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Effect of different protein types on second meal postprandial glycaemia in normal weight and normoglycemic subjects

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Abstract

Background: Diabetes mellitus is a global epidemic affecting 346 million people in the world. The glycemic control is the key for diabetes prevention and management. Some proteins can stimulate insulin release and modulate glycemic response.

Objectives: To assess the effect of the consumption of different types of protein (whey protein, soy protein and egg white) on a second meal postprandial glycaemia in normal weight and normoglycemic subjects.

Methodology: Randomized crossover clinical trial. After an overnight fast of 12-hours, ten subjects attended the laboratory to drink one of the protein shakes (whey, soy or egg white) or the control drink. Thirty minutes later, the subjects consumed a glucose solution (25 g glucose). Glycemic response was monitored at times 0 (before glucose solution) and 15, 30, 45, 60, 90 and 120 min (after glucose solution consumption). Incremental area under the glycemic curve (iAUC) was calculated by the trapezoidal method. Furthermore, glycemic response was assessed by a new method using *iG* equation.

Results: Compared with control, whey and soy protein drinks reduced postprandial iAUC in 56.5% (p = 0.004) and 44.4% (p = 0.029), respectively. Whey protein was the only protein capable of avoiding great fluctuations and a peak in postprandial glycemia. The assessment of glycemic response by iG equation showed positive correlation with iAUC (Pearson 0.985, p < 0.05).

Conclusion: The consumption of whey and soy protein 30 minutes before a glucose load resulted in lower iAUC compared with control drink. Whey protein maintained postprandial glycemia more stable.

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EFECTO DE DIFERENTES TIPOS DE PROTEÍNA EN LA GLICEMIA POSTPRANDIAL DE LA SEGUNDA COMIDA EN INDIVIDUOS DE PESO NORMAL Y NORMOGLICÉMICOS

Resumen

Introducción: La diabetes mellitus es una enfermedad epidémica que afecta a 346 millones de personas en el mundo. El control glicémico es la clave para la prevención y el control de la diabetes. Algunas proteínas pueden estimular la liberación de insulina y modular la respuesta glicémica.

Objetivos: Evaluar el efecto del consumo de diferentes tipos de proteínas (proteína de suero de leche, proteína de soja y la clara de huevo) de la glicemia postprandial en una segunda comida en individuos de peso normal y normoglicémicos.

Metodología: Este fue un ensayo clínico aleatorizado cruzado. Después de un ayuno nocturno de 12 horas, diez individuos asistieron al laboratorio para beber uno de los batidos de proteínas (suero de leche, soja o clara de huevo) o la bebida control. Treinta minutos más tarde, los individuos consumieron una solución de glucosa (25 g de glucosa). La respuesta glicémica fue monitorizada en los tiempos 0 (antes de solución de glucosa) y 15, 30, 45, 60, 90 y 120 min (después del consumo de la solución de glucosa). El área incrementada bajo la curva de glicemia (iAUC) fue calculada por el método trapezoidal. Por otra parte, la respuesta glicémica se evaluó mediante un nuevo método que utiliza la ecuación de iG.

Resultados: En comparación con el control, las bebidas de suero de leche y de proteína de soja reducen iAUC postprandial en 56,5% (p = 0,004) y 44,4% (p = 0,029), respectivamente. La proteína de suero es la única proteína capaz de evitar grandes fluctuaciones y un pico de glicemia postprandial. La evaluación de la respuesta glicémica por la ecuación iG mostró correlación positiva con iAUC (Pearson 0,985, p < 0,05).

Conclusión: El consumo de suero de leche y proteína de soja 30 minutos antes de una carga de glucosa resultó en menor iAUC en comparación con la bebida control. La proteína del suero mantiene la glicemia postprandial más estable.

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Palabras clave: *Metabolismo de glucosa. Diabetes mellitus tipo 2. Proteínas alimentarias. Alimentos y bebidas.*

Abbreviations

β: Beta.
BMI: Body mass index.
CCK: Cholecystokinin.
DPP-4: Dipeptidyl-peptidase 4.
FAO: Food and Agriculture Organization of United Nations.
GIP: Glucose-dependent insulinotropic polypeptide.
GLP-1: Glucagon like peptide-1.
iAUC: Incremental area under the curve.
iG: Equation of incremental glycemia.
IPA: Peptide sequence Ile-Pro-Ala, called β-lactosin A.
T2DM: Type 2 diabetes mellitus.

