U.PORTO

Postprandial glycemic response of snacks made of potato or chickpea

Resposta glicémica após a ingestão de snacks de batata ou de grão-de-bico

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Abstract

Background: The postprandial blood glucose response (PBGR) is well stablished within legumes' varieties. However, PBGR is not accurately defined when legumes' flour is included in *snacks*. **Objective:** This study aims: 1) to assess whether chickpea's low GI is changed when chickpea's flour is incorporated in chickpea-based *snacks* (CPbS) and 2) to compare the glycemic response of CPbS with conventional potato-based snacks (PbS), in healthy individuals. **Design:** A randomized, crossover controlled human trial was performed. The study design included 25 healthy individuals with a Body Mass Index (BMI) of 21.3±2.4 kg/m^2 (mean ± SD BMI) that randomly consumed 25 grams of available carbohydrate (AC) from 44 grams of white bread (control), 35 grams of PbS, and 50 grams of CPbS, in three different occasions with one week apart between each test. Fasting and postprandial blood glucose samples (t=0, t=30, t=60, t=90, and t=120) were measured and used to calculate incremental area under the curve (iAUC) and to estimate glycemic index (GI). **Results:** No significant differences were found in total iAUC values between tested foods (p = 0.982). However, gender was shown to independently affect postprandial glycemic time-trend and iAUC values (p = 0.0237 and p = 0.045, respectively). The hour of experiment showed to affect the postprandial glycemic time-trend (p < 0.0001). **Conclusions:** Processing technologies seem to have impact on molecular structure of food components overlapping positive effects of their content in fiber, protein, lipids and fiber on PBGR.

Keywords: Postprandial blood glucose; glycemic response; legumes; food processing

Resumo

Contexto: A resposta glicémica pós-prandial (RGPP) associada ao consumo de leguminosas está bem estudada e documentada. É necessário, contudo, aprofundar o impacto glicémico da transformação de grão-de-bico em farinhas para a produção de snacks. **Objetivos:** Pretende-se: a) avaliar se o índice glicémico (IG), tipicamente baixo, do grão-de-bico se altera quando se incorpora farinha de grão-de-bico em snacks e 2) comparar a RGPP de snacks de grão-de-bico com a de snacks convencionais de batata, em indivíduos saudáveis. **Desenho do estudo:** Foi realizado um ensaio clínico randomizado e controlado, em crossover. Foram incluídos 25 indivíduos, com um índice de massa corporal (IMC) de 21.3±2.4 kg/m^2 (média ± DP IMC), que consumiram, aleatoriamente, 25 gramas de hidratos de carbono disponíveis presentes em 44 gramas de pão branco (controlo), 35 gramas de snacks de batata e 50 gramas de snacks de grão-de-bico, em 3 ocasiões distintas, com uma semana de intervalo. Os valores de glicose em jejum e pós-prandiais (t=0, t=30, t=60, t=90 e t=120) foram medidos e utilizados para o cálculo da área incremental sob a curva (AISC) e do IG. **Resultados:** Não foram encontradas diferenças significativas entre as AISC dos alimentos testados (p = 0.982). O género mostrou afetar, de forma independente, a tendência glicémica pós-prandial (TGPP) e a AISC (p = 0.0237 e p = 0.045, respetivamente). A hora de ingestão mostrou influenciar a TGPP (p < 0.0001). **Conclusão:** O processamento parece ter impacto na estrutura molecular dos alimentos, podendo este efeito sobrepor-se ao efeito positivo de alguns macronutrientes e fibra na RGPP.

Palavras-chave: resposta pós-prandial; resposta glicémica; leguminosas; processamento

