Impact of food processing on postprandial glycaemic and appetite responses in healthy adults: a randomized, controlled trial.

Maryam S. Hafiz [1,2], Matthew D. Campbell [3,4,5], Nicolas Orsi [6], Georgia Mappa [6], Caroline Orfila [1], Christine Boesch [1]*

[1] School of Food Science and Nutrition, University of Leeds, Leeds, United Kingdom; [2] Faculty of Applied Medical Sciences, Department of Clinical Nutrition, King Abdul-Aziz University, Jeddah, Saudi Arabia; [3] School of Nursing and Health Sciences, Faculty of Health Sciences and Wellbeing, University of Sunderland; [4] Wellcome-MRC Institute of Metabolic Science, University of Cambridge; [5] Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds; [6] Leeds Institute of Cancer & Pathology, St James's University Hospital, Leeds, UK.

*Corresponding author: C Boesch, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, United Kingdom. Tel +44 113 3430268. Email: c.bosch@leeds.ac.uk

ORCID IDs:

Hafiz - 0000-0002-5769-175X

Campbell - 0000-0001-5883-5041

Orsi - 0000-0003-0890-0399

Orfila - 0000-0003-2564-8068

Boesch - 0000-0001-6705-5709

Abbreviations: AUC, area under the curve; BMI, body mass index; CGM, continuous glucose monitor; ChF, chickpea flour pasta; ChPu, chickpeas pureed; ChW, chickpeas whole; Con, control; DPP-IV di-peptidyl peptidase-4; EDTA, Ethylenediamine tetraacetic acid; GI, glycaemic index, GLP-1 glucagon like peptide -1; iAUC, incremental area under the curve; NCDs, non-communicable diseases; PPGR, postprandial glycaemic response; SST, serum separator tubes; T2DM, type 2 diabetes mellitus.

Key words:

Glycaemic response, Continuous glucose monitoring, postprandial interstitial glucose response, satiety, chickpeas, pulses, type 2 diabetes.

ISRCTN registry number: (ISRCTN14869733)

The data of the study is available through University of Leeds repository by reference number DOI_1076.

Abstract

Chickpeas are among the lowest glycaemic index carbohydrate food eliciting protracted digestion and enhanced satiety responses. *In vitro* studies suggest that mechanical processing of chickpeas significantly increases starch digestion. However, there is little evidence regarding the impact of processing on postprandial glycaemic response in response to chickpea intake *in vivo*. Therefore, the aim of this study was to determine the effect of mechanical processing on postprandial interstitial glycaemic and satiety responses in humans. In a randomised crossover design, thirteen normoglycaemic adults attended 4 separate laboratory visits following an overnight fast. On each occasion, one of four test meals, matched for available carbohydrate content and consisting of different physical forms of chickpeas (whole, puree, and pasta) or control (mashed potato), was administered and followed by a subsequent standardised lunch meal. Continuous glucose monitoring captured interstitial glucose responses, accompanied by periodic venous blood samples for retrospective analysis of C-peptide, glucagon like peptide-1 (GLP-1), ghrelin, leptin, resistin, and cortisol. Subjective appetite responses were measured by Visual Analogue Scale (VAS). Postprandial glycaemic responses were comparable between chickpea treatments albeit significantly lower than the control ($p < 0.001$). Similarly, all chickpea treatments elicited significantly lower C-peptide and GLP-1 responses compared to the control ($p < 0.05$), accompanied by enhanced subjective satiety responses ($p < 0.05$), whilst no significant differences in satiety hormones were detected among different intervention groups ($p > 0.05$). Chickpea consumption elicits low postprandial glycaemic responses and enhanced subjective satiety responses irrespective of processing methods.

Introduction

Specific dietary habits, including the regular consumption of ultra-processed food, have been proposed as causative factors of non-communicable diseases (NCDs) such as obesity and type 2 diabetes (T2D) [1-5]. Ultra-processed foods, which are typically high in refined carbohydrates and low in fibre content, induce substantial glucose dysregulation and have been shown to increase appetite and prospective food intake [6-11]. However, emerging evidence suggests that other factors inherent to food, including the type, physical integrity, and viscosity of starch and carbohydrate source, as well as presence of protein also significantly impact postprandial glucose elevation [12-14]. For example, high fibre foods are reported to elicit reduced postprandial glycaemic responses compared to similar carbohydrates with lower fibre content [15], and, the co-ingestion of protein with carbohydrate rich foods has, in some studies, been shown to attenuate postprandial glucose excursions and enhance insulin secretion especially in the presence of secretagogue amino acids [16]. As such, complex carbohydrate rich foods which preserve plant structure, are high in fibre and protein content may result in more favourable postprandial glucose.

Chickpeas *(Cicer arietinum L.)* are pulses rich in slowly digestible carbohydrates, soluble and insoluble dietary fibre, and high quality proteins including bioactive peptides. As a result, chickpeas are widely characterised as having a very low glycaemic index (GI) (reported between 25 to 45) and energy density [17, 18]. Findings of interventional studies suggest a significant attenuation in postprandial glycaemic responses (PPGRs) and suppressed subjective appetite and prospective food intake after chickpea intake when compared to other carbohydrate rich foods with similar amounts of available carbohydrates [19, 20]. Greater intraluminal viscosity, reduced gastric emptying and promotion of incretin secretion are considered as proposed mechanisms by which chickpeas can enhance satiety along with reduction of postprandial glycaemia [21].

Importantly, some *in vitro* studies investigating the effect of mechanical processing of chickpeas, particularly methods that result in cell wall disruption, show a significant increase in the rate of starch digestion and starch release following processing compared to non-processed chickpeas [22, 23]. However, little is known regarding the impact of processing methods on postprandial glucose, and little research has investigated the impact of pulse intake on satiety hormones such as incretins and ghrelin *in vivo* [24, 25].

