The co-ingestion of NUTRALYS® pea protein and a high-carbohydrate beverage influences the glycaemic, insulinaemic, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide -1 (GLP-1) responses: preliminary results of a randomised controlled trial

Pariyarath Sangeetha Thondre[1*], Ifunanya Achebe[1], Alistair Sampson[1], Tyler Maher[1], Laetitia Guérin-Deremaux[2], Catherine Lefranc-Millot[2], Elisabeth Ahlström[1, 3], Helen Lightowler[1]

[1] Oxford Brookes Centre for Nutrition and Health, Faculty of Health and Life Sciences, Oxford Brookes University, Oxford OX3 0BP, UK

[2] Nutrition & Health R&D, Roquette Frères, Rue de la Haute Loge, 62180, Lestrem, France

[3] Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot SL57PY

Short Title: Pea protein, blood glucose and gastrointestinal hormone responses

*Corresponding Author
Dr Pariyarath Sangeetha Thondre https://orcid.org/0000-0003-2065-8443
Oxford Brookes Centre for Nutrition and Health
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus
Oxford OX3 0BP, UK
Tel: +44 (0)1865 483988
E-mail: pthondre@brookes.ac.uk

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Abstract

Purpose: Plant-based proteins may have the potential to improve glycaemic and gastrointestinal hormone responses to foods and beverages. The aim of this study was to investigate the effect of two doses of pea protein on postprandial glycaemic, insulinaemic, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) response following a high carbohydrate beverage intake in healthy individuals. **Methods**: In a single-blind, randomized, controlled, repeat measure, crossover design trial, thirty-one participants were randomly assigned to ingest 50g glucose (Control), 50g glucose with 25 g pea protein (Test 1) and 50g glucose with 50 g pea protein (Test 2) on three separate days. Capillary blood samples (blood glucose and plasma insulin measurements) and venous blood samples (GIP and GLP-1 concentrations) were taken before each test and at fixed intervals for 180 min. Data were compared using repeated-measures ANOVA or the Friedman test. **Results**: Glucose incremental Area under the Curve (iAUC180) was significantly lower ($p < 0.001$) after Test 2 compared with Control (-53%), after Test 1 compared with Control (-31%) and after Test 2 compared with Test 1 (-32%). Insulin iAUC 180 was significantly higher ($p < 0.001$) for Test 1 (+28%) and Test 2 (+40%) compared with Control and for Test 2 (+17%) compared with Test 1 ($p = 0.003$). GIP and GLP-1 release showed no clear difference between control and pea protein drinks. **Conclusion**: The consumption of pea protein reduced postprandial glycaemia and stimulated insulin release in healthy adults with a dose-response effect, supporting its role in regulating glycaemic and insulinaemic responses.

Keywords: Pea protein; Blood glucose; Insulin; Glucose-dependent insulinotropic polypeptide; Glucagon-like peptide-1

Introduction

An increasing body of evidence supports the importance of the glycaemic response (GR) of foods and diets in the prevention and treatment of the major causes of morbidity and mortality in Western countries, including Type 2 diabetes, coronary heart disease and obesity [1-5]. In addition, low-GR foods have been associated with prolonged endurance during physical activity [6], improved insulin sensitivity [7] and increased colonic fermentation [8]. Randomised trials and epidemiological studies have shown that high GR foods can increase the risk of insulin resistance and dyslipidaemia leading to the development of type 2 diabetes and cardiovascular diseases [4-5, 9].

Insulin secretion is elicited primarily by the glycaemic carbohydrates present in food, however, studies have shown that there are other insulinotropic factors such as amino acids, fatty acids and gastrointestinal hormones [10-11], including glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GIP and GLP-1 are secreted from the K-cells and L-cells of the upper and lower intestine respectively, into the blood stream in response to nutrient ingestion. Increases in GIP concentration are correlated with elevated intestinal glucose absorption [12-14], thus assessment of postprandial GIP response may be considered as evidence for the rate of glucose release from a food [14]. As GIP is implicated in liver fat accumulation and the development of impaired glucose tolerance, reducing postprandial GIP response may be a promising approach for the prevention and/or treatment of fatty liver and insulin resistance [15]. GLP-1 has numerous physiological actions including inhibition of gastric emptying, food intake and glucagon secretion, which control glycaemia [16-17].

Pulses are low-glycaemic foods rich in protein and are reported to have significant health benefits, including weight management, improved gastrointestinal function and homeostasis and cardiovascular health [18]. Moreover, previous research has highlighted the potential of plant-based proteins, including pea protein, in improving the glycaemic and satiety response to foods and beverages [19-20]. However, the results for glycaemic and insulinaemic response have not always demonstrated the same trend when different doses of pea protein were included with different test products [20-21].

The aim of this study was to investigate the effect of two different doses of yellow pea protein powder (NUTRALYS® S85 Plus pea protein) on postprandial glycaemic, insulinaemic, GIP and GLP-1 response in healthy individuals. Adding smaller amounts of plant (pea) protein has a positive effect on postprandial glycaemic and satiety responses [19-21], thus the current study aimed to compare the effect of higher doses of pea protein. We

hypothesized that when a control (glucose) beverage is enriched with pea protein, postprandial glycemic response would be reduced and subsequently insulin, GLP-1 and GIP release would be stimulated.