Abbreviations

AC – Available Carbohydrates

BMI – Body Mass Index

CPbS – Chickpea-based Snacks

CVDs – Cardiovascular Diseases

CVe – Cardiovascular events

FCNAUP – Faculty of Nutrition and Food Sciences of University of Porto

FFA – Free Fatty Acids

GI – Glycemic Index

GL – Glycemic Load

GLP1 – Glucagon-like Peptide

GR – Glycemic Response

iAUC – Incremental Area Under the Curve

PBGR – Postprandial Blood Glucose Response

PbS – Potato-based Snacks

SCFA – Short-Chain Fatty Acids

RS – Resistant starch

Table of Contents

Introduction

High Glycemic Index (GI) and Glycemic Load (GL) diets and pronounced postprandial blood glucose response (PBGR) have been broadly studied since they showed to be associated with countless negative effects on human health[1-3], specifically increased mortality, cardiovascular events (CVe) and type 2 diabetes development[4]. At the same time, in the latest years, it was seen an increased production of foods highly rich in carbohydrates. Including these foods in consumers' daily eating habits have grown exponentially since they may have an addicting effect[5, 6]. This kind of products has high GI and high GL, simultaneously, thus producing a rapid glucose excursion and insulin spikes that are risk factors to development of chronic diseases[5-8]. The beneficial potential of low GI foods has been vastly explored and discussed within scientific community. It was suggested that positive effects could be related to intrinsic characteristics of typically low GI foods, such as, vegetables and legumes, and not necessarily due to their isolated impact on blood glucose[9, 10]. Nevertheless, as the existent evidence consistently links low GI foods and low GI diets to health markers this matter should not be neglected.

Choosing food products known to produce modest glycemic response (GR) due to their nutritional content may be of interest to enhance weight loss in overweight or obese people and in pre-diabetic or diabetic people[3, 7, 8, 11]. The foods' effect on blood glucose levels depends not only on individual genetic and physiological characteristics but also on interactions between main compounds of food microstructure such as starch, protein, lipids and fiber. These compounds can induce gastric emptying delay leading to lower release of glucose from food

microstructure in the intestine, lowering glucose absorption rate[11-13]. Fiber is an important feature of foods as its fermentation in colon by saccharolytic bacteria, such as *Bifidobacterium* and *Bacteroides*[14], into short-chain fatty acids (SCFA) (i.e. butyrate, propionate and acetate) is thought to contribute to a greater control of glycemia[15]. Circulating SCFA seem to enhance fatty acid oxidation while inhibiting *de novo* synthesis and lipolysis which in turn reduces free fatty acid (FFA) concentrations, linked to peripheral and hepatic insulin resistance[15-17]. Moreover, SCFA have been linked to increased satiety[18, 19]. Butyrate, for instance, may stimulate enteroendocrine L cells in the gut, because of its association with glucagon-like peptide (GLP1), resulting in an amplified insulin synthesis and release[20, 21]. Resistant starch (RS) and inulin are types of fiber of particular interest in this matter. It has been proposed that human health would benefit from the replacement of refined grains, present in some foods, by RS given the significant reduction seen on glycemic and insulin response[15, 22, 23].

Pulses (or legumes) are nutritionally-dense foods known to have low GI, to be high in plant-based protein, insoluble and soluble fiber, mainly pectin, RS and a wide range of varieties of phytochemicals such as carotenoids and polyphenols[24-26]. Chickpeas (*Cicer Arietinum*), kidney beans (*Phaseolus Vulgaris*) and green peas (*Pisum Sativum*), when boiled, have reported GI values of 28±9, 24±4 and 54±14, respectively[27, 28]. Dietary pulses where shown to increase satiety given their content in macronutrients[29], leading to a modest weight loss both in groups following an isocaloric or hypocaloric diet[30]. Some studies have claimed that high dietary fiber foods, like pulses, increase chyme viscosity (especially pectin, a soluble fiber) and also stimulate cholecystokinin secretion. Both factors decrease gastric motility thus diminishing glucose absorption rate which in turn results in

lower glycemic response[31, 32].Furthermore, pulses' cell wall integrity impacts the range of starch transformation, thus modulating PBGR[33-35]. To address the growing evidence that supports beneficial outcomes of including regularly pulses in diet, pulse industry evolved in order to boost the consumption of this nutritionally dense foods[36]. However, the development of new products with various levels of processing has been thought to impact negatively the effect of intact pulses on GR[31].

The current work aims to address two main objectives: 1) to assess whether chickpea's low GI is changed when this legume's flour is incorporated in chickpea-based *snacks* (CPbS) and 2) to compare the glycemic response of CPbS with conventional potato-based *snacks* (PbS), in healthy individuals. We hypothesize that because of the nutritional composition and apparent low level of processing, CPbS' GI is similar to the reported GI of chickpea and that consuming CPbS produces a lower postprandial GR when compared to PbS.

Materials and Methods

1.1. Participants

Thirty healthy students of the first year of Nutrition Science Bachelors' degree on Faculty of Nutrition and Food Sciences of University of Porto (FCNAUP) aged 18-38 years, in which 6 were males and 24 were females, with a BMI of 21.3±2.4 kg/m^2, were recruited during classes. Exclusion criteria included diabetes mellitus, acromegaly, pituitary dwarfism, congenital metabolic disorders, regular use of glucocorticoids, use of glucocorticoids at the time, allergies or intolerances to the foods that were being tested. The study was

conducted from 24th of February to 11th of March 2020. Together with written consent, a form was asked to be filled by participants on their age, weight, height, type and frequency of physical activity level, current pharmacological therapy and family history of diabetes, obesity, hypertension, thyroid disease and cardiovascular diseases (CVDs).

