visit, participants filled out the Zung self-rating depression scale [27] and the Zung self-rating anxiety scale [28] in order to evaluate the physiological and psychological state of the participants and to ensure that participants would not have additional factors influencing their blood glucose response, including intense psychological distress, depression or anxiety. Each questionnaire comprises of 20 questions. The value range of each questionnaire is between 20 and 80, with higher values indicating worse psychological state and higher stress and the lower values better psychological state and lower stress, accordingly.

2.6. Anthropometric measurements

Height (cm) was measured at baseline. Body weight (kg), waist circumference (cm) and hip circumference (cm) were measured at every visit to ensure body weight stability. BMI (kg/m^2) was calculated.

2.7. Statistical analysis

Data distribution was tested using kernel density plots. Normally distributed continuous variables are presented as mean values ± standard error of the mean (SEM), unless otherwise stated. Differences in baseline continuous variables were evaluated using analysis of variance (ANOVA) for normally distributed continuous variables, Kruskal–Wallis test for skewed continuous data, Pearson chi square test for categorical variables. Between treatments, analysis of variance (ANOVA) for a 2 × 2 crossover study was conducted for each outcome of interest. In a 2 × 2 design we assume that there are no group effects since a complete randomization process was followed for treatment allocation. The models included the factors "subject" (id), "sequence" for inter-subject variation, and "period" & "treatments" to account for intra-subject variability. Time x test meal interaction was evaluated. According to sample size calculation, a total of 13 volunteers was necessary to achieve 80% power, at a two-sided 0.05 significance level, for detecting a clinical difference of 25 (SD 30) mg/dL in mean blood glucose difference between treatments (mixed meal with and without the addition of olive oil). Statistical significance was determined to be $p < 0.05$. All analyses were performed using SPSS software (version 20.0, SPSS Inc., USA).

3. Results

3.1. Participants' characteristics

The mean age of study participants (9 men and 11 women; Table 2) was 35.0 ± 8.9 years and the mean BMI 27.3 ± 4.9 kg/m^2. The diabetes duration was 16.7 ± 10.9 years and they had acceptable blood glucose control (HbA1c 7.5 ± 1.2%). Their total daily insulin dose was 42.2 ± 11.0 IU and their mean calculated carbohydrate (g) to rapid insulin (units) ratio was 9.1 ± 3.9 g to 1 IU.

All participants had a moderate adoption of the Mediterranean diet (mean MedDiet Score 29.6 ± 4.3). Participants had below average score in self-rating depression and anxiety levels throughout the study (Data not shown). BMI, waist and hip circumferences, subjective stress and PAL remained stable throughout the study (Data not shown, all $p > 0.05$).

Table 2
Subject characteristics at baseline.[a].

Age (y)	35.0 ± 8.9
Sex, male/female (n)	9/11
Duration of Diabetes (y)	16.7 ± 10.9
Carbohydrates to insulin ratio (g: insulin units)	9.1 ± 3.9
Smoking, yes (%)	15
Years of education	14.9 ± 3.0
MedDiet Score (0–55)	29.6 ± 4.3
Anthopometrics	
Body mass index (kg/m^2)	27.3 ± 4.9
Waist circumference (cm)	86.7 ± 13.6
Hip circumference (cm)	106.9 ± 8.2
PAL	1.35 ± 0.23
Psychological assessment	
Zung self-rating depression scale (20–80)	37.7 ± 8.4
Zung self-rating anxiety scale (20–80)	35.7 ± 6.2
Biochemical variables	
Glycated Hemoglobin A1c (%)	7.5 ± 1.3
Total cholesterol (mmol/L)	4.3 ± 0.6
LDL (mmol/L)	2.4 ± 0.4
HDL (mmol/L)	1.6 ± 0.4
Triglycerides (mmol/L)	0.8 ± 0.3

Abbreviations: MedDiet: Meditteranean Diet; PAL: Physical Activity Level; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein.
[a] Values are Means ± SD.

3.2. Meal insulin dose

As determined based on the individual insulin to carbohydrate ratio and the carbohydrate grams reported on the food labels of meals, the same insulin dose was administered before meal consumption, without considering the addition of extra virgin olive oil.

