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Title page

Title: Both macronutrient food composition and fasting insulin resistance affect postprandial glycemic responses in senior subjects

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Abstract:

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Introduction: Postprandial hyperglycemia is a risk factor for type 2 diabetes. Insulin resistance (IR) might affect metabolic responses in non-fasting states. Dietary intake and food composition influence postprandial glucose homeostasis. The aims of this study were to evaluate the effects of different test foods varying in macronutrient composition on postprandial glycemic responses and if these outcomes are conditioned by the basal glycemic metabolic status in senior subjects.

Methods: In a randomized, controlled crossover design, thirty-four adults consumed a test food, a high protein product (n = 19) or a high carbohydrate (CHO) product (n = 15), using as a reference the oral glucose tolerance test (OGTT). Blood glucose and insulin were measured at fasting and at 15, 30, 45, 60, 90, and 120 min after starting the food intake. For each food, the incremental area under the curve (iAUC) for glucose and insulin was calculated. IR was measured by the Homeostatic Model Assessment of IR (HOMA-IR).

Results: Consumption of the high protein product significantly lowered the peak and Δ blood glucose concentration compared to the high CHO product ($p<0.001$). Concerning insulin response, no significant differences among both foods were observed. Fasting glucose was positively correlated with glucose iAUC only for the high protein product. Positive associations of both fasting insulin and HOMA-IR with insulin iAUC for all the cases were observed. Linear regression models showed significant positive associations between the glucose iAUC and fasting glucose after adjusting for age and sex. Regarding insulin iAUC, positive associations were found with fasting insulin and HOMA-IR. Regression models also evidenced that both food tests consumed were able to decrease glucose and insulin iAUC when comparing with the OGTT.

Conclusion: Our research found that not only is the nutritional composition of foods important, but also the baseline glycemic state of individuals when assessing glycemic index estimations and addressing precision nutritional strategies to prevent and treat IR-associated disturbances.

Keywords: Postprandial; Glucose; Hyperglycemia; Glucose Metabolism Disorders; Insulin; Food; Protein; Glycemic Index

Main text

Introduction

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Insulin resistance (IR) is a pathological condition where cells fail to respond adequately to insulin.[1] IR is a major risk factor for type 2 diabetes (T2DM) and is associated with various cardiometabolic disturbances, such as hypertriglyceridemia, cardiovascular disease and metabolic syndrome.[2,3] Both genetic and environmental factors contribute to the onset and development of IR.[4] Indeed, obesity and physical inactivity are leading causes of IR condition.[5]

Research in the fasting state have identified a cluster of biomarkers closely linked to IR and predisposing to increased risk for cardiovascular disease.[6] However, early predictive markers of transition from normal to a prediabetes state are unidentified.[7] A large number of postprandial studies have been conducted in individuals suffering from T2DM[7,8], however, limited data are available on individuals without any metabolic alteration. Also, few investigations have been conducted regarding how IR affects metabolic responses in a non-fasting setting, which is the state people are mostly exposed to during waking hours.[8]

Sharp postprandial glycemic peaks and large blood glucose oscillations have been investigated, and they might have a great impact on health, being even more detrimental than an increase in fasting glucose concentrations.[8] Furthermore, postprandial hyperglycemia is an independent risk factor for the development of T2DM, cardiovascular disease and liver cirrhosis and is associated with obesity and enhanced all-cause mortality in both T2DM and cancer.[9]

Dietary intake is a deciding factor for glycemic excursions, especially during the postprandial state.[10] Although fasting blood glucose levels indicate cumulative effects of composite diets and metabolic activity, they do not reflect accurately the impact of individual foods or meals consumed during the day.[11] A reduction in postprandial glycemic responses after meals might be considered a beneficial effect on health, as long as postprandial insulin responses are not largely increased.[12] Interestingly, the glycemic response to meals has been studied widely in subjects affected by diabetes mellitus. Nevertheless, data concerning the glycemic response to foods in healthy population are limited.[13]

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Dietary carbohydrates primarily affect blood glucose response in the postprandial period. Previous studies have confirmed that variation in the glycemic response are to a large extent due to carbohydrate intake.[14] In 1981, the concept of glycemic index (GI) was established to quantify the glycemic response to carbohydrates of a single tested food type.[15] Glycemic load (GL), the mathematical product of the GI of an individual food and its carbohydrate content, was introduced to adjust for serving sizes.[16] In practice, these parameters reflect a total glycemic response to a food or diet independently of the food components responsible or the shape of the glycemic curve. Dietary protein, fat, and fiber can also alter the gastrointestinal transit time, modifying rates of glucose uptake, and specific fatty acids and amino acids can stimulate insulin and glucagon secretions, thereby influencing glucose homeostasis.[17,18,19] Consequently, dietary therapies making food choices that lessen glycemic fluctuations and modulate the postprandial blood glucose levels are needed to ameliorate the health state of populations at cardiometabolic risk.[20]

With this background, the aim of this study was to investigate the effects of different test foods varying in macronutrient composition and if these outcomes are conditioned by the basal glycemic metabolic status in adults aged 50-80 years old.

Materials & methods

Two glycemic response studies with two different test foods, a high protein product (study I) and a high carbohydrate (CHO) product (study II), using as a reference product the Oral glucose tolerance test (OGTT), were conducted at the Centre for Nutrition Research at University of Navarra, each using a randomized, controlled crossover design.