The study was conducted at the Biochemistry Service of the Faculty of Medicine of University of Porto and at FCNAUP, in the context of the curricular unit of Biochemistry I, and according to the guidelines laid down in the Declaration of Helsinki. All procedures involving research study participants were approved by the Ethics Committee Centro Hospitalar São João / Faculty of Medicine, University of Porto.

1.2. Foods

White bread was purchased on a local bakery (Porto); PbS (LAY'S® FORNO ORIGINAIS) is a baked snack made with potato flakes (66%), starch, sunflower oil, sugar, sunflower lecithin, dextrose, salt (1.2%), citric acid and turmeric extract. CPbS (Bean'Go®) is also a baked snack made with chickpea flour (61%), rice flour, olive oil, salt and natural flavorings.

1.3. Study design and interventions

A randomized, crossover controlled human trial was performed. During the three weeks length of the study, once a week, each participant consumed one of the three foods with 25 grams of available carbohydrates (AC): 44 grams of white bread (reference food) or 35 grams of PbS or 50 grams of CPbS. Detailed information about other macronutrients and fiber present in test foods is given in Table I. Recruited individuals would have to drink 150 ml of water while ingesting

test foods. The order in which eligible participants would consume each tested food was settled using an online random number generator.[1]

The assays were conducted during class schedule and they were at either 11 am or 2 pm or 4 pm. Students who measures were made at 11 am, would arrive with a fasting period of 10 hours and the ones measured at 2 pm and 4 pm would be on a 4 hours fasting period, after a light meal.

Table I – Macronutrients, fiber and salt content of tested foods

Nutritional Information	White Bread [a]	Chickpea-based Snacks [b]	Potato-based Snacks [b]
Total Weight (g)	44	50	35
Energy (kcal)	128	197	154
Lipids (g)	1.0	6,5	4.6
Saturated FA (g)	0.2	0,8	0.5
Carbohydrates (g)	25	25	25
Sugars (g)	0.9	1.0	2.4
Protein (g)	3.7	7.5	2.0
Fiber (g)	1.7	5	1.6
Salt (g)	0.7	0.4	0.4

[a.] Based on The Portuguese Food Composition Table. [b.] Based on nutritional information available in snacks' label.

1.4. Blood glucose measurements

Glucose levels measurements were performed in five different moments: before the consumption of any foods (t0, beginning of the class) and in four other moments after the first measurement: thirty minutes after (t30), sixty minutes

[1] Online random number generator can be accessed through https://www.randomizer.org/

(t60), ninety minutes (t90) and, finally, one hundred and twenty minutes after (t120). Peripheral blood samples were collected by fingerstick with a lancet device and using test strips. Then, glucose levels were measured with the Freestyle Precision blood glucose monitoring system (Abbott Laboratories).

1.5. Area Under the Curve and Glycemic Index calculations

After collecting all data about glycemic blood levels, iAUC was calculated using the geometric sums of triangles' and trapezoids' areas above the fasting glucose value, during the two hours in which measurements were made. iAUC values were then used to calculate GI, considering white bread as the reference food according to the following equation, as proposed by Jenkins et al.[37]:

$$GI = AUC_{food}/AUC_{reference} \times 100$$

1.6. Statistical analysis

A Sprent Test, a non-parametric test that identifies outliers, was applied to exclude individuals with non-typical glycemic responses. Five volunteers were excluded because they showed atypical GI values of CPbS and PbS. Baseline characteristics of the eligible study participants are presented in Table II. Normality of iAUC values was tested using Shapiro-Wilk test.

Two approaches were used to assess the effect of foods on glycemic response, the first used the iAUC as an outcome and the second assessed the time-trend of blood glucose levels. For the first approach a two-way repeated measures ANOVA was applied to compare mean values of iAUC between test foods, including gender and hour of experiment as between-subjects factor. For the second

approach, linear mixed-effects models were used to assess the effect of other variables on glycemic time trend. The interaction between time (time and time^2) and the following variables, gender, hour of experiment (11 am, 2 pm or 4 pm), BMI, physical activity level and family history of disease with the glycemia values were studied as fixed effects. It was included the random intercept by participants in all models and in some models the time slope was also included. Maximum Likelihood estimation was used to compare nested models with same fixed effects. Restricted Maximum Likelihood was used to compare models with different random effects with the same fixed effects.

