



Evaluation of the Effect of Macronutrients Combination on Blood Sugar Levels in Healthy Individuals

**Berrak BASTURK¹, Zeynep KOC OZERSON¹, Aysun YUKSEL²*

1. Department of Nutrition and Dietetics, Halic University, Istanbul, Turkey

2. Department of Nutrition and Dietetics, University of Health Sciences, Istanbul, Turkey

*Corresponding Author: Email: berrakerguden@halic.edu.tr

(Received 09 Jul 2020; accepted 21 Sep 2020)

Abstract

Background: The positive effects of blood glucose levels should be demonstrated in healthy or type 2 diabetic individuals who can be recommended to consume macronutrients (protein + fat) with carbohydrates. Therefore, at the end of the research planned with the amount of carbohydrates and fats that can be consumed in a meal, we aimed to recommend the consumption of food with high protein content egg together with the carbohydrate source.

Methods: The study was carried out from Nov 2017 to Apr 2018 by looking at fasting blood glucose levels using feeding 2 different test foods on a minimum of 8 h of fasting in the Halic University Sutluce Campus, Istanbul, Turkey. Before and after the carbohydrate and carbohydrate + protein source, blood glucose was measured from the fingertip for 3 days in 30 min, 60 min and 120 min periods. The average of 3 days was used in the analysis.

Results: Blood glucose values were compared after individuals were given carbohydrate and carbohydrate + protein source. The mean blood glucose value 60 min after the carbohydrate administration was significantly higher than the average blood glucose value 60 min after the carbohydrate + protein administration ($P= 0.006$).

Conclusion: A protein-containing diet positively affects the glycemia response and can recommend it. In individuals with diabetes, they should focus on the effects of proteins to achieve glycemia control.

Keywords: Carbohydrate; Protein; Fasting blood sugar; Glycemic response

Introduction

Maintaining blood glucose balance is important for lifelong health (1). Foods with a low glycemic response are effective in maintaining blood glucose control in individuals, lowering serum lipid levels in individuals with hypertriglyceridemia, increasing high-density lipoproteins (HDL) cholesterol levels and reducing the risk of developing type 2 diabetes and cardiovascular diseases (2). The glycemic index is also associated with many

chronic estern diseases such as insulin resistance and central obesity (3).

Consumption of high protein meal with low-moderate amounts of carbohydrates increases insulin secretion (1). Powerful insulin secretagogues (valine, leucine, isoleucine), glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like Polypeptide-1 (GLP-1) (some amino acids stimulate the incretin system) decreases the rate of gastric leakage, inhibiting glucagon secretion and



stimulating insulin secretion. Increased protein and reduced carbohydrate intake result in improved insulin sensitivity and glucose uptake (1). Dietary proteins and amino acids have an insulino-tropic effect and increase insulin secretion in pancreatic beta cells, ensuring the use of blood glucose by tissues. Co-consumption of carbohydrates and protein stimulates plasma insulin concentrations in healthy individuals (4). When the effect of a meal with high glucose and whey protein content on blood glucose level was compared, the plasma insulin response was higher in the protein-containing meal. Consumption of amino acids (isoleucine, leucine, lysine, threonine and valine), whey and soy proteins have been associated with increased postprandial insulinemia and decreased glycemia in healthy individuals (4).

On the other hand, the excessive amount of proteins taken in meals affects postprandial blood glucose minimally and causes an increase. Dietary proteins have little effects on glucose production and do not increase blood glucose levels (5). Consumption of 12.5-50 g protein just consumption of does not affect blood glucose level, but more than 75 g of protein consumption was found to cause a late and increased glycemic response in postprandial glycemia from 180 min to 300 min (6). Blood glucose levels started to rise after 100 min after excessive protein intake and reached the highest point after the fifth h. In addition, the maximum effect of more than 75 g of protein intake and 20 g of glucose intake on blood glucose level was similar (6).

