Clinical Nutrition Experimental 28 (2019) 111–122

Contents lists available at ScienceDirect

Clinical Nutrition Experimental

journal homepage: http://www.clinicalnutritionexperimental.com

Original Article

Effect of high-protein breakfast meal on within-day appetite hormones: Peptide YY, glucagon like peptide-1 in adults

Hadeel Ali Ghazzawi*, Samar Mustafa

Department of Nutrition and Food Technology, Faculty of Agriculture, The University of Jordan, Amman, 11942, Jordan

ARTICLE INFO

Article history:
Received 14 May 2019
Accepted 8 September 2019
Available online 13 September 2019

Keywords:
Appetite
Hunger
Breakfast
Satiety hormone
High protein meal
High carbohydrate meal

SUMMARY

Background: This study was carried out to investigate the effect of a single high protein breakfast on appetite related hormones (Peptide YY (PYY), and Glucagon-like Peptide-1 (GLP-1)) and perceived appetite sensation among adults. Many studies showed that dietary protein is the most satiating macronutrient, since protein may affect appetite by its involvement with appetite regulating hormones.

Methods: Within-subject crossover design was used, where participants received one of two assigned breakfasts on two different mornings: the first breakfast were a high protein (HP) breakfast that contained (51% protein, 13% CHO, 36% fat), while the second one had a high carbohydrates (HC) breakfast (10% protein, 60% CHO, 30% fat).

Results: The findings showed that PYY levels were significantly higher at ($P = 0.05$) significance level after the HP breakfast than the HC breakfast at 120 min. It also showed that there was an increase in postprandial GLP-1 concentration tended to be greater at significance level ($P < 0.01$) after the HP breakfast than after the HC one at 30, 60, and 120 min. The findings also showed that females had significantly higher GLP-1 levels than men following the HC breakfast, and satiety Visual Analog scales (VAS) scores were significantly higher after the HP breakfast than after the HC one at 120 min.

* Corresponding author. Fax: +962 6 5355 500.
E-mail address: h.ghazzawi@ju.edu.jo (H.A. Ghazzawi).

https://doi.org/10.1016/j.yclnex.2019.09.002

Conclusion: A single HP breakfast of 51% had a greater effect on satiety rather than 10% of energy from protein, meanwhile this effect does not apply to all satiety hormones.

1. Introduction

In the gastrointestinal (GI) tract, there are several signals that control food intake, hunger and satiety, these signals include releasing peptides into circulation before and after food intake. According to the findings of previous studies, these peptides communicate with feeding centers in brain directly or via vagal nerve signaling [1]. The releasing rates of these peptides, and its concentration varies, depending on the composition of the food ingested [1]. Studies also showed that dietary protein is the most satiating macronutrient [2]. It is believed that protein may affect appetite by its involvement with appetite regulating hormones: Cholecystokinin, Ghrelin, Peptide YY, and Glucagon – like peptide-1 and others [2,3]. Several studies found an inverse relationship between body mass index (BMI) and breakfast frequency [4]. Adding to that, cross-sectional studies showed that people who skip breakfast tend to consume higher calories that day, and they tend to have snack more on unhealthy foods and beverages. Breakfast composition has been studied to observe if it influenced appetite and total food intake that day [5,6].

It is known that most Jordanians eat unhealthy snacks during the day mostly high fat snacks [7].

Peptide YY is secreted by the L-cells of the GI tract, and is distributed throughout the gut, levels of PYY immunoreactivity are low in the proximal small intestine, but increase in the ileum, and continue to rise in the large intestine towards the rectum [3]. Studies of PYY actions initially focused on its local effects within the alimentary tract; it delays gastric emptying, and has an inhibitory effect on gall-bladder emptying [8,9].

Glucagon-like peptide-1 (GLP-1) is produced in the intestinal epithelial endocrine L-cells [10,11], its main functions are to stimulate insulin secretion and to inhibit glucagon secretion. GLP-1 derived from proglucagon gene and released from gut-endocrine L-cells, and it has been found that GLP-1 plays a role in decreasing food intake in rodents as well as in humans [12]. GLP-1 inhibits GImotility and secretion, inhibit gastric acid secretion, and gastric emptying, therefore, it appears to be a regulator of appetite and food intake [8,13].

Several studies suggested that changes in the meal composition can influence the release of GI hormones [2,8], since diets with high protein levels elevate circulating PYY and enhance satiety more effectively than other macronutrients. However, previous data suggested that, at a single meal, higher plasma concentrations of PYY were stimulated by high-fat meals, compared with protein or carbohydrate [2]. Several studies showed reduced postprandial GLP-1 release in severely obese subjects, which normalizes with weight loss, while others could not prove that [2,4,8].

A few investigations recommended that adjustments in the meal composition can impact the arrival of GI hormones [2,8], since eating regimens with high protein levels lift flowing PYY and upgrade satiety more adequately than different macronutrients. In any case, past information proposed that, at a single meal, higher plasma groupings of PYY were animated by high-fat meals, contrasted with protein or sugar [2]. A few examinations demonstrated diminished postprandial GLP-1 discharge in seriously obese subjects, which standardizes with weight reduction, while others couldn't demonstrate that [2,4,8].