Introduction

Diabetes mellitus is one of most worldwide epidemic morbidity. It affects 346 million of people in the world.[1] The key for type 2 diabetes (T2DM) prevention and management is glycemic control,[2] which in turn can prevent microvascular complications related to the disease.[3] Beside that, great variabilities in pre and postprandial glycemia increase oxidative stress leading to deleterious effects on health.[4] Therefore, it is recommended the consumption of foods capable to reduce great glycemic fluctuations.[5]

Protein present in foods can stimulate insulin secretion.[6] However, not all protein types are capable to stimulate enough insulin secretion to decrease glycemic response.[7] Proteins are constituted by amino acids and it has been shown that the serum level of isoleucine, leucine, valine and lysine have a strong correlation with insulinemic response.[8] This effect has been linked to an increase in incretins release,[8-11] such as GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon like peptide-1).[9,10] In addition, the rate of protein digestion alters GIP levels, which is an insulinotropic peptide.[11] Thereby, the effects of proteins on glycemia depends on its source, amino acids profile and digestion rate.[12]

Whey protein it is rapidly digested and contains high amounts of isoleucine, leucine, valine and lysine, which are potential modulators of glycemia.[6,13-15] Apparently, whey protein exerts a greater effect on glycemia than do other protein derived from animal or plant sources.

Even though the effect of protein on immediate postprandial glycemia has been well investigated, its impact on glycemia after the consumption of a subsequent meal is not clear.[6] Hence, the aim of the present study was assess the effect of the consumption of different types of protein (whey protein, soy protein and egg white) on a second meal postprandial glycaemia in normal weight and normoglycemic subjects.

Methods

Subjects

Eligibility criteria included normal body weight (Body Mass Index 18,5-24,9 kg/m^2),[16] and normal fasting glucose (glycemia: 70-99 mg/dL). Smokers, pregnant women, people with diabetes, impaired glucose, family history of diabetes and lactose malabsorption, besides the ones using drugs that affects metabolism were excluded from the study. Volunteers were recruited by advertising on university *campus*. During recruitment subjects filled out a form containing personal information, data related to inclusion criteria, life style, familiar and individual medical history. Sample size was calculated[17] considering the incremental area under the curve of glycemic response[14] (iAUC). A statistical power of 90% and an expected difference of 10% in the baseline values were adopted.

The protocol of the study was in agreement with Declaration of Helsinki and approved by the Human Ethical Committee in Scientific Research (protocol number 067/2012) of Universidade Federal de Viçosa, Brazil. All participants were informed about benefits of the study and signed the informed consent before testing began.

Protocol

This is a crossover study in which after a 12 h overnight fasting, subjects reported to the laboratory to participate of four experimental sessions in a random order. In each session, one of the protein drinks or the control drink was consumed within 15 minutes. There was a wash out period of at least one day between sessions. Thirty minutes after consuming one of the previously mentioned drinks, the subjects consumed a 25 g of anhydrous glucose solution and stayed in the laboratory for the next 2 hours for postprandial glycemic response assessment. At the end of experimental session all subjects received a standardized meal and then dismissed to do their daily activities. The experimental design of our study is illustrated in figure 1.

Anthropometric data and Body Composition Assessment

Height and weight were respectively measured[16] using a stadiometer fixed to the wall (SECA 206®, graduation of 0.1 cm) and a platform digital scale (Toledo Brasil 2096PP®, graduation of 50 g). Body composition was assessed by skinfold thickness[18] using a Lange skinfold caliper (accuracy of 0.1 cm). The sum of bicipital, tricipital, subscapular and suprailiac skinfolds was used to estimate body fat percentages.[19]
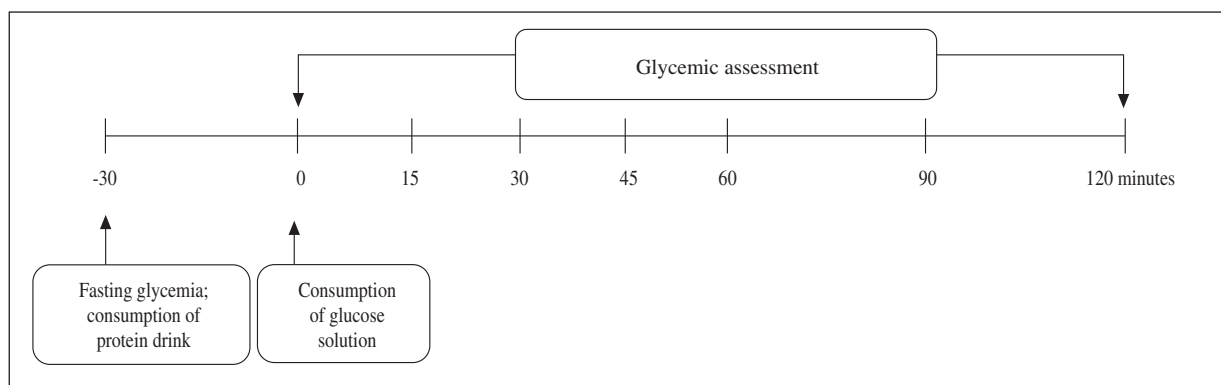
Fig. 1.—*Experimental design of the study. After a 12 h overnight fasting subjects consumed the control or one of the protein drinks (whey protein, egg white or soy protein). Thirty minutes later subjects consumed a glucose solution (25 g of glucose). Finger-stick blood samples were collected in the fasting state (0 min), and 15, 30, 45, 60, 90, 120 min. after the start of glucose solution.*