To suggest consuming protein sources together with carbohydrates to healthy or type 2 diabetic individuals, positive effects on blood glucose level should be demonstrated by clinical studies. Therefore, we aimed to investigate the effect of jam and jam + egg combination on a fasting blood glucose level in healthy individuals and have an ideal body mass index.

Materials and Methods

Study population

This cross-sectional and experimental study was conducted at the Sütluçe Campus in Haliç University, Istanbul, Turkey. Patients' age ranged between 18-65 yr (27.8 ± 3.7). Body Mass Index (BMI) of 20-25 kg / m², without chronic disease and with total of 20 non-smoking individuals. Data were collected during from Nov 2017 to Apr 2018. In this process, one individual was excluded from the study because it was observed that he experienced hypoglycemia after the consumption of jam.

The inclusion criteria were established with the following information:

- Obese individuals, because of the increase in free fatty acid release in enlarged fat cells, liver and the penetration of fatty acids into the peripheral tissues increases, insulin destruction decreases by the liver, and an increase in the circulating insulin level is observed. This affects blood glucose levels (7). High cigarette consumption increases visceral adipose tissue, blood glucose levels and the risk of insulin resistance (8).
- On the other hand, aging causes a decrease in insulin secretion, impaired insulin receptors, an increase in leptin and blood glucose level (9).

Study design and intervention

After signing the voluntary consent form, the research and the questionnaire form were applied to the individuals by informing them about the research.

The survey form consists of five parts:

1. Questions about socio-demographic characteristics (age, gender...),
 2. Questions about eating habits,
 3. Questions about physical activity situations,
 4. Food consumption frequency,
 5. Six-day food consumption registration form
- Individuals weight measurement was made with TANİTA BC 418 MA body measurement device, body fat mass and lean tissue mass were calculated. Harris-Benedict equation was used to calculate the basal metabolic rate of the participants. Taking

into account the physical activity levels of individuals, healthy nutrition programs have been created, containing 55-60% carbohydrates, 12%-15% protein and 25-30% fat. The researcher followed the compliance of the nutritional programs during the period when test food was given to individuals. Individuals were tested on a minimum of 8 h of fasting and averaged by feeding different test foods for 3 d; fasting blood glucose levels were examined.

1. Carbohydrate source: Cherry jam (40 g)
2. Carbohydrate + protein source: Cherry jam (40 g) + egg (50 g)

Blood glucose measurement was performed at 30 min, 60 min, and 120 min after the carbohydrate source was given for 3 d from the tip of the finger. Moreover, the fasting blood glucose was measured.

The same processes were repeated for the carbohydrate + protein source. The average of 3 days was used in the analysis for both test foods. Jam and eggs were provided free of charge by the researcher.

Ethical approval

Ethical approval for study was obtained from the Medipol University Research Ethics Committee (Number: 2018-170). The study was conducted in accordance with the Helsinki World Medical Association Declaration.

Personal Information

A questionnaire developed by the researcher was used to determine the personal characteristics of the individuals. In the questionnaire form, the demographic characteristics (age, gender...), information about nutritional habits and physical activity level of the patients were analyzed. The researcher with the face-to-face interview technique filled in the questionnaire.

Nutritional Habits

Nutritional habits of individuals (main and snack consumption situations, eating habits outside, fast-food preferences, etc.) were questioned, and their food consumption frequency was filled with face-to-face interview technique with the food

consumption frequency form containing 20 food types. In order to determine the carbohydrate level, the “individual food consumption record”, which was taken 1 day before the test foods were consumed, was also done by face-to-face interview technique. Detailed information was given about the amount of carbohydrates they consume daily in the range of 150-300 g and not to consume fast food and processed carbohydrate sources in the days before the measurements.

Blood Glucose Level Measurements

IME-DC brand glucose meter and strips were used to measure blood glucose levels of individuals. During the 6-d period of the study, participants were asked to record their food consumption forms. Fasting and postprandial (30, 60, 120 min) blood glucose level measurements were made and recorded by the researcher.