3.3. Glycemic response

The effectiveness of the carbohydrate counting method in achieving the targeted 2 h postprandial blood glucose levels was high for all test meals and ranged from 85 to 90% when consumed without added extra virgin olive oil (Table 3). When extra virgin olive oil was added to meals, the aforementioned percentages were reduced from 85% to 70% for pasticcio meal; from 90% to 80% for the grilled chicken with boiled vegetables meal; and from 85% to 75% for the baked giant beans meal, however these reductions were not significant (all $p > 0.05$) (Table 3). Regarding mild asymptomatic hypoglycemic events, there were few recorded episodes (chicken with boiled vegetables: 2 out of 20 without added olive oil and 4 out of 20 with added olive oil; baked giant beans: 1 out of 20 without added olive oil and 2 out of 20 with added olive oil; pasticcio: 1 out of 20 without added olive oil and 1 out of 20 with added olive oil). No significant differences in the number of episodes of hypoglycemia between the three meals were observed when meals were consumed without or with added olive oil.

No significant differences were observed on fasting glucose concentrations between the test meals, without and with the addition of olive oil (p for all >0.05; Fig. 1a–c). There was a significant main effect of meal on mean blood glucose concentrations and on 0–3 h iAUC for blood glucose ($p = 0.04$ and $p = 0.04$, respectively). Addition of 11 ml of extra virgin olive oil increased significantly the postprandial blood glucose response only for the pasticcio meal ($p < 0.05$), but did not significantly influence the overall blood glucose profile for the meals grilled chicken with boiled vegetables and giant baked beans (all $p > 0.05$; Fig. 1a–c). Compared to pasticcio without the addition of olive oil, higher

Table 3
Blood glucose evaluation after the consumption of three mixed meals without and with the addition of 11 ml extra virgin olive oil in adults with type 1 diabetes on intensive insulin therapy (N = 20).

	Pasticcio WITHOUT olive oil	Pasticcio WITH olive oil	P-value	Chicken vegetables WITHOUT olive oil	Chicken vegetables WITH olive oil	P-value	Baked Giant Beans WITHOUT olive oil	Baked Giant Beans WITH olive oil	P-value
Preprandial capillary blood glucose (mg/dL)	102.8 ± 6.9	109.7 ± 6.5	NS	114.7 ± 7.9	111.2 ± 6.1	NS	105.3 ± 5.5	107.6 ± 7.6	NS
Mean blood glucose (mg/dL)	119.0 ± 4.6	132.3 ± 5.5	0.04	114.7 ± 5.5	115.3 ± 7.5	NS	129.3 ± 5.4	129.8 ± 7.1	NS
Peak blood glucose (mg/dL)	48.8 ± 8.7	67.5 ± 12.1	NS	32.4 ± 5.2	37.9 ± 8.1	NS	55.2 ± 9.1	52.7 ± 12.1	NS
Peak time blood glucose (min)	108 ± 12.8	126 ± 13.3	NS	107 ± 13.8	108.9 ± 15.8	NS	106.5 ± 10.5	97.5 ± 11.1	NS
0–180 min iAUC for blood glucose (mg*min/dL)	3804 ± 794	5748 ± 1230	0.08	1820 ± 488	2489 ± 725	NS	5244 ± 1024	5374 ± 1632	NS
Blood glucose <180 mg/dl at 120 min, N (%)	17 (85)	14 (70)		18 (90)	16 (80)		17 (85)	15 (75)	

Data are means ± SEM. Episodes of hypoglycemia are presented as actual number. Means of meals without and with the addition of extra virgin olive oil were compared by using 2 × 2 crossover ANOVA for factor "treatment", period and sequence of treatment; *p*-values < 0.05 were considered as significant. Abbreviations: iAUC, incremental area under the curve; NS: not statistically significant.

blood glucose concentrations were observed after the consumption of pasticcio with the addition of olive oil elicited at 150 min and 180 min (p = 0.03 and p = 0.01, respectively; Fig. 1a). Mean blood glucose concentrations were significantly higher after consumption of pasticcio with the addition of olive oil compared to pasticcio without the addition of olive oil (p = 0.04; Table 3). At 3 h postprandial, glucose concentrations tended to be higher compared to baseline after consumption of pasticcio without added extra virgin olive oil, although it did not reach statistical significance (p = 0.051; Fig. 1a). At 3 h postprandial, glucose concentrations were significantly higher compared to baseline after consumption of pasticcio with added extra virgin olive oil (p = 0.009; Fig. 1a). Accordingly, blood glucose 0–3 h iAUC tended to be higher after the pasticcio with added extra virgin olive oil meal compared to that without added olive oil (p = 0.08; Table 3).