Table I
Energy content, macronutrient composition and amino acids profile of protein concentrates (100 g of product) tested in the study

	Whey protein	Egg white	Soy protein
Energy (kJ)	1,548.09	1,464.24	1,464.41
Carbohydrate (g)	3.0	9.37	0
Fat (g)	0.87	0	0
Protein (g)	88.5	78.12	90
Amino acids profile (g/100 g)			
Ala	5.3	4.6	4.2
Arg	2.2	4.4	8.0
Asp	11.9	8.2	12.1
Cys	2.7	2.1	1.4
Glu	18.9	1.0	20.4
Gly	1.7	2.8	4.2
His	2.1	1.8	2.7
Ile	7.2	4.5	4.3
Leu	11.2	6.8	7.8
Lys	8.8	5.4	6.5
Met	2.2	2.7	1.4
Phe	3.1	4.7	5.4
Pro	7.8	3.1	5.3
Ser	5.3	5.5	5.7
Thr	8.0	3.6	3.6
Trp	1.9	1.0	1.0
Tyr	3.2	3.1	4.1
Val	6.3	5.1	4.5

Test drinks

The test drinks were a glucose solution, control drink and three protein drinks. The glucose solution was prepared diluting 25 g of anhydrous glucose (Vetec/Rio de Janeiro) into 200 mL of spring water. Control drink was prepared blending 200 mL of spring water, 2 g of calories-free blackberry powder juice (Clight®). Protein drinks were prepared by adding one of the following protein concentrates (0.5 g·kg⁻¹ of subject body weight) to control drink: whey protein (Diacom®, Belo Horizonte), egg white (Nutryclin®, Viçosa) or soy pro-tein (Nutrysoy®, Paraná). The nutritional composition of the drinks is presented in table I.

Postprandial glycemia assessment

Capillary finger-stick blood samples were taken in the fasting state (0 min) and at 15, 30, 45, 60, 90 and 120 min after the consumption of the glucose solution. Glucose levels were measured using a glucometer (One Touch Ultra II®, LifeScan Inc., Milpitas, CA). The incremental area under the glycemic response curve (iAUC) was calculated by the trapezoidal method[20] using the software SlideWrite 7.0®.

Glycemic response was also assessed by the mean incremental glycemia using the equation *iG = Σ increment of postprandial glycemia (0-2 h)/n*, in which *n* is the number of subjects.

Statistical analysis

Statistical analyses were conducted using SPSS 17 for Windows (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean and standard error of mean (SEM). Data normality and homoscedasticity were assessed by Kolmogorov-Smirnov and Levene tests, respectively. One-Way ANOVA was used to assess significant differences in iAUC an iG. Two-Way Repeated Measures ANOVA was applied to verify the interaction of time and treatment factors, followed by post hoc comparisons using Tukey's tests when necessary. The criterion for statistical significance was $p < 0.05$ (α level of 5%). The association between iAUC and iG was assessed by Pearson's correlation.

Results

A total of ten subjects (4 men and 6 women), mean fasting glycemia 4.78 ± 0.05 mmol/L, BMI 22.0 ± 0.82 kg/m², and 25.6 ± 1.86% body fat participated in the study (table II).

Table II
*Mean ± SE characteristics presented by subjects
at baseline (n = 10)*

	Means	SEM
Age (years)	24.9	0.58
Weight (kg)	61.35	3.05
Height (m)	1.7	0.02
BMI (kg/m²)	22.0	0.82
Body fat (%)	25.6	1.86
Fasting Glucose (mmol/L)	4.78	0.05

BMI: Body Mass Index.