Materials and Methods

Participants

Forty-five healthy male and female adults aged 19 to 55 years were recruited from the staff and student population at Oxford Brookes University and members of the public. Exclusion criteria were: pregnancy or lactating; <18 or >60 years of age; body mass index (BMI) ≥30kg/m^2; fasting blood glucose >6.1 mmol/l; any known food allergy or intolerance; medical condition or medication known to affect glucose regulation or appetite and/or digestion and absorption of nutrients; known history of diabetes mellitus or the use of antihyperglycaemic drugs or insulin to treat diabetes and related conditions; major medical or surgical event requiring hospitalization within the preceding three months; use of steroids, protease inhibitors or antipsychotics. In addition, participants were excluded from the study if they were unable to comply with the experimental procedures or did not follow testing safety guidelines.

Study design

This study was a single-blind, randomised, controlled, repeat measure, crossover design trial conducted at the Oxford Brookes Centre for Nutrition and Health. Participants were randomly assigned to test glucose (Control), glucose with 25 g pea protein (Test 1) and glucose with 50 g pea protein (Test 2) on three separate days.

The study was carried out in accordance with the declaration of Helsinki. Ethical approval was obtained from the University Research Ethics Committee (UREC) at Oxford Brookes University (UREC Registration No: 181259). Participants were given full details of the study protocol and the opportunity to ask questions. All participants gave written informed consent prior to participation. The study was retrospectively registered with Clinical Trials.Gov (NCT04610203).

Anthropometric measurements

Anthropometric measurements were made in the fasted state during the first session. Height was recorded to the nearest centimetre using a stadiometer (Seca Ltd, UK), with participants standing erect and without shoes. Body weight was recorded to the nearest 0.1 kg, with

participants wearing light clothing and no shoes. Body mass index (BMI) was calculated using the standard formula: weight (kg)/height $(m)^2$. Body fat percentage was measured using a body composition analyser (Tanita BC-418 MA; Tanita UK Ltd).

Study protocol

On the day prior to a test, participants were asked to restrict their intake of alcohol and caffeine-containing drinks and to restrict their participation in intense physical activity. Participants were also told not to eat or drink after 21:00 the night before a test, although water was allowed in moderation. In addition, participants were asked to standardize and consume the same foods and drinks and quantities the day before each test and maintain the same physical activity the day before each test.

The Control (50 g glucose) was compared with Test 1 (50 g glucose + 25 g NUTRALYS® S85 Plus pea protein powder) and Test 2 (50 g glucose + 50 g NUTRALYS® S85 Plus pea protein powder). NUTRALYS® S85 Plus pea protein, obtained from the yellow pea (*Pisum sativum*) and designed for protein enrichment and food applications, was provided by Roquette Frères.

On the day of a test, the samples were each mixed with 250 ml water and consumed as beverages; they were all served with an additional 250 ml water. All beverages were tested once in random order on separate days, with at least a seven-day period between measurements to minimise carry over effects. Participants were studied in the morning before 10:00 after a 12-hour overnight fast. Participants consumed the test products at a comfortable pace, within 15 minutes and remained sedentary during each session.

Blood samples were taken at 5 minutes and 0 minutes before consumption of the beverage and the baseline value taken as a mean of these two values. The beverage was consumed immediately after this and further blood samples were taken at different time points after starting to drink.

For blood glucose and plasma insulin measurements, blood was obtained by finger-prick, using the Unistik®3 single-use lancing device (Owen Mumford), at 15, 30, 45, 60, 90, 120, 150 and 180 minutes. Blood glucose was measured using a photometric enzyme coupled assay system (HemoCue Glucose 201 DM analyser, HemoCue® Ltd, Sweden), which was calibrated daily using control solution (GlucoTrol-NG) from the manufacturer. For plasma insulin, 350 µL of capillary blood was collected into chilled microvette® capillary blood collection tubes treated with di Potassium EDTA (CB 300 K2E; Sarstedt Ltd, Germany). The microvette® tubes were centrifuged at 4,000 rpm for 10 minutes and 150 µL of the

supernatant plasma removed. Insulin concentrations in the plasma samples were determined by electrochemiluminescence immunoassay using an automated analyzer (Cobas® E411; Roche diagnostics, Switzerland).

For the GIP and GLP-1 concentrations, 4 ml of venous blood was obtained via cannulation from the antecubital vein in the arm at 0, 30, 60, 90, 120 and 180 minutes. Blood was collected into chilled vacutainer® blood collection tubes treated with EDTA (K3E; Becton, Dickinson and Company, United States). DPP IV inhibitor (Sigma-Aldrich, UK) was added to each vacutainer® tube prior to blood collection. The vacutainer® tubes were centrifuged at 4,400 rpm for 10 minutes and 2 ml of the supernatant plasma subsequently removed. GIP concentrations and GLP-1 concentrations in the plasma samples were determined by enzyme-linked immunosorbent assay (ELISA) (RayBiotech, United States) and read on the ELx800TM absorbance microplate reader (BioTek® Instruments, Inc., United States). Gen5TM software (BioTek® Instruments, Inc., United States) was used for the evaluation of results. Each sample was measured in duplicate and the mean of both measurements was taken for evaluation of results.

