Glycaemic, Gastrointestinal , Hormonal and Appetitive Responses to Pearl Millet or Oats Porridge Breakfasts: a Randomized, Crossover Trial in Healthy Humans

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Abbreviations used: bTFE, balanced turbo field echo; DF, dietary fibre; GIP, Glucose-dependant insulinotropic polypeptide; GLP – 1, Glucagon-like peptide 1; IDF, insoluble dietary fibre; MRI, magnetic resonance imaging; PMP, pearl millet porridge; PYY, Peptide YY; RARE, rapid acquisition with relaxation enhancement; ROI, region of interest; SDF, soluble dietary fibre; SOP, Scottish oats porridge; TE, echo time; TR, repetition time; $T_{50\%}$ time taken for a 50% reduction in stomach contents post meal ingestion; VAS, visual analogue scale.

Supplemental Figures 1 – 6 are available in the Online Supporting Material.

RUNNING HEAD: PEARL MILLET OR OATS RESPONSES

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ABSTRACT

Whole grain cereal breakfast consumption has been associated with beneficial effects on glucose and insulin metabolism as well as satiety. Pearl millet is a popular ancient grain variety that can be grown in hot, dry regions. However, little is known about its health effects. This study investigated the effect of a pearl millet porridge (PMP) compared with a well-known Scottish oats porridge (SOP) on glycaemic, gastrointestinal, hormonal and appetitive responses. In a randomized, two way crossover trial, 26 healthy participants consumed two iso-energetic/volumetric PMP or SOP breakfast meals, served with a drink of water. Blood samples for glucose, insulin, GLP-1, GIP and PYY, gastric volumes and appetite ratings were collected for two hours postprandially, followed by an ad libitum meal and food intake records for the remainder of the day. The incremental area under the curve (iAUC2h) for blood glucose was not significantly different between the porridges ($p > 0.05$). The iAUC2h gastric volume was larger for PMP compared with SOP ($p = 0.045$). The iAUC2h GIP concentration was significantly lower for PMP compared with SOP ($p = 0.001$). Other hormones and appetite responses were similar between meals. In conclusion, this study reports, for the first time, data on glycaemic and physiological responses to a pearl millet breakfast, showing that this ancient grain could represent a sustainable, alternative, with health-promoting characteristics comparable to oats. GIP is an incretin hormone linked to triacylglycerol absorption in adipose tissue, therefore the lower GIP response for PMP may be an added health benefit.

This trial was registered at ClinicalTrials.gov as NCT03068039

Key Words: Breakfast porridges, cereal grains, blood glucose, gastric emptying, magnetic resonance imaging, appetite

INTRODUCTION

Obesity, the prevalence of which is increasing globally [1], is associated with an increased risk of developing chronic diseases such as type 2 diabetes and cardiovascular disease [2; 3]. Diet, amongst other lifestyle factors, potentially contributes to the development of obesity [4]. Cereal consumption at breakfast has been associated with a reduced risk of obesity and related diseases, potentially via improved energy balance regulation and metabolism [5; 6].

Whole - grain cereals provide approximately two-thirds of the energy and protein intake in various countries over the entire world, especially in developing nations [7; 8]. Their consumption is thought to have beneficial health effects [6; 9; 10]. These include blunting postprandial glycaemic and insulinaemic responses [11], lowering blood pressure, improving serum lipid profile [12] and improving long term weight management via satiating properties [13; 14; 15] though there is still need for fully powered randomised controlled trials with longer durations assessing cardiovascular events as well as cardiovascular risk factors [16]. Wholegrain cereals vary in their resilience with respect to growing conditions, an important factor to consider in order to optimize food supply security and sustainability given their key role in the diet. Breakfast cereal porridges, made from a variety of whole grain cereals, are consumed commonly and would be expected to result in varied and complex gastrointestinal, biochemical, and appetitive responses depending on the specific chemical characteristics of the original grain (such as macronutrient composition, amylopectin to amylose ratio and fibre content) and physical characteristics, including differences in the food matrix resulting from various preparation and cooking methods [17; 18; 19; 20]. All potentially modulate, in turn, the glycaemic response, gastrointestinal response and appetitive response. Studying specific whole grains is essential in order to fully understand and exploit the health benefits.

Oats (Avena sativa), is an annual crop used both for human (e.g. breakfast porridges) and animal nutrition that is grown mostly in cool, moist climates being adversely affected by hot, dry weather [21]. Oats are nutritious grains containing most fatty acids including, the essential amino acid linoleic acid [22; 23] and are rich in protein. Whole- grain oats contain dietary fibre, including a high amount of the soluble fibre, β-glucan, varying between 2.3 and 8.5 g/100 g [24; 25]. The dietary fibre (β-glucan) has been suggested to reduce serum cholesterol, a risk factor for chronic heart diseases [26; 27; 28; 29]. In addition, oats contains several antioxidants including vitamin E, phytic acid, phenolic compounds, and avenanthramides; some of which are unique antioxidants that are only present in oats [30; 31].

Pearl millet (*Pennisetum glaucum*) is an ancient, small-seeded grain within the *Poaceae* or *Gramineae* family. Pearl millet is nutritionally comparable to major cereals such as wheat [32] and may have potential health benefits particularly with respect to glucose and insulin metabolism [22; 33; 34; 35]. It has the advantage for some of being gluten free andprovides energy, dietary fibre, proteins and also some vitamins and antioxidants [32; 36]. Furthermore, pearl millet has been targeted for increased iron content and for zinc enhancement [37]. The content of essential amino acids in pearl millet (leucine (10.7 g/100 g protein) and isoleucine (4.4 g/100 g protein)) is higher than that of oats (leucine (7.6 g/100 g protein) and isoleucine (4.1 g/100 g protein)) [14]. However the phytic acid content of pearl millet (varying from 588 mg/100 g to 1382 mg/100g) is also higher than that of oats[38].

Pearl millet production covers about 30 million hectares (ha) in 30 countries spread across Asia, Africa, the Americas and Australia [39]. The largest land use for this crop is India (about 8.5 million ha). Pearl millet ranks third in production after wheat and rice and is a staple food source in economically poor countries [40]. Millet can be grown in areas with water scarcity, low soil fertility and high temperatures [40; 41], which could contribute to a more sustainable and resilient agricultural system, with greater plant and dietary diversity [42].

However there is surprisingly little research available on the physiological responses to pearl millet consumption, particularly as a breakfast cereal.

In a previous pilot study, a pearl millet breakfast porridge appeared to induce lower postprandial blood glucose responses and appetite scores compared with other grains, although the differences were not conclusive [43]. The pilot study was instrumental for the subsequent development of this study, providing a better understanding of issues related to cooking, acceptability of the meals, physical form of the products and participants' reliability in returning the food diaries. Furthermore, the preliminary data collected from the pilot study was used to power this main physiological study. Appetite ratings are only a proxy measure for what people will actually eat later in the day, which led us to introduce an objective assessment of food intake by providing an *ad-libitum* test meal after the consumption of a whole grain porridge. Also, it was recognised that the follow up study should include measurements of insulin and glucose responses as well as the metabolic and appetite related gut hormones such as glucagon-like peptide 1 (GLP-1), glucose-dependant insulinotropic polypeptide (GIP) and peptide YY (PYY). We thus planned a larger study to investigate further the glycaemic, gastrointestinal, hormonal and appetitive responses to consumption of breakfast porridges made from a novel pearl millet flake compared with a commonly consumed porridge oat flakes for which the nutritional composition, as eaten, had been measured. The plasma GLP-1, PYY and GIP concentrations were measured due to their direct physiological effect on gastric emptying, glycaemic response and appetite. Oats porridge was chosen for the comparison food in this study due to its well-known physiological health benefits as well being a commonly consumed porridge [44; 45; 46; 47]. Millet was selected for comparison, because of our previous results, and drawing on the broader context of its potential value due to resilience to harsh environmental growing conditions. The hypothesis underpinning this study was that a pearl millet porridge breakfast will cause a smaller rise in

blood glucose compared with an iso-energetic and iso-volumetric breakfast meal of Scottish oats porridge.

SUBJECTS AND METHODS

Participants

The study was conducted at the Sir Peter Mansfield Imaging Centre located at the University of Nottingham. The study was approved by the University of Nottingham, Medical School Research Ethics Committee (F12072016) and all participants gave written, informed consent.

Participants were recruited between August 2016 to April 2017 from the local student and staff population via a poster advertisement. Those who expressed interest were invited to a screening session to establish whether they met the study inclusion criteria, namely: age 18-65 years old, healthy, BMI $\geq$ 18 and $\leq$ 24.9 kg/m^2 and able to give informed, written consent. Exclusion criteria included: using medication which interfered with study measurements, participating in another nutritional or biomedical trial three months before this study, not being a habitual breakfast consumer, not usually eating at least three meals a day, not being willing to consume all of the foods that would be offered during the study, working night shifts (between midnight and 6.00 am), doing strenuous exercise for >10 h/ week, consuming ser 21 alcoholic drinks in a typical week, following a medically or self-prescribed diet during the two weeks prior to and until the end of this study, contraindications for MRI scanning (e.g. presence of metal implants, an infusion pump and/ or a pacemaker) as assessed by a standard MRI safety questionnaire, pregnancy, inability to lie flat and exceeding the scanner bed weight limit of 120kg.

At the screening visit height was measured to the nearest 0.1 cm with the use of a stadiometer (Seca, Birmingham, UK). Body weight was measured with the use of an electronic scale (Seca, Birmingham, UK) to the nearest 0.1 kg. Body Mass Index (BMI) was calculated as weight (kg) divided by the square of height (m^2).

A total of 34 healthy volunteers were initially assessed for eligibility (**Figure 1**). Seven participants were not eligible; another participant, although initially eligible, did not meet the criteria on the study day. Therefore, 26 participants, 17 females and 9 males, with a mean age of 28.5 (SD 9.6) years old, and with a mean BMI of 23.4 (SD 3.2) kg/m^2 were included in the data analysis. Informed written consent was obtained from each participant before the trial. The format of the site master file and case report forms was informed by Good Clinical Practice (ICH 2016). The study was registered within ClinicalTrials.gov with identifier NCT03068039. The trial registration name was 'Gastrointestinal Responses to **M**illet and **O**ats Breakfast Interventions Assessed by **M**RI (MOM)'.

Experimental design

This study used a single-centre, randomised, two way crossover design that consisted of two separate test days, approximately 1 week apart. Participants consumed their habitual diet between each visit. The randomization scheme was generated with the use of the Second Generator Plan from www.randomization.com. Each study visit lasted from 08:00 am until approximately 13:30. The porridge meals differed in appearance and taste hence participants could not be blind to the intervention although they were not informed of which porridge they were consuming on each visit. The participants were asked to fast overnight (for at least ten hours) but a glass of water was permitted on waking. On arrival they completed the study day eligibility check questionnaire to monitor adherence to the study day restrictions, such as overnight fasting. An MRI scan was done to collect baseline images and to ensure that the

participants' stomach was actually empty at baseline. Measurements were taken at baseline and for up to 2 hours post consumption for gastric emptying, blood glucose, insulin, PYY, GIP, GLP-1 and paper based subjective visual analogue appetite scales were completed **(Figure 2)**. Participants were then given an *ad libitum* test lunch meal to measure intake. After this, but before discharge, they received instructions on how to record in a food diary that was provided, their food and drink intake over the remainder of the day.

All the data except the glucose values was blinded prior to analysis and the blind code was broken only after a blind data review was conducted. The outcome assessor was the one carrying out the finger-prick test so the glucose data could not be blinded.

Breakfast porridge intervention

The two breakfast porridges were made from either Scottish oats (own brand of ASDA, a supermarket chain, United Kingdom) or a novel pearl millet flake (supplied by Unilever, Sharnbrook, UK, under a Material Transfer Agreement). Both products were in the form of steam rolled flakes. Due to the physical size of the grains the millet steam rolled flakes obtained had a smaller in size compared to the oat flakes.

