Progress in Nutrition 2022; Vol. 24, N. 4: e2022132 DOI: 10.23751/pn.v24i4.13111

Original article

Influence of chilling-and-reheating pasta on postprandial glycemic responses and appetite: a randomized control trial

Maha H Alhussain[1], Areej A Almousa[2], Sehad N Alarifi[3], Sahar Abdulaziz AlSedairy[1], Abdulrhamn M. Alhowikan[3]

[1]Department of Food Science and Nutrition, College of Food and Agriculture Sciences, University of King Saud University, Riyadh, Saudi Arabia; [2]Department of Food and Nutrition Science, Al-Quwayiyah College of Sciences and Humanities, Shaqra Univirsity, Saudi Arabia; [3]Department of Physiology, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia

Abstract. *Background and aim:* Starch chilling-and-reheating process can affect starch structures and digestibility. Studies investigating the potential health implications of the chilling-and-reheating process of starchy food are lacking. We aimed to examine the influence of chilling-and-reheating pasta, a starchy food, on postprandial glycemic and insulinemic responses, and appetite. *Methods:* Eight healthy young males participated in this randomized cross-over study. Subjects were provided pasta meals in the laboratory after overnight fasting on two separate occasions, at least 1 week apart. On each laboratory visit, subjects were given either freshly cooked pasta, as a control meal (CM) or cooked, overnight chilled, and reheated pasta as a test meal (TM). Both CM and TM were isocaloric and matched for composition, ingredients, and amount. Blood samples and subjective appetite ratings were collected at fasting and for a period of 3-h after meal consumption. *Results:* TM significantly increased net incremental area under the curve (net iAUC) for satiety (mean ± SD: TM: 10181.25±3140.65 mm; CM: 7132.37±3187.31 mm; p = 0.03) and reduced the desire to eat (TM: −8190.75±4333.34 mm vs. CM: −4594.50±1481.11 mm; p = 0.03). However, no significant differences were found in postprandial glycemic and insulinemic responses between the two meals. *Conclusions*: Chilling-and-reheating pasta was associated with greater satiety and a lower desire to eat and, in a long term, may lead to weight management.

Key words: pasta; resistant starch; dietary fiber; glucose; insulin; appetite; clinical trial

Introduction

Carbohydrates make up a significant proportion of many diets, with starch being the major source of energy for humans. Starch is composed of linear amylose and branched amylopectin chains (1). Amylases are a group of enzymes that are responsible for the digestion of dietary starch. The mechanisms of the action of these enzymes have been explained elsewhere (2). Starch's susceptibility to amylolysis during digestion has a main effect on the postprandial glycemic and insulinemic response (3). Starches can be characterized on the basis of their digestive rate as follows: rapidly digestible starch, slowly digestible starch and undigestible resistant starch (4). From a health perspective, the starch form that is less susceptible to amylolysis is preferred, as it has a low effect on blood glucose. It has been demonstrated that increasing consumption of resistant starch is associated with an improved postprandial metabolic profile including blood glucose and insulin concentrations (1). The mechanism underlying this beneficial effect seems to be that resistant starch are not hydrolyzed in the small intestine and then passes to the colon, where it is fermented by microbes, to produce a range of short-chain fatty acids (acetate, propionate, butyrate, and valerate) and branched short-chain fatty

acids (isobutyrate and isovalerate) (5,6). It has been proposed that resistant starch provides physiological functions similar to those of dietary fiber (7).

There is a great interest in increasing native starches physicochemical properties and resistant features. The chemical structure and digestibility of native starch can be dramatically altered when it is exposed to different food processing including heating (cooking). Cooking starch (50–100°C) in an excess amount of water leads to gelatinization (8). Chilling of gelatinized starch is recognized to form retrograded starches, a type of resistant starch (RS3), which has lower enzyme susceptibility compared with freshly cooked starch (9,10). Chilled storage temperature (4 °C) has been shown to improve the degree of starch retrogradation in comparison to room temperature storage (25-30 °C) and frozen (18 °C) (11).