2. Methodology

2.1. Participants

Thirty healthy, non-smokers participants (12 men and 18 women) aged between 19 and 50 years were recruited using poster advertisement, social media, and word of mouth. Participants met the inclusion criteria: age between 19 and 50, non-smoker, light active (light exercise 1–3 times/week), do not have any chronic disease, not taking any medication or supplements). Participants were informed about the purpose and the protocol of the study, and a written consent was obtained from each participant.

2.2. Study design

The study used within-subject crossover design. Participants visited the University of Jordan students' clinic on two mornings, separated by seven days. They were served a high protein breakfast (egg white, low fat thick yogurt, low fat turkey, cucumber, and an apple) on the first morning, and a high carbohydrates (a dough pie topped with thyme and olive oil, cucumber, and apple) breakfast at the second morning. On the first morning of their visits, weight and height were measured while the participants were in fasted state.

For each testing day, participants attended the students' clinic in the University of Jordan after an overnight fast (10–12 h). Fasting appetite ratings and blood samples were obtained by a trained health care professional (medical laboratory technician) upon arrival (zero time). Each participant was served individually with the testing meal to consume within 20 min. Blood samples were collected after 30, 60, and 120 min of finishing their meal. During this time, the participants stayed in a stress-free room where they were comfortable. Fig. 1 shows study protocol on testing days. Participants were asked to rate their perceptions of hunger. Appetite sensation was measured using validated visual analog scale (VAS) questionnaire, which provides a quantifiable objective measure translated from subjective

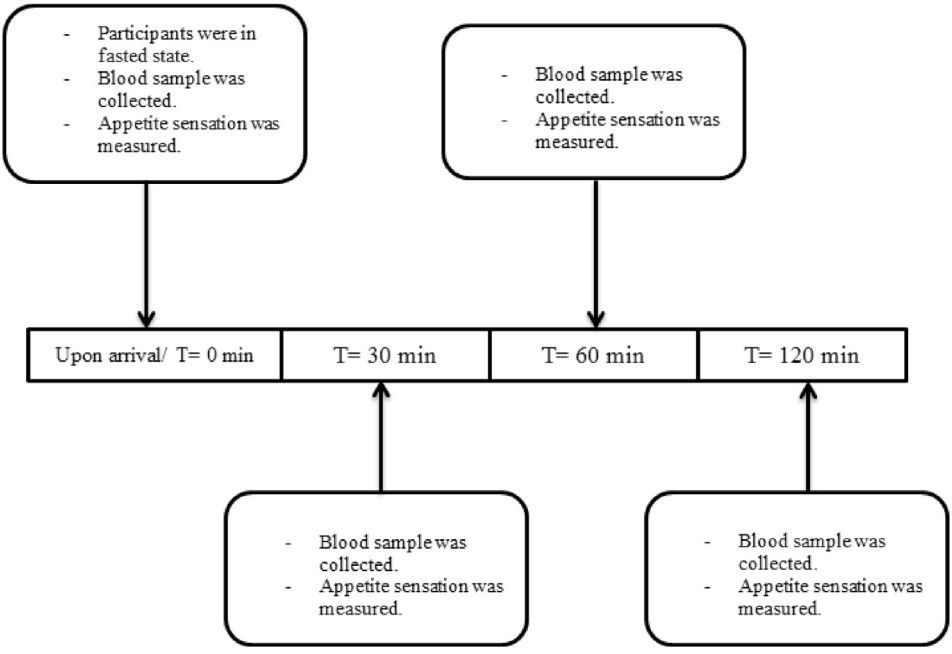

Fig. 1. Diagram of study protocol.

sensations. VAS is self-report methodology to assess subjective appetite sensation [14]. Each VAS consists of a 100 mm horizontal line, where 0 mm represents sensation not felt at all, while 100 mm sensation felt as the greatest. Participants were asked to place a vertical mark on the 100 mm line to indicate how they feel at testing time (0, 30, 60, and 120 min after meal consumption) [15].

2.3. Tested meals

The tested meals were breakfast meals, these meals were based on 2000 calories diet, it represent approximately 20% of total 2000 calories (400 calories), where the first one has a high protein meal (approximately 51% protein, 13% CHO, 36% fat), while the other one has normal protein meal (approximately 10% protein, 60% CHO, 30% fat). Table 1 shows detailed description of the tested meals' composition. Participants were asked to fast for 12 h before eating the testing breakfast meal. Physical activity factor was considered 1.37 (light activity) [16]; since many studies showed physical activity had a significant effect on appetite hormones levels [17,18].

2.4. Blood sample collection

Participants were asked to fast overnight (10–12 h), upon arrival (zero time), where at morning blood sample was obtained by a trained health care professional using 5 ml capacity syringes. Another three blood samples (5 ml each) were taken at 30, 60, 120 min after the completion of ingestion breakfast meal. Blood samples were collected in plain tubes containing separation gel and were left until coagulation; the blood serum was separated by centrifugation for 20 min at 1500 RPM. Serum was transferred into Eppendorf tubes and kept frozen at −20 °C for peptides' analysis according to the recommendations of [17,19] studies.

2.5. Biochemical analysis

All blood samples were analyzed for all four peptides cholecystokinin, peptide YY, glucagon like peptide-1, and ghrelin. Blood samples were analyzed at Al-Khaldi medical center laboratories by the researcher.