Participants

A total of 34 (*n*= 19 study I; *n*= 15 study II) male and female adults aged 50–80 years (BMI ≥18.5 to <30 kg/m^2) were enrolled in the study. Exclusion criteria included pregnancy or breastfeeding, BMI <18.5 kg/m^2 and BMI ≥30 kg/m^2, fasting glucose ≥100 mg/dl or treatment with antidiabetic drugs, history of diabetes mellitus, fasting total cholesterol ≥ 250 mg/dl or specific treatment for lipid abnormality, slimming treatments or hormone replacement therapy, concomitant medications with dose changes in the last three months prior to the start of the study, any chronic disease related to metabolism, smoking and/or follow-up of diets designed for weight loss (last three months). Other exclusion criteria included any serious psychiatric disorders, no autonomy, inability to

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follow the consumption of the product (food allergy or intolerance) as well as difficulties to perform the follow-up.

All the procedures performed were in accordance with the ethical guidelines of the Declaration of Helsinki. The study protocol and informed consent document for both studies were approved by the Research Ethics Committee of the University of Navarra (ref. Study I: 2018/176; ref. Study II: 2018/2154) and were properly registered in www.clinicaltrails.gov (Nutriprecision study; NCT04786925). All participants gave written informed consent prior to inclusion in the study.

Test foods

In the research, two different test foods varying in macronutrient composition were investigated and compared with a reference product, the OGTT. In all the cases, the total amount of available carbohydrates per serving was 25 g, provided by 250 ml of OGTT (25 g of glucose), 100 g of the high protein product and 192 g of the high CHO product. An extruded meat product (high protein product) served with white bread was the designed product for Study I whereas a fruit compote (high CHO product) was the test food developed for Study II. Regarding macronutrient composition, the most important differences between high protein/CHO products lay in protein, carbohydrate and sugar contents, respectively (Supplemental Table 1). As mentioned previously, 40 g of white bread were additionally served together with the extruded meat product in order to reach the grams of available CHO needed to compare all the products. Interestingly, the fiber contained in the high protein product was INNOFIBER 01 (Ensis Sciences), a white fine powdered mix of vegetable fibers. In the extrusion manufacturing process, the powdered fiber was added together with the other ingredients or additives. All the test foods were specifically designed and developed within the framework of the Nutriprecision Project. The extruded meat product was supplied by Hijo de José Martínez Somalo, S.L. (La Rioja, Spain) and analysed by Eolisa Laboratorios (Zaragoza, Spain); the fruit compote was provided by Iberfruta Muerza, S.A (Navarra, Spain) and tested by the National Centre for Food Safety and Technology (CNTA, Spain) and finally, the bread was supplied by Europastry, S.A. (Barcelona, Spain) and analysed by CNTA.

Study design and procedures

Participants were instructed to restrict their intake of alcohol and caffeine-containing drinks and perform of extreme physical activities prior to the test day. Also, participants

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were asked to consume a standardized dinner the night before each test. Participants attended the Nutrition Intervention Unit (Centre for Nutrition Research) in the morning after a 10-12-hours overnight fast. In both studies, participants consumed the test food (high protein/CHO products) and the reference food (OGTT) within 10–15 min and remained sedentary during each session. The reference and test foods were administered once in random order, with a wash-out period between 7 days and 14 days among assays, to minimize carry-over effects. The sequence of product intake was randomized using the ''random between 1 and 3''function in the Microsoft Office Excel 2003 software (Microsoft Ibérica, Spain).

Anthropometry was evaluated at the volunteers first visit. Fasting blood samples were taken at −5 and 0 minutes before food consumption and the baseline value of all the studied variables was taken as a mean of these two values. Afterwards, products were consumed within 15 minutes and further blood samples were taken at 15, 30, 45, 60, 90, and 120 min after starting to drink/eat, as described by Brouns *et al.*[21] Blood samples were obtained by inserting a cannula into the antecubital vein and the blood was collected using EDTA and CLOT tubes. Participants were also asked to fill different questionnaires After 10 minutes of rest and having answered the Mini Nutritional Assessment (MNA) and the Mini-Mental State Examination (MMSE) questionnaires, blood pressure was measured.

Anthropometric, Blood Pressure and Body Composition

Anthropometric measurements (body weight, waist and hip circumference) and body fat percentage (SC-330, Tanita, Tokyo, Japan) were determined in fasting conditions prior to the first test in each of the two studies, following previously described standardized procedures.[22] Height was recorded using a wall-mounted stadiometer (Seca 220, Vogel & Halke, Germany). Body mass index (BMI) was calculated using the standard formula: weight (kg)/height (m)^2. Blood pressure was determined following the World Health Organisation criteria (WHO)[23], using an automatic monitor device (Intelli Sense. M6, OMRON Healthcare, Hoofdorp, the Netherlands).

Biochemical measurements

All serum samples were left at room temperature for 30 minutes before being centrifuged for 15 minutes at 2,013×g (3,500 rpm) at 4°C in a standard centrifuge (Eppendorf 5804R, Hamburg, Germany). On the other hand, plasma samples were centrifugated immediately.

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The blood samples were then pipetted to obtain plasma and serum aliquots which were then stored at -80°C until the analyses were performed.

Serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG) were measured by specific calorimetric assays in an autoanalyzer Pentra C200 (Horiba ABX Diagnostics, Montpellier, France). Insulin concentrations were quantified using specific Enzyme-Linked ImmunoSorbent Assay (ELISA) kits (Demeditec; Kiel-Wellsee, Germany) in a Triturus auto-analyzer (Grifols, Barcelona, Spain). The low-density lipoprotein (LDL-c) levels were calculated using the Friedewald formula: LDL-c = TC − HDL-c − TG/5.[24] On the other hand, the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = (fasting insulin (μU/mL) × fasting glucose (mmol/L))/22.5.[25] The triglycerides-glucose (TyG) index was calculated as Ln (TG (mg/dL) x glucose (mg/dL)/2)[26] whereas the TG/HDL-c index was determined as TG (mg/dL) divided by HDL-c (mg/dL).[27]

AUCi calculations

The incremental area under the curve (AUCi) for glucose was calculated via the geometric sums of the areas of the triangles and trapezoids above the fasting glucose concentration over a 2-h period as previously described.[28] Similar calculations were done to obtain the insulin AUCi.