Statistical analysis

Sample size calculation was based on published glycaemic response data of soup with added pea protein [21]. To detect a 72.7 mmol/l/min (SD 76.2) reduction in postprandial glucose iAUC with a two-sided α-level of 5% and a power of 90%, a sample size of at least 30 participants was necessary. To account for any dropouts, 45 participants were recruited for the current study.

Data were analysed using the IBM Statistical Package for the Social Sciences 25 (SPSS Inc., Chicago, Illinois). Data are presented as mean, standard deviation (SD) and standard error of the mean (SEM) values. Prior to statistical analysis, the normality of the data was tested using the Shapiro-Wilk statistic. For blood glucose, plasma insulin, plasma GIP and plasma GLP-1, the repeated measures ANOVA test (for normally distributed data) and non-parametric Friedman test (where data were not normally distributed) were used to compare concentrations at each time point, iAUC (at 60, 90 120 and 180 minutes), peak concentrations (blood glucose and plasma insulin only) and time of the peak concentrations (blood glucose and plasma insulin only) between the test products and glucose reference. Post-hoc analyses were performed using the Bonferroni correction for parametric data and the Wilcoxon signed-rank test for non-parametric data.

193 Statistical significance was set at $p<0.05$ for all tests, with the exception of the
194 Wilcoxon signed-rank test (where required), which was conducted with a Bonferroni
195 correction applied, resulting in a significance level set at $p<0.017$.
196
197 **Results**
198 Out of the forty-five participants recruited, twelve withdrew from the study and two
199 participants were excluded as they were either unable to comply with experimental
200 procedures or no longer eligible for the study. Therefore, the complete GR, IR, GIP and GLP-
201 1 data are reported for thirty-one participants. The physical characteristics of these
202 participants are presented in Table 1.
203
204 ***Glycaemic and insulinaemic response***
205 There was a significant difference in the change in blood glucose (A) and plasma insulin (B)
206 from baseline between Control, Test 1 and Test 2 at various time points (Fig.1). Table 2
207 shows the blood glucose and plasma insulin iAUC for the three beverages. Control blood
208 glucose iAUC was significantly higher compared to both Test 1 and Test 2 ($p < 0.001$). In
209 addition, blood glucose iAUC for Test 2 was significantly lower compared to Test 1 ($p <$
210 0.05) suggesting a dose related effect. Compared to Control, there was 31% and 53%
211 reduction in the mean glucose iAUC-180 for Test 1 and Test 2, respectively. There was a
212 32% reduction in the mean glucose iAUC-180 for Test 2 in comparison with Test 1.
213 Insulin iAUC was significantly higher with Test 1 and Test 2 compared to Control ($p <$
214 0.001). In addition, plasma insulin iAUC-180 for Test 2 was significantly higher compared to
215 Test 1 ($p < 0.05$) suggesting also a dose related effect. Compared to Test 1 and Test 2, there
216 was 28% and 40% reduction in the mean insulin iAUC-180 for Control, respectively. There
217 was a 17% reduction in the mean insulin iAUC-180 for Test 1 in comparison with Test 2.
218 There was a significant difference ($p < 0.001$) in the mean peak blood glucose between
219 Control and Test 1, Control and Test 2 and Test 1 and Test 2 (Table 3). There was a
220 significant difference in the mean peak plasma insulin between Control and Test 1 and
221 Control and Test 2 ($p < 0.001$; Table 3).
222
223 ***GIP and GLP-1 response***
224 GIP release was lower with pea protein and GLP-1 release was higher compared to Control
225 (Fig. 2). Table 2 shows the plasma GIP and GLP-1 iAUC for the three test beverages. There
226 was no significant difference in the mean GIP iAUC between the three test beverages at any

time point ($p > 0.05$). However, the peak plasma GIP was significantly different between the three test drinks ($p < 0.05$; Table 3). Pairwise comparisons showed significantly lower peak for Test 2 compared to Control ($p = 0.001$). There was no significant difference in the peak time for plasma GIP between the three tests beverages at any time point ($p > 0.05$).

There was no significant difference ($p > 0.05$) in the mean GLP-1 iAUC between the three tests beverages at any time point (Table 2). Peak plasma GLP-1 for Test 1 and Test 2 were significantly higher than Control ($p < 0.05$; Table 3). The time of peak for Control was significantly earlier than for Test 1 and Test 2 ($p < 0.05$).

Discussion/Conclusion

The aim of this study was to investigate the effect of two doses of NUTRALYS® pea protein (25 g and 50 g) on postprandial glycaemic, insulinaemic, GIP and GLP-1 responses in healthy individuals. It was hypothesised that a high-carbohydrate beverage enriched with pea protein would reduce postprandial glycaemic response and stimulate insulin, GLP-1 and GIP release compared to a control (glucose) beverage. Overall, the results of the current study support a role for pea protein in regulating glycaemic and insulinaemic response.