Manipulating food processing (i.e., chilling-and-reheating process) that could improve metabolic biomarkers is of great importance for the promotion of public health. Pasta is a widely consumed food product in many countries including Saudi Arabia. Few studies have investigated the potential health implications of chilling-and-reheating pasta and revealed mixed findings. A recent study showed that postprandial glycemic response was significantly lower in the cooked and reheated pasta compared with the hot condition (12). On the other hand, another study reported no significant differences between postprandial glycemic responses to freshly cooked pasta compared with chilled and reheated pasta (13). The effect of chilling-and-reheating pasta on appetite has yet to be investigated. More studies are still needed to elucidate the effects of chilling-and-reheating pasta on health. Therefore, the present study aimed to confirm the effects of chilling-and-reheating of pasta meal, a starchy food, on glycemic and insulinemic responses among healthy adult males. The effects of chilling-and-reheating of pasta meals on subjective appetite and ghrelin were also investigated.

Materials and methods

Subjects

The study was conducted at a human studies laboratory in the Physiology Department, King Khalid University Hospital, College of Medicine Riyadh, Saudi Arabia. Data were collected between November 2019 and March 2020. A total of eight subjects were recruited through an advertisement that was distributed online through social media platforms.

The inclusion criteria were as follows: healthy males (females were not included in order to avoid possible influences of the menstrual cycle on study outcomes), aged 18-35 years, normal weight (BMI 18.5–24.9 kg/m^2), no history of serious disease or currently taking any medications, not dieting or seeking to lose weight. The exclusion criteria included: smokers, allergy to gluten-containing products, lactose intolerance or dairy allergy or a particular dislike to any of the foods provided during the study.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Institutional Review Board at King Saud University (Ref. No. 19/01008 / IRB). Signed consent forms were obtained from all subjects after the study protocol had been explained to them in writing and orally at the screening visit. The study protocol was registered on ClinicalTrials.gov with the registration number NCT05108142.

Screening visit

Before commencing the study, all study subjects attended a screening visit to provide informed consent and to establish that they met the study inclusion criteria. During the screening visit, height and weight were measured following standard procedures (e.g., without shoes, and wearing light clothes). BMI was calculated using the standard formula [weight (kg)/height (m^2)].

Study design

This study was a single-blind, randomized, crossover study. Subjects were asked to attend a laboratory visit on two separate occasions, at least 1 week apart. Subjects were randomly assigned to begin the study with either the CM visit or the TM visit. The randomization scheme was carried out using the Second Generator Plan from randomization.com before the start of the study. On each visit, the subjects arrived

at the laboratory following an overnight fast (>10 h). Subjects were instructed to consume an identical evening meal on the day before the laboratory visits to exclude any effect of the evening meal on metabolic biomarkers (e.g., blood glucose and insulin) at these visits.

The two meals, (CM and TM) consisted of the same ingredients but were prepared in different ways. CM consisted of hot, freshly cooked pasta, and TM consisted of reheated pasta that had been cooked the previous day and chilled overnight at 4°C. Subjects were instructed to consume the provided meal over a period of 10 min and kept to the same start and finish times on both visits. Water consumption during the laboratory visit was allowed, but the total volume of water consumed was recorded and repeated on the second visit for consistency. Blood samples were obtained from each subject at the fasted state and during the next 3 h of meal consumption. Subjective appetite was also assessed using a visual analogue scale (VAS) both before the meal and for 3 h following the meal consumption.

Study meals

Meals were isocaloric, providing 954 kJ/100 g with 14%, 33%, and 53% of the energy provided by protein, fat, and carbohydrate, respectively. One portion of each meal contained dried pasta (125 g; Goody®), cheddar cheese (40 g; Forsan®), olive oil (8 g; Freshly®) and tomato pasta sauce with cream (170 g; Puck®).

On the day of the laboratory visit, CM was cooked in 800 ml boiling water in a full-power 900 W microwave for 13 min and stirred midway through the cooking period. The pasta was drained, then mixed with the other ingredients (cheddar cheese, olive oil, and tomato pasta sauce) and served directly to the subjects. The pasta of TM was cooked (in a full power 900 W microwave for 13 min and stirred midway, then the pasta was drained, cooled rapidly using cold water) and chilled one day prior to the laboratory visit at 4 °C. On the day of the study, just before the meal was consumed, the cooked and chilled pasta was reheated in the microwave for 2 min and then mixed with the other ingredients before being served to the subjects.