Therefore, this study aimed to assess the acute postprandial interstitial glycaemic and satiety responses to chickpea ingestion following different processing methods in healthy adults. We used a continuous glucose monitoring (CGM) as a less invasive method to collect glycaemic information over the intervention period, including post-meal effects.

Methodology

Study design

This study followed a randomised, crossover, controlled design to assess the postprandial glucose response to chickpeas that were differently processed in normoglycaemic adults. Experimental procedures consisted of four visits; and randomisation was conducted using an online programme (http://www.randomization.com).

Participants were screened for eligibility and recruited for the trial at the human study facility in the School of Food Science and Nutrition at the University of Leeds. The included participants were healthy adults aged 18-65 years, presenting with fasting blood glucose < 5.6 mmol/L and body mass index (BMI) 18-29.9 kg/m^2. The exclusion criteria for the study were BMI ≥ 30 kg/m^2 (obese), fasting blood glucose > 5.5 mmol/L, the presence of disease, allergies, or medication use known to impact food digestion, appetite, food sensory, or glucose metabolism. Written informed consent was obtained from all participants prior to participation and the study procedures were conducted according to the guidelines laid down in the

Declaration of Helsinki. All procedures were approved by the Mathematics and Physical Sciences and Engineering Joint Faculty Research Ethics Committee at the University of Leeds (Ethics reference MEEC 18-035). The study was prospectively registered at www.isrctn.com as ISRCTN14869733.

Study procedure

Nineteen participants were recruited between 15 August to 20 December 2019. Participants attended four sessions to assess the postprandial responses to four different meals (three different chickpea meals and one control meal). The sessions were conducted over a two week period with a minimum of two days between visits allowing for washout [26]. The order of the interventions was random as per pre-generated sequences (Supplemental table 1). Each session commenced on the morning at 9:00, after an overnight fast (10-12 hours). One day prior to the first experimental visit, participants were fitted with a Continuous Glucose Monitor (FreeStyle LibrePro, Abbott, Wiesbaden, Germany), which was placed on the upper arm as previously described [27]. The monitor remained in place for the duration of the two week intervention period. Interstitial glucose values were obtained by reading the CGM glucose sensors that recorded values every 15 minutes over the two week period. The participants were blinded from the data collection.

Participants were requested to avoid legume and alcohol intake, and limit vigorous exercise for a minimum of 24 h before each experimental visit, and to otherwise maintain their dietary habits and physical activity constant throughout their visits to minimise variations due to these factors. Participants were asked to record dietary intake in the 24 h period before each visit.

Upon arrival, participants assumed a seated rested position whilst an intravenous cannula was inserted in the forearm for the periodic collection of venous blood samples. Stylets were used to keep the vein patent for during the 3 h observation window. Following a resting blood

95 sampling, test meals were provided along with one cup of water, and volunteers were asked to
96 consume their meals (see below) within 15 minutes. Participants remained seated throughout
97 the three hour observation window, and intravenous blood samples were obtained every 30
98 minutes from the inserted cannulas. Subjective appetite levels were also recorded at baseline
99 and over three hours after meal intake using a visual analogue scale (VAS) on 100 mm line
100 with intervals describing individual's perception of hunger fullness and prospective food intake
101 [28]. After 3 h, cannulas were removed, and participants were given a standardised lunch meal to
102 be consumed within one hour following discharge.
103 Blood samples were collected in serum separator tubes (SST, BD Vacutainer) for serum
104 isolation and in ethylenediamine tetraacetic acid (EDTA, BD Vacutainer) tubes for plasma
105 collection. Plasma samples were treated with the addition of two protease inhibitors: di-
106 peptidyl peptidase-4 (DPP-IV) and aprotinin at a final concentration of 1 mg/mL to preserve
107 GLP-1, ghrelin, and leptin [29]. Blood samples were kept on ice and centrifuged within 30
108 minutes at 2000 rpm for 10 minutes at 4° C for plasma separation and 2000 rpm for 15 minutes
109 at 25° C for serum, and subsequently stored in aliquots at -80° C until analysis.

110 ***Study food***

111 The experimental test meals comprised of three differently processed chickpea foods: whole
112 chickpeas (250 g), pureed chickpeas (250 g), and fusilli made out of chickpea flour (217 g),
113 each providing 50 g available carbohydrates, mainly as starch. The control intervention was
114 Smash® instant mashed potatoes (425 g, providing 50 g available carbohydrates). All
115 experimental foods were matched in total available carbohydrates, which was analytically
116 estimated by using an Available Carbohydrate kit (KACHDF), Megazyme International (Bray,
117 Ireland). Fat and salt contents were equalized by addition of olive oil and table salt. The
118 nutrition information of all intervention foods is shown in table 1. Whole chickpeas were

obtained from ready to eat tins of chickpeas (Sainsbury's, UK), which were rinsed with tap water and drained for 5 minutes, before weighing. Pureed chickpeas were also prepared using the same canned chickpeas (Sainsbury's, UK), pureed using an electric blender for 5 minutes to obtain an incorporated texture. Chickpea fusilli (Ugo) was cooked freshly on the day; the pasta was boiled for 3 minutes in water and drained for 5 minutes. Smash® instant mashed potatoes was freshly prepared by mixing with boiling water according to instructions on the packaging. All test meals were served at room temperature.

The lunch meals consisted of a cheddar cheese sandwich (Morrison's, UK), salted crisps (Sainsbury's, UK), and 150 mL of carbonated soft drink (Coca-Cola, UK). The nutritional content of lunch food is described in Supplemental table 2.