Human PYY Elisa kit (catalog no. MBS005003; Mybiosource, California, USA), Human GLP-1 Elisa kit (catalog no. MBS760336; Mybiosource, California, USA), Human Elisa kit (catalog no. MBS765132; Mybiosource, California, USA) were used in the analysis, where these kits were based on sandwich enzyme-linked immune-sorbent assay technology. Well plates were pre-coated with anti-hormone (PYY, and GLP-1) antibody. As a detection antibodies, biotin conjugated anti-hormone antibody was used.

The same procedure was applied for testing hormones: peptide YY, cholecystokinin, and glucagon like peptide-1.

Table 1
Detailed composition of the tested breakfasts.

Very high protein meal	Food item	Serving size	Calories	Protein (g)	CHO (g)	Fat (g)
	Egg white	4	70	14	0	1
	Thick yogurt	4 Tbsp	110	14	0	6
	Processed turkey (used in sandwiches) with 3 g of fat/oz	90 g	165	21	0	9
	Vegetables	1 cup	25	2	5	0
	Apple	½ medium	30	0	8	0
Total			**400**	**51**	**13**	**16**
High CHO meal	dough pie topped with thyme and olive oil	1 medium (110 g)	330	9	45	12
	Apple	1 medium	60	0	15	0
	vegetables	½ cup	13	1	3	0
Total			**403**	**10**	**63**	**12**

As the manual attached with the kit instructed, 25× wash buffer solution was prepared by adding 4 ml of 25× washing buffer to 96 ml of distilled water. 100 μl of sample was added into sample wells. The plate was sealed with a cover and incubated at 37 °C for 90 min. The plate content was discarded, and then the wells were washed with the wash buffer two times.

To each well 100 μl of Biotin-labeled antibody working solution was added. The plate was covered and incubated at 37 °C for 60 min, and washed 3 times with wash buffer. To each well 100 μl of HRP–streptavidin conjugate (SABC) working solution was added. The plate was covered and incubated at 37 °C for 30 min, and washed with the wash buffer 5 times. 90 μl of TMB Substrate was added to each well, and then the plate was covered and incubated at 37 °C for 15–30 min. The wells (not all) displayed blue color. 50 μl of stop solution was added into each well. Immediate changes were observed into yellow color, which means the tested hormone was detected. Then, the plate was put in Multiskan FC Microplate Elisa Reader and the absorbance was read at 450 nm.

To each well 50 μl of Standard or Sample were added, where 50 μl of Biotin-detection antibody working solution was added to each well. The plate was sealed with a cover and incubated at 37 °C for 45 min. The plate content was discarded, and then the wells were washed with the wash buffer for 3 times. 100 μl of HRP–Streptavidin Conjugate (SABC) working solution was added to each well. The plate was covered and incubated at 37 °C for 30 min. The wells were washed with the wash buffer for 5 times. 90 μl of TMB Substrate was added to each well, and then the plate was covered and incubated at 37 °C for 15–20 min. 50 μl of stop solution was added each well. The plate was put in Multiskan FC Microplate Elisa Reader and the absorbance was read at 450 nm.

To calculate each tested hormone concentration; standard curve was created by plotting the absorbance for each standard at the y-axis and the concentration at x-axis, a best fit curve was drawn and the best fitting equation was calculated using Microsoft Office Excel 2010.

2.6. Statistical analysis

Statistical Package for Social Science software (SPSS, version 22.0, 2013) was used to perform the statistical analysis. All results were presented as mean ± standard error of the mean (SEM). Repeated-measures ANOVA were used to evaluate VAS scores and hormones' concentration with time and treatment as factors. Independent-sample t-test was used to test if there were any statistical differences at ($P \leq 0.05$) statistical level in the magnitude of the response in hormones between males and females.

3. Results

3.1. Participants

A total of 30 healthy non-smokers participants were served with high protein breakfast on one morning, and high carbohydrate breakfast on the second morning, separated by 7 days washout period including: 18 females and 12 males. As Table 2 shows, total mean age was 25.4 ± 7.6 years (mean age for female is 24.28, and male is 27.08, at P-value = 0.192), and mean of BMI was 25.7 ± 0.9 kg/m^2. All participants were not on a specific diet, did not exercise regularly, did not take any medication, and they did not have any allergies. Four participants (13.3%) skipped breakfast usually in their daily life, ten

Table 2
Mean (± SEM) of age and anthropometric measurements of the study participants.

	Female (18)	Male (12)	Total participants (30)
Age (years)	24.28 ± 5.1	27.08 ± 10.2	25.4 ± 7.6
Weight (kg)	67.5 ± 3.2	79.5 ± 3.9	72.3 ± 2.7
Height (cm)	162.7 ± 1.5	174.7 ± 1.5	167.5 ± 1.5
BMI (kg/m^2)	25.6 ± 1.3	26.0 ± 1.2	25.7 + 0.9

Table 3
Mean concentration (± SEM) of measured hormones before and after high protein and high carbohydrate breakfasts.