The glycemic response produced after the consumption of grilled chicken with boiled vegetables did not differ when consumed without and with the addition of olive oil (all p > 0.05; Fig. 1b). At 3 h postprandial, blood glucose concentrations did not differ compared to baseline after consumption of grilled chicken with boiled vegetables either without added extra virgin olive oil (p > 0.05) or with added extra virgin olive oil (p > 0.05; Fig. 1b). Mean blood glucose, 0–3 h iAUC for blood glucose, peak blood glucose levels, and blood glucose levels peak time did not differ between grilled chicken with boiled vegetables without and with added extra virgin olive oil (all p > 0.05; Table 3). Similarly, the glycemic response produced after the consumption of giant baked beans did not differ when consumed without and with the addition of olive oil (all p > 0.05; Fig. 1c). At 3 h postprandial, blood glucose concentrations were significantly higher compared to baseline after consumption of baked giant beans without and with the addition of extra virgin olive oil, without differences between them (all p > 0.05; Fig. 1c). Mean blood glucose, 0–3 h iAUC for blood glucose, peak blood glucose levels and blood glucose levels peak time did not differ between baked giant beans without and with added extra virgin olive oil (all p > 0.05; Table 3).

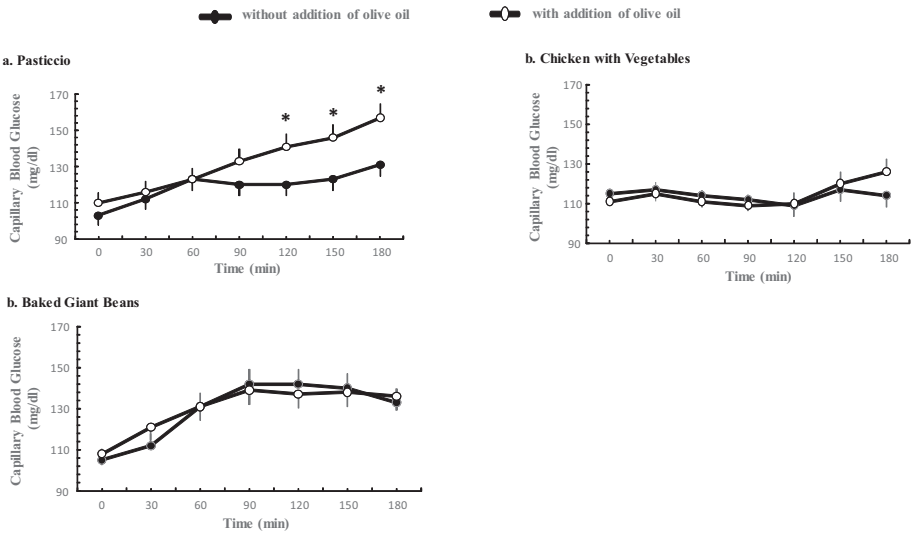

Fig. 1. Comparisons of mean blood glucose concentrations after the consumption of: a) pasticcio meal without and with the addition of 11 ml extra virgin olive oil; b) grilled chicken with boiled vegetables without and with the addition of 11 ml extra virgin olive oil; and c) baked giant beans without and with the addition of extra virgin olive oil. Data are means ± S.E.M. An asterisk indicates a significant difference (p < 0.05) between test meals at specific time points, determined by a 2 × 2 crossover ANOVA for factor "treatment", period and sequence of treatment. Post hoc Tukey test, with Bonferroni correction, was conducted to determine test meal differences at east specific time. There was a significant main effect of test meal on blood glucose concentrations (p = 0.04). Compared to pasticcio without the addition of olive oil, higher blood glucose concentrations were observed after the consumption of pasticcio with the addition of olive oil elicited at 150 min and 180 min (p = 0.03 and p = 0.01, respectively). At 3 h postprandial glucose concentrations returned to baseline after consumption of pasticcio without added extra virgin olive oil, but continued to rise after consumption of pasticcio with added olive oil. No differences were observed between grilled chicken with boiled vegetables and baked giant beans, without and with added extra virgin olive oil at any time point.