Biochemical analysis of blood markers

Plasma C-peptide, ghrelin, leptin, resistin, cortisol, and GLP-1 were measured using a commercially available fluid phase multiplex immunoassay kit as per manufacturer's instructions (Invitrogen ProcartaPlex Human metabolism/obesity panel, Fisher Scientific, Leicestershire, UK) using a Luminex 200 ™, Houston, Texas. The intra-assay variation was < 15% for each analyte.

Statistical analysis

The primary objective of the trial was to compare differences in postprandial interstitial glycaemic responses determined by continuous glucose monitoring system, after consuming pulses with different processing in comparison to a high GI control food. Secondary outcomes were serum C-peptide, incretin, appetite hormones, as well as subjective appetite response and the subsequent meal's glycaemic response. The sample size was calculated to detect differences of at least one standard deviation of PPGR between intervention arms. According to the calculation, a total of 18 participants would be required for this crossover study for a

significance level of 0.05 and a probability of 80%. However, previous acute studies have shown that ten participants on average are sufficient to detect a minimum difference of 1 mmol/L of postprandial glucose peak response [30, 31].

The effect of intervention food on peak postprandial interstitial glycaemic and blood insulinaemic rise (c-max) along with other biomarkers was assessed using a two factors repeated measure ANOVA and comparisons were conducted using Bonferroni's test, where a significant difference was observed. Postprandial interstitial glycaemic and blood insulinaemic incremental area under the curves (iAUCs) were calculated using the trapezoidal rule, omitting values below the baseline, over 120 and 180 minutes after consuming intervention and control foods, and the data were analysed using one-way ANOVA. In outcomes where values below the baseline were of interest such as satiety responses, total area under the curves (tAUCs) was calculated in place of iAUC [32].

Subjective hunger, fullness, and prospective food intake scores were analysed for differences using one-way ANOVA along with their tAUCs, and post hoc analysis using Bonferroni's test where a significant difference was detected.

All statistical analyses were performed using SPSS (version 26, IBM), with a statistical difference of $p < 0.05$ considered as significant.

Results

In total, 30 volunteers were initially screened for participation in the trial, 19 volunteers initiated their visits out of which 13 completed all four study visits (figure 1), 4 males and 9 females. Baseline characteristics of study participants are shown in table 2.

Postprandial interstitial glycaemic responses

All participants on all study visits presented with fasting interstitial glucose values below 5.5 mmol/L, with no significant differences between the intervention arms in baseline values of interstitial glucose, and there was no effect of gender, age, or BMI on the fasting interstitial glucose status of volunteers. A significant time x intervention interaction effect was observed when assessing postprandial interstitial glucose concentration in response to test meals ($p < 0.001$). Interstitial glucose increased after breakfast consumption in all groups (time $p < 0.001$), with the greatest temporal rise observed after ingestion of *Con* (intervention $p < 0.001$) when assessed as absolute concentrations and iAUC ($p < 0.001$). Postprandial interstitial glucose peak (c-max) was comparable across chickpea conditions, and significantly lower compared to *Con* ($p < 0.001$); no differences were observed in time to peak with peak glucose occurring at 45-minutes post-consumption under all conditions.

Interstitial glucose levels were significantly higher after intake of *Con* compared to all treatments from 30 to 90 minutes ($p < 0.05$). Following intake of *ChF*, glucose values were gradually lowered back to baseline values at 75 minutes after following peak at 45 minutes, before rising to a second peak at 90 minutes, while other chickpea treatments (*ChW* and *ChPu*) showed a slower reduction in glucose concentrations with no significant differences among chickpea treatments. Mean glucose iAUCs (0-3 h) were significantly lower after intake of all forms of chickpea breakfasts in comparison to *Con* ($p < 0.001$), however there were no significant differences among chickpea processing methods.

Subsequent meals' glycaemic response

Following the standardised lunch, glucose peak (c-max) occurred at 45 minutes under all conditions. Peak glucose was significantly attenuated under both *ChW* and *ChPu* ($p = 0.049$), as compared to *Con* condition, but not *ChF* ($p = 0.156$). Total glucose exposure expressed as

188 average iAUCs of interstitial glucose during this period was comparable between *ChW*, *ChPu*,
189 and *ChF* and was lower than *Con* ($p = 0.01$) (figure 3).

190 ***Subjective appetite responses***

191 Average subjective appetite responses of all participants are shown in table 3, with no
192 significant differences between the interventions arms in baseline values of hunger, fullness,
193 and prospective food intake. There were high interpersonal variabilities observed in reporting
194 the subjective responses, however, results remained robust following adjustment for potential
195 confounders. Subjective responses of hunger at the end of the visit and total (AUC 0-3 h) were
196 significantly greater for *Con* compared to all forms of chickpeas ($p < 0.05$); and responses of
197 fullness (AUC 0-3 h) after ingesting *Con* were significantly lower compared to all chickpea
198 meals ($p < 0.05$). There was no significant difference between conditions observed for
199 prospective food intake. However, we observed significantly lower hunger ratings in normal
200 weight individuals at 60 min after *ChF* ($p = 0.04$), and at 180 min after *ChW* ($p = 0.03$) in
201 comparison to overweight participants. There was no significant gender x intervention
202 interaction for any related to hunger, fullness, or prospective food intake.

203 ***Plasma hormonal responses***

204 There was a trend for mean postprandial GLP-1 responses to be lower after *ChW* intake
205 compared to all other conditions, although these results were not statistically significant (Figure
206 4A). When comparing postprandial iAUCs of GLP-1, significantly higher iAUCs were
207 observed after intake of *Con* compared to all other treatments ($p = 0.041$). A similar pattern
208 was noted in postprandial plasma C-peptide levels that were significantly lower following
209 intake of all chickpea interventions compared to *Con* after both 30 ($p = 0.05$) and 60 minutes
210 ($p < 0.001$) (Figure 4B). Similarly, iAUC 0-3h postprandial C-peptides levels were also
211 significantly lower for all chickpea treatments ($p < 0.001$).