Determination of Body Weight and Body Composition

Body weight measurements of the patients were made on average three h after going to the toilet, eating, and excessive fluid intake with bare and dry feet. Segmental analysis of the patients was performed using a bioelectrical impedance analyzer (TANITA BC 418 MA). The device works with Bio Impadence Analysis and sends 8 kHz electric current to five different body regions. The ratio of fat in arms, legs and trunk, lean mass and muscle weight are analyzed with this method.

Statistical analysis

SPSS (Chicago, IL, USA) version 22 was used to perform all statistical analyses and data are presented as means \pm standard error. Statistical significance level was evaluated as $P < 0.05$ in the tests. Descriptive statistics such as frequency, percentage, arithmetic mean, standard error, minimum and maximum were used in the analysis of the data. Parametric tests were used after the normality tests were performed in the analysis of the data. Independent Sample *t* test for comparing the means of two independent groups, One-way ANOVA test in comparison of more than two independent groups, Pearson Correlation test was

used in the relationship analysis of the scales. Dependent sample *t* test and repeated measures were used for more than two dependent groups.

Results

The mean age of the sample was 27.8±3.7 years old. Of these 50% were men. The minimum and maximum of participants' body mass index (BMI) values respectively; 20 kg / m² and 25 kg / m² (Table 1).

The mean percentage of body fat was 25.9 ± 4.9% while the average of male individuals was 14.0 ± 2.9%. Individuals participating in the study according to the frequency of food consumption consumed often milk and dairy products; red meat and poultry at least 3-5 times a week; fish and seafood at least once every 15 days; processed meat 1-2 times a week; eggs almost every day; dry legumes 3-5 times a week; vegetables at least 1-2 times a week; fruit 3-5 times a week; Dried fruit at least once in 15 days; rice, pasta and bulgur at least

once a week; white bread and brown bread at least 1-2 times a week; at least 1-2 times per week sugar, chocolate, carbonated beverages, bakery products milk sweet syrup. It was determined that the fasting blood glucose level of 5 participants was higher than the targeted level. There was no significant difference between the mean fasting blood glucose value and gender of the participants in the study (*t*= -0.525; *P*=0.606).

The fasting blood sugar change graph is shown according to the time intervals of the people and the test foods consumption (Fig. 1). The blood glucose value was determined as the highest value 30 min after consuming the jam and jam + egg and the lowest value after 120 min after consuming the jam. The blood glucose value 30 min after the jam and jam+egg consumption was significantly higher than the blood glucose value after 60 min; the blood glucose value after 60 min was also significantly higher than the blood glucose value after 120 min (*P*=0.001)(Tables 2,3).

Table 1: General characteristics of individuals (n=20)

<i>Parameters</i>	<i>Mean±SD</i>	<i>Minimum</i>	<i>Maximum</i>
Age (yr)	27.8±3.7	22	36
Height (cm)	170.6±10.1	150	184
Body Weight (kg)	66.0±11.3	45	82
BMI (kg/m ²)	22.4±1.7	20	25

*BMI: Body Mass Index

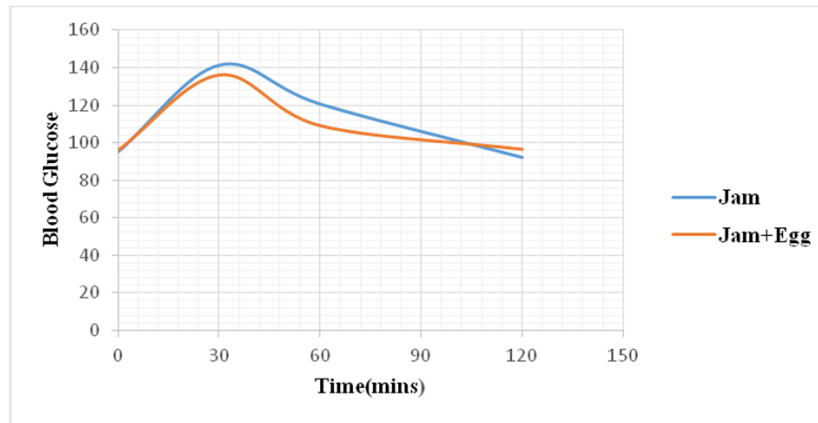


Fig. 1: Blood glucose values after jam and jam + egg consumption

The average blood glucose value after 60 min after jam consumption was significantly higher than the average blood glucose value after 60 min after jam+egg consumption ($P=0.006$). There was no