Table II - Baseline characteristics of eligible study participants

Variable	Value
N	25
Age, y	22 ± 5.8[a]
Women, n (%)	20 (80)
Weight, kg	59 ± 9.9
Height, m	1.70 ± 0.1
BMI, kg/m	21.5 ± 2.6
Physical Activity[b]	
Low, n (%)	4 (16)
Moderate, n (%)	3 (12)
Hight, n (%)	16 (64)
Family history of diseases 1, n (%)[c]	3 (12)
Family history of diseases 2, n (%)[d]	20 (80)
Time of the Day	
At 11 am, n (%)	13 (52)
At 2 pm, n (%)	6 (24)
At 4 pm, n (%)	6 (24)

[a.] Mean ± Standard Deviation (all such values). [b.] Physical activity level of individuals was defined as low, moderate or high according to *International Physical Activity Questionnaire* (IPAQ).[38] [c.] Family history of diseases 1 refers to individuals whose first-degree relatives suffer from diabetes, obesity, hypertension, thyroid disease and CVDs. [d.] Family history of diseases 2 refers to individuals whose any of the second-degree relatives suffers from diabetes, obesity, hypertension, thyroid disease and CVDs.

Using the fixed effect estimates from the previous models, the glycemic peak time and the corresponding glycemic value was calculated.

All analysis presented in the study were performed with a significance level of $p < 0.05$.

Data was analyzed using IBM® SPSS® Statistics version 26.0 for Windows Software, of Microsoft Excel 2016 and R version 4.0.0.

3. Results

3.1. Glycemic response curve

Figures I, II and III shows the average postprandial glycemic responses of the different foods when tested at 11 am, 2 pm and 4 pm, respectively. Table III presents the results of linear mixed-effects model analysis. The first model (model 1), including only the effect of time, suggests an increment in blood glucose levels until reaching a maximum at t = 64 min (117 mg/dL). The inclusion of gender (model 2) was shown to affect significantly the trend of the curve ($p = 0.0237$), meaning that females and males have different GR to tested foods. Although the average time to reach the peak between females (t = 64.2 min) and males (t = 63.9 min) was similar, average glycemic value at that time was lower in male (120 versus 105 mg/dL). When an adjustment of the model (model 1) was made to the hour of experiment (model 3), a statistically significant difference was observed ($p < 0.0001$). This variable seems to affect both the time to reach the peak and the corresponding glycemic value. Throughout the day, it seems to take longer to achieve the maximum blood glucose value (t=58 min, t=64 min and t=79 min). Additionally, a higher average glycemic peak was attained within individuals who were measured at 2 pm (129 mg/dL) compared with individual measured at 11 am

and 4 pm, both groups with maximum glycemic peak of 114 mg/dL. Other variables didn't show a significant interaction. The standard deviation for the random effect for time and time^2 was 0.33 and 0.002, respectively (Model 4, p = 0.0009). These values show that blood glucose levels trends highly depend on the individuals. The following models (Model 5 and Model 6) included these random effects. Gender and hour of experiment were included together in model 5, showing that both variable effect the glycemic time trend (p = 0.0079). Model 6 showed that the food items ingested did not affect the glycemic time trend after adjusting for gender and hour of experiment (p = 0.4869).

3.2. Area Under the Curve

Data on average and standard deviation of iAUC values of tested foods as well as the hypothesis tests results are presented in Table IV. The relatively high standard deviations point to the large variability observed among the 25 participants. Using the Shapiro-Wilk test, normality was observed on total iAUC of white bread, PbS and CPbS (p = 0.850, p = 0.559, and p = 0.168, respectively). No significant differences were observed in total iAUC values between test foods (p = 0.912). The inclusion of gender and hour of experiment as between subject factors showed a significant effect of gender (p = 0.045) but not of hour of experiment (p = 0.104). No combind effects of gender and time of experiment on the iAUC were observed (p = 0.760). Bonferroni-adjusted paired t-tests resulted in significant differences between males and females iAUC values.