Postprandial plasma resistin levels in *Con* were significantly higher at 30 minutes compared to *ChW* ($p = 0.05$), and at 60 minutes compared to *ChW* and *ChF* ($p = 0.02$). However, this could be due to unexplained slightly higher baseline values in the *Con* group, although the difference was not statically significant when comparing baseline values of all treatments ($p = 0.061$) (Figure 4D).

No significant differences were observed in postprandial leptin, ghrelin, and cortisol values between all conditions ($p > 0.05$) (Figure 4).

Discussion

The present study was designed to determine the effects of different chickpea processing methods on subsequent postprandial interstitial glycaemic and appetite responses. The outcomes of the study indicate a comparable attenuation in postprandial interstitial glycaemic and appetite responses after chickpea intake irrespective of their physical form compared to the reconstituted mashed potato control. Average peak glucose was numerically higher after *ChF* compared to *ChW* (mean difference of ~ 0.12 mmol/L in maximum glucose rise), although differences failed to reach statistical significance and the magnitude of the difference is largely negligible. Likewise, peak glucose levels were higher after lunch intake in the *ChF* group, but the difference was not statistically significant owing to substantial variations within the group. Our outcomes are in contrast with some previous findings showing that ingestion of pulse flour based meals led to significantly higher postprandial glycaemic responses compared with whole pulses [33-35]. This discrepancy is likely to be due to divergent test meals, specifically the use of pulse flour based pasta in the present study as opposed to other test meals made from pulse flour such as bread. White pasta is generally considered to elicit a lower glycaemic response compared to white bread, despite both being produced from refined wheat flour [36]. Commercial dried pasta is manufactured industrially using an extrusion process that results in a dense

product which reduces the digestive enzyme accessibility and thus elicits substantially lower postprandial glucose responses [27]. The structure of pulse pasta was described as quite a compact protein/starch network which may limit access to digestive enzymes [37]. Moreover, different varieties within a given pulse type have demonstrated compositional differences that lead to significantly different glycaemic responses when given the same amount of carbohydrates [38]. It was not possible, as part of our trial, to keep the variety of chickpea seeds constant since we used commercial products. Our findings are consistent with another study reporting that pureeing pulses or grinding them to flour does not impact on immediate blood glucose levels [24]. Above mentioned discrepant findings are likely to be due to differences in the degree of processing applied in flour preparations, which may have resulted in differences in cell wall integrity and hence starch bioaccessibility [22,23]. The extent of intracellular starch digestion from chickpeas is largely dependent on cell wall integrity that act as a barrier regulating hydration and controlling the permeability to α-amylase. Consequently, the starch granules in intact chickpea cells are generally less susceptible to gelatinization and amylolysis highlighting the underpinning mechanism to their lower postprandial glucose response [23]. We observed intact chickpea cells in *ChW* and *ChPu* samples hence explaining the lower glycaemic response. In the case of *ChF*, we did not observe intact cells, but a dense network of what appeared to be starch, protein and cell wall material. This dense structure appears to compensate for the lack of intact cells, since this sample also showed an attenuated postprandial glycaemic response. On the other hand, *Con* consisted of rehydrated potato flakes which form a hydrated, easily accessible starch matrix lacking in cellular or native starch structures. We have found this food to be a good control in glycaemic studies since it is easy to prepare consistently prior to consumption, is well accepted by participants and leads to consistent glycaemic responses between participants.

We have also shown that the beneficial effect of chickpeas on glycaemic responses was extended to the subsequent meal as made evident by lower glycaemic responses following intake of the standardised lunch. Interestingly, the attenuated postprandial glucose effect following subsequent feeding was limited to *ChW* and *ChPu* only, which might be attributed to the larger pulse particle size and the presence of intact cells in those treatments [14]. This finding is consistent with a study showing that only whole pulses are effective in reducing glucose concentrations in response to subsequent feeding in normoglycaemic adults [24]. The exact mechanisms behind the beneficial effect of pulses on reduced glycaemic response following a second meal are yet to be elucidated. The effect of short chain fatty acids resulting from the fermentation of indigestible carbohydrates in suppressing glucose metabolism is a proposed mechanism [39, 40]. Furthermore, intact cells have been demonstrated to promote different microbes compared to isolated resistant starches [41]. These short chain fatty acids can be detected in blood as early as three hours following food ingestion, and might therefore affect glucose metabolism [31]. Another proposed mechanism is slow, albeit sustained, release of glucose through the slowly digestible starch present in less processed chickpeas [42, 43]. Food items containing high amounts of slowly digestible starch ingested at breakfast are suggested to induce slow glucose appearance throughout the day [42-45]. The slow digestion of these starches is proposed to induce a delayed and prolonged response of incretin (180 to 300 minutes following slowly digestible starch intake), which in turn affect the digestion rate and glucose appearance following intake of a subsequent meal [46].

In line with postprandial glycaemic responses, insulin (as represented by C-peptide) and incretin responses (as represented by GLP-1) were significantly lower after ingestion of all chickpea treatments compared to *Con,* with no significant differences between different processing methods. We noted peak glucose and GLP-1 responses at 45 minutes following breakfast ingestion, followed by a c-peptide peak at 60 minutes, reinforcing the insulinotropic

activity that is mediated by incretin, in agreement with previous findings correlating blood insulin levels with GLP-1 [47].

The results of the study also show a significant increase in postprandial satiety as represented by significantly higher subjective fullness scores, and significantly lower hunger and prospective food consumption scores after ingestion of chickpea foods compared to *Con*. However, the effect on satiety was not paralleled by appetite hormone response. We found higher secretion of the anorexic hormone GLP-1 after *Con* ingestion compared to other groups, however, no differences were detected in postprandial leptin and in the orexigenic gut hormone, ghrelin. A previous trial investigating the impact of incorporating chickpea flour in flat breads reported no effects on GLP-1 levels although significantly higher levels of ghrelin were measured as a result of the intervention [48]. However, the incorporated chickpea flour only amounted to 30% in the intervention meals, accounting for consistency in both glucose and insulin responses [48].