Laboratory visit

Subjects were asked to fast overnight (> 10 h). On arrival at the laboratory, subjects rested, after which two finger-prick capillary blood samples were taken, five minutes apart in the fasting state to measure the blood glucose. In addition, a 20-gauge cannula (BD) was inserted into an antecubital vein for subsequent blood sampling. Blood samples were drawn from a 2-way tap, and the first 2 ml of each sample was discarded to avoid contamination with the saline. Two blood samples (5 ml each) were collected in the fasting state for assessment of the mean values of insulin and ghrelin.

After consumption of the meal, capillary blood samples were taken from the fingertip using single-use lancet every 15 min for 3 h, and venous blood samples (5 ml each) were taken at 30-min intervals for 3 h. Venous blood samples were dispensed into serum-separating tubes (and allowed to clot for 30 min at room temperature before centrifugation) for serum insulin samples. Plasma ghrelin samples were dispensed into EDTA-coated tubes containing 50 μL aprotinin (Thermo Scientific). All samples were centrifuged for 10 min at 3000 x g at 4 ° C. The supernatant fluid was then transferred into plastic tubes and kept at -80°C until further analysis.

Blood analysis

Whole blood glucose concentrations were determined using (Accu-Chek® Instant glucometer, Roche Diabetes Care, Mannheim, Germany). Insulin concentrations were measured with commercially available enzyme-linked immunosorbent assays (ELISA) (Millipore, Catalog No.: EZHI-14K). Ghrelin concentrations were measured with commercially available ELISA kits (Millipore, Catalog No.: EZGRT-89K).

Appetite rating assessments

Subjective appetite rating was evaluated using an anchored 100 mm visual analogue scale (VAS) for hunger, fullness, desire to eat, and prospective food consumption(14). VAS consisted of 100 mm horizontal lines with labels anchored at the extremities indicating the most positive and negative sensations. Subjects

were asked to draw a vertical line on the horizontal line corresponding to their sensation. Distances on the VAS were converted into scores between 0 and 100 mm.

The VAS was translated from English into Arabic by two independent translators. The two translated versions were compared and then a preliminary translated version was developed. Finally, a blind back-translation of the preliminary translated version from Arabic to English was made to validate the English to Arabic translation.

Subjects were instructed to mark a cross at the point on the scale where they felt the cross best represented their appetite at the time the VAS was completed. Subjective appetite was evaluated once in the fasting state and then every 30 min over a 3 h period after consumption of the meal.

Statistical analyses

All statistical analyses were conducted using SPSS software (version 22 for Windows; SPSS). Data were checked for normality using Shapiro-Wilk's test. Data were expressed as mean ± SD unless otherwise stated. The net incremental area under the curve (net iAUC) for postprandial (the 3-h measurement periods) glucose, insulin, ghrelin and VAS responses was calculated for each subject as the difference between the integrated area of the response curve and the rectangular area determined by baseline values (fasting values). To determine the statistical significance of the outcome measures between the visits, a paired t-test was used. To determine statistical significance between individual time points, the two-way repeated measures ANOVA was used, and if a significant interaction was found, follow-up Bonferroni correction for multiple comparisons was made to identify specifically where statistically significant differences were. Statistical significance was accepted at the 5% level.

Results

Characteristics of the study subjects

The characteristics of the eight subjects who successfully completed the two visits of the study are shown in Table 1. The subjects were young, adult males with normal BMI and normal fasting blood glucose values.

Blood variables

Table 2 shows the blood variables across the study visits. No significant differences were observed in the fasting values for blood glucose between the two visits.

Table 1. Descriptive characteristics of the study subjects[1].

Characteristics	Mean ± SD
Age (years)	28.75 ± 4.53
Height (cm)	172.50 ± 6.52
Weight (kg)	71.25 ± 5.42
BMI (kg/m^2)	23.93 ± 0.80
Fasting Glucose (mg/dl)	97.3 ± 4.6

[1]N = 8. BMI, Body Mass Index; SD, standard deviation.