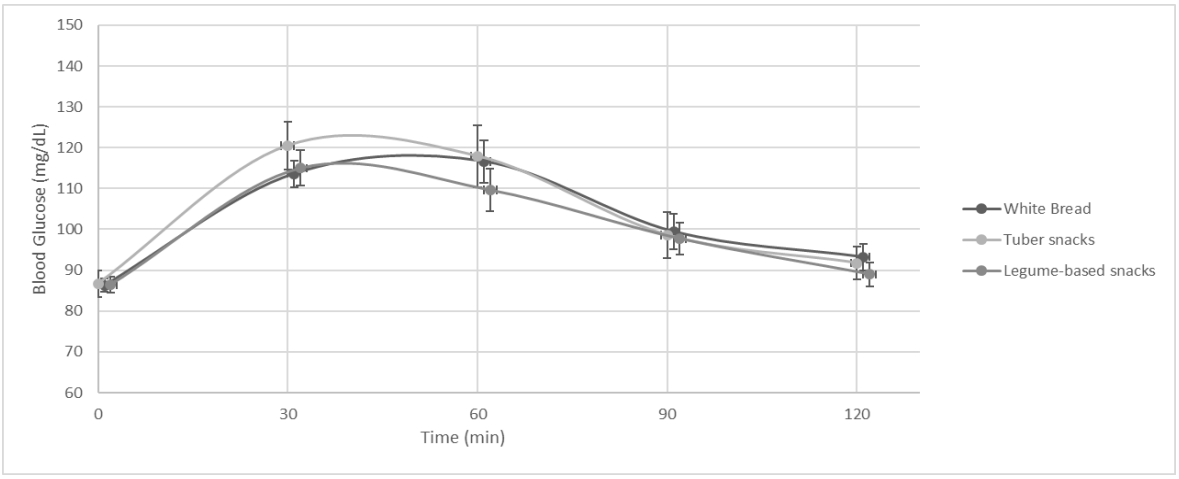

Figure I. Average glycemic response curves obtained when glucose measurements were made at 11 am (n = 13)

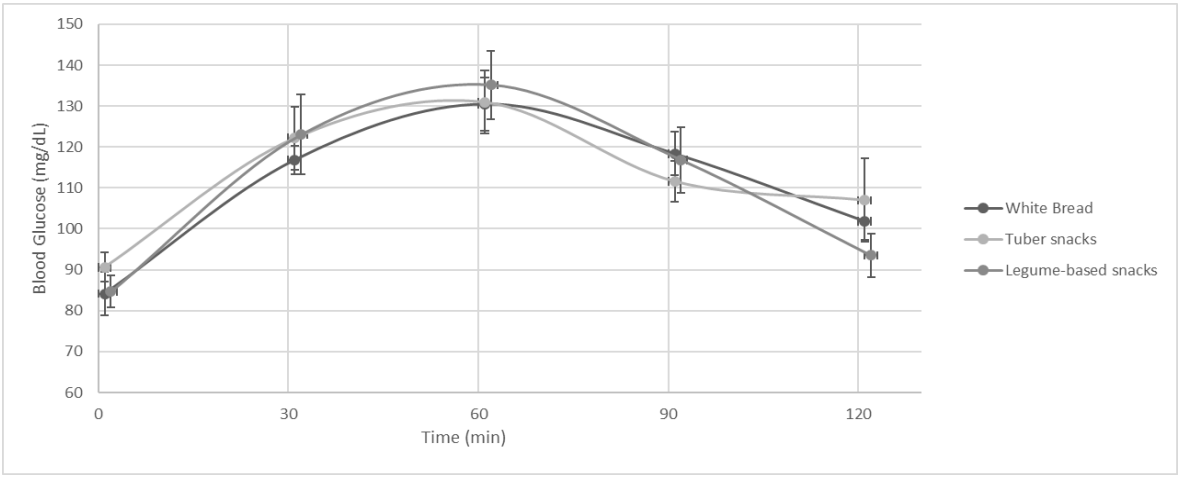

Figure II. Average glycemic response curves obtained when glucose measurements were made at 2 pm (n = 6)