To the best of our knowledge, no other studies have assessed the acute postprandial responses of GLP-1, ghrelin, and leptin after pulse intake. The effect of protein intake on postprandial ghrelin secretion is still controversial, with some studies suggesting enhanced secretion while others reported reduced levels after protein inclusion in meals [49, 50]. However, findings of previous trials showed that the administration of high fibre and/or high protein diets trigger the secretion of incretin hormones in both acute and long-term settings [51-53]. The proposed mechanism is that fibre can lead to increases in incretin secretion, principally through short chain fatty acid production after fermentation of non-digestible carbohydrates in the colon [51]. This can explain the lower responses observed in our trial as we only investigated 3-hour responses following a meal intake.

A major strength of our trial lies with quantifying the amount of available carbohydrates in our laboratories rather than relying on food labels in which carbohydrates are often calculated by difference. Also, use of a standardised CGM system allowed us to comprehensively profile individual glucose responses throughout the course of a protracted observation period. Moreover, we assessed the hormonal responses following intervention in order to clarify the mechanism(s) underpinning the regulation of glucose levels. However, caution is warranted when comparing the present outcomes with the literature. Firstly, as CGM systems do not measure glucose in blood but in interstitial fluid, a delay of 4.5 minutes relative to circulatory levels has been estimated. Further, interstitial glucose levels could be up to 11.4% lower mean absolute relative difference compared to reported capillary blood glucose values and 12% in comparison to venous blood glucose analysed by Yellow Springs Instrument [54]. Secondly, the test foods used in the trial are not made from the same chickpea variety. While the use of store brand products is more realistic, it does introduce variation due to potential varietal and therefore compositional differences (e.g. carbohydrate and protein content), which in turn might affect postprandial responses. This was partially mitigated by measuring carbohydrate content experimentally. Thirdly, it cannot be excluded that the two day washout period as part of the crossover design, despite randomisation, might have introduced carryover effects and hence influenced the subsequent sessions' responses, although it has been shown in the literature that no carryover effects were detected in glucose values after 48 hours of chickpea consumption [26]. Finally, although our sample size was sufficient to detect clinical significant differences in our primary outcome, a larger sample may be necessary to detect differences in our secondary outcomes.

In conclusion, this study showed that postprandial interstitial glucose levels and incretin hormones are unaffected by chickpea processing methods. However, the presence of intact cells appear to have effects on the glycaemic response to the subsequent meal. The use of CGM

provides more information on subsequent meal effects that would be impractical to obtain otherwise.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgments

We acknowledge the volunteers participated in our study. This research was funded by King Abdul-Aziz University, Jeddah, Saudi Arabia, through a PhD scholarship to M.S.H.

Author contributions

The authors' contributions were as follow: M.S.H., M.D.C., C.O., and C.B. designed the trial; M.S.H. and M.D.C. conducted the study; N.O. and G.M. conducted the biochemical analyses; M.S.H., and C.B. performed the statistical analysis; M.S.H. wrote the manuscript; and M.D.C., C.O., and C.B. supervised data analysis and contributed toward the writing and reviewing of the manuscript.