The test meals prepared for the study were iso-energetic (220 kcal each) and iso-volumetric (640 mL each) (**Table1**). Both porridges were cooked in the same way, in that 40 g of flakes were placed in an open glass bowl, gently mixed with 270 mL water at room temperature and heated in a 900W microwave. This procedure was repeated in parallel using an identical second open glass bowl and a second identical microwave. The porridges were heated for 2 minutes at full power, stirred gently with a spoon and left to rest for one minute, heated again for 2 minutes at full power, stirred gently with a spoon and left to cool for 6 minutes. By this point the water from the cooking had all been absorbed into the cooked product. The contents of the 2 bowls were then combined before a set weight of porridge was

given to the participants to eat, namely 400g for SOP and 415g for PMP. This was done to match the energy content of the cooked product, flakes plus cooking water, to 220kcal. The study meals were consumed with a glass of water at room temperature and the volume of water provided to the participants was used to compensate for volume differences in the cooked iso-energetic product portions. Therefore 240 mL of water was provided in a glass with SOP and 304 mL of water was provided in a glass with PMP, making the total volumes matched to 640 mL. The drinking of the glass of water was not standardised in aliquots, but the participants were asked to consume all of the porridge and all of the drink within 15 minutes. The manner and timing of the way the participants drank the water was not formally recorded but they mostly drank the water whilst eating the porridge, as opposed to consuming all of the water at the end. Other meal characteristics such as appearance and weight necessarily differed between meals **(Table 1).**

The composition of the cooked products was analysed for fibre, protein, fat and moisture (Table 1). Fibre analysis was performed using AOAC Method 991.43 using a three-stage enzymatic hydrolysis by heat-resistant α-amylase, protease and amyloglucosidase. After hydrolysis the soluble and insoluble fractions were separated using filtering crusible (Celatom® bed). The insoluble dietary fibre (IDF) content was measured using gravimetric analysis. The filtrate fraction containing soluble dietary fibre (SDF) fraction was precipitated using 4x volume of 60 °C 95% (v/v) ethanol. The ethanol precipitation of SDF in the pearl millet fraction was observed to be markedly different to that in the Scottish oats. Upon addition of ethanol to the pearl millet SDF fraction a very fine colloid suspensions was formed. In order to enhance the recovery of precipitated fibre two methods were applied; first, we reduced the volume of the filtrate using a rotary evaporator (60 °C, 100 mBar, Rotavapor® R-300, Büchi). Upon evaporation the higher concentration of solids was achieved which facilitated the precipitation process. The second method used was to employ a

high speed centrifuge to separate the SDF precipitate (10,000 g, Jouan CR3i Multifunction Centrifuge, ThermoFisher Scientific). Both methods gave comparable results and further analysis was performed using the centrifugation method for both oat and millet samples. The SDF precipitate was washed with ethanol, redispersed in de-ionised water, freeze-dried and the amount of SDF was determined using the gravimetric method.

The β-glucan content was measured using Megazyme ® β-Glucan Assay Kit (K-BGLU, Megazyme, Bray, Ireland) which follows the AOAC Method 995.16. The method is based on a two-stage enzymatic hydrolysis using lichenase and β-glucosidase, with subsequent determination of the reaction products using UV/VIS spectrophotometry.

Available carbohydrate was calculated as the difference between total carbohydrate and fibre (measured by the AOAC method). Total carbohydrate per 100g was calculated by difference (100 - (moisture / 100 g + ash/100g + fat / 100 g + protein / 100g) [48]. The total energy was calculated assuming that the energy provided by protein, fat, available carbohydrate and fibre is 4 kcal /g, 9 kcal /g, 4 kcal /g and 2 kcal /g respectively (analysis and estimations provided by Campden BRI, Chipping Campden, UK).

Outcome measures

Finger-prick blood glucose

The blood glucose incremental area under the curve (iAUC) is the primary outcome for this study. Capillary blood samples were collected at the fasting baseline (t = 0), immediately after feeding (t = 15) and every 15 minutes thereafter until t = 135 min **(Figure 2)**. The capillary blood samples were collected by finger prick using single-use lancets (Unistix Owen Mumford, Oxfordshire, United Kingdom). The capillary blood glucose was measured using a hand-held device (Accu-check, Roche Diagnostics, USA) [49]. Participants were requested to warm their hands before the finger prick in order to increase the blood flow. To extract the

blood, the fingertips were gently massaged from the base of the hand, moving towards the tips in order to minimise the plasma dilution.

The glycaemic response was calculated using the protocol described by Brouns *et al.* [50] which is in line with techniques recommended by the World Health Organization (WHO) / Food and Agricultural Organization (FAO 1998).

MRI of gastric volumes

Magnetic resonance imaging (MRI) was carried out on a research-dedicated 1.5T Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands). Participants lay in the supine/oblique position with a 16 element receiver coil wrapped around their abdomen. MRI scans were collected at baseline (t = 0 min), immediately post-consumption (t = 15 min) and at 30 minutes intervals until t = 135 min **(Figure 2).**

Gastric volumes of the meal and emptying were measured using a balanced turbo field echo (bTFE) sequence. A total of 25 axial slices (10 mm thick) were acquired within one breath hold for 10 seconds. Gastric volume was manually measured by a single operator by tracing a region of interest around the meal within the stomach using an intensity-based region-growing algorithm developed in-house and summing the volume across slices [51]. The gastric half emptying times ($T_{50\%}$) were calculated for each individual and then averaged [52].

Blood sampling and analysis of peptides

The sampling and assay protocols were similar to previous work [53]. Briefly: on arrival, a 20-G cannula (Intron Saety 3, B Braun Melsungen AG) was sited in a forearm vein of the participants to allow serial blood sampling. Blood samples were collected at fasting baseline (t = 0), immediately after feeding (t = 15) and every 15 minutes thereafter until t = 135 min for plasma insulin, plasma GLP-1, plasma GIP and plasma PYY. The initial 2 mL dead-space

blood sample was discarded to avoid contamination with the saline flush and the 6 mL experimental sample was then drawn into a vacutainer tube (K2E EDTA, BD, UK) containing 0.5 ml of aprotinin (3-7 TIU / mg protein, A6279 Sigma Aldrich, UK) added on the morning of the test. The cannula was flushed with 5 mL 0.9% Sodium chloride (BD PosiFlush^{TM} SP, UK). Blood samples were centrifuged for 10 minutes before being stored on ice. The plasma was immediately aspirated from the centrifuge tubes and divided into 3 aliquots that were stored in a (-20°C) freezer within 2 h of being taken and transferred to a -80°C freezer at the end of the MRI study day until subsequent analysis. Plasma insulin and PYY concentrations were measured using RIA kits (Millipore, Missouri 63304 USA). Total GLP-1 and total GIP concentrations were each measured with the use of a specific ELISA kit (both kits from EMD Millipore Corporation, Missouri 63103 USA).

Appetite ratings

Subjective feelings of hunger, satisfaction, fullness, desire to eat and prospective food consumption ratings were assessed using paper-based 100 mm VAS [54; 55]. Each end of the line was anchored by statements expressing the extreme for the sensation. For example, 'not hungry at all' and 'more hungry than have ever been" (**Supplemental Figure 1)**. To avoid bias from previous answers the participants were presented with a new VAS sheet at each time point, and this was removed immediately after completion. Every time they came out of the MRI scanner room **(Figure 2)**, the participants were requested to make a vertical mark on each scale at the point that best matched how they felt at that time.

A composite satiety score was calculated for each individual at each time point, without adjusting for baseline, using the formula:

composite satiety score = [hunger + (100 – satisfaction) + (100 –fullness) + desire to eat + prospective consumption]/5.

The range for the composite satiety score was therefore between 0 and 100 with lower composite scores being in the 'beneficial' direction (low hunger, high fullness, low desire to eat) and higher composite scores being in the 'non beneficial' direction (high hunger, low fullness, high desire to eat) in this context [56; 57].

Ad libitum test meal

A pasta based test meal consisting of a single large quantity was served at lunch time to assess *ad libitum* food intake [58]. The *ad libitum* meal consisted of tomato and mozzarella pasta bake (Tesco, United Kingdom). The nutritional composition table indicated that it had 129 kcal per 100 gram provided by 5.5 protein, 17.0 g carbohydrate, 3.6 g fat and 3.0 g of fibre.

Three semi-fresh pasta bake packs (450 g each) were heated in a microwave (900 W) at full power for a total of 10 minutes and stirred at the end of the period. Participants were given a single weighed portion of approximately 1300 g and a 200 mL glass of water. They were told that this portion was deliberately much larger than that normally consumed, and to eat from the bowl until satisfied. They were also told to drink the water when they wanted with the pasta but that they had to finish the entire amount of water. The amount of pasta left over was removed and weighed and the energy intake was calculated from the amount consumed as an objective measure of food consumption [58].

Food diaries

Food diaries were given to the participants before discharge from the MRI unit. They were instructed to provide a detailed record of food and beverages consumed over the remainder of the day. They were required to include information such as portion sizes, product brand names, and cooking and preparation methods. Furthermore, if the participants prepared composite dishes at home, then they were requested to provide the recipe and portion size.

Nutritics software (Nutritics Ltd, Dublin, Ireland) was used to analyse the food intake from the food diaries. If not on the database, food items were added manually using information on nutrition labels which was converted to database equivalent values by the software. Recipes were added to the database, with adjustment made for water and nutrient loss during cooking.

Statistical analysis

Prism version 6.07 (Graph Pad Software Inc., La Jolla, CA) was used to undertake descriptive and statistical analyses. All data are presented as mean±SE unless otherwise indicated. The data were assessed for normality using the Shapiro-Wilk's test. Most data were normally distributed and were analysed using parametric methods; the GLP-1, insulin and composite satiety data were non-normally distributed and were analysed using non-parametric methods.

The sample size was calculated using fingerprick glucose pilot data from the previous study on similar porridge breakfasts [43]. Using a crossover, paired design it would be possible to detect a change of 27.4 mmol·min/L (or 33%) in iAUC2h blood glucose with alpha=0.05 and a power of 80% using n=26 participants. This change is of the same order of magnitude as that reported in a published study comparing a rye versus an oat breakfast.

Values for the iAUC blood glucose, gastric volumes, gut hormones and appetite ratings were calculated with the use of differences from baseline. Values were considered positive when they were greater than baseline values and considered negative when they were less than baselines values. The area above or below baseline was calculated with the use of the trapezoid rule [59]

Comparisons of blood glucose, gastric volume, the gut hormones, the composite satiety score, intake of the ad libitum test meal and self-reported daily energy intake between SOP and PMP were made with the use of Student's paired t test (2 tailed).

Two-factor repeated-measure ANOVAs (factor 1:meal, 2 levels; factor 2: time,10 levels) were used to for blood glucose, gastric volumes, the gut hormones and the composite satiety score. When an interaction was identified, simple main effects were explored with the use of pairwise comparisons for the different time points, and a one way ANOVA for within each treatment. When no interaction was seen, main effects were compared.

An exploratory investigation of correlation was undertaken between gastric volume and glycaemic and insulinaemic responses, gut hormones, and appetite scores. Differences were considered significant at $p < 0.05$.

RESULTS

In this study, the effects of porridges made from pearl millet and oats, on glycaemic, gastrointestinal (gastric volume), hormonal (insulin, GLP-1, GIP and PYY) and appetite responses were measured. The study procedures were well tolerated and all 26 subjects completed the two study days. There were no adverse events during the study. The MRI scanner broke down (quenched) causing exclusion from analysis of 3 MRI data sets. Failure to sample bloods caused exclusion of 4 peptide data sets. The composition of the products, as served is given in Table 1. The behaviour of the SDF under conditions of ethanol precipitation was markedly different for pearl millet and Scottish oats.

Glycaemic response

Fasting baseline glucose levels between study arms were not significantly different, as expected. The glucose level rose rapidly after feeding and declined towards baseline level at t = 135 min (**Figure 3**). There was no significant difference between the meals for iAUC glucose (paired t test, $P > 0.05$), which was the primary outcome for this study.

The glucose levels peaked at 7.9 ± 0.2 mmol / L for pearl millet and 7.4 ± 0.1 mmol / L for oats porridge, a modest but significant difference (paired t test, $P < 0.05$). The ANOVA showed a significant interaction between factors. Glucose levels were higher for the PMP breakfast meal at t = 15 min and at t = 30 min ($P < 0.05$).

Appearance of the gastric content and gastric volumes

Figure 4 shows the appearance of the gastric content for SOP and PMP immediately after consumption (t = 15 min). Both porridges showed clear layering (phase separation), with a brighter layer on top (consistent with a more liquid phase in this type of moderately T2-weighted images) and a darker layer at the bottom (consistent with thicker / particulate material in this type of moderately T2-weighted images). The two layers were present also at t = 45 min. However, at later time points (t = 75 min to t = 135 min) the top layer was no longer visible.