Table 2. Blood variables during the study visits[1].

Blood variables		CM	TM	P-value[2]
Glucose (mg/dL)	Fasting	98.06 ± 7.76	97.81 ± 4.56	0.76
	Peak	151.37 ± 18.87	148.50 ± 21.06	0.66
	net iAUC (over 3h)	4893.50 ± 1964.62	4982.75 ± 1933.08	0.89
Insulin (µU/mL)	Fasting	11.15 ± 5.22	8.59 ± 5.69	0.17
	Peak	101.00 ± 44.78	95.54 ± 43.67	0.68
	net iAUC (over 3h)	10806.12 ± 5262.67	10128.12 ± 4826.19	0.53
Ghrelin (pg/mL)	Fasting	317.19 ± 114.18	374.48 ± 161.14	0.29
	net iAUC (over 3h)	-48713.50 ± 11799.07	-49044.12 ± 10473.08	0.89

[1] Data are presented as means ± SD. N = 8. [2] P-value was tested by Paired sample t-test.
iAUC, incremental area under the curve; CM, control meal, TM, test meal, SD, standard deviation.

Neither peak values nor net iAUC for blood glucose differed significantly between the two visits.

Following meal consumption during the two visits, blood glucose levels reached a maximum value at 30 min (Figure 1). Blood glucose levels remained above fasting level until the last sampling time point (180 min) in the two visits. No significant interaction of the visit by time was observed in post-prandial blood glucose (Two-factor repeated measure ANOVA, P > 0.05).

No significant differences between the two visits were observed in the fasting levels and net iAUC for insulin (Table 2). As shown in Figure 2, insulin levels peaked at 30 min after each of the two meals and no significant difference between the peak levels in the two visits was noted. Post-prandial insulin level did not show significant interactions for the visit by time (Two-factor repeated measure ANOVA, P > 0.05).

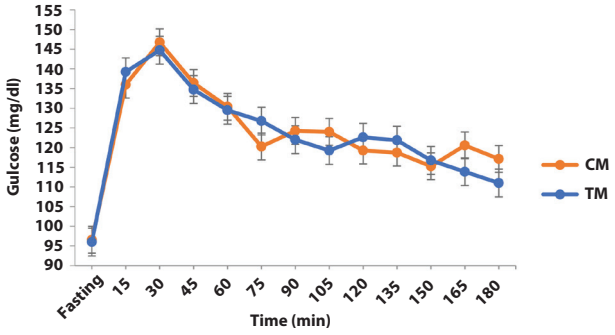

Figure 1. Mean ± SD for glucose levels over 3 h following meal consumption in the two visits. N=8. CM, control meal and TM, test meal.

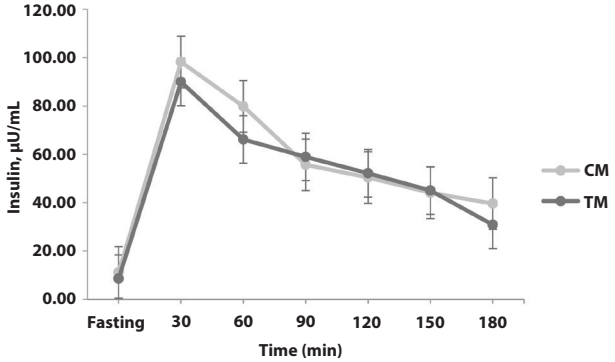

Figure 2. Mean ± SD for insulin levels over 3 h following meal consumption in the two visits. N = 8. CM, control meal and TM, test meal.

No significant differences were found in fasting, peak and net iAUC values for plasma ghrelin between the two visits (Table 2). No significant interaction of the visit by time was observed in post-prandial ghrelin (Two-factor repeated measure ANOVA, P > 0.05).