References

1. M. H. Forouzanfar, A. Afshin, L. T. Alexander, Z. A. Bhutta, S. Biryukov, M. Brauer, R. Burnett, K. Cercy, F. J. Charlson, A. J. Cohen, L. Dandona, K. Estep, A. J. Ferrari, J. J. Frostad, N. Fullman, P. W. Gething, M. Griswold, S. I. Hay, Y. Kinfu, H. H. Kyu, X. Liang, A. D. Lopez, L. Marczak, G. A. Mensah, A. H. Mokdad, M. Moradi-Lakeh, M. Naghavi, B. Neal, M. B. Reitsma, G. A. Roth, J. A. Salomon, T. Vos, J. A. Wagner, H. Wang, M. Zhou, G. M. Aasvang, A. A. Abajobir, K. H. Abate, C. Abbafati, F. Abd-Allah, S. F. Abera, B. Abraham, I. A. Adedeji, Z. Ademi, J. C. Adsuar, E. E. Agardh, A. Agarwal, A. Agrawal, A. A. Kiadaliri, O. N. Ajala, T. F. Akinyemiju, Z. Al-Aly, K. Alam, S. F. Aldhahri, R. W. Aldridge, Z. A. Alemu, R. Ali, F. Alla, P. Allebeck, U. Alsharif, K. A. Altirkawi, E. A. Martin, N. Alvis-Guzman, A. T. Amare, A. K. Amegah, H. Amini, W. Ammar, H. H. Andersen, C. A. T. Antonio, J. Ärnlöv, A. Artaman, R. Assadi, S. Atique, E. F. G. A. Avokpaho, A. Awasthi, B. P. A. Quintanilla, P. Azzopardi, U. Bacha, M. C. Bahit, K. Balakrishnan, A. Barac, R. M. Barber, S. Barquera, L. Barregard, S. Basu, C. Batis, S. Bazargan-Hejazi, J. Beardsley, N. Bedi, E. Beghi, M. L. Bell, A. K. Bello, D. A. Bennett, I. M. Bensenor, A. Berhane, E. Bernabé, B. D. Betsu, A. Bhansali, S. Biadgilign and B. Bikbov, Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015, *The Lancet (British edition)*, 2016, **388**, 1659-1724.
2. M. Nardocci, J. Y. Polsky and J.-C. Moubarac, Consumption of ultra-processed foods is associated with obesity, diabetes and hypertension in Canadian adults, *Can J Public Health*, 2020, 1-9.
3. J. M. Poti, B. Braga and B. Qin, Ultra-processed Food Intake and Obesity: What Really Matters for Health—Processing or Nutrient Content?, *Curr Obes Rep* , 2017, **6**, 420-431.
4. J. E. Yardley and M. D. Campbell, Moving Toward Precision Medicine with Diabetes, Exercise and Physical Activity, *Can J Diabetes*, 2020, **44**, 679.
5. M. D. Campbell, T. Sathish, P. Z. Zimmet, K. R. Thankappan, B. Oldenburg, D. R. Owens, J. E. Shaw and R. J. Tapp, Benefit of lifestyle-based T2DM prevention is influenced by prediabetes phenotype, *Nat Rev Endocrinol*, 2020, **16**, 395-400.
6. A. Fardet, Minimally processed foods are more satiating and less hyperglycemic than ultra-processed foods: a preliminary study with 98 ready-to-eat foods, *Food Funct*, 2016, **7**, 2338-2346.
7. S. H. Holt and J. B. Miller, Particle size, satiety and the glycaemic response, *Eur J Clin Nutr*, 1994, **48**, 496-502.
8. A. Pan and F. B. Hu, Effects of carbohydrates on satiety: differences between liquid and solid food, *Curr Opin Clin Nutr Metab Care* , 2011, **14**, 385-390.
9. J. Slavin and H. Green, Dietary fibre and satiety, *Nutr Bull*, 2007, **32**, 32-42.
10. K. D. Hall, A. Ayuketah, R. Brychta, H. Cai, T. Cassimatis, K. Y. Chen, S. T. Chung, E. Costa, A. Courville, V. Darcey, L. A. Fletcher, C. G. Forde, A. M. Gharib, J. Guo, R. Howard, P. V. Joseph, S. McGehee, R. Ouwerkerk, K. Raisinger, I. Rozga, M. Stagliano, M. Walter, P. J. Walter, S. Yang and M. Zhou, Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake, *Cell Metab*, 2019, **30**, 226.
11. M. S. Hafiz, M. D. Campbell, L. L. O'Mahoney, M. Holmes, C. Orfila and C. Boesch, Pulse consumption improves indices of glycemic control in adults with and without type 2 diabetes: a systematic review and meta-analysis of acute and long-term randomized controlled trials, *Eur J Nutr* , 2021, DOI: 10.1007/s00394-021-02685-y.
12. J. C. Brand-Miller, K. Stockmann, F. Atkinson, P. Petocz and G. Denyer, Glycemic index, postprandial glycemia, and the shape of the curve in healthy subjects: analysis of a database of more than 1000 foods, *Am J Clin Nutr*, 2009, **89**, 97-105.

13. D. M. Allerton, M. D. Campbell, J. T. Gonzalez, P. L. S. Rumbold, D. J. West and E. J. Stevenson, Co-Ingestion of Whey Protein with a Carbohydrate-Rich Breakfast Does Not Affect Glycemia, Insulinemia or Subjective Appetite Following a Subsequent Meal in Healthy Males, *Nutrients*, 2016, **8**, 116.
14. E. Howard, A. Attenbourgh, L. L. O'Mahoney, A. Sakar, L. Ke and M. D. Campbell, Postprandial vascular-inflammatory and thrombotic responses to high-fat feeding are augmented by manipulating the lipid droplet size distribution, *Nutr Metab Cardiovasc Dis*, 2021, DOI: 10.1016/j.numecd.2021.05.021.
15. G. Livesey and H. Tagami, Interventions to lower the glycemic response to carbohydrate foods with a low-viscosity fiber (resistant maltodextrin): meta-analysis of randomized controlled trials, *Am J Clin Nutr*, 2009, **89**, 114-125.
16. D. G. King, M. Walker, M. D. Campbell, L. Breen, E. J. Stevenson and D. J. West, A small dose of whey protein co-ingested with mixed-macronutrient breakfast and lunch meals improves postprandial glycemia and suppresses appetite in men with type 2 diabetes: a randomized controlled trial, *Am J Clin Nutr*, 2018, **107**, 550-557.
17. J. Wood and M. Grusak, in *Chickpea breeding and management*, eds. S. S. Yadav, R. J. Redden, W. Chen and B. Sharma, 2007, DOI: 10.1079/9781845932138.005, pp. 101-142.
18. A. K. Jukanti, P. M. Gaur, C. L. L. Gowda and R. N. Chibbar, Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review, *Br J Nutr*, 2012, **108**, S11-S26.
19. M. A. McCrory, B. R. Hamaker, J. C. Lovejoy and P. E. Eichelsdoerfer, Pulse Consumption, Satiety, and Weight Management, *Adv Nutr*, 2010, **1**, 17-30.
20. T. A. Zafar and Y. Kabir, Chickpeas suppress postprandial blood glucose concentration, and appetite and reduce energy intake at the next meal, *J Food Sci Technol*, 2017, **54**, 987-994.
21. N. Becerra-Tomás, C. Papandreou and J. Salas-Salvadó, Legume Consumption and Cardiometabolic Health, *Adv Nutr*, 2019, **10**, S437-S450.
22. S. Dhital, R. R. Bhattarai, J. Gorham and M. J. Gidley, Intactness of cell wall structure controls the in vitro digestion of starch in legumes, *Food Funct*, 2016, **7**, 1367-1379.
23. C. H. Edwards, P. Ryden, G. Mandalari, P. J. Butterworth and P. R. Ellis, Structure–function studies of chickpea and durum wheat uncover mechanisms by which cell wall properties influence starch bioaccessibility, *Nat Food*, 2021, **2**, 118-126.
24. G. H. Anderson, Y. Liu, C. E. Smith, T. T. Liu, M. F. Nunez, R. C. Mollard and B. L. Luhovyy, The acute effect of commercially available pulse powders on postprandial glycaemic response in healthy young men, *Br J Nutr*, 2014, **112**, 1966-1973.
25. P. Binou, A. E. Yanni and V. T. Karathanos, Physical properties, sensory acceptance, postprandial glycemic response, and satiety of cereal based foods enriched with legume flours: a review, *Crit Rev Food Sci Nutr*, 2020, DOI: 10.1080/10408398.2020.1858020, 1-19.
26. F. Madrid-Gambin, C. Brunius, M. Garcia-Aloy, S. Estruel-Amades, R. Landberg and C. Andres-Lacueva, Untargeted 1H NMR-Based Metabolomics Analysis of Urine and Serum Profiles after Consumption of Lentils, Chickpeas, and Beans: An Extended Meal Study To Discover Dietary Biomarkers of Pulses, *J Agric Food Chem*, 2018, **66**, 6997-7005.
27. C. S. Brennan and C. M. Tudorica, Evaluation of potential mechanisms by which dietary fibre additions reduce the predicted glycaemic index of fresh pastas, *IJFST*, 2008, **43**, 2151-2162.
28. A. Flint, A. Raben, J. E. Blundell and A. Astrup, Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies, *Int J Obes*, 2000, **24**, 38-48.
29. M. Bielohuby, S. Popp and M. Bidlingmaier, A guide for measurement of circulating metabolic hormones in rodents: Pitfalls during the pre-analytical phase, *Mol Metab*, 2012, **1**, 47-60.
30. F. Brouns, I. Bjorck, K. N. Frayn, A. L. Gibbs, V. Lang, G. Slama and T. M. Wolever, Glycaemic index methodology, *Nutr Res Rev*, 2005, **18**, 145-171.