Gastric volumes at fasted baseline (t = 0) for both meals were not significantly different, as expected. Gastric volumes rose immediately after feeding for both meals and then the volumes declined with time (**Figure 5**). The ANOVA showed a significant interaction between factors. Gastric volumes were higher or the PMP breakfast meal at t = 15 min and at t = 45 min ($P < 0.05$). The iAUC for gastric volumes were significantly different between the meals, although both meals were iso- volumetric at ingestion (paired t test, $P < 0.05$). PMP meal had larger gastric volumes compared with SOP (**Table 2**). The half gastric emptying time ($T_{50\%}$) of SOP and PMP were however similar at 47 ± 4 min and 47 ± 3 min respectively (paired t test, $P > 0.05$).

Blood peptides

Insulin

Plasma insulin concentrations increased markedly after both PMP and SOP up to t = 45 min and declined afterwards towards baseline at t =120 min (**Figure 6**). There were no significant differences either by iAUC or ANOVA for insulin concentration between PMP and SOP ($P > 0.05$).

Total GLP-1

Plasma GLP-1 concentrations following SOP rose quickly at t = 15 min compared with PMP. Thereafter, at t = 30 min, the concentration declined below the fasting value (**Figure 7**). There were no significant differences either by iAUC or ANOVA between porridges for GLP-1 concentration between SOP and PMP ($P > 0.05$).

Total GIP

Plasma GIP concentrations rose rapidly from baseline after feeding for both SOP and PMP. At t = 30, the two curves separated with the peak GIP for SOP being 23% higher than for PMP. GIP remained higher for SOP than for PMP throughout the remainder of the sampling period, the difference being significant (ANOVA, $P < 0.05$) (**Figure 8**). Accordingly, there was a significant difference in iAUC 2h GIP concentration between the two porridge breakfasts (paired t test, $P < 0.05$) with SOP being higher. The ANOVA showed a significant interaction between factors. GIP was lower for the PMP breakfast meal at all time points between t = 30 min and t = 135 min ($P < 0.05$).

PYY

Plasma PYY concentrations for SOP increased slightly from baseline upon feeding at t = 15 min and remained at the same level until t = 90 min, then dropped to the baseline level

399 (**Figure 9**). Plasma PYY concentrations for PMP remained at the same level as baseline, until
400 t = 30 min when the concentration increased rapidly, before returning to the baseline values at
401 t = 60 min. There were no significant differences either by iAUC or ANOVA for PYY
402 concentration between SOP and PMP ($P > 0.05$).
403
404 **Appetite ratings**
405 As predicted, the feelings of hunger, desire to eat and prospective food consumption all
406 decreased from the fasting baseline following consumption of the breakfast porridges and
407 returned to baseline two hours later, whereas the feeling of fullness and satisfaction increased
408 after feeding and returned to baseline after two hours. There were no significant differences
409 either by iAUC or ANOVA between porridges for the specific appetite ratings ($P > 0.05$). The
410 composite satiety scores for both meals were not statistically different **(Figure 10)** either by
411 iAUC or ANOVA. The iAUC for the subjective appetite rating are summarized in **Table 4**.
412 Data for hunger, fullness, satisfaction, desire to eat and prospective food consumption are
413 shown in supplementary materials.
414
415 ***Ad libitum* test meal**
416 There was no significant difference in the energy intakes from the *ad libitum* pasta bake meal
417 following consumption of the PMP and SOP (paired t test, $P > 0.05$) (**Table 4**).
418
419 **Food intake**
420 The recorded intake of food consumed during the remainder of the day (**Table 4**) was not
421 significantly different between the two arms of the study ($P > 0.05$). There were no significant
422 differences in the self-reported percentage of total energy from carbohydrate, protein and fat
423 following the two meals (paired t test, $P > 0.05$). The total daily energy intake including the

porridge breakfast, *ad libitum* pasta meal and recorded intake for the reminder of the day (**Table 4**), was again not significantly different (paired t test, $P > 0.05$).

Correlations

For PMP there was a significant correlation between gastric volume iAUC and the iAUCs for satisfaction (r = 0.49, $P = 0.03$), fullness (r = 0.48, $P = 0.04$) and desire to eat (r = -0.54, $P = 0.02$). For SOP there was a significant correlation between gastric volume iAUC and the iAUCs for fullness (r = 0.47, $P = 0.04$) and desire to eat (r = -0.53, $P = 0.02$).

DISCUSSION

This study has assessed the nutritional composition and glycaemic, gastrointestinal, hormonal and appetitive responses of iso-energetic and iso-volumetric breakfast porridge meals made from novel pearl millet flakes compared with standard, commercial oat flakes. Oats were chosen as the comparator as they are a common breakfast grain with recognised health-promoting characteristics [25; 60; 61] Millet was chosen as the intervention because of potential health benefits indicated by our previous work, the potential to exploit human consumption more fully in developed countries, and the broader context of resilience with respect to growing conditions enabling it to contribute potentially to improving food security and sustainability [62]. This study is the first randomised controlled trial of a pearl millet breakfast intervention.

The nutritional composition of the two porridges, as served, was established in order to the ensure that the energy content of the two meals was identical. For fibre, the composition of two porridge preparations was markedly different; while the total dietary fibre content of both cereals was comparable, the insoluble dietary fibre (DF) in pearl millet was measured to

be almost two times higher compared with Scottish oats. By contrast, the soluble DF content was measured to be higher in Scottish oats. The β-glucan content was in parallel with the soluble DF content, with the amount in pearl millet found to be approximately two times lower compared with Scottish oats. It is important to note that the SDF under conditions of ethanol precipitation behaved differently for pearl millet and Scottish oats, which promotes the hypothesis that SDF in these two grains may be markedly different in terms of the molecular weight, the ratio of 1→3/1→4 linkages, as well as polymer structure, which reflects the distribution of 1→3/1→4 linkages within the polymer molecule. Future studies may include more elaborate analysis of β-glucan structure and that of other SDF components as well as IDF, which is a composite structure of plant cell walls containing cellulosic components as well as insoluble glucans and xylans and some soluble fibre trapped within the cellulosic matrix and hence not accessible to enzymes [63].

No significant differences were seen in the glycaemic responses between PMP and SOP either in terms of capillary blood glucose, or insulin response. The glycaemic response is influenced by many factors, however in this study there were similar glucose and insulin iAUC responses between PMP and SOP. Pearl millet showed a higher glucose peak value than oats, although the difference was modest. Considering that the two meals were well matched for energy and volume and that most of the other responses were very similar, one could speculate that the smaller particle size of the PMP flakes compared with the SOP flakes may have offered an increased surface area for digestion[28; 61]. Other factors, such as total fibre content, were fairly similar, however the grains contained different types of fibre, potentially explaining the slightly different physiological response [64; 65]. The macronutrient composition of both meals was comparable (**Table 1**). The glycaemic response after oats is in agreement with many studies that have shown similar peak blood glucose value around 7

mmol/L [38], which is also in agreement with our pilot studies. To our knowledge these are the first human data on the glycaemic response after pearl millet flakes [48].

The gastric appearance of both meals was similar with two separated layers being apparent immediately after feeding. The layers comprised of an upper liquid phase and a lower solid/viscous phase that could be seen in the stomach. An hour later, the liquid phase was no longer visible for both meals, suggesting that gastric sieving promoted the emptying of the liquid component of the stomach contents [52]. These results with flakes are similar to those reported by Mackie *et al.* [61]. The half gastric emptying times were also similar for SOP and PMP. This could well relate to the iso-energetic nature of both meals, as energy content may drive gastric emptying to a greater extent than volume [66; 67].

Although both meals were iso-energetic and iso-volumetric, iAUC gastric volumes after PMP were significantly higher than after SOP. This is counter-intuitive because the total meal volume was matched by requiring the participants to consume more water volume with PMP because the cooked volume of the iso-energetic pearl millet porridge product was smaller. The water was not blended into the cooked porridge because of the desire to keep a more ecological validity with participants able to drink with a meal. Blending would have also required additional stirring with possible changes in the food matrix. The additional water volume could be expected to sieve rapidly from the stomach but this would have resulted in lower volumes for PMP. Larger gastric volumes after PMP could well be due to the characteristics of the meal. It may also be possible that the PMP flakes underwent further absorption of water in the gastric lumen, thus causing some additional swelling of the PMP volume, though from the MRI images it was not possible to dissect this. An alternative hypothesis could be put forward that the presence of IDF in PMP can stimulate (mechanically) the gastric wall, resulting in the increased release of mucus, which can associate with the meal and increase its gastric volume [68]. The gastric volume results are in

keeping with the previous pilot study, which showed a significant difference in gastric volume between different porridges [43]. The reasons for this remain to be understood. Increased wall stretch and tension is known to result in increased feeling of fullness [69] which correlates with gastric volumes [52] and reduces short-term food intake. Positive significant correlations were found here between gastric volumes and appetite ratings.

The plasma GLP-1 and PYY concentrations were measured due to their direct physiological effect on gastric emptying and appetite [45; 46; 47]. However, we were not able to measure other hormones such as CCK, active form of GLP-1 or active form of GIP, which may also have effects on gastric emptying and appetite.

GLP-1 is an incretin hormone released from L cells located in both the small and the large intestine in response to food intake[46]. Plasma GLP-1 levels are at their lowest in the fasting state (after overnight fast). The plasma levels rise rapidly during meals and usually remain above the baseline (the morning levels) between meals [46; 53]. PYY is also secreted from L cells that are located in the small and large intestine [45]. PYY inhibits gastric motility and increases water and electrolyte absorption in the colon. It has been shown to reduce appetite [45]. In this study the GLP-1 responses were consistent with plasma insulin concentrations which were comparable following both meals. PYY was not significantly different between the two meals.

The differences in GIP responses between meals are instead marked, with GIP being significantly lower after pearl millet compared with oats. GIP is secreted from intestinal K-cells [70] in response to the absorption of glucose and fat. More specifically, GIP release is stimulated by the rate of nutrient absorption rather than the presence of nutrients in the intestine [70]. The primary role of GIP is that of an incretin hormone, in that it binds to its specific receptor on pancreatic β-cells, and enhances glucose-dependent insulin secretion [70]. Although some studies reported that plasma GIP profiles are consistent with insulin profiles,

in the current study we found that GIP profiles behaved differently. Insulin concentrations were comparable between meals, however, GIP was significantly different between meals. GIP in combination with hyperinsulinaemia and hyperglycaemia has been shown to promote triacylglycerol absorption in adipose tissue [71], with high plasma levels of GIP associated with unhealthy body fat distribution [72]. The lower GIP response from the PMP meal may therefore suggest an added health benefit if taken on a regular basis, although further studies would be needed to confirm this.

The subjective appetite responses, the *ad libitum* pasta meal intake and the food intake for the reminder of the day were similar. Therefore the two porridges had similar effects on appetite and satiety in this acute test day setting.

The strengths of the study included the direct analysis of the porridge meals, as served, having carefully controlled for differences in the degree of processing including manufacturing a novel pearl millet steamed rolled flake. Both grain flakes were cooked identically and in plain water as different cooking methods may have an effect on the degree of starch gelatinization **[73; 74]** and also to avoid macronutrient confounders from added milk or jam. The exploration of pre and post absorptive variables, subsequent appetitive perceptions and behaviours presented here is unique in relation to the study of millet. It is worth noting that the structure of β-glucan is poorly characterised in pearl millet, though some of its properties are similar to those of sorghum [75]. Therefore, the mass content of β-glucan alone may not reflect fully its functional role. The health benefits of millets can be related also to the nature and characteristics of their starches, proteins and lipids [76].

Although the participants were of different body sizes, and hence would have had different energy requirements, the test meal portion given was the same for all participants and so would have been a higher proportion of total energy intake for some. This may have reduced the potential for differences in energy intake at the lunch in the participants with a

lower energy requirement. Matching for energy, rather than other micronutrients, meant that slight differences in, for example, fat composition may have confounded the results. However this was felt to be the most clinically relevant approach.