Past literature has shown that adding plant (pea) protein has a positive effect on postprandial glycaemic response [19-22]. Most studies have compared different plant proteins or smaller amounts [10-30 g] of pea protein, thus the current study aimed to compare the effect of a higher dose of pea protein. The results from the current study showed that both 25 g pea protein and 50 g pea protein produced a significantly lower glycaemic response when added to a control (glucose) beverage; the glucose iAUC was significantly lower with 50 g pea protein (Test 2) compared with 25 g pea protein (Test 1), with a 41% reduction at iAUC-120. This is in contrast to the study of Re et al. [21] which found no significant difference in glucose AUC between 15 g pea protein and 30 g pea protein; however, this may be attributed to the different levels of carbohydrate in the test meals used in the Re et al. [21] study. The test soup used with 15g pea protein had 28.7g carbohydrate whereas the soup with 30 g pea protein consisted of only 12.1 g carbohydrate, which may have been inadequate to demonstrate the effect of the higher dose of pea protein [21]. Therefore, the current study is a direct comparison between test foods containing two different doses of pea protein in the presence of identical carbohydrate content.

It has been shown that incorporating protein into a carbohydrate food or beverage increases the insulinaemic response [22-23] as proteins stimulate the release of insulin. The findings in the current study that the addition of pea protein to a control (glucose) beverage

significantly stimulated insulin release is consistent with previous research [20]. Moreover, 25 g and 50 g pea protein produced significantly different insulin responses at 120, 150 and 180 minutes, with 50 g resulting in higher plasma insulin concentrations compared to the lower dose. Overstimulation of insulin by protein has been reported to be beneficial in insulin resistance [24]. However, long-term hyperinsulinemia may have detrimental effects on insulin sensitivity in healthy individuals [25]. Whilst a positive association between animal protein intake and insulin resistance has been reported previously due to high levels of branched chain amino acids, plant protein consumption has not been linked to insulin resistance [26]. Therefore, including pea protein with high glycaemic foods may be a safe and useful strategy to manage blood glucose levels.

Previous research has shown that GIP release was increased following the consumption of pea protein, consistent with a higher insulin release [27]; however, it may be noted that for the test food in that study, pea protein was provided with mixed meals [27] rather than with glucose. Moreover, the participants were individuals with type 2 diabetes, reported to have enhanced GIP response [28]. In contrast to this, the results of the current study in healthy individuals showed no differences in GIP response to 25 g and 50 g pea protein compared to Control. Moreover, the results showed an insignificant reduction in GIP release after consumption of pea protein, with 50 g pea protein producing the lowest response. This is in agreement with Kahleova et al [29] who reported decreased postprandial GIP, yet increased insulin levels following a plant-based meal consisting of a tofu burger. Therefore, pea protein may exert metabolic effects similar to soy protein, which is the major plant-based protein used worldwide for food product development. Considering the inverse correlation between plasma GIP levels and insulin sensitivity [30], pea protein may be a promising ingredient to prevent the development of impaired glucose tolerance and insulin resistance.

Previous research has indicated that pea protein modulates GLP-1 levels and induces a plasma rise of GLP-1 [20-21]. In the current study, consumption of 25 g and 50 g pea protein resulted in increased levels of GLP-1 compared with the Control, although these trends were not significant. This finding is consistent with previous studies, which demonstrated an increase in GLP-1 concentrations with higher levels of pea protein [20-21]. Furthermore, the postprandial increase in insulin secretion observed in this study can be attributed to the incretin effect of GLP-1 following plant protein consumption [29]. A significant increase in peak GLP-1 value after pea protein consumption can delay gastric emptying, increase satiety and inhibit glucagon secretion, further contributing to glycaemic control [16-17].

It is well documented that protein stimulation of insulin secreting β-cells and the effects of protein on slowing gastric emptying may be the mechanisms responsible for the effects of pea protein on reducing postprandial glycaemia [19]. Pea protein used in this study is a fast protein (personal communication) with similar digestibility properties as whey protein [21], which has been associated with stimulation of plasma GLP-1 [30]. Although gastric emptying was not measured in this study, the faster absorption of amino acids from pea protein combined with the augmented GLP-1 response may be the mechanisms behind the dose-dependent insulinotropic effects seen in this study. The ability of higher doses of pea protein to modulate postprandial excursions of GIP following a high carbohydrate meal, as demonstrated in this study indicates potential for future therapeutic uses.

The glycaemic response of food depends on many factors, such as particle size, cooking and food processing, other food components (e.g. fat, protein, dietary fibre) and starch structure [32]. NUTRALYS® S85 Plus is a pea protein powder; while processing generally affects the starch in pulses and can alter their biological effects, previous research has shown that commercial processing of pulses to a powder form does not alter their low glycaemic characteristics [33], thus highlighting the potential role for pea protein powder in improving postprandial glycaemic and insulinaemic control. A recent review has highlighted regular high protein intake as a nutritional strategy to improve glycaemic control in older adults with pre-diabetes or type 2 diabetes [34]. Unlike the current study, majority of the research so far have been using high protein from animal sources or soy with low carbohydrate/low caloric diets [34]. Therefore, it is pertinent to conduct long term studies using novel plant protein such as pea protein in individuals at risk of insulin resistance.