4. Discussion

In the present study we confirmed the effectiveness of the carbohydrate counting method after the consumption of 3 typical Greek mixed meals with varied macronutrient profiles, mimicking real life conditions in adults with T1D on intensive insulin therapy with multiple insulin injections using pen. The meals were consumed without and with the addition of extra virgin olive oil. Adding small amounts of dietary fat to low fat meals did not significantly alter the postprandial response in the first 3 h. However, the added small amount of extra virgin olive oil caused a sustained increase in postprandial blood glucose concentrations only to the high energy density meal (i.e. the pasticcio meal) which had also the highest fat content of the test meals (49 g of fat).

Achievement of within targeted threshold postprandial blood glucose levels in T1D is a complex issue, because blood glucose concentrations are significantly influenced by multiple factors such as preprandial blood glucose levels, timing and mode of insulin administration, insulin sensitivity, insulin units, exercise, stress, other medications and illness [29]. The effectiveness and efficiency of the carbohydrate counting method in T1D are well established [3,5,30,31]. When the individualized carbohydrate to insulin ratio is accurately estimated, the likelihood for on target insulin doses ranges between 50.1% and 98.5% [32]. In our study, the calculation of the premeal insulin dose was based on the amount, but not the type, of carbohydrates and was proven sufficient for achieving on target postprandial blood glucose levels in nearly 80% of the cases. Moreover, use of the carbohydrate counting method is associated with minimal incidence of hypoglycaemia, as shown in our study as well as in others [3,4,7,8]. In contrast, one study reported increased frequency (nearly 36%) of hypoglycemic episodes (blood glucose <70 mg/dl) when rapid insulin units were added for dietary protein and fat vs only 10% for carbohydrates alone [18].

Regarding the effects of dietary protein and fat on postprandial glucose concentrations, there are still a lot of conflicting opinions and data. Even the official Diabetes Associations seem to take different stands on this issue with the American Diabetes Association suggesting that “individuals who consume meals containing more protein and fat than usual may also need to make mealtime insulin dose adjustments to compensate for delayed postprandial glycemic excursions” [4] and the U.K. Diabetes Association suggesting that “considering the strength of the current evidence and the magnitude of the effect of fat and protein on postprandial glucose, more evidence is needed to justify routine prioritization of fat and protein counting in clinical practice” [3]. Briefly, some intervention studies suggest that dietary protein does not increase blood glucose concentrations within 2–3 h postprandially [22,33]; but it may increase the duration of postprandial hyperglycemia [13,14,22,34], others have shown that meals containing between 28 and 57 g of protein produce significantly higher postprandial glycemic excursions and insulin requirements 2–5 h postprandially compared to standard meals [34–37]; whereas Paterson et al. [15] recently showed a dose-dependent and bi-phasic effect of protein on postprandial glycemic control. Regarding the effects of fat on glycemic response, some studies have suggested that dietary fat, alone or in combination with protein, may cause acute insulin resistance [17], an initial decreased glycemic response [16] and a delayed sustained hyperglycemia 3–6 h post-meal consumption [13,17,22,34], whereas recently, also the fat quality was explored in an RCT demonstrating that the addition of 37 g of extra virgin olive oil to high glycemic index meals, attenuated the early (up to 3 h) postprandial glucose response observed, when these meals were consumed with either a low fat content (8 g extra virgin olive oil) or 43 g of butter [38].

Accordingly, mixed meal studies have produced inconclusive and conflicting results with meals such as pizza, carbonara and fast food items (i.e. hamburger and fries), with some showing that these meals require higher [17,18,39–41], than anticipated by carbohydrate counting alone, rapid insulin units, others showing lower [20,42] than anticipated rapid insulin units and the rest showing that carbohydrate counting alone is a sufficient and efficient method [17,20,42]. In our study, the meals used varied in terms of both protein (protein range 20–49 g) and fat (range 10–38 g, without the addition of olive oil) to explore the effectiveness of counting within a range of protein and fat content and fat content was further increased with the addition of olive oil leading up to a greater range of fat intake (22–49) g. According to our results, the addition of a small amount of fat affected only the glucose response after the consumption of the high energy density, already high in fat (49 g per typical portion serving) and protein (42 g per typical portion serving), meal (pasticcio), suggesting that there might be a fat or even energy threshold after which fat exerts an increased glucose response. It may then be that only very high intakes of non-carbohydrate calories coming from fat and protein induce the sustained or delayed hyperglycemia and not dietary fat and protein per se, particularly when these are consumed in smaller amounts. The pasticcio meal is close, fat and protein wise, to the pizza and carbonara meals [20,41,42]. Furthermore, in our study grilled chicken with boiled vegetables had the highest protein content (i.e. 49 g) however blood glucose levels were within the anticipated limits during the 3 h postprandial period. Whether this was noticed because no extra insulin is needed for protein or to the differential insulin needs between starchy and non-starchy carbohydrates (as in the case of boiled vegetables in this meal), remains to be elucidated.