In conclusion, this trial has investigated for the first time the glycaemic, gastrointestinal, hormonal and appetite responses of a pearl millet breakfast porridge intervention compared with a common oats porridge. PMP elicited glycaemic, insulinaemic, GLP-1, PYY and appetite responses comparable to a known breakfast grain with recognised health-promoting characteristics. In addition, PMP had a larger iAUC gastric volume and a lower GIP responses compared with that of SOP. Pearl millet could therefore represent an alternative breakfast food with similar beneficial effects to those of oats and also sustainable and resilient agricultural credentials.

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Authors' contributions

The authors' responsibilities were as follows: JA, MAT, HFJB and LM designed the study with contribution from, RCS on gastroenterology, PAG on imaging, IAM on metabolic physiology, GEY on dietary fibre analysis and GPA on liver metabolism. CLH set up the MRI sequences and analysis. JA, EW and SEP ran the study days and collected and analyzed

573 data. KH and EB collected blood samples. SMC carried out the plasma assays. GEY
574 performed dietary fibre and β-glucan analysis, JA drafted the manuscript. All authors read and
575 approved the final manuscript.
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TABLES

TABLE 1

Breakfast porridge test meal characteristics per served portion[1]

	SOP	PMP
Weight (g) of cooked product served	400	415
Volume of Water drunk with cooked product served (mL)	240	304
Total volume (mL) = volume of cooked product served + water drunk (mL)	640	640
Energy (kJ)	920	920
Energy (kcal)	220	220
Protein (Kjeldahl, g)	7.2	6.6
Total carbohydrate (by difference, g)	42.0	44.4
Carbohydrate (avail, g)	34.0	37.4
Total sugars (enzymic, g)	1.6	1.7
Fat (Weibull-Stoldt, g)	4.4	3.3
Saturates (g)	0.8	0.8
MUFA (cis, g)	2.0	0.8
PUFA (cis)	1.2	1.7
Trans fatty acids (g)	0.4	0.4
Insoluble fibre (g)	3.1	6.4
Soluble fibre (g)	4.9	3.0
β-glucan (g)	2.9	1.6
Total fibre (AOAC, g)	8.0	9.4
Moisture (oven102°C)	345.2	359.4
Ash (at 525°C)	1.2	1.1
Protein N Factor	6.3	6.3
Equivalent salt (g)	0.4	0.4

[1] SOP, Scottish oats porridge and PMP, pearl millet porridge

TABLE 2

Glucose, insulin, GIP, GLP-1 and PYY concentrations measured from healthy participants who were fed two different breakfast porridge test meals[1]

	SOP	PMP	*P* < [2]
Fasting glucose (mmol / L)	5.1 ± 0.1	5.1 ± 0.1	0.627
Glucose peak (mmol / L)	7.4 ± 0.1	7.9 ± 0.2	0.010
Glucose iAUC 2h (mmol/L min)	100 ± 11	125± 14	0.106
Insulin iAUC 2h (mIU/L·min)	2885 ± 189	2759 ± 202	0.503
GIP iAUC 2h (pg / mL·min)	21643 ± 1375	15796 ± 858	0.001
GLP-1 iAUC 2h (pM·min)	3670 ± 370	3467 ± 334	0.121
PYY iAUC 2h (pg / mL·min)	15337±811	14971 ± 956	0.127

[1]All values are mean ± SEM. n = 26 for blood glucose, n = 22 for insulin, GIP, GLP-1 and PYY concentrations. SOP, Scottish Oats porridge and PMP, pearl millet porridge.

[2] Paired t test of difference between SOP and PMP.

TABLE 3

Post-prandial gastric volumes measured by MRI in healthy participants who were fed two different breakfast porridge test meals[1]

	SOP	PMP	*P* < [2]
The half gastric emptying time, $T_{50\%}$ (min)	45 ± 17	47 ± 18	0.918
Gastric volumes iAUC 2h (mL min)	23340 ± 1639	26779 ± 1774	0.045

[1] All values are mean ± SEM. n = 23. SOP, Scottish oats porridge and PMP, pearl millet porridge.

[2] Paired t test of difference between SOP and PMP.

TABLE 4

Subjective appetite scores by question, energy intake from *ad libitum* meal and daily energy intakes from healthy participants who were fed two different breakfast porridge test meals[1]

	SOP	PMP	P < [2]
Hunger iAUC 2h (mm / min)	4049 ± 356	4484 ± 289	0.271
Satisfaction iAUC 2h (mm / min)	8311 ± 330	8137 ± 334	0.685
Fullness iAUC 2h (mm / min)	8487 ± 347	8261 ± 314	0.412
Desire to eat iAUC 2h (mm / min)	4708 ± 375	4722 ± 357	0.812
Prospective food consumption iAUC 2h (mm / min)	5630 ± 387	5711 ± 332	0.985
A composite appetite score iAUC 2h (mm / min)	4918 ± 296	5066 ± 274	0.708
Energy intake from *ad libitum* meal (kcal)	863 ± 78	900 ± 76	0.328
Self-reported energy intake over the remainder of the day (kcal)	1166 ± 105	1076 ± 106	0.468
Self-reported protein intake over the remainder of the day (g)	53 ± 7	50 ± 7	0.408
Self-reported fat intake over the remainder of the day (g)	45 ± 4	40 ± 6	0.353
Self-reported carbohydrate intake over the remainder of the day (g)	132 ± 14	117 ± 11	0.394
The total daily energy intake (kcal)	1753 ± 138	1818 ± 135	0.506

[1] All values are mean ± SEM. n = 26 for appetite scores, energy intake from *ad libitum* meal and self-reported daily energy intakes. SOP, Scottish oats porridge and PMP, pearl millet porridge

[2] Paired t test of difference between SOP and PMP.

LEGENDS FOR FIGURES

Figure 1. Study participant flow diagram.

Figure 2. Diagram of the study day protocol.

Figure 3. Plot of the blood glucose values with time for healthy participants after they consumed two different breakfast porridge test meals.  , Scottish oats porridge (SOP) and  , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26.

Figure 4. Representative example of axial MRI images through the same location in the abdomen of a healthy participant who consumed Scottish oats porridge (SOP) or pearl millet porridge (PMP) test meals on two different occasions. Images were taken at t = 15 min after feeding. Anatomical landmarks such as the liver, spine and spleen are indicated by the white arrows, whereas the stomach is circled in blue on the panel on the right. Both porridges showed clear layering (phase separation), with a darker layer at the bottom of the stomach (circled in yellow on the panel on the left) and a brighter layer at the top of the stomach (circled in red on the panel on the left).

Figure 5. Plot of the gastric volume with time for healthy participants after they consumed two different breakfast porridge test meals.  , Scottish oats porridge (SOP) and  , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 23. There was a significant differences in gastric volume iAUC 2h between the meals (paired t test, $P < 0.05$).

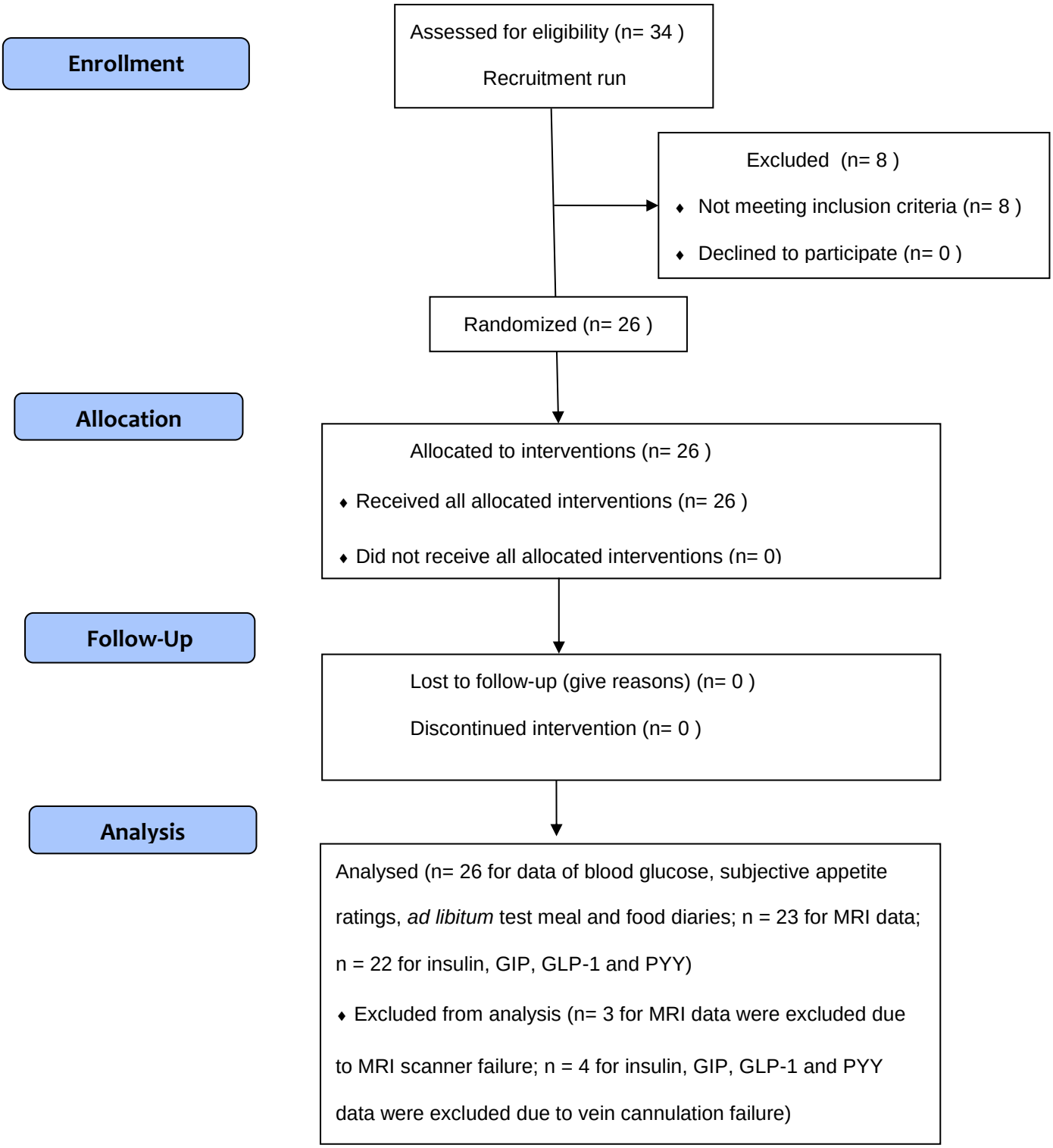
Figure 6. Plot of the plasma insulin concentrations with time for healthy participants after they consumed two different breakfast porridge test meals.  , Scottish oats porridge (SOP) and  , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 22.

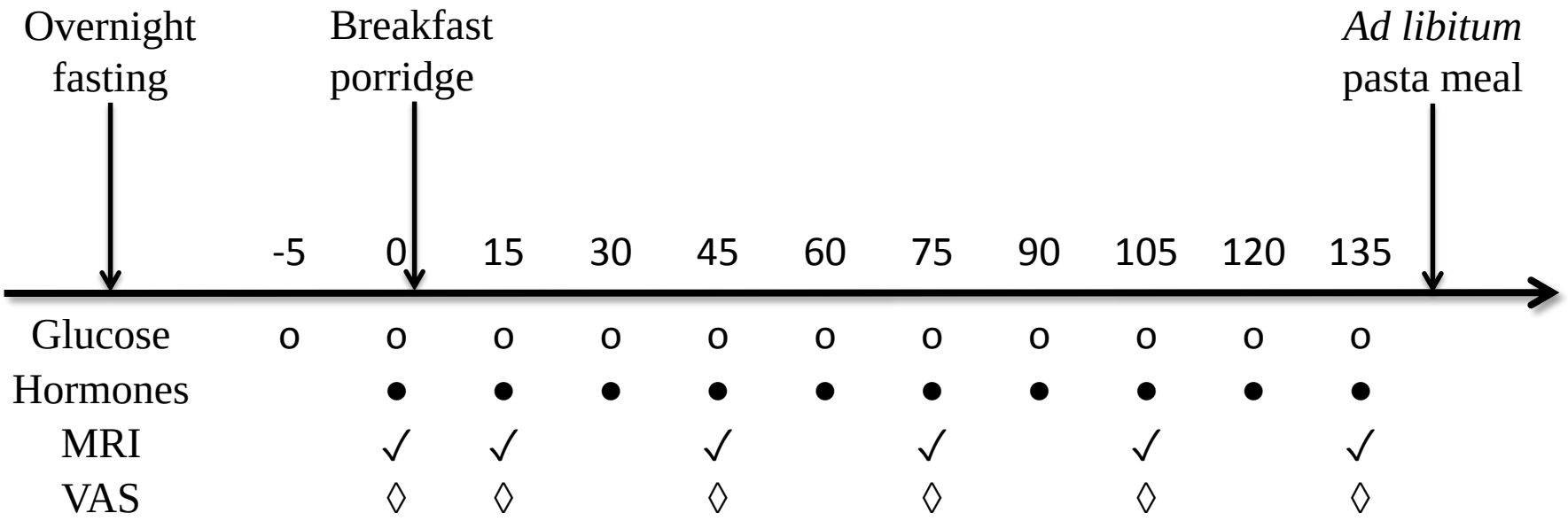
Figure 7. Plot of the plasma GLP-1 concentrations with time for healthy participants after they consumed two different breakfast porridge test meals.  , Scottish oats porridge (SOP) and  , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 22.