The main strengths of this study were the direct comparison of two different doses of pea protein showing a clear dose response impact and the use of glucose as the test beverage. Thus, carbohydrate levels were the same in all test beverages and there was no interference from other nutrients, such as fat and fibre. The main limitation of the study was the high variation seen in GIP and GLP-1 response, although high variation has been seen in other previous studies [20, 21]. For this reason, we did not see a statistically significant difference in the GLP-1 iAUC after pea protein even though they appeared to be lower than the Control.

Although the current study had sufficient statistical power to detect a significant difference in glycaemic response, a larger sample size is deemed necessary for detecting differences in the appetite hormone response. The present study measured the post-prandial effect of pea protein for 3 hours; however, extending the study duration beyond 180 minutes

may provide an insight into any delayed effects of pea protein on blood glucose, plasma insulin and incretin hormones.

In conclusion, this study demonstrated that the addition of pea protein to a glucose beverage reduced postprandial glycaemia and stimulated insulin release with a dose-response effect, supporting a role for pea protein in regulating glycaemic and insulinaemic response. Additionally, it highlights the use of pea protein in lowering glycaemic response to simple sugars without a disproportionate increase in GIP hormone levels. Unlike protein of animal origin that has been linked to insulin resistance, pea protein may therefore be a safer alternative to manage blood glucose levels. Considering the increasing popularity of plant proteins due to the environmental impact of whey protein and soy protein, future research could investigate the implications of long-term consumption of pea protein on metabolic markers.

Declarations

Funding

This study was supported by funding from Roquette Frères, France.

Conflicts of interest/Competing interests

L Guérin-Deremaux and C Lefranc-Millot are employees of Roquette Frères. Roquette Frères did not play a part in the execution of the study or analysis of the results.

Ethics approval

The study was carried out in accordance with the declaration of World Medical Association Declaration of Helsinki. Ethical approval was obtained from the University Research Ethics Committee (UREC) at Oxford Brookes University (UREC Registration No: 181259).

Consent to participate

Participants were given full details of the study protocol and the opportunity to ask questions. All participants gave written informed consent prior to participation

Consent for publication

Not applicable

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

H Lightowler, PS Thondre, L Ahlstrom, L Guérin-Deremaux and C Lefranc-Millot contributed to the development of the study protocol. Material preparation and data collection were performed by PS Thondre, I Achebe, A Sampson and T Maher. Data analysis was performed by PS Thondre, I Achebe and H Lightowler. The first draft of the manuscript was written by H Lightowler. PS Thondre, L Guérin-Deremaux and C Lefranc-Millot revised the subsequent drafts of the manuscript. All authors have read and approved the final manuscript.

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Figure Legends

Fig. 1. Glycaemic (A) and insulinaemic (B) response curves for 50 g glucose, Test 1 (50 g glucose + 25 g NUTRALYS® S85 Plus pea protein), and Test 2 (50 g glucose + 50 g NUTRALYS® S85 Plus pea protein). Data are presented as mean and SEM (*n* 31). [a] Significantly different from Glucose (repeated measures ANOVA: $p<0.05$; Friedman test: $p<0.017$); [b] Significantly different from Test 1 (repeated measures ANOVA: $p<0.05$; Friedman test: $p<0.017$)

Fig. 2. Plasma GIP (A) and plasma GLP-1 (B) response curves for 50 g glucose, Test 1 (50 g glucose + 25 g NUTRALYS® S85 Plus pea protein), and Test 2 (50 g glucose + 50 g NUTRALYS® S85 Plus pea protein). Data are presented as mean and SEM (*n* 31).

477 **Table 1** Physical characteristics of the included study population (mean ± SD)

	All participants (*n* 31)
Age (y)	27.6 ± 7.7
Height (m)	1.7 ± 0.1
Weight (kg)	66.6 ± 11.6
BMI (kg/m^2)	22.9 ± 2.6
Fat mass (%)	24.9 ± 8.1
Lean body mass (kg)	49.7 ± 8.7

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480 **Table 2** Mean (± SD) iAUC blood glucose, iAUC plasma insulin, iAUC plasma GIP and
481 iAUC plasma GLP-1 at 60, 90, 120 and 180 min after consumption of 50 g glucose
482 (Control), 50 g glucose + 25 g pea protein (Test 1) and 50 g glucose + 50 g pea
483 protein (Test 2)