Hormone	Type of diet	HP				P-value*	HC				P-value*
	Time (min)	0	30	60	120		0	30	60	120	
PYY (pg/ml)	Female	30.99 ± 0.43	32.59 ± 1.28	41.34 ± 7.23	37.34 ± 2.35	**0.016**	31.45 ± 0.38	31.3 ± 0.32	32.06 ± 0.76	34.76 ± 2.81	0.257
	Male	32.36 ± 0.68	37.5 ± 3.21	35.98 ± 2.65	44.66 ± 4.6	**0.025**	31.88 ± 0.68	31.79 ± 0.83	32.84 ± 1.36	32.8 ± 1.38	0.820
	Total	31.54 ± 0.39	34.55 ± 1.53	39.2 ± 4.44	40.27 ± 2.37	**0.001**	31.62 ± 0.35	31.49 ± 0.38	32.37 ± 0.7	33.98 ± 1.76	0.187
GLP (ng/ml)	Female	14.25 ± 1.59	17.9 ± 1.5	18.19 ± 1.36	18.61 ± 1.37	**0.016**	14.49 ± 1.6	12.21 ± 1.75	13.58 ± 1.73	11.38 ± 1.91	**0.028**
	Male	8.74 ± 1.66	14.71 ± 1.24	14.79 ± 1.57	14.42 ± 1.54	**0.011**	8.09 ± 1.74	6.46 ± 1.23	6.37 ± 1.21	6.15 ± 1.08	0.275
	Total	12.04 ± 1.25	16.62 ± 1.05	16.83 ± 1.06	16.93 ± 1.08	**0.000**	11.93 ± 1.3	9.91 ± 1.26	10.7 ± 1.31	9.29 ± 1.3	**0.014**

Bold values represents the significance of P-values.

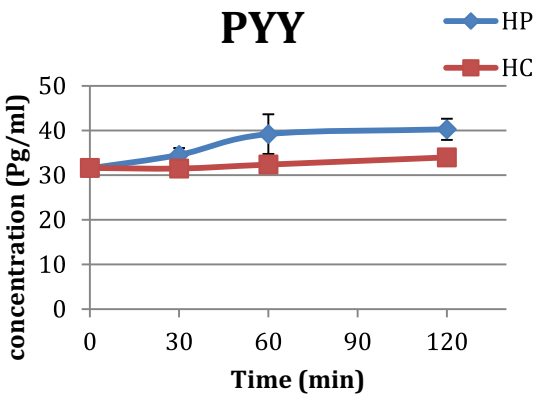

Fig. 2. Mean serum of peptide YY (PYY) response for high protein (HP) and high carbohydrate (HC) breakfast meal. High protein meal shows significantly differences through out the time line (P value = 0.001). For HP meals the standards deviations were: SD at 0 times = ±0.39; SD at 30 min = ±1.53; SD at 60 min = ±4.44; SD at 120 min = ±2.37. While for HC breakfast the SDs were SD at 0 times = ±0.35; SD at 30 min = ±0.38; SD at 60 min = ±0.7; SD at 120 min = ±1.76.

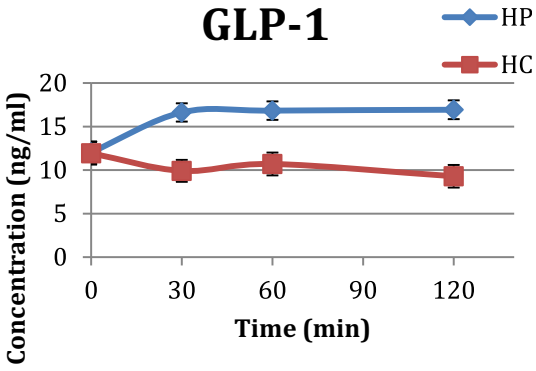

Fig. 3. Mean serum of Glucagon like peptide-1 (GLP-1) response for high protein (HP) and high carbohydrate (HC) breakfast meal. High protein meal shows significantly differences through out the time line (P value = 0.00). For the HP breakfast the SD at 0 times = ±1.25; SD at 30 min = ±1.05; SD at 60 min = ±1.06; SD at 120 min = ±1.08. While for HC breakfast the SDs were: SD at 0 times = ±1.3; SD at 30 min = ±1.26; SD at 60 min = ±1.31; SD at 120 min = ±1.3.

participants (33.3%) used to eat breakfast on daily basis, eleven participants (36.7%) used to eat breakfast 4–6 days/week, and five of them (16.7%) used to eat breakfast 1–3 days/week.

3.2. Appetite hormones

There were no significant difference in the hormones' levels at the fasting state (0 min) between high carbohydrate and high protein breakfasts at ($P \leq 0.05$). Table 3 shows Mean concentrations of hormones measured before and after high protein and high carbohydrate breakfasts intake.

For peptide YY, Fig. 2 showed that there was a significant difference only at 120 min between HP and HC breakfasts at ($P \leq 0.05$) significance level. PYY levels rose at 30, 60, and 120 min for both tested meals, and PYY levels were higher after at all postprandial measuring points following the HP breakfast when compared to HC breakfast.