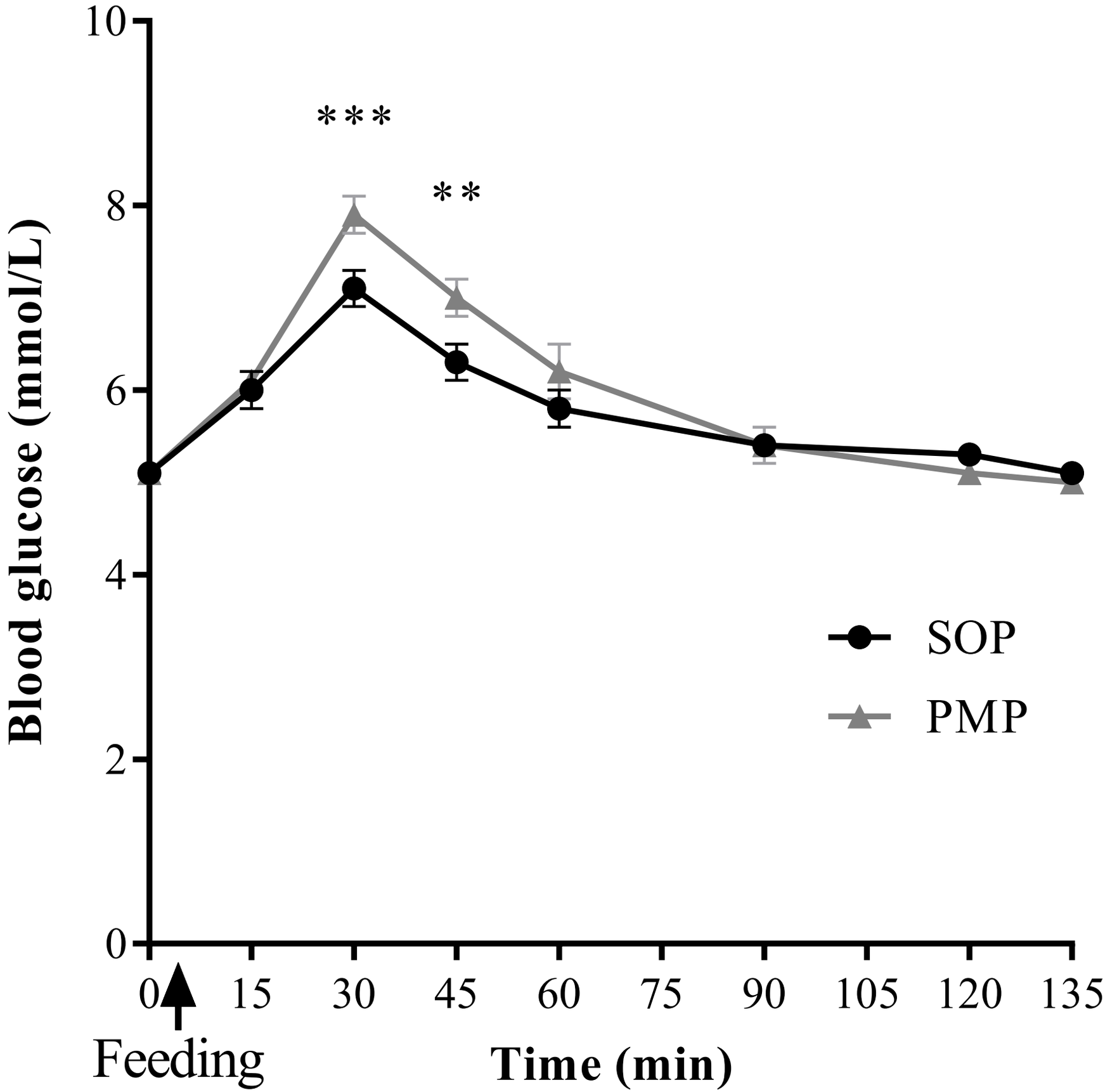
Figure 8. Plot of the plasma GIP concentrations with time for healthy participants after they consumed two different breakfast porridge test meals.  , Scottish oats porridge (SOP) and  , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 22. There was a significant difference in GIP iAUC 2h between the breakfast meals (paired t test, $P < 0.05$). * significant difference between SOP and PMP, $P < 0.05$.

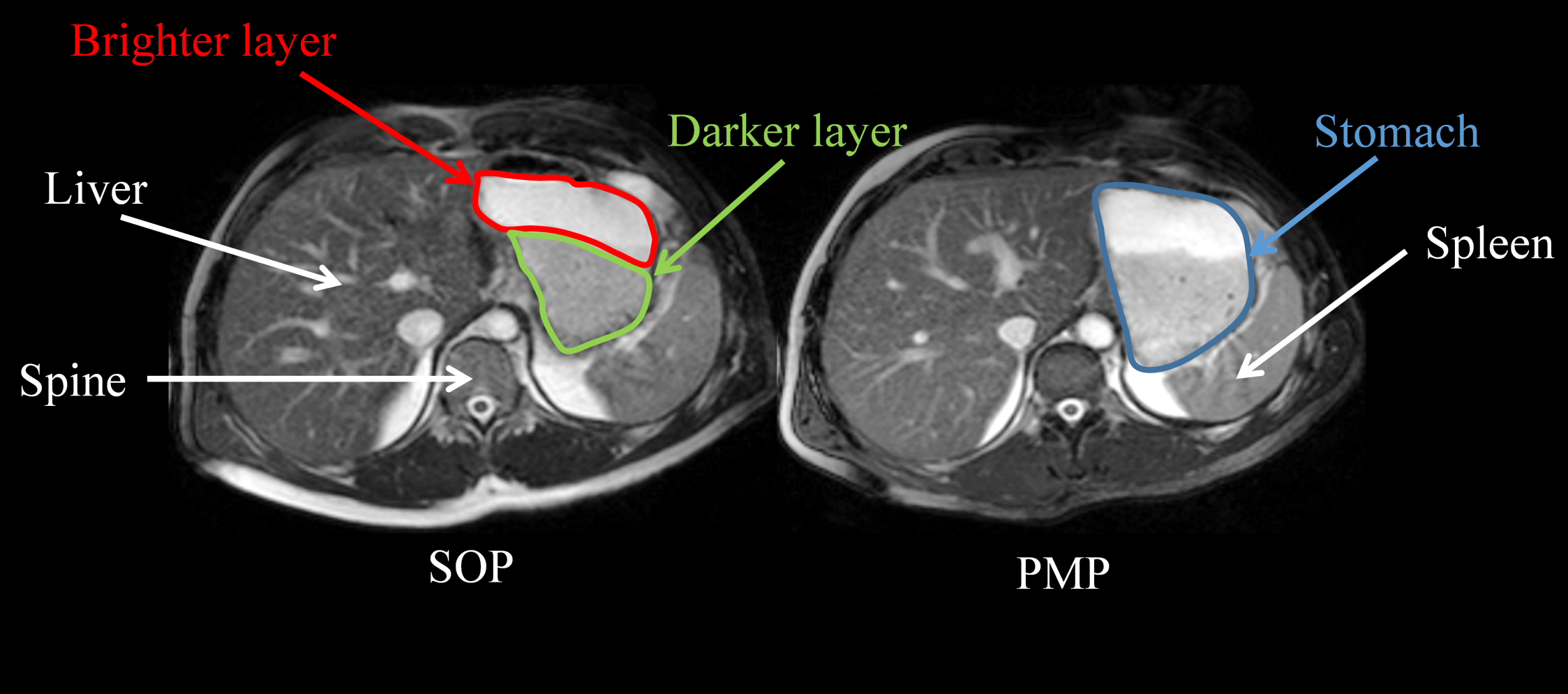
Figure 9. Plot of the plasma PYY concentrations with time for healthy participants after they consumed two different breakfast porridge test meals.  , Scottish oats porridge (SOP) and  , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 22.

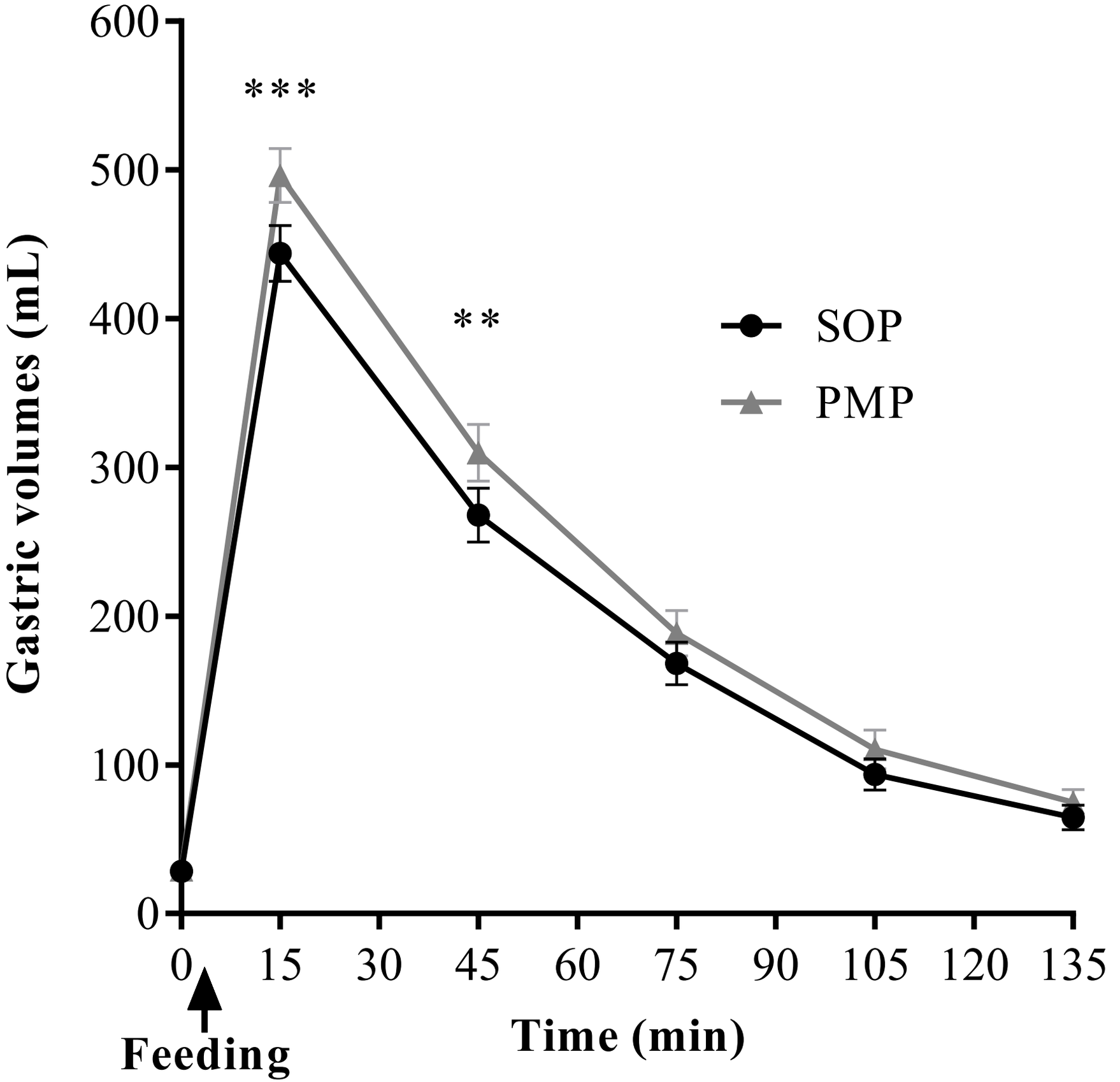
Figure 10. Plot of the composite appetite score with time for healthy participants after they consumed two different breakfast porridge test meals.  , Scottish oats porridge (SOP) and  , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26.