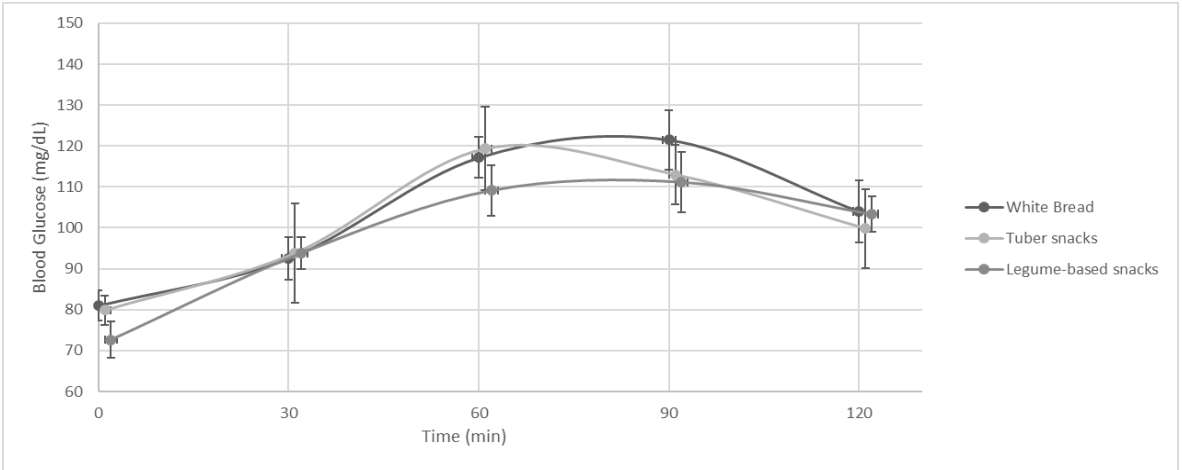

Figure III. Average glycemic response curves obtained when glucose measurements were made at 4 pm (n = 6)

Table IV. iAUC values (mg/dL·min^{-1}) of tested foods (total, by gender and by hour of test). Mean ± Standard Deviation. Two-way ANOVA with repeated measures.

	Total	11 am		2 pm		4 pm			
		Female	**Male**	**Female**	**Male**	**Female**	**Male**		
White Bread	2668 ± 1535	2163 ± 1622	488	3599 ± 1364	2625	3975 ± 1356	2715 ± 256		
PbS	2704 ± 1488	2591 ± 1536	589	3166 ± 759	2595	3874 ± 2184	2072 ± 1240		
CPbS	2653 ± 1179	2500 ± 993	611	3076 ± 1127	2596	3955 ± 1657	2052 ± 260		
	Food[a]							**Gender**[b]	**Hour**[b]
p	0.912							0.045	0.104

[a.] within subjects; [b.] between subjects; PbS, potato-based snacks; CPbS, chickpea-based snacks

3.3. Glycemic Index

Assuming GI value of white bread as 100, an average GI of 106 ± 48 and 101 ± 44 was obtain, for PbS and CPbS, respectively.

4. Discussion

Nowadays, eating habits are changing rapidly mainly to maintain or increase work and personal life pace. This raises important questions: Is it possible to keep a healthy diet when having a busy life? Can industries conceive products that suit consumers' lifestyles without compromising their health?

Legumes are known to have a low GI, strongly related to the effect of their structural and nutritional components, responsible for slowing digestive processes

and for extending colonic fermentation. The present study was designed to determine whether macronutrients and fiber content are, in fact, the most important features to modulate PBGR or if processing technologies applied to chickpea when formulate CPbS influence the GI of native chickpea.

Attained results indicate that the type of food eaten does not affect neither GR time trends nor glycemic iAUC. These findings lead to assume that the processing methods (milling, kneading, extrusion and baking) used to obtain the CPbS (with about 60% of chickpeas flour) can indeed increase the GI when compared with boiled chickpeas, overlapping the effect of desirable macronutrients and fiber content. Countless studies have emphasized the influence of starch gelatinization, promoted by wet heating, on starch enzymatic hydrolysis during digestion[34, 35]. Starch digestion rate is increased when gelatinization occurs together with other transformations in legumes' cell walls integrity, which in turn depends on the length and type of processing[24]. Studied CPbS are made with chickpea's flour that are already a transformed product. Additionally, their processing comprise kneading, extrusion and baking technologies where ingredients suffer a pre-treatment in order to form a dough-like material, that is then shaped into a thin layer followed by high temperature baking[39]. This may explain the enhanced PBGR in opposition to what was expected when analysis the high level of chickpea incorporated in the formulation of the CPbS. Consistent with these results, Johnson et al. did not found any significant differences in PBGR of extruded chickpea bread against white bread (control).[40] Thus, it is reasonable to consider that food matrix complexity and integrity are, indeed, essential PBGR modulators.

Interestingly, the GI values of CPbS are very close to the GI value observed for PbS, which are made of potato flakes (66%), an apparently highly processed ingredient.