The effect of meal composition was not significant at 30, 60 min measuring points at ($P \leq 0.05$) significance level. Meanwhile, PYY levels rose at 30, 60, and 120 min to both test meals, HP breakfast

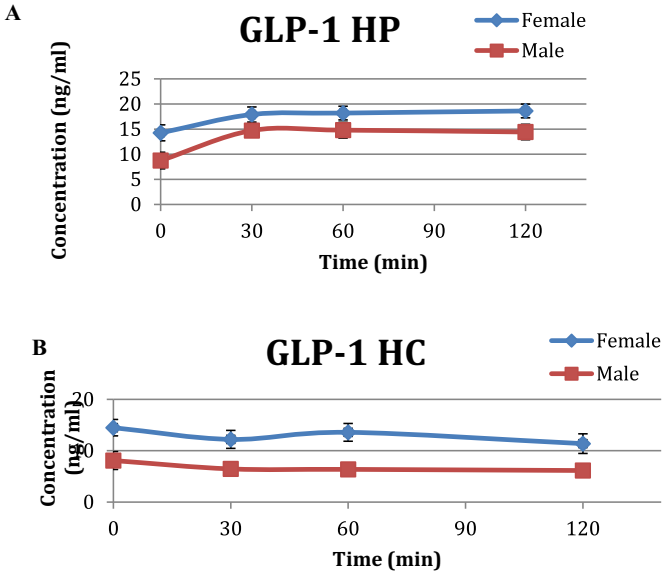

Fig. 4. (A) Mean serum of Glucagon like peptide-1 (GLP-1) response after high protein breakfast for males and females. For the HP breakfast the SD among females at 0 times = ±1.59; SD at 30 min = ±1.5; SD at 60 min = ±1.36; SD at 120 min = ±1.37. While for males the SDs were at 0 times = ±1.66; SD at 30 min = ±1.24; SD at 60 min = ±1.57; SD at 120 min = ±1.54. (B) Mean serum of Glucagon like peptide-1 (GLP-1) response after high carbohydrates breakfast for males and females. For the HC breakfast the SD among females at 0 times = ±1.6; SD at 30 min = ±1.75; SD at 60 min = ±1.73; SD at 120 min = ±1.91. While for males the SDs were at 0 times = ±1.74; SD at 30 min = ±1.23; SD at 60 min = ±1.21; SD at 120 min = ±1.08.

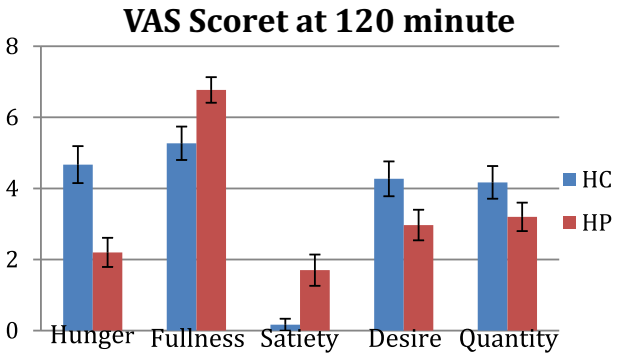

Fig. 5. mean VAS scores at 120 min for both breakfasts high protein (HP) and high carbohydrates (HC).

had greater rising effect on PYY levels at all postprandial measuring points when compared with HC. At 120 min there were significant differences in PYY levels between HP and HC breakfasts at ($P \leq 0.05$) significance level. It also found that there were significant differences in PYY levels between 0 min and 120 min following HP breakfast at (P-value = 0.01), meanwhile there were no significant differences between 0 min and 120 min following HC breakfast at (P-value = 0.187).

For GLP-1, the effect of meal composition was statistically significant at all measuring points after both HP and HC ($P \leq 0.05$) significance level. Fig. 3 showed that there were a significant difference in GLP-1 levels between 0 min and 120 min following both HP and HC breakfasts at ($P \leq 0.01$ and