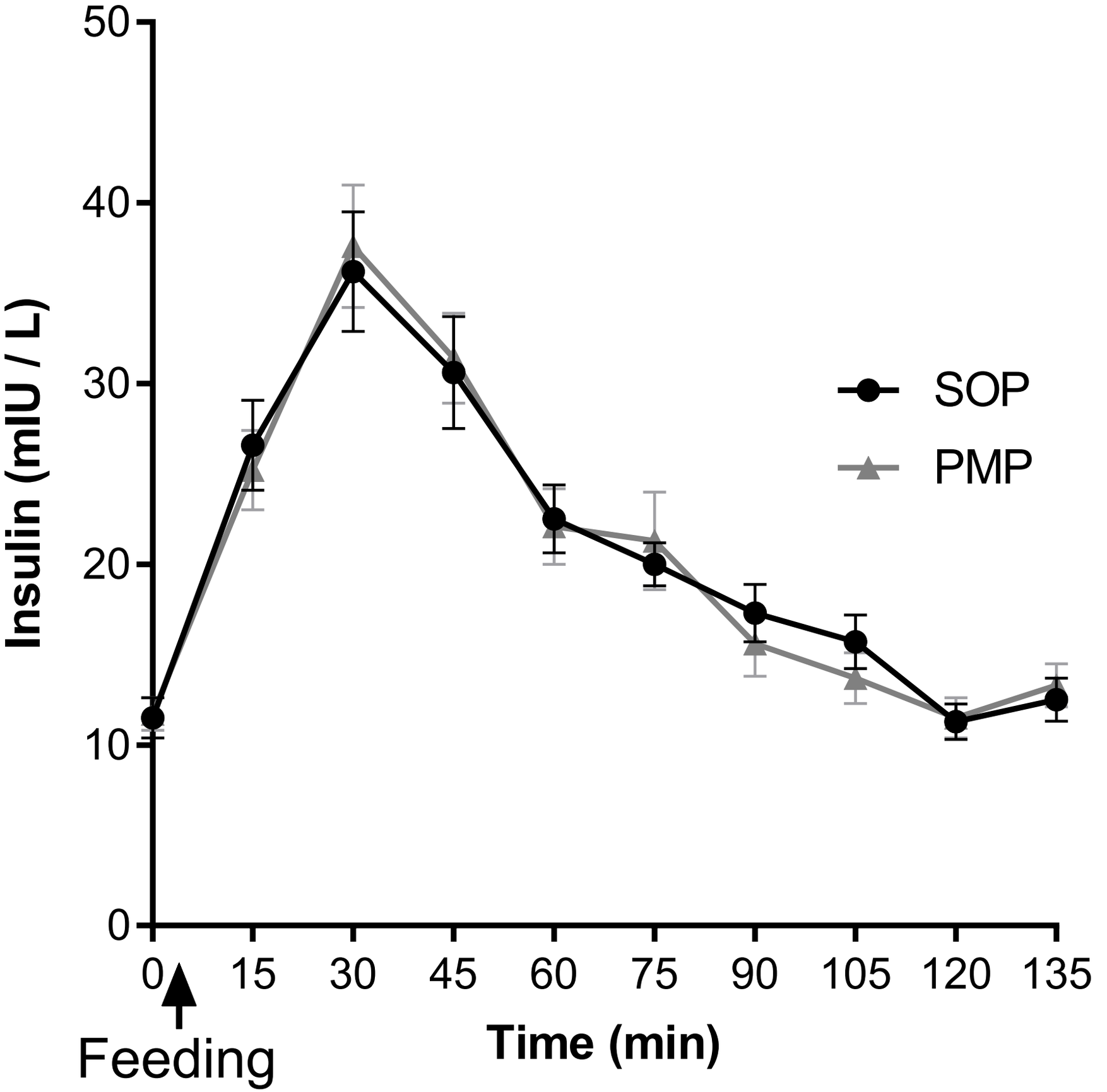

Enrollment
Assessed for eligibility (n= 34)
Recruitment run
Excluded (n= 8)
♦ Not meeting inclusion criteria (n= 8)
♦ Declined to participate (n= 0)
Randomized (n= 26)
Allocation
Allocated to interventions (n= 26)
♦ Received all allocated interventions (n= 26)
♦ Did not receive all allocated interventions (n= 0)
Follow-Up
Lost to follow-up (give reasons) (n= 0)
Discontinued intervention (n= 0)
Analysis
Analysed (n= 26 for data of blood glucose, subjective appetite ratings, *ad libitum* test meal and food diaries; n = 23 for MRI data; n = 22 for insulin, GIP, GLP-1 and PYY)
♦ Excluded from analysis (n= 3 for MRI data were excluded due to MRI scanner failure; n = 4 for insulin, GIP, GLP-1 and PYY data were excluded due to vein cannulation failure)

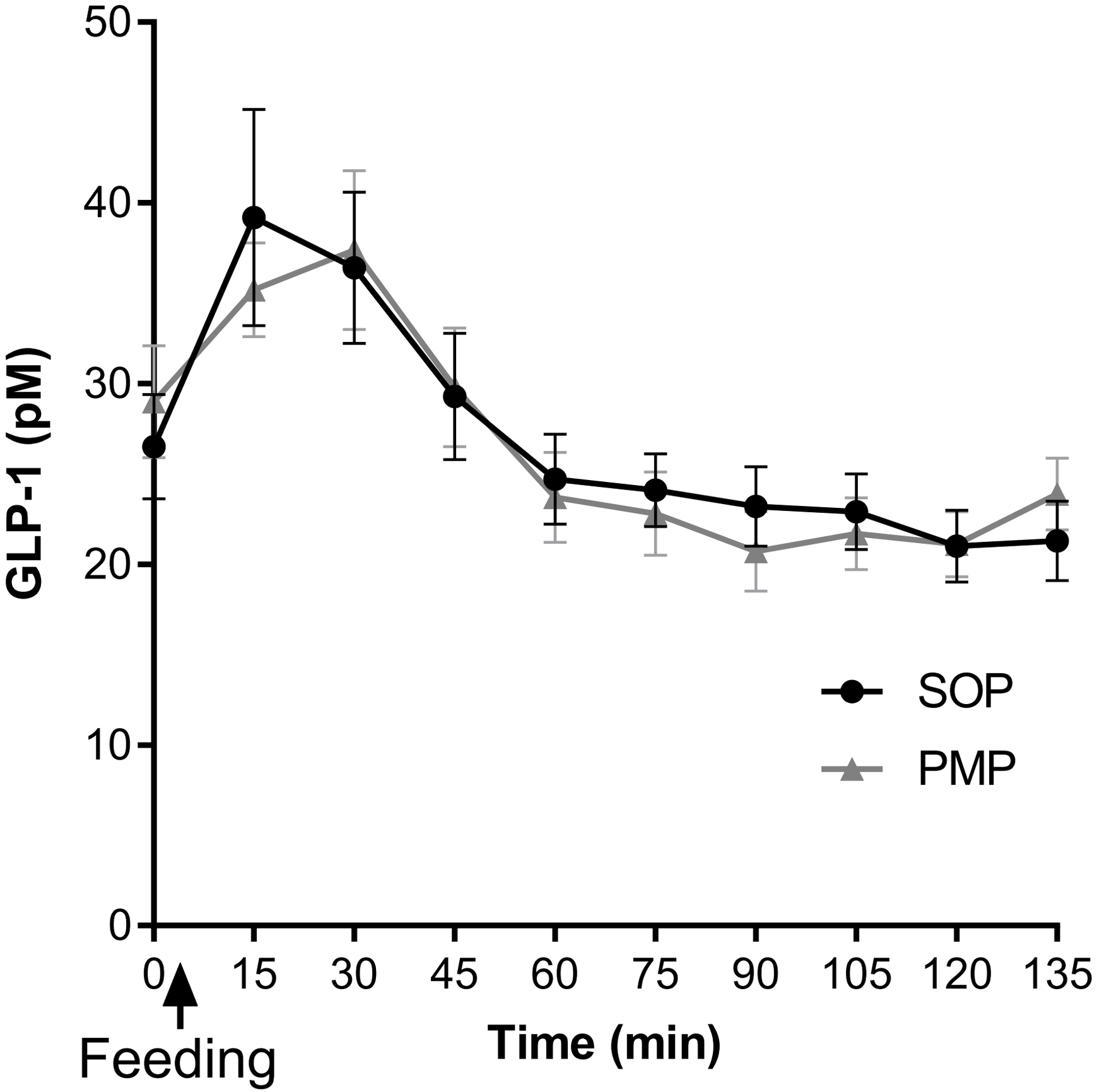

Overnight fasting
Breakfast porridge
Ad libitum pasta meal
-5
0
15
30
45
60
75
90
105
120
135
Glucose
Hormones
MRI
VAS

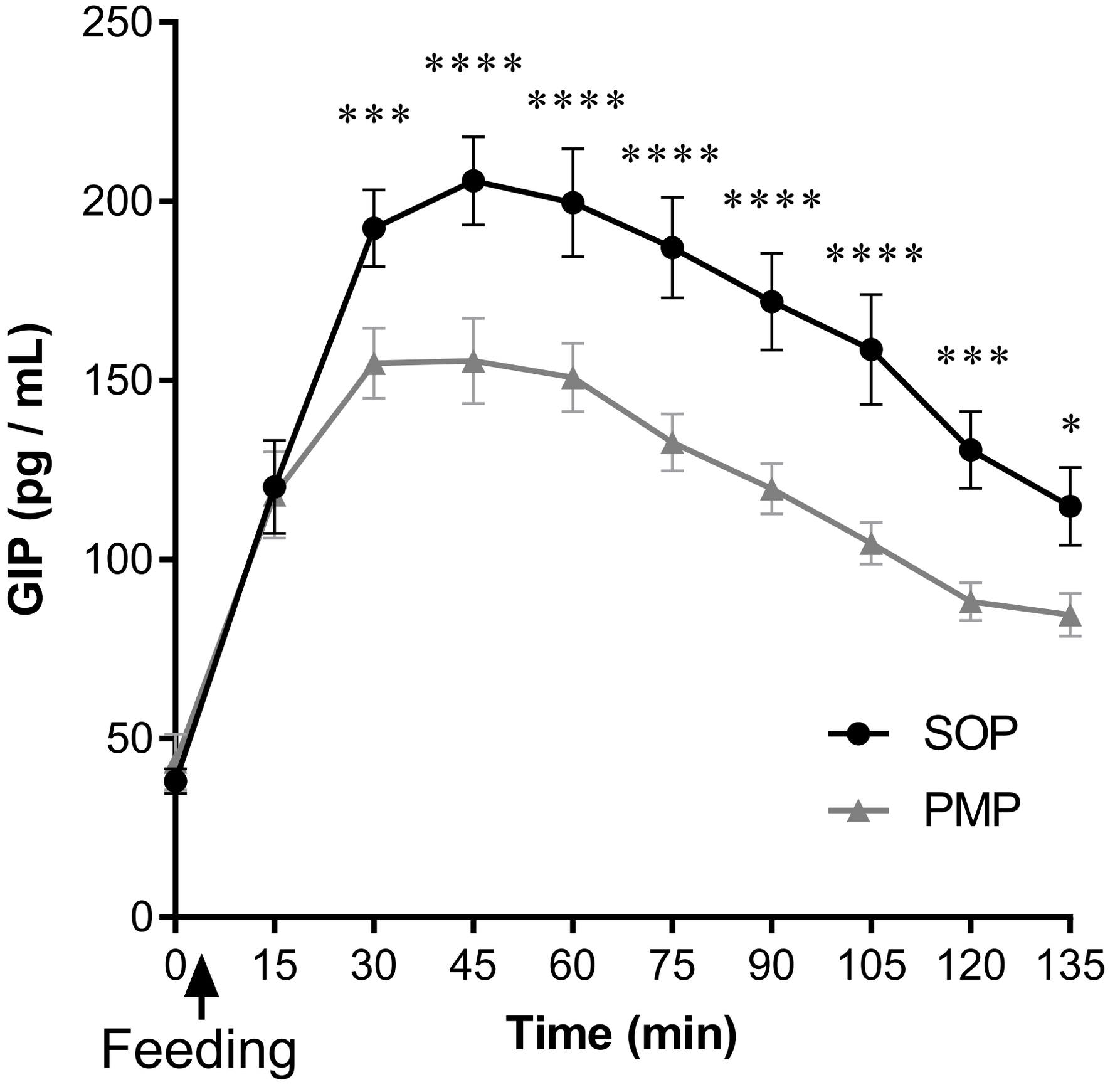

10
8
6
4
2
0
***
**
Blood glucose (mmol/L)
SOP
PMP
0
15
30
45
60
75
90
105
120
135
Feeding
Time (min)