Gender, independently, do seem to affect GR time trends and glycemic iAUC. Females showed to have a more pronounced GR. Other covariates like body weight, 56±5 kg in women vs 76±7 kg in men, can be influencing the amount of ingested carbohydrates per kg of body weight and confounding the observed gender effect. However, a more robust discussion on this result is hampered by the high discrepancy between the number of males (n = 5) and females (n = 20) in the sample.

The hour of experiment, independently, affect GR time trends. Glycemia starts to decrease earlier when foods are consumed in the morning than when consumed during the afternoon. It has already been reported that throughout the day, there is a decrease in glucose tolerance as these levels are regulated by the circadian rhythms[41]. Consistent with these findings, it was previously discussed that ß cells responsiveness tend to decrease along the day and a decreased insulin action and increased hepatic insulin extraction in the meals following breakfast was observed[42]. Hence, it is well stablished that the composition of previous meals affects the response to following meal.

When the random effects were included in the model (model 4), it was observed that blood glucose time trend highly depends on other non-studied participants characteristics, probably including genetic or physiological traits[43].

It should be considered that some factors related to study design and sample size may have had an influence on the obtained results. A reduction on

study participants' number, due to atypical glycemic responses as well as the short amount of AC of test food portions (25 grams) might have enhanced inter- and intra-individual variability. Accounting to the fact that glucose tolerance is not stable at different moments of the day, to decrease the variability observed PBGR, blood glucose levels measurement should have been made consistently at the same hour in all participants. Individuals who started the evaluation at 2 pm or 4 pm were instructed to have a light standard meal, at least 4 hours before the experiment. However, the compliance with this instruction could have been different between individuals resulting in possible diverse nutritional content within previous meals, thus, probably affecting the blood response to the tested foods [44, 45].

In the current study, insulin and insulin markers were not measured. Particularly, the measurement of C-peptide, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), together with insulin, would be valuable to understand blood glucose influx rate to peripheral tissues. Participants included in the study are normoponderal and so, the effect of BMI class on the GR was not possible to assess[46, 47]. Also, it would have been interesting to assess satiety levels between tested foods as it is not totally dependent on GI, as previously discussed.

5. Conclusions

GI of CPbS is higher than boiled chickpeas and not different from white bread or PbS, probably due the effect of chickpea processing on cell wall integrity and starch gelatinization. However, other features of the CPbS can be highlighted, when compared with the white bread or PbS, namely, its high content in fiber and protein. The incorporation of a vegetable protein source in snacks can also

contribute to reduce the intake of animal-based protein sources with higher environmental impact and detrimental health effects.

The lower glycemic response of tested food in males, when compared to females, and the effect of the time of consumption on the PBGR, deserves further attention in future studies.

Efforts should be made in order to continue evolving to develop practical and healthy food products to consumers through innovation. In fact, nowadays, foods choices are no longer ruled only by taste and immediate nutritional needs. Consumers look for products that improve their health and bring them welfare.

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ANNEXES

Table of contents – Annexes

Annex A

Table III - Linear mixed-effects models of GR over time

	Maximum Likelihood			
	β	95% CI	p-value	L.Ratio p-value
Model 1				
Intercept *(0 Ref.)*	86.2	[81.4, 91.0]	<0,0001	-
Time	0.97	[0.84, 1.09]	<0,0001	
Time^2	-0.0076	[-0.0086, -0.0066]	<0,0001	
Model 2	Maximum Likelihood			
Intercept *(Female Ref.)*	87.4	[82.2, 92.3]	< 0.0001	
Time	1.03	[0.89, 1.17]	< 0.0001	
Gender	-6.0	[-17.6, 5.6]	0.3013	
Time^2	-0.0080	[-0.0091, -0.0069]	< 0,0001	
Interaction				
Time x Gender	-0.29	[-0.60, 0.01]	0.0638	
Time^2 x Gender	0.0023	[- 0,0002, 0.0047]	0.8880	
Model 1 vs Model 2				0.0237
Observations, n	365			
Voluntaries, n	25			

Table III. Linear mixed-effects models of GR over time (continuation)