significant difference between the mean blood glucose values after 30 min, 120 min after jam consumption and the mean blood glucose values after 30, 120 min after jam+egg consumption.

Table 2: Blood glucose values after jam consumption

<i>Time (mins)</i>	<i>Jam</i>	
	<i>Mean ± SD</i>	<i>P</i>
30	141.3 ± 18.8	0.001*
60	120.7 ± 18.3	
120	92.2 ± 14.5	

*Wilks' lambda (Λ): 79,236

*Statistically significant ($P<0.05$)

Table 3: Blood glucose values after jam + egg consumption

<i>Time (mins)</i>	<i>Jam+Egg</i>	
	<i>Mean ± SD</i>	<i>P</i>
30	136.2 ± 24.8	0.001*
60	109.0 ± 19.4	
120	96.3 ± 9.3	

*Wilks' lambda (Λ): 39,747

*Statistically significant ($P<0.05$)

Discussion

Postprandial glucose deviations cause glucose toxicity. Bozbulut et al showed that dietary fat, protein may affect postprandial glycemic response and insulin release (10). Gastric emptying rate is a major determinant of the postprandial glycemia. Adding fat sources to the meal slows down the gastric emptying rate of carbohydrates. In this way, the glycemic response decreases (10). The protein source is sufficient insulin secretion minimal increase of postprandial blood glucose causes (11).

The recommended level of fasting blood glucose is ≤ 100 mg/dl. According to the American Diabetes Association (ADA), fasting plasma glucose 100-126 mg / dl; 2-h postprandial blood glucose of 140-200 mg/dl impaired glucose tolerance (IGT) and diabetes is diagnosed when fasting plasma glucose is >140 mg/dl and 2 h blood glucose is >200 mg/dl (12).

The mean fasting blood glucose levels of the individuals in our study were 96.0 ± 8.5 mg/dl (min: 82.5 max: 96.0). Because individuals pay attention to their nutrition programs, fasting and postprandial blood glucose levels remained close to the targeted levels.

In the study of glucose intolerance, atherogenic lipid profile, hypertension, abdominal obesity incidence, individuals, women were higher than men (13). The reason for this situation is the advancement in the technological field, making life easier and not spending time on sports activities (13). In our study, fasting blood glucose levels of male and female individuals were not found to be significantly different ($P > 0.05$).

Adult mammalian adipocytes and adipocyte secreted from the connective tissue in some proteins (adipokines) was the endocrine effects. In cases where the fat mass increases, the amount of these proteins also increases. Tumor Necrosis Factor (TNF), interleukin-6 and resistin are one of these

proteins play an important role in the emergence of insulin resistance in obesity, causing increase blood glucose levels (14). There was relationship between fat percentages and fasting blood glucose values. BMI's of the individuals in our study are in the normal range. For this reason, percentages of body fat and fasting blood glucose are also at the targeted values.

Macronutrients are important for the glucose response; carbohydrates act quickly on blood glucose and increase insulin secretion (15). Dietary proteins stimulate different levels of insulin and glucagon secretion. Post-prandial amino acid increase depends on the digestion rate of proteins. Insulin secretion is an important incretin hormone in response to the increase the end products of protein digestion in intestine. This increase is influenced by the circulating concentration, not the total level of the amino acid (16).