GI and GL calculations

GI is calculated as the incremental area under the blood glucose response curve during 2 hours after intake of a 25 g carbohydrate portion of the test food and expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject.[15] Glucose was used as standard as mentioned before. GL was also calculated as the amount of glycemic carbohydrate in a food times the GI of the food/100.[16]

Sample size calculation

Sample size calculation for both studies was based on published Glycemic Index data.[21] To detect a reduction in postprandial glycemia with a two-sided α-level of 5% and a

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power of 80%, a sample size of 12 participants was estimated. Expecting a dropout rate of 20 %, the total number of participants needed in each study was established in 15.

Statistical analysis

The normal distribution of the continuous variables was assessed using the Shapiro–Wilk test. The data were expressed as a mean ± standard deviation for continuous traits and percentage for categorical variables. Participants were classified according to BMI, HOMA-IR and TC medians (BMI: 25 kg/m^2; HOMA-IR: 1.2; TC: 213.5 mg/dL) as well as the study they belonged. Differences between groups (< or ≥ the median) were assessed by the Student´s t-test and the Mann-Whitney U test for quantitative parametric and non-parametric variables, respectively. Regarding categorical variables, differences in the frequency distribution among groups were assessed by means of chi-squared test. ANOVA and Post hoc tests (Bonferroni) were performed to compare the peak, Δ and iAUC values of blood glucose and insulin among the reference test and high protein/CHO products. Spearman correlations were performed to further assess the association between baseline IR markers and the postprandial glucose and insulin response to the test foods. Multivariable linear regression analyses were performed to investigate the effect of cardiometabolic risk factors, including the different treatments, on both glucose and insulin response to the test products after adjusting for potential confounders (age, sex). Statistical calculations and graphs were performed with Stata version 12.1 (StataCorp 2011, College Station, TX, USA). All p values presented are two-tailed, and differences were considered statistically significant at $p<0.05$.

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Results

Baseline data of participants is given in Table 1. The average age of participants was 60 ± 8 years old and 59 % were women. The mean BMI of the studied population was 25 ± 3 kg/m² with a waist circumference of 88 ± 9 cm. Subjects were categorized according to the study they belonged (study I or II). No significant differences were observed in any variable between participants of Study I and II (Table 2).

The analysis of the glycemic response after ingestion of the different test foods are depicted in Table 2. The glucose iAUC was significantly lower after consumption of the high protein product compared to the OGTT ($p < 0.01$). Likewise, both peak and Δ blood glucose concentration following ingestion of the high protein product were significantly lower in comparation with the high CHO product and the reference test. By contrast, no notable differences between both products were found in GI and GL values. On the other hand, insulin iAUC was lower following ingestion of both high protein/CHO products when comparing with the reference test. No significant differences among products were observed concerning peak and Δ blood insulin values.

Postprandial serum glucose and insulin levels during 2 h after food test consumption are depicted in Figure 1. As shown, glucose concentrations were significantly lower after the high protein product intake at 15 and 30 minutes compared with the OGTT and the high CHO and 45 min in comparation with the OGTT (Figure 1).

Likewise, the relationship between fasting glucose metabolism related variables and both glucose and insulin responses for test products was assessed (Table 3). Fasting glucose was positively correlated with Glucose iAUC only for high protein product. Also, relevant positive associations between fasting insulin and insulin iAUC for OGTT and high protein/CHO products were observed. About HOMA-IR, significant associations were found with insulin iAUC for the three test foods.

Linear regression models were set up with glycemic response (both glucose and insulin iAUC) to test foods as the dependent variable and cardiometabolic variables such as waist circumference, type of treatment and IR markers (glucose, insulin or HOMA-IR) as independent factors (Table 4). Both age and sex adjusted models showed significant positive associations between the Glucose iAUC and fasting glucose. Also, the type of treatment influenced the glucose iAUC, being the high protein product significantly lower when compared with the reference product, OGTT. Regarding insulin iAUC, significant

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positive associations were found with WC, when fasting glucose was introduced in the model as an IR independent factor. Interestingly, both fasting insulin and HOMA-IR were positively associated with insulin iAUC when introduced as independent factors (Table 4, Figure 2). The type of treatment also had a significant effect on insulin iAUC, when comparing both high protein/CHO products with the OGTT (Table 4, Figure 2).

Discussion

This research confirmed that macronutrient composition largely determines the glycemic response to foods. Consumption of the high protein product significantly lowered the glucose response compared to the high CHO product. Regarding insulin response, no significant differences have been shown between both test foods. Our results also demonstrated the association of fasting insulin and HOMA-IR with insulinemic responses independently of the test food consumed in adults.

Postprandial glucose and insulin excursions might be early signs of diabetes development in normoglycemic subjects.[9] Glycemic fluctuations in the non-diabetic population are closely modulated by non-modifiable factors (physiopathological mechanisms, genetic background, age, sex), and also by modifiable factors such as lifestyle choices (physical activity, smoking, alcohol, drug therapy, dietary intake).[29] IR leads to a higher pancreatic production of insulin, required to allow the entrance of glucose into the cells. If the pancreas can produce enough insulin to overcome the weak response of cells to insulin, blood glucose levels will remain within a normal physiological range.[1,30] Our study demonstrated that individuals with higher glycemic and insulinemic excursions had a worse fasting glucometabolic status. Interestingly, our results showed the association of baseline insulin and HOMA-IR with insulinemic response but not with postprandial glucose to test foods. These results were in line with multiple studies[31-34], who reported that the plasma glucose shape during an oral glucose tolerance test (OGTT), commonly used to identify high-risk individuals, depends on glucose tolerance. In addition, genetic factors and sex seem to play a crucial role too.[34] Our study thus reveals early differences in glucose metabolic responses in adults with normal weight/overweight and no metabolic alterations. So, our findings are key to strengthen the importance of discriminating normoglycemic individuals with impaired postprandial glucose metabolism, as they might have increased risk of developing IR and ultimately, T2DM.