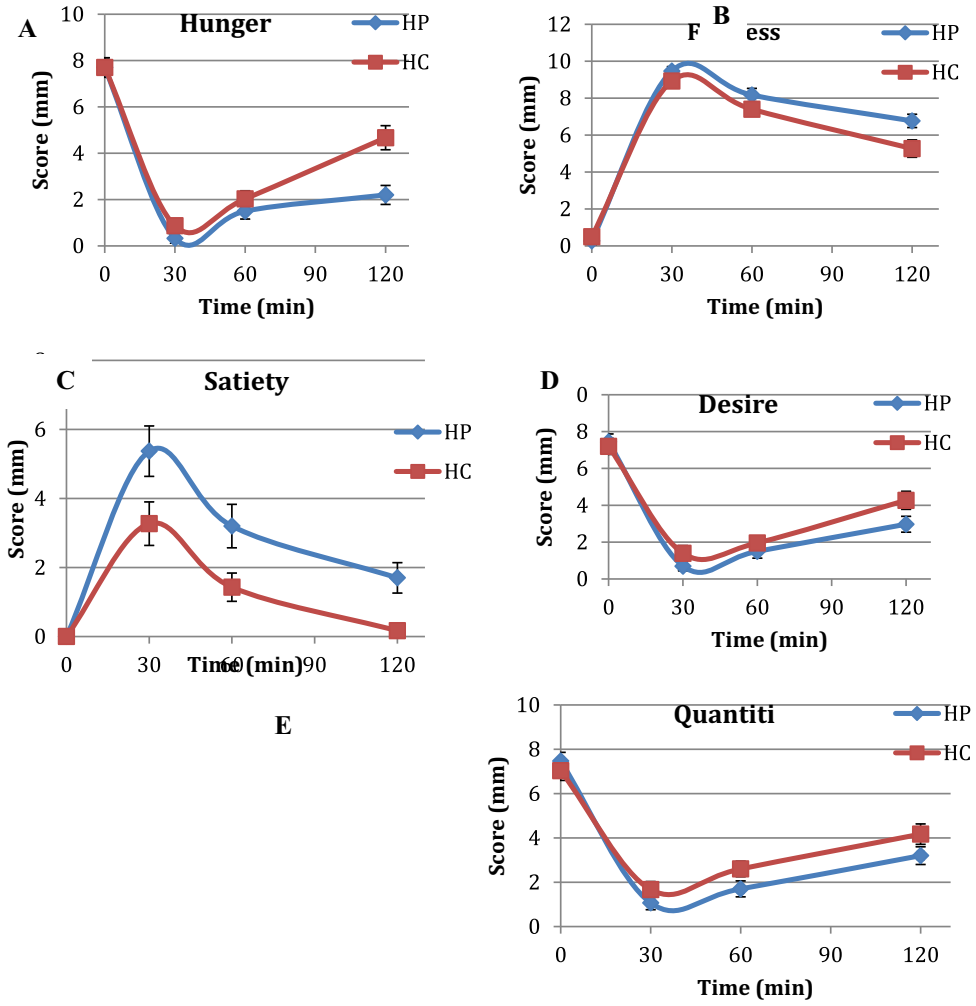

Fig. 6. Scores (mean ± SEM) for hunger, fullness, satiety, desire to eat, and quantity following ingestion of test meals either high protein or high carbohydrates. (A) Participants reported less hunger after HP meal with $P < 0.05$ at 120 min between HC and HP. (B) Fullness was greater after HP meal with $P < 0.05$ at 120 min between HC and HP. (C) Satiety was greater after HP meal at all postprandial measuring points with $P < 0.05$ at 120 min between HC and HP. (D) Desire to eat decreased directly after ingestion of both meals, it started to rise 60 min postprandial with higher desire after HC, with $P < 0.05$ at 120 min between HC and HP. (E) Quantity participants were able to eat after both meals was nothing at 30 min, increased slightly at 60, and 120 min with higher amount after HC meal $P < 0.05$ at 120 min between HC and HP.

$P \leq 0.05$) significance levels in sequence. HP meal had a rising effect on GLP-1 levels at all measuring points, but HC meal had a suppressive effect at 30 min measuring point, then a rising effect at 60 min. At 120 min GLP-1 levels dropped again. It also found that there were a significant difference at ($P \leq 0.05$) significance levels between males and females in GLP-1 levels after HC breakfast, while there were no significant differences at ($P \leq 0.05$) significance levels between males and females in GLP-1 levels after HP breakfast. It was also found that females had higher concentration levels of GLP-1 in both cases than males (See Fig. 4A and B).

3.3. Subjective appetite sensation

There were no significant differences at ($P > 0.05$) significance level between meal composition and subjective appetite ratings (hunger, fullness, satiety, desire to eat, and prospective consumption) at: baseline (0 min), 30 min and 60 min. And there were significant differences at 120 min for all (hunger, fullness, satiety, desire to eat, and prospective consumption) at ($P \leq 0.05$) significance level between HP and HC. Fig. 5 shows VAS scores at 120 min postprandial for both HP and HC, where participants reported an increase in their hunger and desire to eat, less fullness and no satiety on HC day than on HP day at all postprandial measuring points (See Fig. 6).

4. Discussion

This study aimed at observing the acute effect of macronutrients consumed at breakfast on appetite hormones and perceived appetite sensation. In general different studies had showed that high-protein diets support the maintenance of lean body mass and promote weight loss. Protein diet has been found to reduce risks of cardiovascular disease in obese subjects [20,21]. Weight management could be easier if appetite and satiety are controlled or regulated, where several studies showed that high protein breakfast may lead to a reduction in total energy intake, decreases hunger and desire to eat, and reduces body weight more than normal-protein breakfast [4,22]. Literature reviewed involvement of protein and specific amino acids in the regulation of appetite over the years through different mechanisms, from taste to action in the brain. There was no specific known mechanism to control the secretion and release of appetite hormones [19].

Most studies that diagnosed the effect of HP meal on satiety and gut hormones were conducted on a long-term period, where participants consumed the tested meals for several days, weeks or even months. Meanwhile, this study observed the immediate effect of one breakfast meal for 2 h postprandial. In this study, consumption of breakfast led to increase in perceived fullness and reduction in perceived hunger, desire to eat and prospective food consumption. Furthermore, there was a postprandial increase in PYY, GLP-1 levels. While, HP breakfast led to greater reduction in perceived hunger, and desire to eat, this reduction lasted longer on HP day than HC day, and so did the increase in perceived fullness.