31. R. C. Mollard, C. L. Wong, B. L. Luhovyy, F. Cho and G. H. Anderson, Second-meal effects of pulses on blood glucose and subjective appetite following a standardized meal 2 h later, *Appl Phsiol Nutr Metab* , 2014, **39**, 849-851.
32. T. M. J. B. J. o. N. Wolever, Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values, *Br J Nutr,* 2004, **91**, 295-300.
33. D. J. Jenkins, M. J. Thorne, K. Camelon, A. Jenkins, A. V. Rao, R. H. Taylor, L. U. Thompson, J. Kalmusky, R. Reichert and T. Francis, Effect of processing on digestibility and the blood glucose response: a study of lentils, *Am J Clin Nutr*, 1982, **36**, 1093-1101.
34. K. O'Dea and S. Wong, The rate of starch hydrolysis in vitro does not predict the metabolic responses to legumes in vivo, *Am J Clin Nutr*, 1983, **38**, 382-387.
35. J. Tovar, Y. Granfeldt and I. M. Bjoerck, Effect of processing on blood glucose and insulin responses to starch in legumes, *J Agric Food Chem*, 1992, **40**, 1846-1851.
36. L. Chiavaroli, C. W. C. Kendall, C. R. Braunstein, S. Blanco Mejia, L. A. Leiter, D. J. A. Jenkins and J. L. Sievenpiper, Effect of pasta in the context of low-glycaemic index dietary patterns on body weight and markers of adiposity: a systematic review and meta-analysis of randomised controlled trials in adults, *BMJ Open*, 2018, **8**, e019438.
37. A. Bresciani, S. Iametti, D. Emide, A. Marti and A. Barbiroli, Molecular features and cooking behavior of pasta from pulses, *Cereal Chem*, 2021, **n/a**.
38. D. D. Ramdath, Q. Liu, E. Donner, A. Hawke, D. Kalinga, J. Winberg and T. M. S. Wolever, Investigating the relationship between lentil carbohydrate fractions and in vivo postprandial blood glucose response by use of the natural variation in starch fractions among 20 lentil varieties, *Food Funct*, 2017, **8**, 3783-3791.
39. A. C. Nilsson, E. M. Ostman, Y. Granfeldt and I. M. Björck, Effect of cereal test breakfasts differing in glycemic index and content of indigestible carbohydrates on daylong glucose tolerance in healthy subjects, *Am J Clin Nutr*, 2008, **87**, 645-654.
40. K. Verbeke, V. Ferchaud-Roucher, T. Preston, A. C. Small, L. Henckaerts, M. Krempf, H. Wang, R. J. Vonk and M. G. Priebe, Influence of the type of indigestible carbohydrate on plasma and urine short-chain fatty acid profiles in healthy human volunteers, *Eur J Clin Nutr*, 2010, **64**, 678-684.
41. Y. Huang, S. Dhital, F. Liu, X. Fu, Q. Huang and B. Zhang, Cell wall permeability of pinto bean cotyledon cells regulate in vitro fecal fermentation and gut microbiota, *Food Funct*, 2021, **12**, 6070-6082.
42. S. Vinoy, M. Laville and E. J. M. Feskens, Slow-release carbohydrates: growing evidence on metabolic responses and public health interest. Summary of the symposium held at the 12th European Nutrition Conference (FENS2015), *Food Nutr Res*, 2016, **60**, 31662-31662.
43. T. M. Wolever, D. J. Jenkins, A. M. Ocana, V. A. Rao and G. R. Collier, Second-meal effect: low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response, *Am J Clin Nutr*, 1988, **48**, 1041-1047.
44. D. J. Jenkins, T. M. Wolever, R. H. Taylor, C. Griffiths, K. Krzeminska, J. A. Lawrie, C. M. Bennett, D. V. Goff, D. L. Sarson and S. R. Bloom, Slow release dietary carbohydrate improves second meal tolerance, *Am J Clin Nutr*, 1982, **35**, 1339-1346.
45. H. Liljeberg and I. Björck, Effects of a low-glycaemic index spaghetti meal on glucose tolerance and lipaemia at a subsequent meal in healthy subjects, *Eur J Clin Nutr*, 2000, **54**, 24-28.
46. R. E. Wachters-Hagedoorn, M. G. Priebe, J. A. Heimweg, A. M. Heiner, K. N. Englyst, J. J. Holst, F. Stellaard and R. J. Vonk, The rate of intestinal glucose absorption is correlated with plasma glucose-dependent insulinotropic polypeptide concentrations in healthy men, *J Nutr*, 2006, **136**, 1511-1516.
47. K. S. Juntunen, L. K. Niskanen, K. H. Liukkonen, K. S. Poutanen, J. J. Holst and H. M. Mykkänen, Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects, *Am J Clin Nutr*, 2002, **75**, 254-262.