Dietary factors also determine glycemic variations, modulating the duration and the intensity of the postprandial response.[10,35] Glucose response was primarily related to

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carbohydrate content of foods/meals.[36] Additionally, carbohydrate quality is also a key determinant of glucose and insulin metabolism.[14] Previous studies have confirmed that carbohydrates could reliably predict glucose values in the acute response. Wolever *et al*. reported that both carbohydrates and glycemic index explained about 90% of the variation in the glycemic response.[37] Concerning dietary GI and GL, a considerable body of work[38-40] has investigated associations between average GI and GL values and chronic disease risk with data inconsistency. Although some studies have found associations of low-GI or low-GL diets with reduced risk of cardiovascular disease and diabetes, other findings have stated no associations.

On the other hand, dietary fat and protein also affect postprandial glycemic fluctuations.[17] In this context, numerous studies concluded that high-protein foods attenuate postprandial glycemic excursions by delaying gastric-emptying rates and enhancing gut hormones secretion including cholecystokinin, gastric inhibitory polypeptides, and glucagon like peptide-1.[12,41] Protein ingested in combination with carbohydrates may also reduce blood glucose rises by stimulating β-cell function and insulin secretion.[42] Indeed, previous studies reported the insulinotropic potential of specific amino acids, as they can directly and indirectly (via incretin release) stimulate insulin release.[43,44] However, some studies[45-47] found that addition of protein to a carbohydrate meal does not reduce the plasma glucose area above the baseline in normal subjects. They also noted little difference in insulin response as the protein content was increased up to as much as 25 g. When a greater amount of protein was given, both glucose and insulin responses were increased.[47] Additionally, several studies stated that protein-rich foods may lead to delayed hyperglycemia by gluconeogenesis and increased glucagon secretion.[45]

Our results showed that the glucose peak was reduced after ingestion of the high protein product when comparing with the high CHO product and glucose load test. iAUC glucose was also lower when consuming the high protein test food in comparison with the OGTT. As mentioned before, these results could be explained by the effect of protein slowing gastric emptying. Regarding insulin, no differences were shown between foods. One possible explanation could be that the protein content of the extruded food was lower than 25 g and then small differences in postprandial insulin were found, as numerous studies with similar amounts of protein reported.[45-47] Nevertheless, the insulin secreted in response to the mixture of protein and CHO content was enough to reduce the postprandial glucose rise. Also, the different content in sugars between both food tests

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could explain these findings since the high CHO product contains more sugars than the high protein food test, and sugars fastly rise postprandial glucose concentrations.[12]
On the other hand, energy density and food appearance need to be also taken into consideration when developing products to manage glycemic responses, as they could influence nutrient bioavailability and metabolic consequences.[41]

Therefore, dietary interventions represent an important strategy to attenuate these oscillations and improve postprandial glycemia.[12,48]

The strength of this research is the controlled nature of the food challenges in terms of the environment, the time of day when the tests were conducted, and the standardization of the test foods. Participants have been also well characterized and selected. However, this study was not devoid of limitations. Firstly, all study participants were presenior Spanish, without baseline impaired fasting glucose and diabetes mellitus, among others. In this context, it is not likely that our results are generalizable to other groups with different clinical and metabolic features. Secondly, we did not analyse insulin values of 15´,30´,45´and 90´of the high protein product, and this could interfere in the accuracy of the results regarding the insulin related variables of this food test. Thirdly, the sample size is relatively low, but the results are plausible.

Conclusion

Current findings confirmed that both macronutrient composition of foods and IR condition have significant effects on glucose and insulin responses.

Our research found that foods with different content in protein and sugars but similar

fiber amount induce differential glucose responses with no differences in postprandial insulin, probably due to the modest protein quantity of the high protein product (not more than 25 g). We also demonstrated that basal insulin and HOMA-IR modulate insulinemic responses independently of the type of food ingested. Our findings are key to reinforce the importance of identifying impaired postprandial glucose metabolism in apparently metabolically healthy adults, which might lead to an increased risk of developing hyperglycemia and finally, T2DM.

In order to address precision nutritional strategies to prevent and treat IR-associated disturbances, it is important to consider not only the nutritional composition of foods, but also the baseline glycemic state of individuals.

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Conflicts of interest

There are no conflicts of interest to declare.

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Author Contributions

"Conceptualization, C.G., S.N.-C., C.J.G.-N., J.A.M., M.A.Z. and I.A.; methodology, C.G., S.N.-C., C.J.G.-N., J.A.M., M.A.Z. and I.A.; validation, C.G., S.N.-C., C.J.G.-N., J.A.M., M.A.Z. and I.A.; formal analysis, C.G., J.A.M., M.A.Z. and I.A.; investigation, C.G., S.N.-C., C.J.G.-N., J.A.M., M.A.Z. and I.A.; resources, C.G., S.N.-C., C.J.G.-N.,

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References

1. S. Schinner, W. A. Scherbaum, S. R. Bornstein, A. Barthel, Molecular mechanisms of insulin resistance, *Diabet Med.*, 2005, **22**, 674–682.
2. S. Sookoian, M. S. Rosselli, A. L. Burgueño, T. Fernández Gianotti, G.O. Castaño, et al., Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: impact of liver methylation of the peroxisome proliferator-activated receptor γ coactivator 1α promoter, *Hepatology*, 2010, **52**, 1992-2000.
3. J. Zhao, J. Goldberg, J. D. Bremner, V. Vaccarino, Global DNA methylation is associated with insulin resistance: a monozygotic twin study, *Diabetes,* 2012, **61**, 542–546.
4. J. B. Meigs, M. K. Rutter, L. M. Sullivan, C. S. Fox, R. B. D'Agostino Sr, et al., Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome, *Diabetes Care*, 2007, **30**, 1219–1225.
5. G. Reaven, Insulin resistance, type 2 diabetes mellitus, and cardiovascular disease: the end of the beginning, *Circulation,* 2005, **112**, 3030–3032.