iAUC	Control	Test 1	Test 2	*P* value
Blood glucose (mmol/l/min)				
iAUC-60	119.0 ± 34.1	87.2 ± 33.3[a]	51.7 ± 25.3[a,b]	**<0.001***
iAUC-90	165.4 ± 55.7	116.1 ± 51.8[a]	63.6 ± 35.7[a,b]	**<0.001***
iAUC-120	188.9 ± 72.7	130.3 ± 64.0[a]	76.7 ± 45.2[a,b]	**<0.001***
iAUC-180	198.0 ± 82.1	137.0 ± 72.5[a]	93.3 ± 58.3[a,b]	**<0.001***
Plasma insulin (µU/ml/min)				
iAUC-60	2200.2 ± 832.9	2954.1 ± 1210.5[a]	2848.1 ± 1205.6[a]	**<0.001***
iAUC-90	2976.9 ± 1182.0	4093.6 ± 1630.0[a]	3973.5 ± 1801.0[a]	**<0.001***
iAUC-120	3369.6 ± 1516.5	4668.7 ± 1904.6[a]	4952.8 ± 2382.5[a]	**<0.001***
iAUC-180	3515.7 ± 1738.3	4867.9 ± 2011.0[a]	5849.1 ± 3008.4[a,b]	**<0.001***
Plasma GIP (pg/ml/min)				
iAUC-60	725.7 ± 940.4	467.8 ± 995.3	415.0 ± 502.6	0.743
iAUC-90	1281.7 ± 1515.2	899.4 ± 2126.6	720.1 ± 846.2	0.798
iAUC-120	1810.7 ± 2005.9	1251.7 ± 2554.6	1063.6 ± 1198.2	0.657
iAUC-180	2824.6 ± 3050.0	1895.7 ± 3499.2	1730.0 ± 1838.9	0.508
Plasma GLP-1 (pg/ml/min)				
iAUC-60	2364.0 ± 9496.8	5923.7 ± 23818.0	5982.5 ± 20510.8	0.225
iAUC-90	4397.4 ± 11874.9	8161.7 ± 24701.4	24950.4 ± 73671.9	0.117
iAUC-120	6044.8 ± 15486.1	10344.9 ± 25605.9	45525.2 ± 134219.3	0.141
iAUC-180	6863.8 ± 17288.1	13297.1 ± 28531.7	67383.9 ± 223780.2	0.140

484 *Statistically significant difference ($p<0.05$)

485 [a] Significantly different from Control (repeated measures ANOVA: p<0.05; Friedman test: p<0.017)

486 [b] Significantly different from Test 1 (repeated measures ANOVA: p<0.05; Friedman test: p<0.017)

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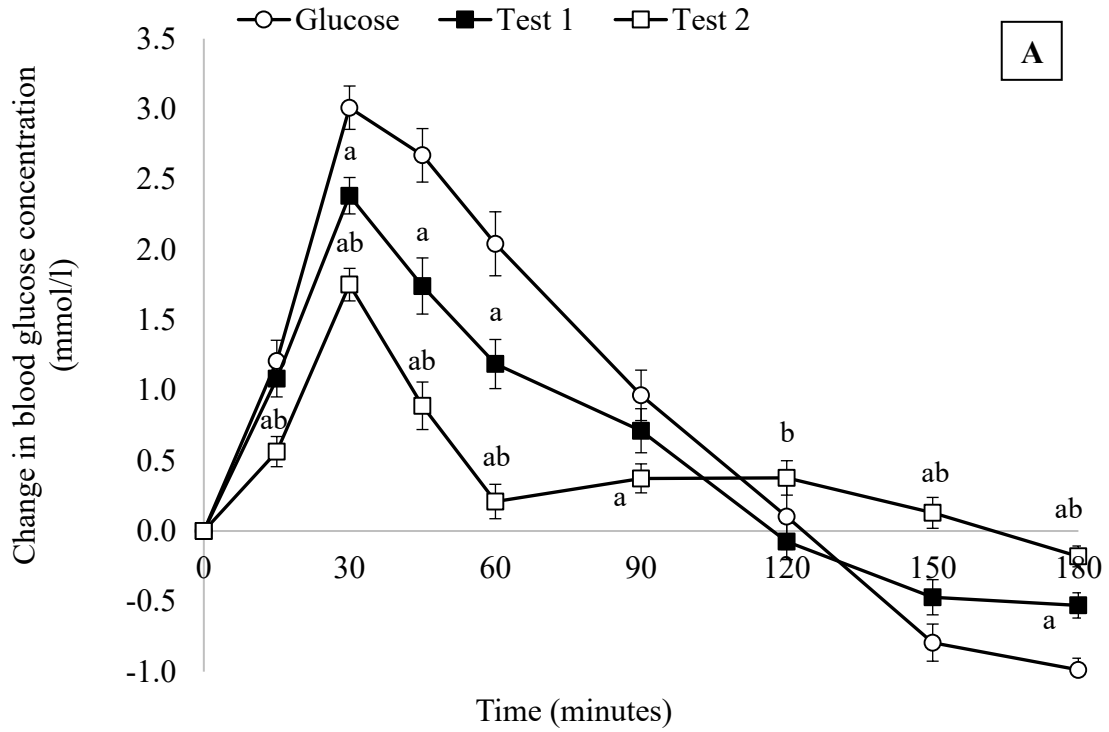
Table 3 Mean (± SD) peak value and time of peak for blood glucose, plasma insulin, plasma GIP and GLP-1 after consumption of 50 g glucose (Control), 50 g glucose + 25 g pea protein (Test 1) and 50 g glucose + 50 g pea protein (Test 2)