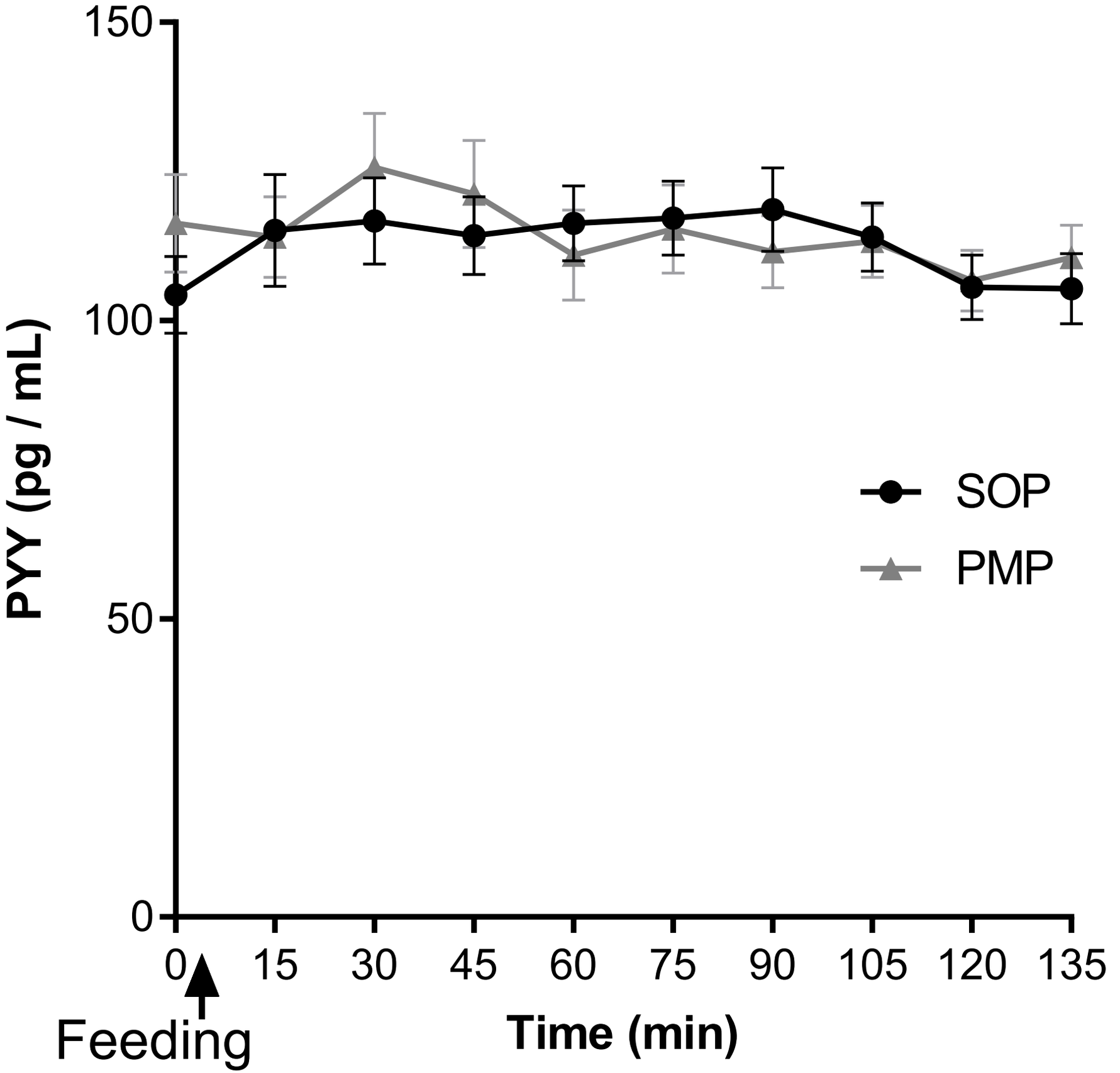

Brighter layer
Darker layer
Liver
Spine
SOP
Stomach
Spleen
PMP

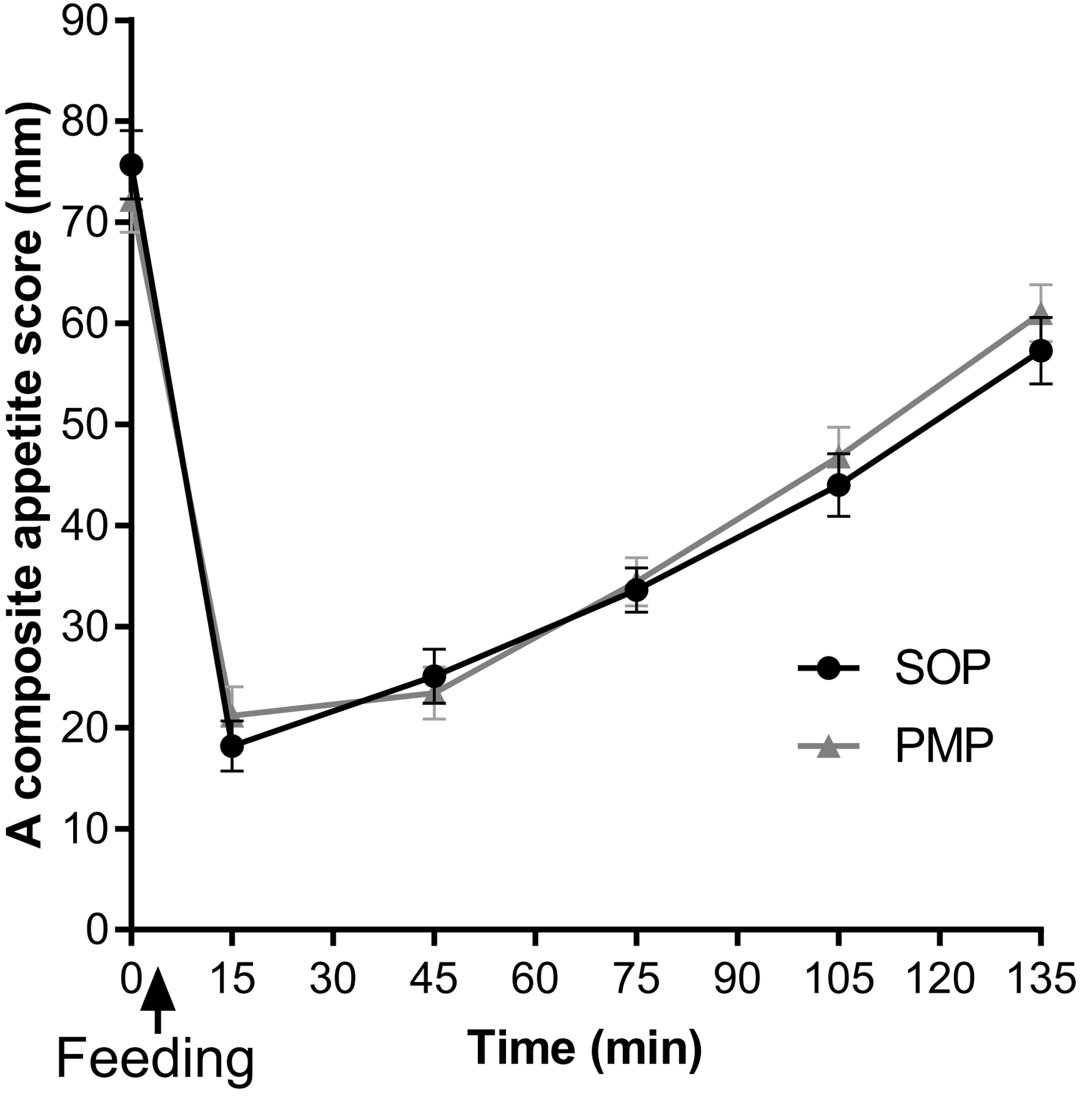

600
500
400
300
200
100
0
Gastric volumes (mL)
***
**
SOP
PMP
0
15
30
45
60
75
90
105
120
135
Feeding
Time (min)


50
40
30
20
10
0
Insulin (mIU / L)
SOP
PMP
0
15
30
45
60
75
90
105
120
135
Feeding
Time (min)


50
40
30
20
10
0
GLP-1 (pM)
0
15
30
45
60
75
90
105
120
135
Time (min)
Feeding
SOP
PMP

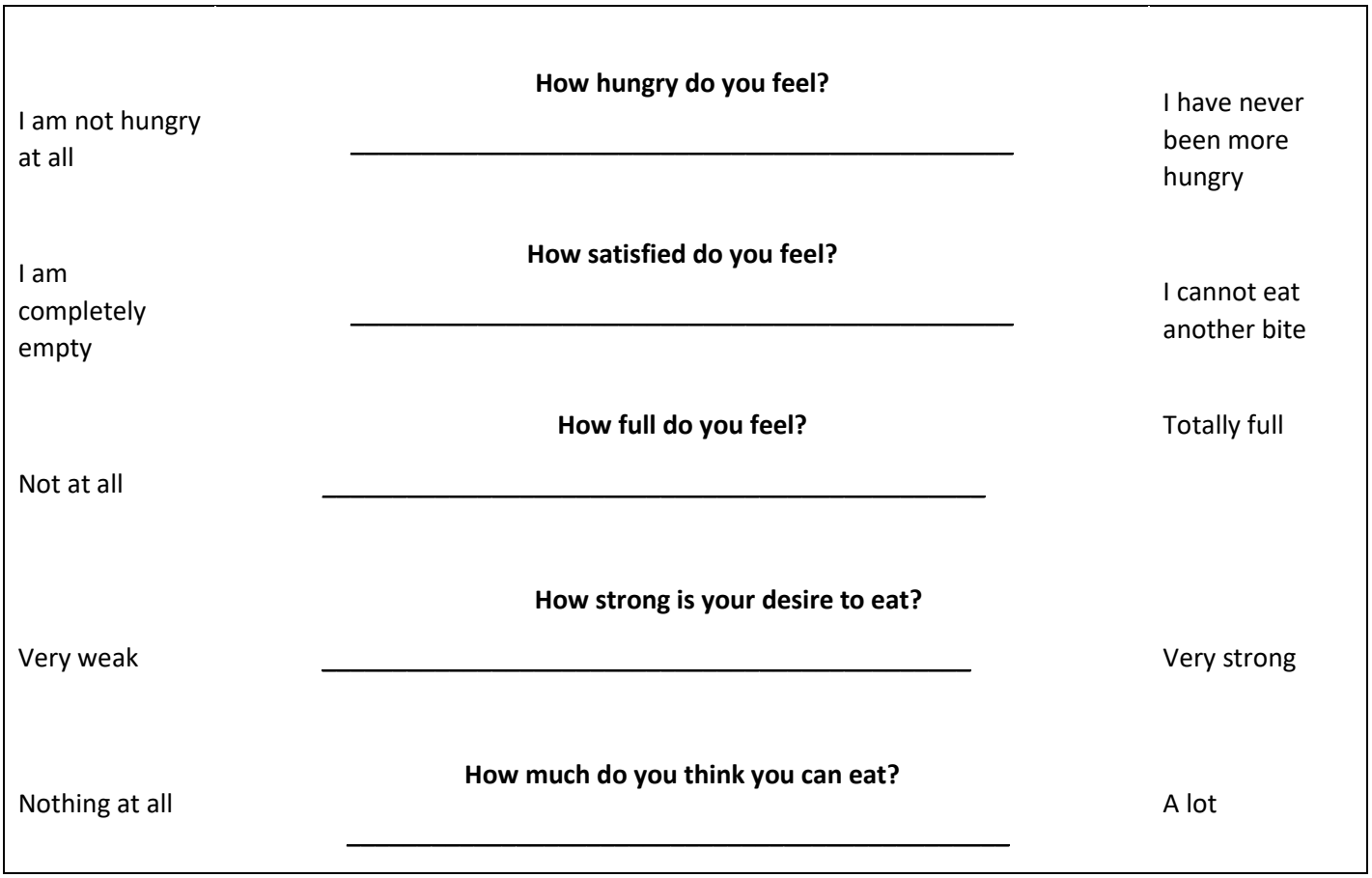

250
200
150
100
50
0
GIP (pg / mL)
***
****
****
****
****
****
***
*
0
15
30
45
60
75
90
105
120
135
Time (min)
Feeding
SOP
PMP