The strengths of this study include the randomized, crossover design. Moreover, we tested the effectiveness of the carbohydrate counting method using meals typical of the Greek cuisine and tried to further explore the effects of a small amount of added extra virgin olive oil in these meals on the acute glycemic response as per the carbohydrate counting method guidelines. Among the limitations of the study one could mention that the 3 h blood glucose recordings is not long enough to detect the full effect of dietary protein and/or fat on induced hyperglycemia beyond the 3 h interval. Furthermore, it would have been preferable if we had measured plasma glucose concentrations using an automated analyzer instead of self-monitored blood glucose measurements. Moreover, test sessions where the participant experienced hypoglycaemia were terminated immediately so that the hypoglycemic episode could be treated appropriately. In these cases, the last recorded blood glucose value was carried forward. Alternatively, we could have chosen to assume a consistent gradient in blood glucose concentrations over the remainder of the recording period. However, imputing any value for missing data raises doubts about the generalizability of the findings. Although we tried to control for several factors known to affect blood glucose levels (i.e. stress, exercise, illness) and the crossover design of the study also contributed to that; remaining confounding factors cannot be ruled out.

In conclusion, the results of our study confirmed the effectiveness of the carbohydrate counting method in achieving on-target postprandial blood glucose concentrations within a 3 h period after consumption of mixed meals with various macronutrient content. Adding small amounts of dietary fat to low fat meals did not significantly alter the postprandial response in the first three hours, however, no conclusions can be drawn about high fat meals, protein or longer timeframes. Based on the current results and the relevant literature, whether adding dietary protein and fat to the puzzle of premeal insulin calculation, is clinically meaningful to patients

with T1D, remains obscure. Instead, frequent blood glucose testing every 2–3 h postprandial for patients on multiple injection schemes, as suggested by many scientific associations, should be recommended.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Author contribution

EP and MDK conceptualized and designed the study and drafted the manuscript. KP and CM collected the data and conducted the statistical analyses. EM served as counselor and helped with the statistical analyses; SB helped with patient recruitment and served as scientific counselor to the project. AK, AS and MP served as medical team of the study. SP served as scientific counselor to the project and critically revised the manuscript. All authors contributed to the writing and editing of this manuscript according to their area of expertise and all authors approved it.