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6. I. Martín-Timón, C. Sevillano-Collantes, A. Segura-Galindo, F. J. Del Cañizo-Gómez, Type 2 diabetes and cardiovascular disease: have all risk factors the same strength?, *World J Diabetes.*, 2014, **5**, 444-470.
7. A. A. Kumar, G. Satheesh, G. Vijayakumar, M. Chandran, P. R. Prabhu et al., Postprandial Metabolism is Impaired in Overweight Normoglycemic Young Adults without Family History of Diabetes, *Sci Rep*, 2020, **15**, 353.
8. A. Ceriello, K. Esposito, L. Piconi, M. A. Ihnat, J. E. Thorpe, 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**, 1349-1354.
9. E.E. Blaak, J.M. Antoine and D. Benton, I. Björck, L. Bozzetto, et al., Impact of postprandial glycaemia on health and prevention of disease, *Obes Rev.*, 2012, **13**, 923-984.
10. D. Zeevi, T. Korem, N. Zmora, D. Israeli, D. Rothschild, Personalized nutrition by prediction of glycemic responses, *Cell*, 2015, **163**, 1079-1094.
11. Q. Wang, J. Jokelainen, J. Auvinen, K. Puukka, S. Keinänen-Kiukaanniemi, et al., Insulin resistance and systemic metabolic changes in oral glucose tolerance test in 5340 individuals: an interventional study, *BMC Med.*, 2019, **17**.
12. H. Meng, N. R. Matthan, L. M. Ausman, A.H. Lichtenstein, Effect of prior meal macronutrient composition on postprandial glycemic responses and

Food & Function Accepted Manuscript

glycemic index and glycemic load value determinations, *Am J Clin Nutr.*, 2017, **106**, 1246-1256.

13. M. González-Rodríguez, M. Pazos-Couselo, J. M. Garciá-López, S. Rodríguez-Segade, J. Rodríguez-García, Postprandial glycemic response in a non-diabetic adult population: the effect of nutrients is different between men and women, *Nutr Metab (Lond).*, 2019, **16**.
14. K. L. Pearce, M. Noakes, J. Keogh, P. M. Clifton, Effect of carbohydrate distribution on postprandial glucose peaks with the use of continuous glucose monitoring in type 2 diabetes, *Am J Clin Nutr.*, 2008, **87**, 638-644.
15. D. J. Jenkins, T. M. Wolever, R. H. Taylor, H. Barker, H. Fielden, et al., Glycemic index of foods: a physiological basis for carbohydrate exchange, *Am J Clin Nutr.*, 1981, **34**, 362-366.
16. J. C. Brand-Miller, M. Thomas, V. Swan, Z. I. Ahmad, P. Petocz, et al., Physiological validation of the concept of glycemic load in lean young adults, *J Nutr.,* 2003, **133**, 2728–2732.
17. W. R. Russell, A. Baka, I. Björck, N. Delzenne, D. Gao, et al., Impact of diet composition on blood glucose regulation, *Crit Rev Food Sci Nutr.*, 2016, **56**, 541-590.
18. J. Pavlisova, O. Horakova, V. Kalendova, J. Buresova, K. Bardova, et al., Chronic n-3 fatty acid intake enhances insulin response to oral glucose and elevates GLP-1 in high-fat diet-fed obese mice, *Food Funct.*, 2020, **11**, 9764-9775.

Food & Function Accepted Manuscript

19. G. Zhang, L. Y. Hasek, B. -H. Lee, B. R Hamaker, Gut feedback mechanisms and food intake: a physiological approach to slow carbohydrate bioavailability, *Food Funct.*, 2015, **6**, 1072-1089.
20. C. Galarregui, M. Á. Zulet, I. Cantero, B. A. Marín-Alejandre, J. I. Monreal, et al., Interplay of glycemic index, glycemic load, and dietary antioxidant capacity with insulin resistance in subjects with a cardiometabolic risk profile, *Int J Mol Sci.*, 2018, **19**, 3662.
21. F. Brouns, I. Bjorck, K.N. Frayn, A. L. Gibbs, V. Lang, et al., Glycaemic index methodology, *Nutr Res Rev*, 2005, **18**, 145-171.
22. M. A. Zulet, I. Bondia-Pons, I. Abete, R. de la Iglesia, P. López-Legarrea, et al., The reduction of the metabolic syndrome in Navarra-Spain (RESMENA S) study: A multidisciplinary strategy based on chrononutrition and nutritional education, together with dietetic and psychological control, *Nutr Hosp.*, 2011, **26**, 16–26.
23. J. A. Whitworth, J. Chalmers, World health organisation-international society of hypertension (WHO/ISH) hypertension guidelines, *Clin Exp Hypertens.*, 2004, **26**, 747-752.
24. W. T. Friedewald, R. I. Levy, D. S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, *Clin Chem.*, 1972, **18**, 499–502.
25. D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, et al., Homeostasis model assessment: insulin resistance and beta-cell

Food & Function Accepted Manuscript

function from fasting plasma glucose and insulin concentrations in man, *Diabetologia*, 1985, **28**, 412-419.