150
100
50
0
PYY (pg / mL)
SOP
PMP
0
15
30
45
60
75
90
105
120
135
Feeding
Time (min)

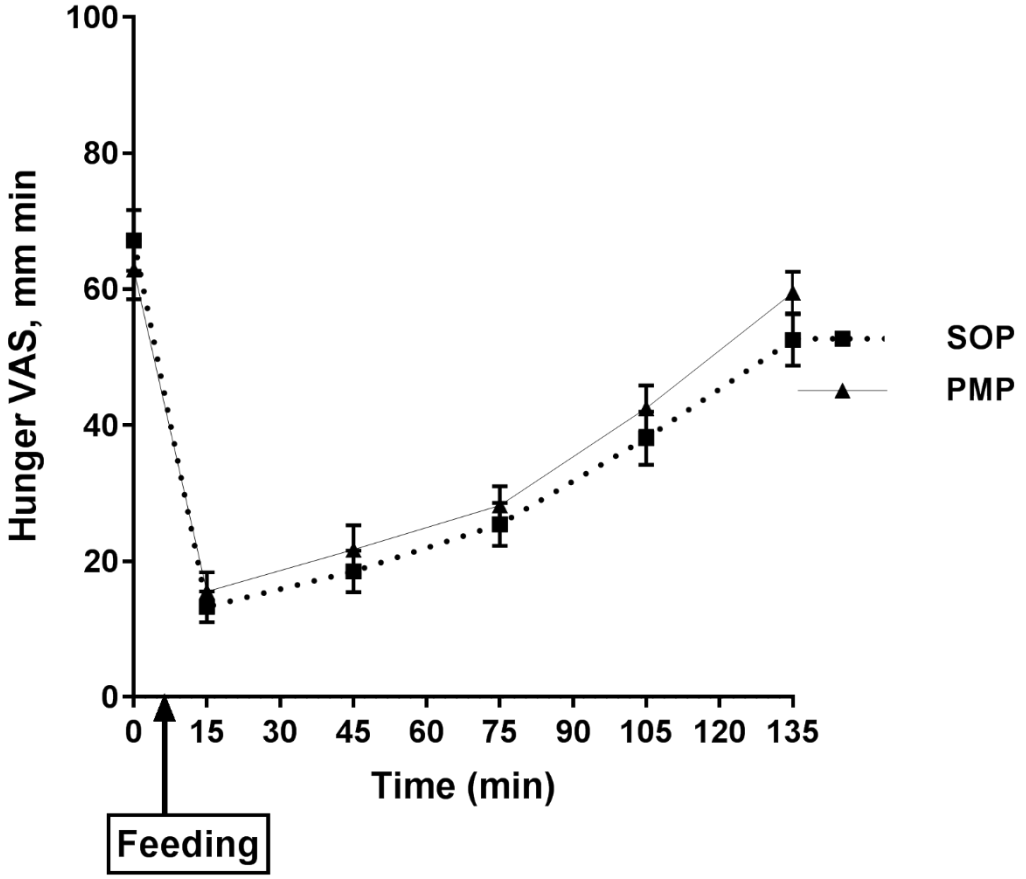

A composite appetite score (mm)
90
80
70
60
50
40
30
20
10
0
0
15
30
45
60
75
90
105
120
135
Feeding
Time (min)
SOP
PMP

Supplementary material

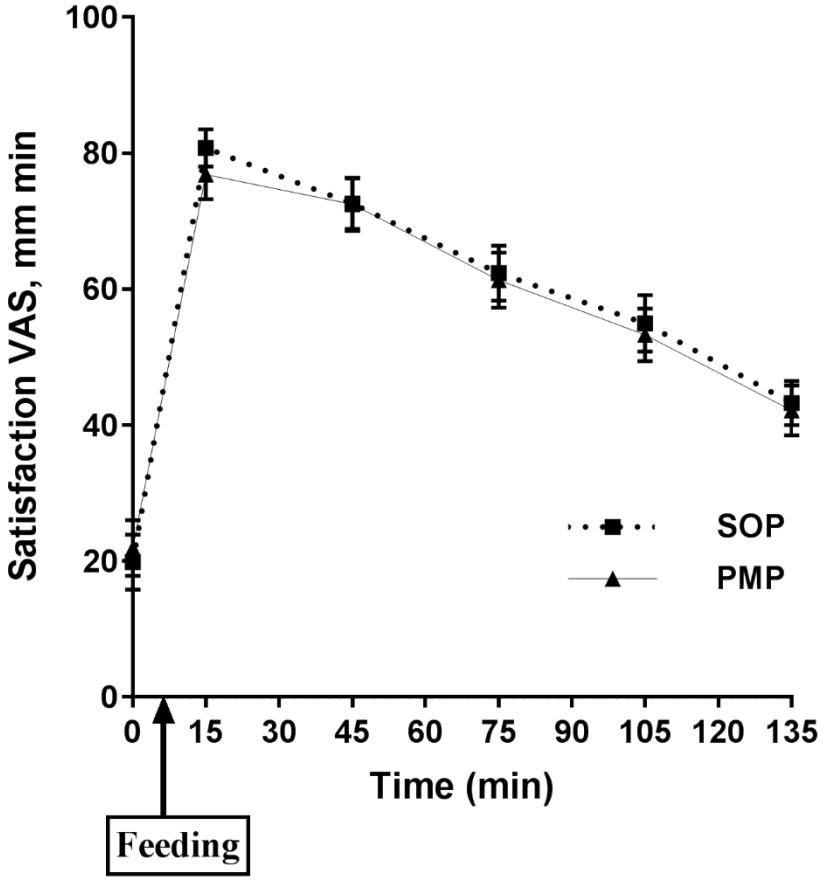
Supplementary Figure 1: Subjective appetite ratings VAS.



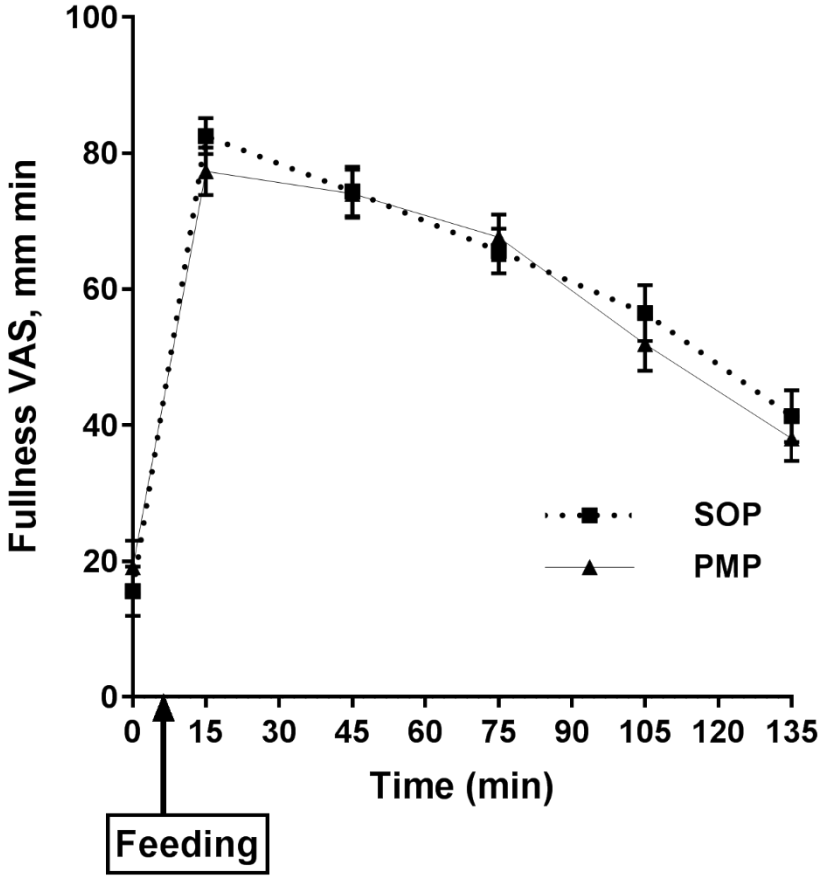
Supplementary Figure 2. Plot of hunger with time for healthy participants after they consumed two different breakfast porridge test meals. , Scottish oats porridge (SOP) and , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26.



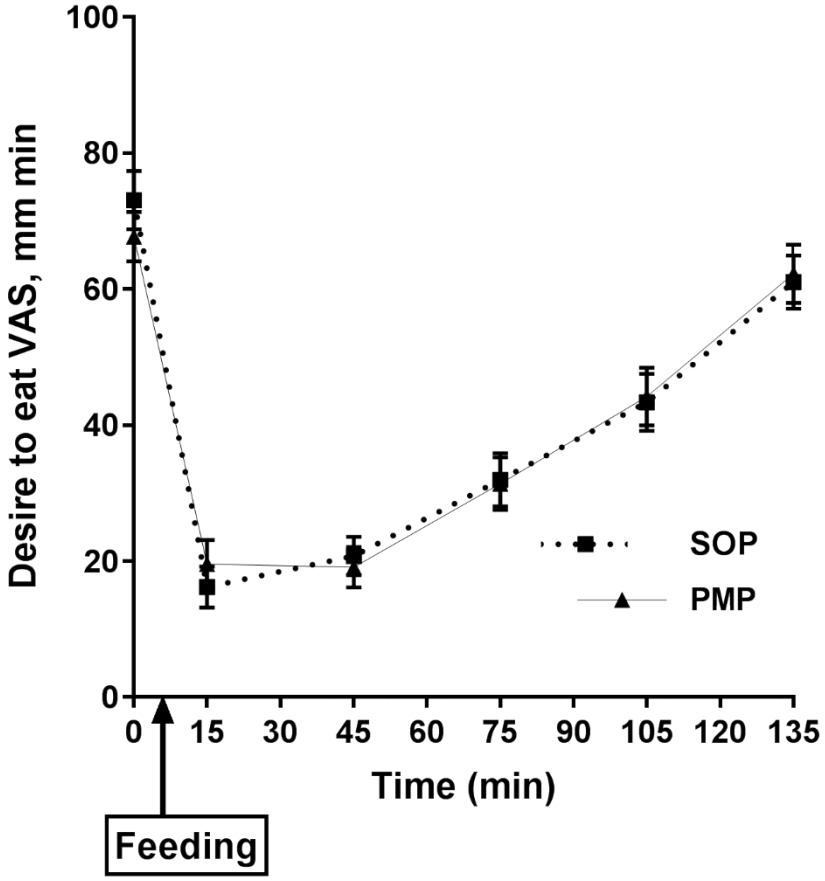
Supplementary Figure 3. Plot of satisfaction with time for healthy participants after they consumed two different breakfast porridge test meals. , Scottish oats porridge (SOP) and , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26.



Supplementary Figure 4. Plot of fullness with time for healthy participants after they consumed two different breakfast porridge test meals. , Scottish oats porridge (SOP) and , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26.



Supplementary Figure 5. Plot of desire to eat with time for healthy participants after they consumed two different breakfast porridge test meals. , Scottish oats porridge (SOP) and , pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26.



Supplementary Figure 6. Plot of prospective to food consumption with time for healthy participants after they consumed two different breakfast porridge test meals. ●—●, Scottish oats porridge (SOP) and ▲—▲, pearl millet porridge (PMP). The arrow on the horizontal axis indicates the meal start time. Values are mean ± SEM, n = 26.