Acknowledgments

The authors would like to thank the “ATCARE Ltd” company (http://www.atcare.gr) for providing us with the automatic lancets, glucose meters and glucose strips (FORA COMFORT lux GD50) and the supermarket chain “Sklavenitis SA” (Peristeri, Greece; http://www.sklavenitis.gr) for providing us part of the test meals, as well as information on their nutrient content. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes 2008;57(5):1349–54.
[2] Monnier L, Colette C, Owens DR. Integrating glycaemic variability in the glycaemic disorders of type 2 diabetes: a move towards a unified glucose tetrad concept. Diabetes Metab Res Rev 2009;25(5):393–402.
[3] Dyson PA, Twenefour D, Breen C, Duncan A, Elvin E, Goff L, et al. Diabetes UK evidence-based nutrition guidelines for the prevention and management of diabetes. Diabet Med 2018;35(5):541–7.
[4] American Diabetes Association. Lifestyle Management: Standards of medical care in diabetes—2018. Diabetes Care 2018;41(Supplement 1):S38–50.
[5] Kawamura T. The importance of carbohydrate counting in the treatment of children with diabetes. Pediatr Diabetes 2007;8(Suppl. 6):57–62.
[6] Meng H, Matthan NR, Ausman LM, Lichtenstein AH. Effect of macronutrients and fiber on postprandial glycemic responses and meal glycemic index and glycemic load value determinations. Am J Clin Nutr 2017;105(4):842–53.
[7] Maahs DM, Higgins J. Is carbohydrate counting enough? Towards perfection or unwanted complexity? Diabetes Technol Ther 2012;14(1):3–5.
[8] Schmidt S, Schelde B, Norgaard K. Effects of advanced carbohydrate counting in patients with type 1 diabetes: a systematic review. Diabet Med 2014;31(8):886–96.
[9] Bell KJ, Barclay AW, Petocz P, Colagiuri S, Brand-Miller JC. Efficacy of carbohydrate counting in type 1 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol 2014;2(2):133–40.
[10] Fu S, Li L, Deng S, Zan L, Liu Z. Effectiveness of advanced carbohydrate counting in type 1 diabetes mellitus: a systematic review and meta-analysis. Sci Rep 2016;6:37067.
[11] Souto DL, Zajdenverg L, Rodacki M, Rosado EL. Impact of advanced and basic carbohydrate counting methods on metabolic control in patients with type 1 diabetes. Nutrition 2014;30(3):286–90.
[12] Hommel E, Schmidt S, Vistisen D, Neergaard K, Gribhild M, Almdal T, et al. Effects of advanced carbohydrate counting guided by an automated bolus calculator in Type 1 diabetes mellitus (StenoABC): a 12-month, randomized clinical trial. Diabet Med 2017;34(5):708–15.
[13] Bell KJ, Smart CE, Steil GM, Brand-Miller JC, King B, Wolpert HA. Impact of fat, protein, and glycemic index on postprandial glucose control in type 1 diabetes: implications for intensive diabetes management in the continuous glucose monitoring era. Diabetes Care 2015;38(6):1008–15.
[14] Paterson MA, Smart CE, Lopez PE, McElduff P, Attia J, Morbey C, et al. Influence of dietary protein on postprandial blood glucose levels in individuals with Type 1 diabetes mellitus using intensive insulin therapy. Diabet Med 2016;33(5):592–8.
[15] Paterson MA, Smart CEM, Lopez PE, Howley P, McElduff P, Attia J, et al. Increasing the protein quantity in a meal results in dose-dependent effects on postprandial glucose levels in individuals with Type 1 diabetes mellitus. Diabet Med 2017;34(6):851–4.
[16] Lodefalk M, Aman J, Bang P. Effects of fat supplementation on glycaemic response and gastric emptying in adolescents with Type 1 diabetes. Diabet Med 2008;25(9):1030–5.
[17] Wolpert HA, Atakov-Castillo A, Smith SA, Steil GM. Dietary fat acutely increases glucose concentrations and insulin requirements in patients with type 1 diabetes: implications for carbohydrate-based bolus dose calculation and intensive diabetes management. Diabetes Care 2013;36(4):810–6.
[18] Kordonouri O, Hartmann R, Remus K, Blasig S, Sadeghian E, Danne T. Benefit of supplementary fat plus protein counting as compared with conventional carbohydrate counting for insulin bolus calculation in children with pump therapy. Pediatr Diabetes 2012;13(7):540–4.
[19] Pankowska E, Blazik M. Bolus calculator with nutrition database software, a new concept of prandial insulin programming for pump users. J Diabetes Sci Technol 2010;4(3):571–6.
[20] MacDonald K, Lowe JM, Barker D, Mensch M, Attia J. Effect of popular takeaway foods on blood glucose levels in type 1 diabetes mellitus patients on intensive insulin therapy. Int J Clin Pract 2009;63(2):189–94.