Fasting glycemia did not differ between study sessions ($p < 0.05$). There was an effect of time ($p < 0.001$) and treatments ($p < 0.004$), and an interaction of time and treatment ($p < 0.001$) in postprandial glycemia. Glycemia thirty minutes after the consumption of the protein drinks or the control drink (0 min) did not differ (fig. 2A). However, whey protein drink resulted in lower glycemic response compared with soy and egg white drinks at 15 minutes ($p = 0.007$). At 30 minutes, whey protein led to lower glycemic values than soy, control and egg white ($p = 0.001$) drinks. Lower glycemic values for whey protein was also observed at 45 minutes compared with control ($p < 0.001$) and egg white ($p = 0.02$) drinks. Whey and soy protein drinks resulted in lower glycemic response ($p = 0.02$ and $p = 0.001$, respectively) than the control, at 60 minutes. No differences were detected at times 90 and 120 minutes ($p > 0.05$) (fig. 2A). Whey protein resulted in a more stable response during the 120 min in which glycemia was assessed (fig. 2A).

Whey and soy protein drinks reduced postprandial iAUC in 56.5% ($p = 0.004$) and 44.4% ($p = 0.029$) respectively, compared with control. However, postprandial iAUC did not differ between the protein drinks (whey, soy and egg white) ($p > 0.05$) (fig. 2B).

There was a positive correlation between the glycemic response assessed by the iG equation and by the iAUC (0.985, $p < 0.05$). Furthermore, whey (3.97 mmol/L) and soy (5.52 mmol/L) proteins mean incremental glycemias were significant lower ($p < 0,04$) than the ones obtained for egg white (5.89mmol/L) and control (9.85 mmol/L).

Discussion

Glycemic control is the main objectives of nutritional intervention in diabetics and pre diabetics. Therefore, glycemia should be as close as possible to normal levels to avoid the manifestation of diabetes in predisposed subjects and the development of comorbidities related to T2DM.[21] Thus, it has been recommended the adoption of therapeutic strategies capable to prevent the occurrence of glycemic peaks.[5]

In the present study, the protein drinks and control drink was consumed 30 minutes before assessing the glycemic response to the glucose solution. This procedure is necessary to ensure that the observed response reflects the production and release of insulin stimulated by protein load.[10] Monitoring glycemic response in shorter time period (less than 30 minutes) would reflect the effect of stimulus of previous meal, not of the protein load.[22]

The impact of consuming 50 g of protein (whey protein, tuna, turkey or egg white) for 12 weeks 30 minutes before two daily main meals on postprandial glycemia and insulinemia was assessed in 22 healthy men.[15] Whey protein decreased iAUC compared to turkey and egg white, and increased insulinemic response compared with all the other protein tested.[15] It has been proposed that the reduction of glycemia is due to increase of insulin releasing and of incretins production and also a reduction in gastric emptying rate.[6,7,12,14]

Amino acids exert different stimulus on postprandial insulinemia.[23,24] Leucine, isoleucine, lysine and valine are in high concentrations in whey protein. Due to their insulinotropic properties, these amino acids can increase insulinic release and sensitivity, contributing to reduce glycemic response.[24] We observed that whey protein has approximately 62% more of those amino acids than soy protein and egg white, and may have favored our results. However, in our study, we did not monitor the postprandial insulinemic responses.

The stimulus for insulin release mediated by whey protein is complex. Its mechanisms may reflect a synergistic effect of amino acids (such leucine, isoleucine, lysine and valine and threonine) and also the activation of incretins (ex: GIP) effect on pancreatic β-cells.[12,25]

It has been proposed that whey protein stimulates incretins release due to the presence of Ile-Pro-Ala a peptide sequence, called β-lactosin A, identified from hydrolyzed of β-lactoglobulin[26]. The results of studies have shown the inhibitory role of IPA over dipeptidyl-peptidase 4 (DPP-4) activity, an enzyme responsible of GIP and GLP-1 degradation, extending the half-life of these incretins in the bloodstream.[8,26] Moreover, GIP level in the blood could increase concomitantly with an increase in insulin levels, after consuming meals containing whey protein.[7,8,12] Whey protein intake can slow gastric emptying rate, reducing glycemic response of the next meals. This effect increases the postprandial release of cholecystokinin (CCK), GLP-1 and GIP[12].