	Control	Test 1	Test 2	*P* value
Blood glucose				
Peak (mmol/l)	7.9 ± 0.8	7.0 ± 0.7[a]	6.3 ± 0.6[a,b]	**<0.001***
Time of peak (min)	39.7 ± 18.8	31.9 ± 9.3	37.7 ± 26.8	**0.037***
Plasma insulin				
Peak (μU/ml)	69.7 ± 26.3	86.7 ± 26.9[a]	93.1 ± 37.5[a]	**<0.001***
Time of peak (min)	34.8 ± 10.5	37.7 ± 11.5	32.9 ± 8.1	0.147
Plasma GIP				
Peak (pg/ml)	143.0 ± 10.4	125.4 ±14.0	106.4 ± 12.8[a]	**0.009***
Time of peak (min)	91.0 ± 10.2	89.0 ± 9.8	80.3 ± 10.9	0.976
Plasma GLP-1				
Peak (pg/ml)	742.6 ± 364.2	2986.6 ± 1587.0[a]	3750.3 ± 1998.9[a]	**0.026***
Time of peak (min)	12.6 ± 5.0	51.3 ± 11.4 [a]	55.2 ± 11.8 [a]	**0.013***

*Statistically significant difference ($p<0.05$)

[a] Significantly different from Control (repeated measures ANOVA: p<0.05; Friedman test: p<0.017)

[b] Significantly different from Test 1 (repeated measures ANOVA: p<0.05; Friedman test: p<0.017)



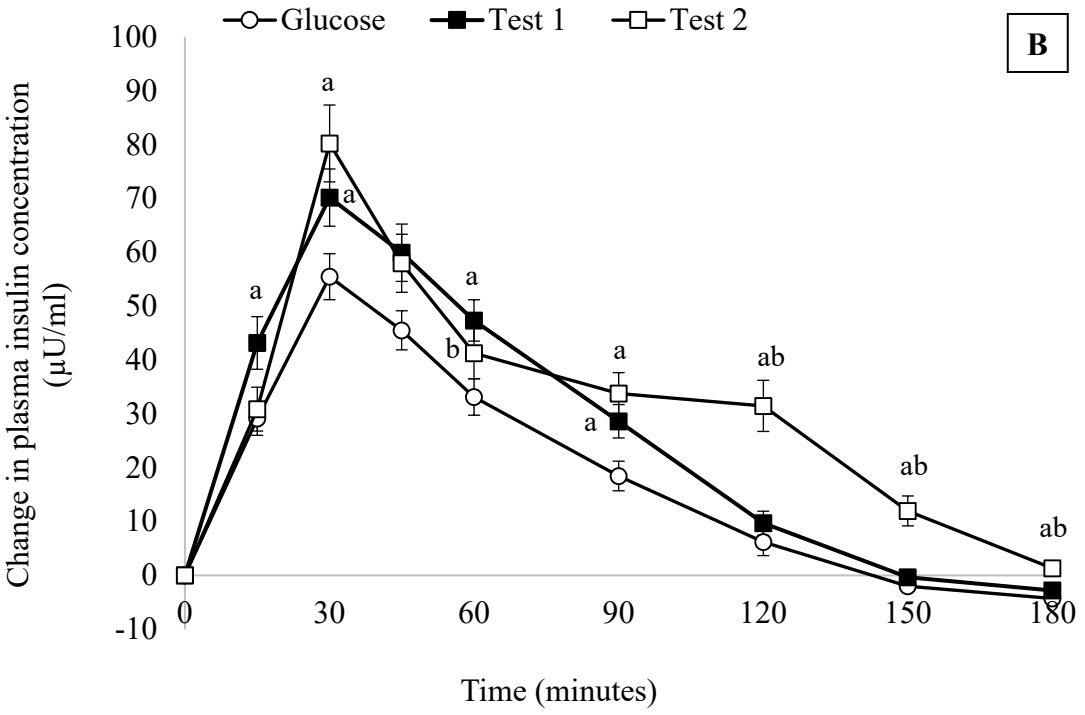
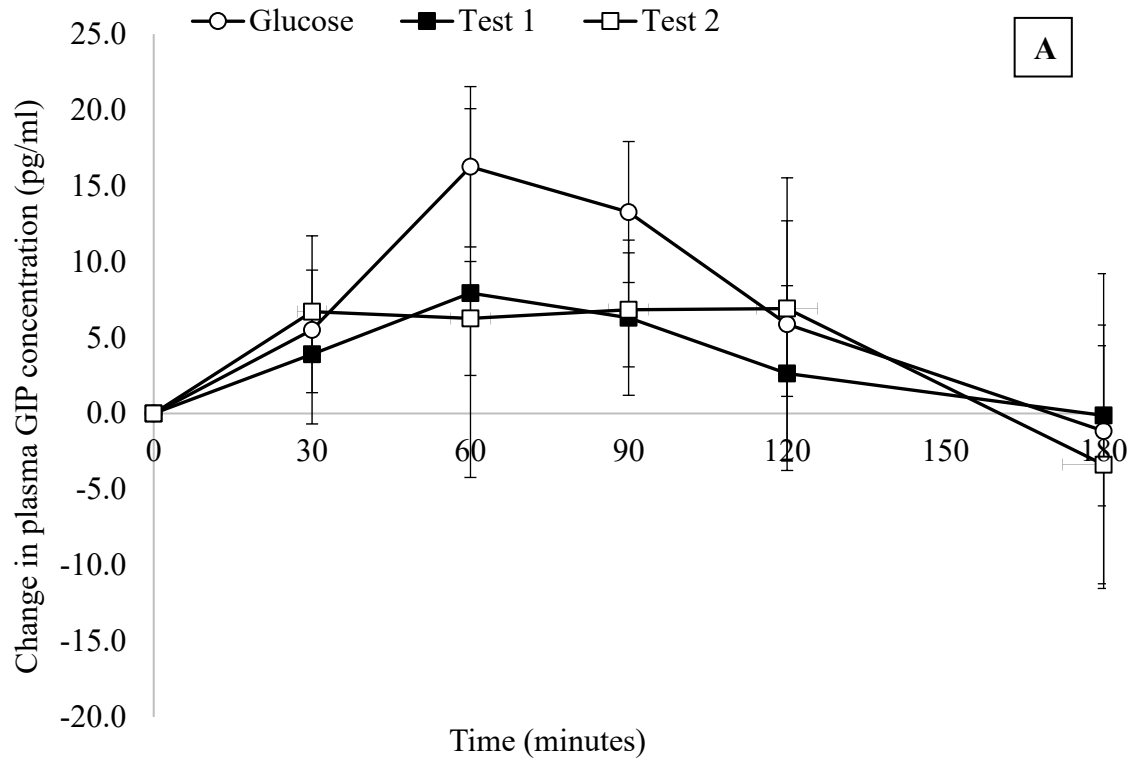