[21] Bell KJ, Gray R, Munns D, Petocz P, Howard G, Colagiuri S, et al. Estimating insulin demand for protein-containing foods using the food insulin index. Eur J Clin Nutr 2014;68(9):1055–9.
[22] van der Hoogt M, van Dyk JC, Dolman RC, Pieters M. Protein and fat meal content increase insulin requirement in children with type 1 diabetes - role of duration of diabetes. J Clin Transl Endocrinol 2017;10:15–21.
[23] American Diabetes Association. Glycemic targets: Standards of medical care in diabetes—2018. Diabetes Care 2018;41(Suppl. 1):S55–64.
[24] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499–502.
[25] Panagiotakos DB, Pitsavos C, Stefanadis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis 2006;16(8):559–68.
[26] Kavouras SA, Maraki MI, Kollia M, Gioxari A, Jansen LT, Sidossis LS. Development, reliability and validity of a physical activity questionnaire for estimating energy expenditure in Greek adults. Sci Sports 2016;31:e47–53.
[27] Fountoulakis KN, Lacovides A, Samolis S, Kleanthous S, Kaprinis SG, St Kaprinis G, et al. Reliability, validity and psychometric properties of the Greek translation of the Zung depression rating scale. BMC Psychiatry 2001;1:6.
[28] Samakouri M, Bouhos G, Kadoglou M, Giantzelidou A, Tsolaki K, Livaditis M. Standardization of the Greek version of Zung's self-rating anxiety scale (SAS). Psychiatriki 2012;23(3):212–20.
[29] Bevier WC, Zisser H, Palerm CC, Finan DA, Seborg DE, Doyle FJ, et al. Calculating the insulin to carbohydrate ratio using the hyperinsulinaemic-euglycaemic clamp-a novel use for a proven technique. Diabetes Metab Res Rev 2007;23(6):472–8.
[30] Tascini G, Berioli MG, Cerquiglini L, Santi E, Mancini G, Rogari F, et al. Carbohydrate counting in children and adolescents with type 1 diabetes. Nutrients 2018;10(1).
[31] Scavone G, Manto A, Pitocco D, Gagliardi L, Caputo S, Mancini L, et al. Effect of carbohydrate counting and medical nutritional therapy on glycaemic control in Type 1 diabetic subjects: a pilot study. Diabet Med 2010;27(4):477–9.
[32] Virdi NS, Mahoney JJ. Importance of blood glucose meter and carbohydrate estimation accuracy. J Diabetes Sci Technol 2012;6(4):921–6.
[33] Klupa T, Benbenek-Klupa T, Matejko B, Mrozinska S, Malecki MT. The impact of a pure protein load on the glucose levels in type 1 diabetes patients treated with insulin pumps. Int J Endocrinol 2015;2015:216918.
[34] Smart CE, Evans M, O'Connell SM, McElduff P, Lopez PE, Jones TW, et al. Both dietary protein and fat increase postprandial glucose excursions in children with type 1 diabetes, and the effect is additive. Diabetes Care 2013;36(12):3897–902.
[35] Peters AL, Davidson MB. Protein and fat effects on glucose responses and insulin requirements in subjects with insulin-dependent diabetes mellitus. Am J Clin Nutr 1993;58(4):555–60.
[36] Winiger G, Keller U, Laager R, Girard J, Berger W. Protein content of the evening meal and nocturnal plasma glucose regulation in type-I diabetic subjects. Horm Res 1995;44(3):101–4.
[37] Garcia-Lopez JM, Gonzalez-Rodriguez M, Pazos-Couselo M, Gude F, Prieto-Tenreiro A, Casanueva F. Should the amounts of fat and protein be taken into consideration to calculate the lunch prandial insulin bolus? Results from a randomized crossover trial. Diabetes Technol Ther 2013;15(2):166–71.
[38] Bozzetto L, Alderisio A, Giorgini M, Barone F, Giacco A, Riccardi G, et al. Extra-virgin olive oil reduces glycemic response to a high-glycemic index meal in patients with type 1 diabetes: a randomized controlled trial. Diabetes Care 2016;39(4):518–24.
[39] Pankowska E, Szypowska A, Lipka M, Szpotanska M, Blazik M, Groele L. Application of novel dual wave meal bolus and its impact on glycated

hemoglobin A1c level in children with type 1 diabetes. Pediatr Diabetes 2009;10(5):298–303.
[40] Bao J, Gilbertson HR, Gray R, Munns D, Howard G, Petocz P, et al. Improving the estimation of mealtime insulin dose in adults with type 1 diabetes: the Normal Insulin Demand for Dose Adjustment (NIDDA) study. Diabetes Care 2011;34(10):2146–51.
[41] Ahern JA, Gatcomb PM, Held NA, Petit Jr WA, Tamborlane WV. Exaggerated hyperglycemia after a pizza meal in well-controlled diabetes. Diabetes Care 1993;16(4):578–80.
[42] De Palma A, Giani E, Iafusco D, Bosetti A, Macedoni M, Gazzarri A, et al. Lowering postprandial glycemia in children with type 1 diabetes after Italian pizza "margherita" (TyBoDi2 Study). Diabetes Technol Ther 2011;13(4):483–7.