Belza et al. (2013) tested HP meal, provided 50% of energy from protein [8]. However, this study shared similar results with Belza et al. (2013) that indicated an increase in PYY levels postprandial of HP and HC [8]. This would support that PYY is released in response to the ingestion of macronutrients, where some studies [3,19] proclaimed that greater release happens to protein ingestion, followed by carbohydrates, and fat came last. While Gibbons et al. (2013) claimed the greater response of PYY release is to fat [13]. Same as Brennan et al. (2012) findings that showed no significant differences between HP and HC at 30 and 60 min, however, PYY levels were higher after the HP meal at all postprandial measuring points [23]. These differences may be contributed to the fact that the percentage of protein (51%) in the meal was not as high as meals presented in the other studies; where at Klaauw et al. (2013) study, HP meal had 60% protein. This study showed significant differences at 120 min between HP and HC in PYY levels [3]. These differences could be attributed to Batterham et al. (2006) and Lobley et al. (2015) findings which showed that protein has lower rate of digestion and absorption along the digestive tract, which would fit with the theory that PYY release continue for several hours after ingestion of a meal [19,25]. One possible explanation is that the initial elevation in PYY levels may be related to the rapid absorption of macronutrients, such as digestible carbohydrate, while the second rise may involve more slowly absorbed nutrients, like protein.

GLP-1 is a satiety hormone; its secretion pattern is similar to that of PYY [24]. GLP-1 was found to delay gastric emptying, and therefore decrease food intake [26]. In agreement with Belza et al. (2013) and Klaauw et al. (2013) [3,8] findings, the present study showed significant increase in GLP-1 levels after the HP breakfast, and there were significant differences in GLP-1 levels between HP and HC. This result suggests that protein stimulation of GLP-1 secretion, therefore its effect on reducing gastric emptying rate. The decline in GLP-1 levels after HC breakfast in this study contradicted with the published literature; where several studies showed elevation in GLP-1 levels after meal ingestion specifically by carbohydrates [27,28]. This response in GLP-1 concentration might be related to the

effect of peripheral GLP-1 on satiety, and may be influenced by the central sensitivity for GLP-1, or interactions with other hormones.

This study showed significant differences in GLP-1 levels between males and females after the HC breakfast; where females had higher GLP-1 levels. It was noticed that females also had higher levels of GLP-1 than males after HP, but these differences were not statistically significant. Minimum number of the conducted research studied gender as a factor, such as Vaag et al. (1996) and Adam and Westerterp-Plantenga (2005), reported that gender had great impact on GLP-1 levels, with higher levels in females compared to males [29,30]. The explanation for this is unclear, one explanation could be related to the possibility of delayed gastric emptying and/or intestinal motility in females compared with males. Other explanation could be partly due to the relationship between body-fat mass and GLP-1, since an indirect effect was mediated through neural pathways that increased satiety and reduced gastric emptying [31].

4.1. Limitations

One meal would not have a strong effect on the whole day satiety, hence it is recommended to study the whole day hormones concentration after a specific meal. In addition; few days (few breakfasts) trail affect would also be a suggestion for further studies. Recruiting participants is one issue to consider in this kind of research due to the restricted requirement the study demanded. Some participants were reluctant to come back because of the invasive blood collection the study required, and the inconvenience of spending 3–4 h in the clinic. The study required participants to come on week-days because the clinic was closed on weekends, which was inconvenient for some of them.

5. Conclusion

In conclusion, a single HP breakfast of 51% of total energy from protein rather than 10% of energy from protein has a greater effect on satiety. The effects of a single HP meal in the postprandial state are mediated by increased plasma GLP-1 and PYY concentrations. Other factors, which may explain the HP effect on satiety, may be other metabolites. Males and females presented different concentrations of GLP-1 following HC meal.

Ethical Issues

Participation in the study was completely voluntary, since participants were fully informed about the purpose of the study, and how their collected data will be used. They also had been given the opportunity to ask questions in order to increase their comfort level. This study was approved by Deanship of Academic Research and Quality Assurance in the University of Jordan, and was conducted under the supervision of a licensed and approved by the University of Jordan Hospital physician in the students' clinic in the University of Jordan. Institutional Review Board (IRB) was obtained from the ethics committee in the Jordan University Hospital (1782/2017/49). Information collected from participants was confidential and was only used to serve the objective of this study.

Conflict of interest

None.

References

[1] Blundell J, De Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al. Appetite control: methodological aspects of the evaluation of foods. Obes Rev 2010;11(3):251–70.
[2] Crowder CM, Neumann BL, Baum JI. Breakfast protein source does not influence postprandial appetite response and food intake in normal weight and overweight young women. J Nutr Metab 2016;2016. https://doi.org/10.1155/2016/6265789.
[3] Klaauw AA, Keogh JM, Henning E, Trowse VM, Dhillo WS, Ghatei MA, et al. High protein intake stimulates postprandial GLP1 and PYY release. Obesity 2013;21(8):1602–7.