The mean incremental glycemia (iG) we used showed to be a good method to assess the glycemic response because it presented a good correlation with the traditional method recommended by FAO.[20] The assessment of the effect of each protein shake on glycemia by iG was easier and faster than the traditional methodology and it does not require the use of specific software.
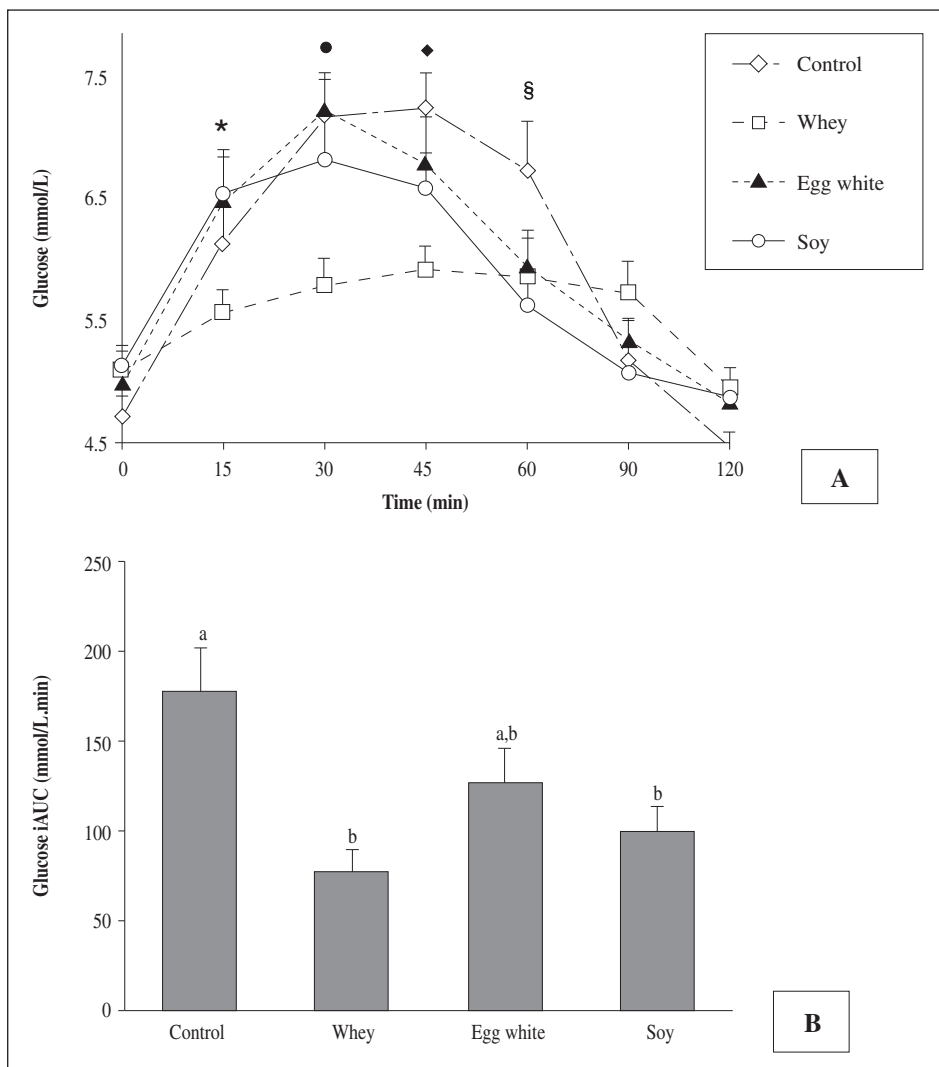
Fig. 2.—Mean ± SE post-prandial glucose response (0-120 min.) (A), and postprandial incremental area under the glycemic response curve (iAUC) (0-120 min.) (B) in response to the consumption of control and of three different types of protein drinks (whey protein, egg white and soy). *Whey protein drink mean values are significantly lower (p = 0.007) than the ones obtained for egg white and soy protein drink. •Whey protein drink mean values are significantly lower (p = 0.001) than control, egg white and soy. ◆Whey protein drink mean values are significantly lower (p ≤ 0.02) than control and egg white protein drinks. §Soy and whey protein drink mean values are significantly lower than control (p = 0.001 and p = 0.02, respectively) (A). Bars followed by different letters differ significantly from each other (B).

Conclusion

The consumption of whey and soy protein 30 minutes before a glucose load resulted in lower iAUC compared with control drink. Furthermore, whey protein maintained postprandial glycemia more constant. The effects of whey protein chronic consumption on T2DM prevention and management of T2DM should be assessed in long-term feeding trials.

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