Fig. 1 Glycaemic (A) and insulinaemic (B) response curves for glucose, Test 1 (glucose + 25 g NUTRALYS® S85 Plus pea protein), and Test 2 (glucose + 50 g NUTRALYS® S85 Plus pea protein) Data are presented as mean and SEM (*n* 31). [a] Significantly different from Control (repeated measures ANOVA: $p<0.05$; Friedman test: $p<0.017$); [b] Significantly different from Test 1 (repeated measures ANOVA: $p<0.05$; Friedman test: $p<0.017$)



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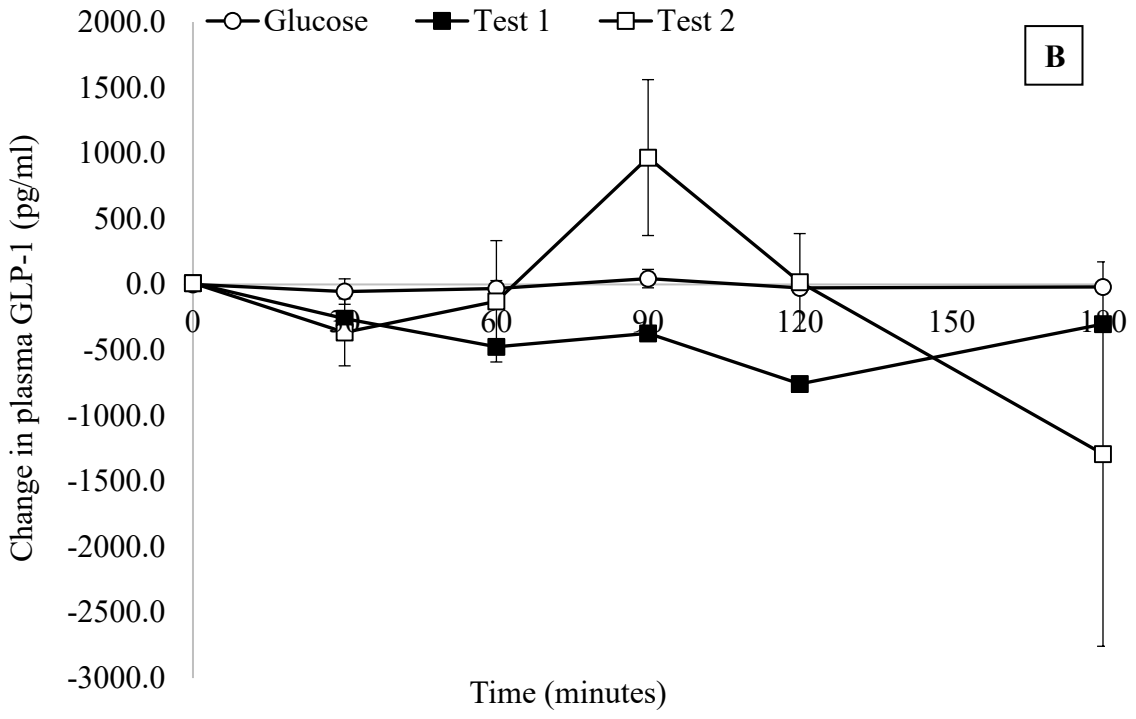

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508 **Fig. 2** Plasma GIP (A) and plasma GLP-1 (B) response curves for glucose, Test 1 (glucose +
509 25 g NUTRALYS® S85 Plus pea protein), and Test 2 (glucose + 50 g NUTRALYS®
510 S85 Plus pea protein) Data are presented as mean and SEM (*n* 31)

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