Subjective appetite assessment

Table 3 and Figure 3 illustrate fasting and iAUC for subjective appetite ratings in the study visits. As shown in the table, there were no significant differences in fasting values for subjective appetite ratings between CM and TM visits. A significant increase in iAUC for satiety following the TM consumption compared with the CM was observed (p > 0.05, paired t-test). The iAUC for the desire to eat was significantly decreased following consumption of the TM meal compared with the CM (p < 0.05, paired t-test). On the other hand, no significant differences in iAUC for hunger, fullness, and prospective food consumption were observed.

Discussion

The present study aimed to examine the effects of chilling and reheating cooked pasta on postprandial glycemic and insulinemic responses as well as appetite. Our findings showed greater satiety and a reduced desire to eat following TM consumption compared with CM. However, there were no significant differences in blood glucose and insulin between the TM and CM visits.

Pasta, typically rich in starches, is a principal food item in many countries, including Saudi Arabia. Chilling-and-reheating process, can reduce carbohydrate availability by changing both starch structure and resistance to digestion (3). Chilling of gelatinized starch promotes the formation of retrograded amylose. Retrograded starch resists digestion in the small intestine because it is physically inaccessible (1). However, it is unclear if the retrograded starch changes drive the glucose responses to pasta.

In a line with our findings of postprandial glycemic responses, Alzaabi et al. (13) reported no significant differences between postprandial glycemic responses

Table 3. Fasting and iAUC for subjective appetite ratings in the study visits.

Variables		CM	TM	P-value[2]
Hunger (mm)	Fasting	58.25 ± 24.58	67.25 ± 23.60	0.51
	net iAUC (over 3h)	-7069.62 ± 3093.63	-8859.25 ±3468.93	0.35
Satiety (mm)	Fasting	27.00 ± 23.77	26.00 ± 27.15	0.88
	net iAUC (over 3h)	7132.37 ± 3187.31	10181.25 ± 3140.65	**0.03***
Fullness (mm)	Fasting	26.12 ± 26.21	20.25 ± 15.07	0.56
	net iAUC (over 3h)	6638.62 ± 3428.59	9067.50 ± 3625.52	0.13
Desire to eat (mm)	Fasting	43.75 ± 20.26	59.50 ± 28.14	0.31
	net iAUC (over 3h)	-4594.50 ± 1481.11	-8190.75 ± 4333.34	**0.03***
Prospective food consumption (mm)	Fasting	54.12 ± 24.31	69.87 ± 21.25	0.27
	net iAUC (over 3h)	-6989.50 ± 4509.42	-6993.12 ± 4961.64	0.99

[1] Data are presented as means ± SD. N = 8. [2] P-value was tested by Paired sample t-test.
iAUC, incremental area under the curve; CM, control meal, TM, test meal, SD, standard deviation.

to freshly cooked pasta compared with chilled and reheated pasta among ten overweight and obese adults. In contrast, a recent study conducted on forty-five participants reported that the AUC for blood glucose was significantly lower in the reheated pasta that was previously cooked and chilled for 24 hours overnight at 4°C, than the freshly cooked pasta (12). The authors proposed that the mechanisms behind the changes in postprandial blood glucose were due to modifications in the starch structure and the subsequent influence on the glycemic response. Another study included ten healthy participants showed a significant difference in glucose iAUC between freshly cooked pasta and chilled and reheated pasta (15). Variations in findings may be due to variations in the methods used to determine blood glucose. It has been suggested that chilling and reheating processes have limited potential influence on glycaemic responses (3). Further, it has been shown that the impacts of particle size on starch digestibility are greater than subtle changes resulting from chilling and reheating processes (3). The other non-starch components in pasta meals could also have an important role in influencing starch susceptibility to amylolysis after chilling and reheating processes.

Appetite is a complex behavior, involves links between peripheral physiology, brain processes and metabolism. To the best of our knowledge, no previous studies investigated the effect of chilling and reheating pasta on appetite. In the present study a greater iAUC for satiety and a lower iAUC for desire to eat were noted following TM consumption compared with CM. These findings suggest that the adoption of the chilling and reheating process for cooked starch may be a promising strategy for weight management. Replacing rapidly digestible starch with resistant starch can reduce the energy density of the diet because of its lower calorie content (16). It has been reported that reducing diet energy density increases satiety and, consequently and weight loss (17, 18). Additionally, feelings of satiety may increase by incorporating resistant starch into a meal, although its effect on satiety is less clear compared with that of dietary fiber.