Moghaddam et al. (17) investigated the effect of 50 g of glucose plus different levels of fat and protein on the glycemic index in a randomized controlled study of non-diabetic humans, 10 with a fasting plasma insulin (FPI) < 40 pmol/L and 10 with FPI >40 pmol/L. As a result, high fat intake together with glucose was not found to decrease the blood glucose level, the glycemic response of the protein had 2-3 times more effect than the fat, and the combination of 0 g fat and 30 g protein significantly reduced the glycemic response (17).

The effect of 10 different protein-rich foods on glycemic response curve relative to reference food (1000 kJ of glucose) was examined (18). These protein rich foods (chocolate biscuits, chocolate and peanut butter balls, nuts and chocolate muffin, chocolate almond bar, chocolate drink, apple and cinnamon biscuit, pizza dough, rosemary bread-crumbs and 2 different pasta) were isocaloric energy content (1000 kJ portion). The first stage of the study took 3 weeks. During these 3 weeks, participants were asked to consume reference food glucose once a week. After the 3rd week, 2 different test foods were studied in each week. Two different test foods studied in the same week were consumed on different days. All tested foods and reference food glucose were consumed within 10 minutes. Measurements were taken at 15, 30, 45,

60, 90 and 120-min periods (18). Measurements were taken at 15, 30, 45, 60, 90 and 120-min periods. The most glycemic responses were chocolate, peanut butter balls and pasta types. The glycemic response created by foods with high protein content was lower. It was determined that the increase in blood glucose level was faster in the transition from the 15th min to the 30th min and reached the highest level in the 30th min (18). Similarly, in our study, the blood glucose value reached the highest level in the 30th min after giving jam (mean 141.1 mg/dl) and jam and eggs (mean 136.3 mg/dl) (Tables 2,3).

In another study, 48 healthy individuals with a BMI in the normal range (BKI $23 \pm 1 \text{ kg / m}^2$) were served breakfast options with 390 kcal energy and the same fat content and their glycemic effects were evaluated. Options included white toast + cereal flakes (10 g protein); egg + white toast (30 g protein) and whole grain toast + egg (20 g protein + 7 g fiber). Study resulted in 30 g protein and 20 g of egg protein + 7 comprising fibers containing g breakfast options were found to attenuate the postprandial glycemic response by 10 g protein ($P < 0.05$). The highest level of glycemic response ($143 \pm 3 \text{ mg/dl}$) was the option with lower protein (10 g) and high carbohydrate (55 g) compared to other options. The glycemic responses of the alternatives of breakfasts glycemic response are at 120 min are similar and are close to the initial blood glucose value (19). Similarly, in our study, the highest glycemic response ($141.35 \pm 18.84 \text{ mg/dl}$) produced 40 g of jam with a high carbohydrate content in the 30th min (Fig. 1). The effect of jam ($92.2 \pm 14.5 \text{ mg/dl}$) and jam egg ($96.3 \pm 9.28 \text{ mg/dl}$) on blood glucose level in the 120th min is similar and has a baseline value (respectively 95.4 mg/dl , $95, 9 \text{ mg/dl}$).

Limitations

1. The number of participants was restricted due to the inclusion criteria and the test food, followed by frequent periodic intervals, and blood glucose monitoring.

2. Since individuals could not be observed during the application of diets, their compliance with

their diets was monitored through food consumption records.

3. One individual has been left from study to hypoglycemia after consumption of the jam.

Conclusion

A protein-containing diet in healthy individuals can be recommended as it positively affects the glycemia response. In individuals with diabetes, they should focus on the effects of proteins on glycemia to achieve control of glycemia. Similar studies should be conducted with diabetic patients to see the effects of protein on blood glucose more clearly. In order to see the differences of the effects of jam and jam + egg on blood glucose more prominently in healthy individuals, it is recommended to increase the amount of test food (especially protein) or to plan food combinations with different participants for a longer period of time. Prevention of some disease and maintain and development of public health. It is important to plan new researches that will decrease the risk of increase in blood glucose level, decrease in insulin response, which is a public health problem, especially in obese individuals.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