48. S. Dandachy, H. Mawlawi, M. Chedid, C. El-Mallah and O. Obeid, Impact of Pre-Processed Chickpea Flour Incorporation into "Mankoushe" on Appetite Hormones and Scores, *Foods*, 2018, **7**, 173.
49. J. Erdmann, R. Töpsch, F. Lippl, P. Gussmann and V. Schusdziarra, Postprandial Response of Plasma Ghrelin Levels to Various Test Meals in Relation to Food Intake, Plasma Insulin, and Glucose, *J Clin Endocrinol Metab*, 2004, **89**, 3048-3054.
50. W. A. M. Blom, A. Lluch, A. Stafleu, S. Vinoy, J. J. Holst, G. Schaafsma and H. F. J. Hendriks, Effect of a high-protein breakfast on the postprandial ghrelin response, *Am J Clin Nutr*, 2006, **83**, 211-220.
51. A. M. Bodnaruc, D. Prud'homme, R. Blanchet and I. Giroux, Nutritional modulation of endogenous glucagon-like peptide-1 secretion: a review, *Nutr Metab*, 2016, **13**, 92.
52. J. Ma, J. E. Stevens, K. Cukier, A. F. Maddox, J. M. Wishart, K. L. Jones, P. M. Clifton, M. Horowitz and C. K. Rayner, Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes, *Diabetes Care*, 2009, **32**, 1600-1602.
53. M. A. Nauck and J. J. Meier, Incretin hormones: Their role in health and disease, *Diabetes Obes Metab*, 2018, **20 Suppl 1**, 5-21.
54. T. Bailey, B. W. Bode, M. P. Christiansen, L. J. Klaff and S. Alva, The performance and usability of a factory-calibrated flash glucose monitoring system, *Diabetes Technol Ther*, 2015, **17**, 787-794.

Table 1 Macronutrient composition of the intervention and control food.

Nutrition information	***ChW***	***ChPu***	***ChF***	***Con***
Weight, g	250.0	250.0	217.0	425.0
CHO, g[1]	50.0 (57%)	50.0 (57%)	50.0 (56%)	50.0 (68%)
Fibre, g	15.3	15.3	12.4	4.7
Fat, g[2]	8.0 (20%)	8.0 (20%)	8.0 (20%)	8.0 (24%)
Protein, g[3]	19.3 (23%)	19.3 (23%)	21.3 (24%)	6.2 (8%)
Salt, g	0.8	0.8	0.8	0.8
Energy, kJ	1460.6	1447.6	1497.4	1241.9

ChW, chickpeas whole; ChPu, chickpeas pureed; ChF, pasta made of chickpea flour; Con, mashed potatoes
1 values in the brackets present the percentage contribution of the carbohydrate toward total energy of the meal
2 values in the brackets present the percentage contribution of the fat toward total energy of the meal
3 values in the brackets present the percentage contribution of the protein toward total energy of the meal

Table 2 Participant characteristics.

	Mean	SD
Age (y)	28.7	6.6
females (n)	9	-
Smoking, yes (n)	3	-
Height (cm)	164.5	10.6
Weight (kg)	63.6	11.1
Body mass index (kg/m2)	23.2	2.5
Fasting glucose (mmol/L)[1]	4.1	0.5
Glycated haemoglobin A1c (%)[1]	4.48	0.22

1 measured by continuous glucose monitors

Table 3 Incremental subjective appetite responses as measured by visual analogue scale over 3 hours after intervention [1].

	ChW		*ChPu*		*ChF*		*Con*		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Hunger score 60 min	23.8	13.6	20	8.2	22.3	11.5	28.5	11.2	0.255
Hunger score 180 min	36.2[a]	17.7	33.8 [a]	13.5	36.2 [a]	15.3	48.5 [b]	11.4	0.045
Hunger total AUC0–3h, mm × h	91.2 [a]	37.7	85.4 [a]	23.4	89.6 [a]	30.9	113.5 [b]	26.8	0.035
Fullness score 60 min	43.1	15.3	43.1	11.7	40.8	8.6	33.8	9.6	0.137
Fullness score 180 min	31.5	15.9	30.8	11.9	26.9	12.9	20	12.7	0.095
Fullness total AUC0–3h, mm × h	107 [a]	37.2	107 [a]	26.0	101 [a]	25.2	80 [b]	25.2	0.012
Prospective food intake score 60 min	26.9	17.6	26.9	16.4	26.2	12.7	36.2	8.7	0.208
Prospective food intake score 180 min	41.5	19.8	39.2	11.3	38.5	11.1	50	11.2	0.123
Prospective food intake total AUC0–3h, mm × h	104	41.1	102	34.0	98.1	32.9	126	25.6	0.165

ChW, chickpeas whole; ChPu, chickpeas pureed; ChF, pasta made of chickpea flour; Con, mashed potatoes.

[1] n = 13.

Different superscript letters indicate significant differences within means in a row (Bonferroni's post hoc test, p<0.05)