26. D. Navarro-González, L. Sánchez-Íñigo, J. Pastrana-Delgado, A. Fernández-Montero, J. A. Martinez, Triglyceride-glucose index (TyG index) in comparison with fasting plasma glucose improved diabetes prediction in patients with normal fasting glucose: The Vascular-Metabolic CUN cohort, *Prev Med.*, 2016, **86**, 99-105.
27. M. Dobiášová, J. Frohlich, The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate inapob-lipoprotein-depleted plasma (FERHDL), *Clin Biochem.*, 2001, **34**, 583-588.
28. T. M. Wolever, D. J. Jenkins, The use of the glycemic index in predicting the blood glucose response to mixed meals, *Am J Clin Nutr.*, 1986, **43**, 167-172.
29. H. Kolb, S. Martin, Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes, *BMC Med.,* 2017, **15**, 131.
30. G. Wilcox, Insulin and insulin resistance, *Clin Biochem Rev.*, 2005, **26**, 19-39.
31. M. A. Abdul-Ghani, V. Lyssenko, L. Groop, T. Tuomi, R. A. Defronzo, The shape of plasma glucose concentration curve during OGTT predicts future risk of type 2 diabetes, *Diabetes Metab Res Rev.*, 2010, **26**, 280–286.

Food & Function Accepted Manuscript

32. X. Cheng, N. Yang, Y. Li, Q. Sun, L. Qiu, The shape of the glucose response curve during an oral glucose tolerance test heralds β-cell function in a large Chinese population, *BMC Endocr Disord.*, 2019, **19**, 119.
33. M. Manco, G. Nolfe, Z. Pataky, L. Monti, F. Porcellati, et al., Shape of the OGTT glucose curve and risk of impaired glucose metabolism in the EGIR-RISC cohort, *Metabolism,* 2017, **70**, 42–50.
34. O. Tschritter, A. Fritsche, F. Shirkavand, F. Machicao, H. Häring, et al., Assessing the shape of the glucose curve during an oral glucose tolerance test, *Diabetes Care*, 2003, **26**, 1026-1033.
35. B. A. Marin-Alejandre, I. Abete, I. Cantero, J. I. Monreal, M. Elorz, et al., The Metabolic and Hepatic Impact of Two Personalized Dietary Strategies in Subjects with Obesity and Nonalcoholic Fatty Liver Disease: The Fatty Liver in Obesity (FLiO) Randomized Controlled Trial, *Nutrients*, 2019, **11**, 2543.
36. I. Ibero-Baraibar, M. Cuervo, S. Navas-Carretero, I. Abete, M. A. Zulet, et al., Different postprandial acute response in healthy subjects to three strawberry jams varying in carbohydrate and antioxidant content: a randomized, crossover trial, *Eur J Nutr.*, 2014, **53**, 201–210.
37. T. M. Wolever, M. Yang, X. Y. Zeng, F. Atkinson, J.C. Brand–Miller, Food glycemic index, as given in glycemic index tables, is a significant

Food & Function Accepted Manuscript

determinant of glycemic responses elicited by composite breakfast meals, *Am J Clin Nutr.*, 2006, **83**, 1306–1312.

38. I. Abete, D. Parra, J. A. Martinez, Energy-restricted diets based on a distinct food selection affecting the glycemic index induce different weight loss and oxidative response, *Clin Nutr.*, 2008, **27**, 545–551.
39. M. A. Martínez-González, C. I. Fernandez-Lazaro, E. Toledo, A. Díaz-López, D. Corella, et al., Carbohydrate quality changes and concurrent changes in cardiovascular risk factors: a longitudinal analysis in the PREDIMED-Plus randomized trial, *Am J Clin Nutr.*, 2020, **111**, 291–306.
40. T. M. Larsen, S. M. Dalskov, M. van Baak, S. A. Jebb, A. Papadaki, et al., Diets with high or low protein content and glycemic index for weight-loss maintenance, *N Engl J Med.,* 2010, **363**, 2102–2113.
41. S. Shafaeizadeh, L. Muhardi, C. J. Henry, B. J. M. van de Heijning, E. M. van der Beek, Macronutrient Composition and Food Form Affect Glucose and Insulin Responses in Humans, *Nutrients*, 2018, **10**, 188.
42. L. J. van Loon, W. H. Saris, A. J. Wagenmakers, Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate, *Am J Clin Nutr.*, 2000, **72**, 96–105.
43. W. A. Blom, A. Lluch, A. Stafleu, S. Vinoy, J. J. Holst, et al., Effect of a high-protein breakfast on the postprandial ghrelin response, *Am J Clin Nutr.*, 2006, **83**, 211–220.

Food & Function Accepted Manuscript

44. A. T. Hutchison, D. Piscitelli, M. Horowitz, K. L. Jones, P. M. Clifton, et al., Acute load-dependent effects of oral whey protein on gastric emptying, gut hormone release, glycemia, appetite, and energy intake in healthy men, *Am J Clin Nutr.*, 2015, **102**, 1574–1584.
45. K. J. Bell, C. E. Smart, G. M. Steil, J. C. Brand-Miller, B. King, et al., Impact of fat, protein, and glycemic index on postprandial glucose control in type 1diabetes: implications for intensive diabetes management in the continuous glucose monitoring era, *Diabetes Care*, 2015, **38**, 1008-1015.
46. A. Neu, F. Behret, R. Braun, S. Herrlich, F. Liebrich, et al., Higher glucose concentrations following protein- and fat-rich meals - the Tuebingen Grill Study: a pilot study in adolescents with type 1 diabetes, *Pediatr Diabetes.*, 2015, **16**, 587–591.
47. C. E. Smart, M. Evans, S. M O'Connell, P. McElduff, P. E. Lopez, 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**, 3897-3902.
48. C. Galarregui, I. Abete, S. Navas Carretero, G. Reglero, A. Ramírez de Molina,et al., Estrategias de guía e ingredientes dietéticos de precisión para enfermedades crónicas en población pre-sénior y sénior [Precision dietary guidelines and ingredients for chronic diseases in pre-senior and senior populations], *An Sist Sanit Navar.*, 2018, **41**, 227–242.