Ghrelin is a peripheral hormone that stimulates appetite and food intake and decreases post-prandially (19). Although in our study the TM resulted in greater satiety and a lower desire to eat than CM, no significant difference in iAUC for ghrelin was observed, likely due to the small number of subjects. This aspect of the study warrants further research with larger sample size. Given our findings, it would be interesting to know if the pasta chilling-and-reheating process evokes a response in the other incretin hormones including GLP-1 and peptide YY (PYY).

The current study has several limitations. The relatively small sample size is acknowledged as a major limitation of the study and may increase the probability of type II errors. The short period of the postprandial testing duration (3 h) is also a limitation as the

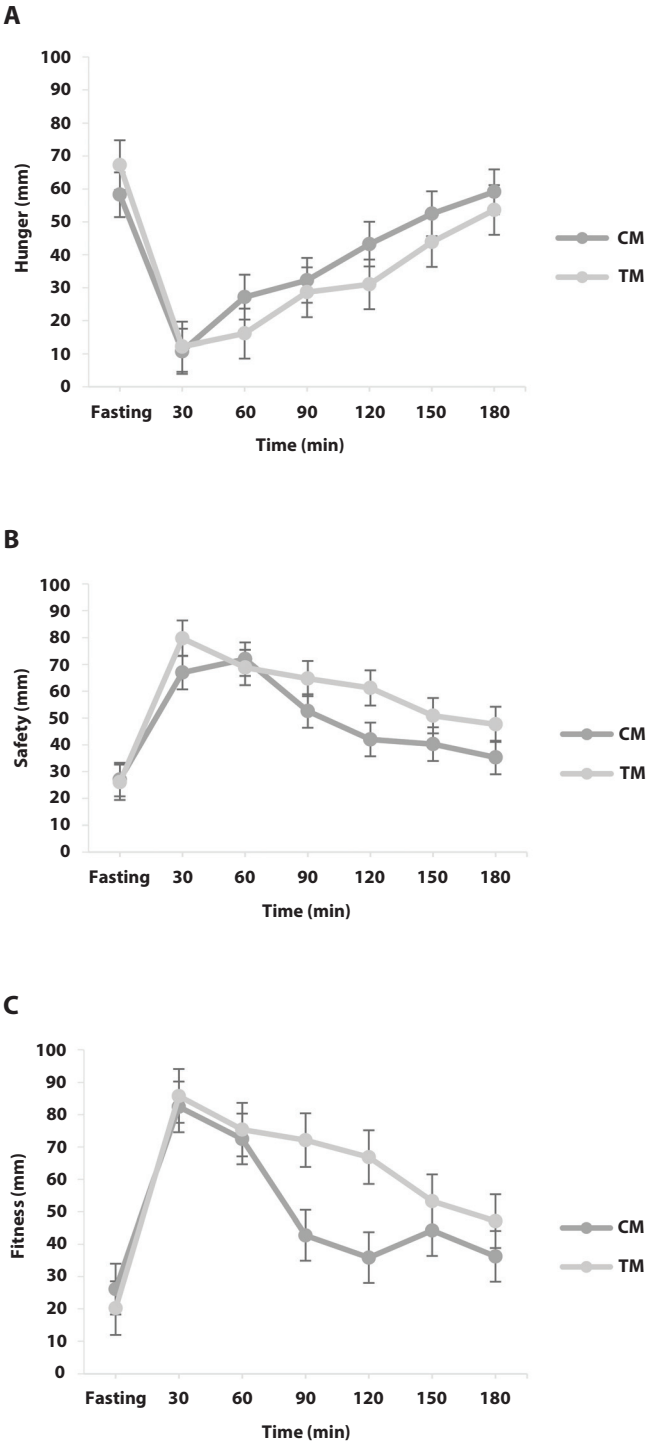

Figure 3. Mean ± SD for subjective appetite ratings (assessed by visual analogue scales) over 3 h following meal consumption in the two visits. Hunger (A), satiety (B), fullness (C), desire to eat (D) and prospective food consumption (E). (A) A rating of 0 is 'I am not hungry at all' and a rating of 100 is 'I have never been more hungry'; (B) A rating of 0 is 'I am completely empty' and a rating of 100 is 'I cannot eat another bite'; (C) A rating of 0 is 'Not at all full' and a rating of 100 is 'Totally full'; (D) A rating of 0 is 'Very weak' and a rating of 100 is 'Very strong'; (D) A rating of 0 is 'Nothing at all' and a rating of 100 is 'A lot'. N= 8. CM, control meal and TM, test meal.