Model 3	Maximum Likelihood			
Intercept *(11am Ref.)*	90.4	[85.3, 96.5]	< 0.0001	
Time	0.82	[0.65, 0.98]	< 0.0001	
Hour (2pm)	-2.4	[-13.8, 9.2]	0.6796	
Hour (4pm)	-15.0	[-26.4, -3.6]	0.0131	
$Time^2$	-0.0070	[-0.0083, -0.0057]	< 0.0001	
Interaction				
Time x Hour (2pm)	0.48	[0.19, 0.77]	0.0016	
Time x Hour (4pm)	0.17	[-0.12, 0.45]	0.2527	
$Time^2$ x Hour (2pm)	-0.0031	[-0.0054, -0.0008]	0.0100	
$Time^2$ x Hour (4pm)	0.0008	[-0.0015, 0.0031]	0.5041	
Model 1 vs Model 3				< 0,0001

Model 4	Restricted Maximum Likelihood [a]			
Intercept *(11am Ref.)*	90.4	[85.8, 95.0]	< 0.0001	
Time	0.82	[0.65, 0.98]	< 0.0001	
Hour (2pm)	-2.6	[-11.3, 6.1]	0.6796	
Hour (4pm)	-15.0	[-23.6, -6.5]	0.0131	
$Time^2$	-0.0070	[-0.0088, -0.0052]	< 0.0001	
Interaction				
Time x Hour (2pm)	0.49	[0.07, 0.91]	0.0016	
Time x Hour (4pm)	0.17	[-0.25, 0.59]	0.2527	
$Time^2$ x Hour (2pm)	-0.0032	[-0.0063, -0.0000]	0.0100	
$Time^2$ x Hour (4pm)	0.0008	[-0.0023, 0.0039]	0.5041	
σ (time)	0.33	[0.20, 0.54]		
σ ($time^2$)	0.002	[0.001, 0.004]		
Model 3 vs Model 4				0.0009
Observations, n	365			
Voluntaries, n	25			

Table III. Linear mixed-effects models of GR over time (continuation)

Model 5	Maximum Likelihood			
Intercept *(Female 11am Ref.)*	90.4	[86.0, 94.7]	< 0.0001	
Gender	0.77	[-8.25, 9.79]	0.8633	
Time	0.85	[0.64, 1.06]	< 0.0001	
Hour (2pm)	-2.5	[-10.8, 5.7]	0.5375	
Hour (4pm)	-15.3	[-24.2, -6.4]	0.0020	
$Time^2$	-0.0072	[-0.0088, -0.0056]	< 0.0001	
Interaction				
Time x Hour (2pm)	0.53	[0.16, 0.90]	0.0062	
Time x Hour (4pm)	0.35	[-0.05, 0.75]	0.0935	
Time x Gender	-0.43	[-0.84, -0.02]	0.0429	
$Time^2$ x Hour (2pm)	-0.0034	[-0.0063, -0.0004]	0.0261	
$Time^2$ x Hour (4pm)	-0.0001	[-0.0033, 0.0030]	0.9307	
$Time^2$ x Gender	0.0022	[-0.0010, 0.0054]	0.1863	
Model 4 vs Model 5				0.0079
Observations, n	365			
Voluntaries, n	25			

Table III. Linear mixed-effects models of GR over time (continuation)

Model 6	Maximum Likelihood			
Intercept *(Female bread 11am Ref.)*	90.8	[82.8, 98.7]	< 0.0001	
Gender	0.8	[-8.2, 9.8]	0.8636	
Food	-0.20	[-3.51, 3.11]	0.9071	
Time	0.87	[0.53, 1.20]	< 0.0001	
Hour (2pm)	-2.5	[-10.8, 5.7]	0.5378	
Hour (4pm)	-15.4	[-24.2, -6.4]	0.0021	
$Time^2$	0.0071	[-0.0091, -0.0052]	< 0.0001	
Interaction				
Time x Hour (2pm)	0.53	[0.16, 0.90]	0.0065	
Time x Hour (4pm)	0.35	[-0.05, 0.75]	0.0953	
Time x Gender	-0.43	[-0.84, -0.02]	0.0440	
Time x Food	-0.01	[-0.14, 0.12]	0.9003	
$Time^2$ x Hour (2pm)	-0.0034	[-0.0063, -0.0004]	0.0268	
$Time^2$ x Hour (4pm)	-0.0001	[-0.0033, 0.0030]	0.9305	
$Time^2$ x Gender	0.0022	[-0.0010, 0.0054]	0.1885	
$Time^2$ x Food (PbS)	0.0000	[-0.0011, 0.0011]	0.9947	
$Time^2$ x Food (CPbS)	-0.0002	[-0.0023, 0.0019]	0.8724	
Model 5 vs Model 6				0.4869
Observations, n	365			
Voluntaries, n	25			

PbS, potato-based snacks; CPbS, chickpea-based snacks