[4] Neacsu M, Fyfe C, Horgan G, Johnstone AM. Appetite control and biomarkers of satiety with vegetarian (soy) and meat-based high-protein diets for weight loss in obese men: a randomized crossover trial. Am J Clin Nutr 2014;100(2):548–58.
[5] Buosi W, Bremner DM, Horgan GW, Fyfe CL, Johnstone AM. Effect of high-protein breakfast meals on within-day appetite and food intake in healthy men and women. Food Nutr Sci 2015;6(3):386.
[6] Rains TM, Leidy HJ, Sanoshy KD, Lawless AL, Maki KC. A randomized, controlled, crossover trial to assess the acute appetitive and metabolic effects of sausage and egg-based convenience breakfast meals in overweight premenopausal women. Nutr J 2015;14(1). https://doi.org/10.1186/s12937-015-0002-7.
[7] Bawadi H, Al-Hamdan Z, Ershidar O, Hammad F, Agraib L. Cultural eating practices among Jordanians. Food Nutr Sci 2012;3(6):790–5.
[8] Belza A, Ritz C, Sørensen MQ, Holst JJ, Rehfeld JF, Astrup A. Contribution of gastroenteropancreatic appetite hormones to protein-induced satiety. Am J Clin Nutr 2013;97(5):980–9.
[9] Chaudhri O, Small C, Bloom S. Gastrointestinal hormones regulating appetite. Philos Trans R Soc B Biol Sci 2006;361(1471):1187–209.
[10] Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold RE, et al. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Investig 1992;22(4):283–91.
[11] Wettergren A, Wøjdemann M, Meisner S, Stadil F, Holst JJ. The inhibitory effect of glucagon-like peptide-1 (GLP-1) 7-36 amide on gastric acid secretion in humans depends on an intact vagal innervation. Gut 1997;40(5):597–601.
[12] Badman M,K, Flier J,S. The gut and energy balance: visceral allies in the obesity wars. Science 2005;307(5717):1909–14.
[13] Gibbons C, Caudwell P, Finlayson G, Webb DL, Hellström PM, Näslund E, et al. Comparison of postprandial profiles of ghrelin, active GLP-1, and total PYY to meals varying in fat and carbohydrate and their association with hunger and the phases of satiety. J Clin Endocrinol Metab 2013;98(5):E847–55.
[14] Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes 2000;24(1):38–48.
[15] Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M, et al. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. Br J Nutr 2000;84(04):405–15.
[16] Gerrior S, Juan W, Peter B. An easy approach to calculating estimated energy requirements. Prev Chronic Dis 2006;3(4):A129.
[17] Hallworth JR, Copeland JL, Doan J, Hazell TJ. The effect of exercise intensity on total PYY and GLP-1 in healthy females: a Pilot study. J Nutr Metab 2017;2017. https://doi.org/10.1155/2017/4823102.
[18] Ueda SY, Miyamoto T, Nakahara H, Shishido T, Usui T, Katsura Y, et al. Effects of exercise training on gut hormone levels after a single bout of exercise in middle-aged Japanese women. Springerplus 2013;2(1):83. https://doi.org/10.1186/2193-1801-2-83.
[19] Lobley GE, Holtrop G, Horgan GW, Bremner DM, Fyfe C, Johnstone AM. Responses in gut hormones and hunger to diets with either high protein or a mixture of protein plus free amino acids supplied under weight-loss conditions. Br J Nutr 2015;113(08):1254–70.
[20] Anderson GH, Moore SE. Dietary proteins in the regulation of food intake and body weight in humans. J Nutr 2004;134(4):974S–9S.
[21] Layman D,K, Boileau R,A, Erickson D,J, Painter J,E, Shiue H, Sather C, et al. A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. J Nutr 2003;133(2):411–7.
[22] Leidy HJ, Racki EM. The addition of a protein-rich breakfast and its effects on acute appetite control and food intake in 'breakfast-skipping'adolescents. Int J Obes 2010;34(7):1125.
[23] Brennan I,M, Little T,J, Feltrin K,L, Smout A,J, Wishart J,M, Horowitz M, et al. Dose-dependent effects of cholecystokinin-8 on antropyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men. Am J Physiol Endocrinol Metab 2008;295(6):E1487–94.
[24] Feinle-Bisset C, Patterson M, Ghatei MA, Bloom SR, Horowitz M. Fat digestion is required for suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid. Am J Physiol Endocrinol Metab 2005;289(6):E948–53.
[25] Batterham R,L, Cohen M,A, Ellis S,M, Le Roux C,W, Withers D,J, Frost G,S, et al. Inhibition of food intake in obese subjects by peptide YY3–36. N Engl J Med 2003;349(10):941–8.
[26] Blom WA, Lluch A, Stafleu A, Vinoy S, Holst JJ, Schaafsma G, et al. Effect of a high-protein breakfast on the postprandial ghrelin response. Am J Clin Nutr 2006;83(2):211–20.
[27] Lejeune MP, Westerterp KR, Adam TC, Luscombe-Marsh ND, Westerterp-Plantenga MS. Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber–. Am J Clin Nutr 2006;83(1):89–94.
[28] Smeets AJ, Soenen S, Luscombe-Marsh ND, Ueland Ø, Westerterp-Plantenga MS. Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. J Nutr 2008;138(4):698–702.
[29] Vaag A,A, Holst J,J, Vølund A, Beck-Nielsen H. Gut incretin hormones in identical twins discordant for non-insulin-dependent diabetes mellitus (NIDDM) evidence for decreased glucagon-like peptide 1 secretion during oral glucose ingestion in NIDDM twins. Eur J Endocrinol 1996;135(4):425–32.
[30] Adam TC, Westerterp-Plantenga MS. Nutrient-stimulated GLP-1 release in normal-weight men and women. Horm Metab Res 2005;37(02):111–7.
[31] Stouwe J,G, Aeschbacher S, Krisai P, Schoen T, Meyre P, Todd J, et al. Plasma levels of glucagon-like peptide 1 and markers of obesity among young and healthy adults. Clin Endocrinol 2015;83(5):636–42.