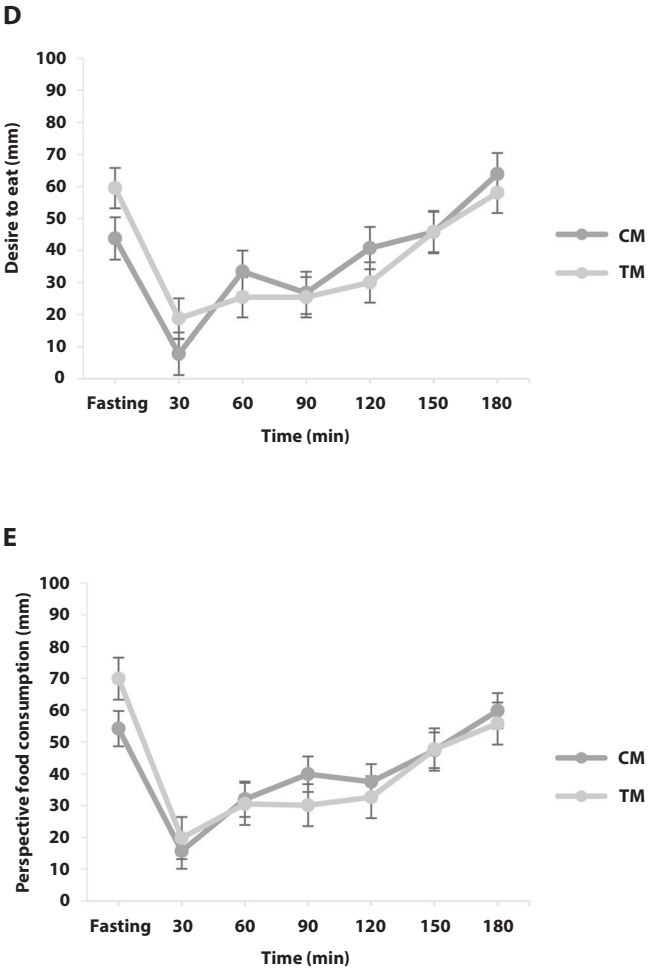

Figure 3. (Continued)

analysis of the effects of colonic fermentation takes about 6-8 h for food to reach the large intestine (20). Furthermore, subsequent food intake was not measured to assess whether it was associated with increased satiety or decreased desire to eat found after pasta meal consumption. Finally, although participants were blinded to which type of pasta meal was being served, some participants were able to distinguish between the two meals, based on experience.

Conclusions

Although, in the current study, no significant difference in postprandial glycaemic response was observed between freshly cooked and reheated pasta, chilling-and-reheating pasta increased satiety and reduced desire to eat which might favor weight management in the long term. These findings suggest that food processing of starchy foods (e.g. pasta) should be considered when planning weight-reduction regimes. More studies with a larger sample size are needed. In addition, long-term studies on individuals with metabolic syndrome including obesity and insulin resistance are more appropriate, and may provide additional insights, for evaluating the beneficial effects of the starch chilling-and-reheating process.

Acknowledgments: The authors would like to thank all subjects for their participation in this study.

Conflicts of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Funding: Researchers Supporting Project Number (RSP-2021/338), King Saud University, Riyadh, Saudi Arabia.

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Correspondence:
Maha H Alhussain, PhD
Department of Food Science and Nutrition, College of Food and Agriculture Sciences, University of King Saud University, Riyadh, Saudi Arabia
Phone: 00966118058126
E-mail: mhussien@ksu.edu.sa