The study was self-funded.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. De Oliveira FE, Volp AP, Alfenas RC (2011). Impact of different protein sources in the glycemic and insulinemic responses. *Nutr Hosp*, 26(4): 669-676.
2. Jenkins DJ, Kendall CW, Augustin LS, et al (2002). Effect of wheat bran on glycemic control and risk factors for cardiovascular disease in type 2 diabetes. *Diabetes Care*, 25(9): 1522-8.
3. Jenkins DJ, Kendall CW, Augustin LS, et al (2002). Glycemic index: overview of implications in health and disease. *Am J Clin Nutr*, 76(1):266S-73S.
4. Azzout-Marniche D, Gaudichon C, Tomé D (2014). Dietary protein and blood glucose control. *Curr Opin Clin Nutr Metab Care*, 17(4): 349-54.
5. Nuttall FQ, Gannon MC (2013). Dietary protein and the blood glucose concentration. *Diabetes*, 62(5): 1371-1372.
6. Nansel TR, Gellar L, McGill A (2008). Effect of varying glycemic index meals on blood glucose control assessed with continuous glucose monitoring in youth with type 1 diabetes on basal-bolus insulin regimens. *Diabetes care*, 31(4):695-7.
7. Goossens GH, Blaak EE, Amer P, et al (2007). Angiotensin II: A Hormone That Affects Lipid Metabolism In Adipose Tissue. *Int J Obes (Lond)*, 31(2):382-4.
8. Karakoc E, Yalcin BM, Yalcin E (2017). The comparison of insulin levels between overweighted and non-obese smokers. *Journal of Experimental & Clinical Medicine*, 34(1): 67-71.
9. Savas HB, Gultekin F (2017). Insulin resistance and clinical significance. *Med J SDU*, 24(3):116-125.
10. Bozbulut R, Sanlier N (2016). The effect of dietary fat, protein and glycemic index on glycemic control and its use in insulin applications in Type 1 Diabetes. *Turkey Clinical J Endocrin*, 11(2): 46-54.
11. Ekici M, Nergiz-Unal R (2017). Association Between Insulin Resistance, Nutrition, Fatty Meal Desire and CD36 Receptor. *Turkish Journal of Agriculture: Food Science and Technology*, 5(9): 1108-1118.
12. Hsueh WA, Orloski L, Wyne K (2010). Prediabetes: the importance of early identification and intervention. *Postgrad Med*, 122(4): 129-43.
13. Kitiş Y, Bilgili N, Hisar F, et al (2010). Prevalence of metabolic syndrome in women aged 20 and

- above and factors affecting it. *Anatolian J Cardiology*;10(2).
14. Erin E Kershaw, Jeffrey S Flier (2004). Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*, 89(6):2548-56.
 15. Bao J, Atkinson F, Petocz P, et al (2011). Prediction of postprandial glycemia and insulinemia in lean, young, healthy adults: glycemic load compared with carbohydrate content alone. *Am J Clin Nutr*, 93(5): 984-96.
 16. He T, Giuseppin ML (2014). Slow and fast dietary proteins differentially modulate postprandial metabolism. *Int J Food Sci Nutr*, 65(3): 386-90.
 17. Moghaddam E, Vogt JA, Wolever TM (2006). The effects of fat and protein on glycemic responses in nondiabetic humans vary with waist circumference, fasting plasma insulin, and dietary fiber intake. *J Nutr*, 136(10): 2506-11.
 18. Lodi A, Karsten B, Bosco G, et al (2016). The effects of different high-protein low-carbohydrates proprietary foods on blood sugar in healthy subjects. *J Med Food*, 19(11): 1085-1095.
 19. Angela L, Bonnema AL, Altschwager BDK, et al (2016). The effects of the combination of egg and fiber on appetite, glycemic response and food intake in normal weight adults – a randomized, controlled, crossover trial. *Int J Food Sci Nutr*, 67(6): 723-31.