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Tables

Table 1. Baseline characteristics of the study participants.

Parameters	All	Study I	Study II	*P*-value
	(*n*=34)	(*n*=19)	(*n*=15)	
Sex (men/women)	14/20	9/10	5/10	ns
Age (years)	59.9 (8)	59.9 (7)	59.9 (8)	ns
BMI (kg/m^2)	24.6 (3)	24.2 (3)	25.1 (3)	ns
WC (cm)	88.3 (9)	89.3 (10)	87.0 (9)	ns
Total fat mass (%)	30.5 (11)	29.4 (13)	31.9 (8)	ns
Insulin Resistance				
Glucose (mg/dL)	92 (7)	92.5 (5)	92.4 (9)	ns
Insulin (mU/L)	5.7 (2)	6.0 (3)	5.3 (2)	ns
HOMA-IR	1.3 (1)	1.4 (0.7)	1.2 (0.5)	ns
TyG index	8.3 (0.3)	8.3 (0.4)	8.3 (0.3)	ns
TG/HDL-c index	1.7 (0.8)	1.7 (0.9)	1.7 (0.7)	ns
Lipid Metabolism				
TG (mg/dL)	94 (32)	93.7 (36)	94.3 (27)	ns
TC (mg/dL)	220 (28)	224.0 (35)	215.0 (16)	ns
LDL-c (mg/dL)	141 (23)	143.9 (28)	137.4 (15)	ns
HDL-c (mg/dL)	60 (15)	61.4 (17)	58.7 (11)	ns
LDL-c/HDL-c ratio	2.5 (0.7)	2.5 (0.7)	2.4 (0.6)	ns

[1] Values are represented as Mean (SD). Abbreviations: BMI: body mass index; HDL-c: high-density lipoprotein cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; TC: total cholesterol; TG: triglycerides; TG/HDL-c index: Triglyceride/high-density lipoprotein cholesterol index; TyG index: Triglyceride-glucose index; LDL-c: low-density lipoprotein cholesterol; WC: waist circumference. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, ns: non-significant.

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Table 2. Blood glucose and insulin response to the oral glucose solution (*n*=34), high protein product (Study 1: *n*=19) and high carbohydrate (CHO) product (Study 2: *n*= 15).

	OGTT (n=34)	**High Protein Product** (*n*=19)	**High CHO Product** (*n*=15)	**P-value**
Glucose iAUC (mg/dL ×min)	14000 (2340)	11923 (1709) *	12771 (1942)	0.003
Δ Glucose (mg/dL)	56.1 (20.0)	19.0 (20.3) *	45.8 (21.8) †	<0.001
Glucose peak (mg/dL)	151.1 (21.6)	111.7 (21.3) *	139.5 (21.4) †	<0.001
GI	100	87.8 (11)	89.9 (10)	0.565
GL	25	21.9 (3)	22.5 (2)	0.565
Insulin iAUC (mU/L ×min)	2665 (959)	1453 (715) *	1874 (705) #	<0.001
Δ Insulin (mU/L) ‡	37.8 (15.4 §	25.6 (13.7)	26.4 (15.4)	0.063
Insulin peak (mU/L) ¥	43.6 (16.8) §	31.4 (15.0)	32.3 (17.6)	0.076

Abbreviations: CHO: carbohydrate; iAUC: incremental Area Under Curve; GI: Glycemic Index; GL: Glycemic Load; OGTT: Oral Glucose Solution; * p was significant between Oral Glucose Solution and High Protein Product; # p was significant between Oral Glucose Solution and High CHO Product; † p was significant between High Protein Product and High CHO Product.

‡ Highest increase of insulin concentrations during the insulin curve.

¥ Highest insulin value during the insulin curve.

§ n=15

Table 3. Correlation analysis between baseline insulin resistance markers and the postprandial glucose and insulin response to the oral glucose solution (OGTT) (*n*=34), high protein product (*n*=19) and high carbohydrate (CHO) product (*n*= 15).

	OGTT				**High Protein Product**				**High CHO Product**			
	Glucose iAUC		**Insulin iAUC**		**Glucose iAUC**		**Insulin iAUC**		**Glucose iAUC**		**Insulin iAUC**	
Glucose Metabolism	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Fasting Glucose (mg/dL)	0.293	0.092	0.036	0.837	**0.598**	**0.007**	0.284	0.238	0.364	0.182	-0.132	0.639
Fasting Insulin (mU/L)	0.067	0.706	**0.452**	**0.007**	0.251	0.300	**0.756**	**<0.001**	-0.068	0.810	**0.621**	**0.013**
Baseline HOMA-IR	0.102	0.566	**0.471**	**0.005**	0.296	0.218	**0.761**	**<0.001**	-0.025	0.929	**0.518**	**0.048**

Abbreviations: CHO: carbohydrate; HOMA-IR: homeostatic model assessment of insulin resistance; iAUC: incremental Area Under Curve; OGTT: oral glucose solution.

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Table 4. Linear regression models assessing the relationship between cardiometabolic risk factors, including the different treatments (independent variable) and blood glucose and insulin response to the test products (dependent variables).

Variables	Model 1		Variables	Model 2		Variables	Model 3	
	β	*P*-value		β	*P*-value		β	*P*-value
Glucose iAUC (mg/dL ×min)			**Glucose iAUC (mg/dL ×min)**			**Glucose iAUC (mg/dL ×min)**		
WC (cm)	35.4	0.222	WC (cm)	25.2	0.338	WC (cm)	37.3	0.187
Baseline HOMA-IR	230.1	0.595	Baseline glucose	**121.4**	**0.004**	Baseline insulin	48.9	0.615
Treatment			**Treatment**			**Treatment**		
OGTT	1.00 (reference)		OGTT			OGTT		
High Protein Product	**-2189.8**	**<0.001**	High Protein Product	**-2353.3**	**<0.001**	High Protein Product	**2178.8**	**<0.001**
High CHO Product	-1086.6	0.086	High CHO Product	-760.1	0.204	High CHO Product	-1102.5	0.053
Insulin iAUC (mU/L ×min)			**Insulin iAUC (mU/L ×min)**			**Insulin iAUC (mU/L ×min)**		
WC (cm)	17.8	0.144	WC (cm)	**25.4**	**0.049**	WC (cm)	23.4	0.060
Baseline HOMA-IR	**554.7**	**0.005**	Baseline glucose	20.5	0.271	Baseline insulin	**91.9**	**0.035**
Treatment			**Treatment**			**Treatment**		
OGTT	1.00 (reference)		OGTT *			OGTT		
High Protein Product	**-1322.0**	**<0.001**	High Protein Product	**-1321.7**	**<0.001**	High Protein Product #	**-1279.7**	**<0.001**
High CHO Product	**-791.3**	**0.003**	High CHO Product	**-794.4**	**0.006**	High CHO Product ‡	**-819.1**	**0.003**

Models adjusted for both age and sex. Abbreviations: CHO: carbohydrate; HOMA-IR: homeostatic model assessment of insulin resistance; iAUC: incremental Area Under Curve; OGTT: oral glucose solution; WC: waist circumference.

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Figure legend

Figure 1. Postprandial serum glucose levels during 2 h after food test consumption (Study I: *n* = 19; Study II: *n*=15).* p < 0.05; ** p < 0.01; *** p < 0.001 OGTT vs High Protein Product; a p < 0.05; aa p < 0.01; aaa p < 0.001 High Protein Product vs High CHO Product; b p < 0.05; bb p < 0.01; bbb p < 0.001 OGTT vs High CHO Product. Abbreviations: OGTT: oral glucose solution.

Figure 2. Regression analysis with insulin iAUC and HOMA-IR. All variables were adjusted by age and sex. A) insulin iAUC and HOMA-IR; B) High Protein Product insulin iAUC and HOMA-IR; C) High CHO Product insulin iAUC and HOMA-IR.

Figures

Fig.1

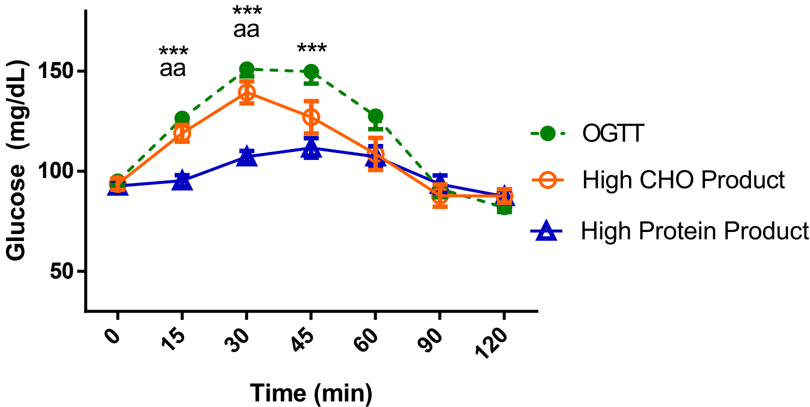

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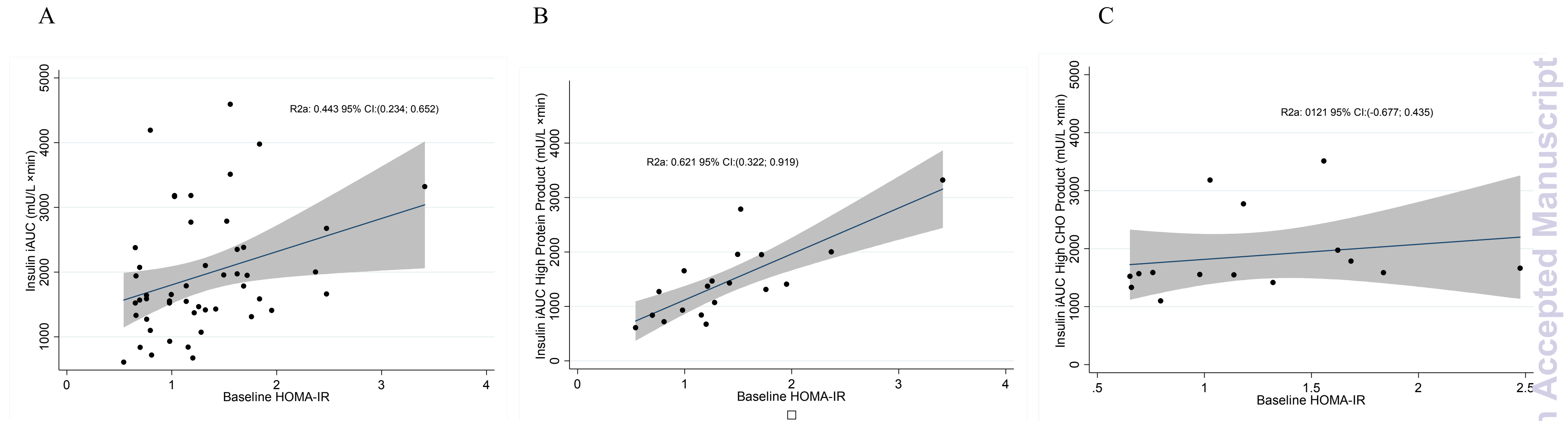
Fig.2



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