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Augmented gut hormone response to feeding in older adults exhibiting low appetite.

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ABSTRACT

Age-related changes in gut hormones may play a role in anorexia of ageing. The aim of this study was to determine concentrations of ghrelin, PYY, and GLP-1 in older adults exhibiting an anorexia of ageing phenotype. Thirteen older adults with healthy appetite (OA-HA; 8f, 75±7 years, 26.0±3.2 $kg \cdot m^{-2}$), fifteen older adults with low appetite (OA-LA; 10f, 72±7 years, 23.6±3.1 $kg \cdot m^{-2}$), and twelve young adults (YA; 6f, 22±2 years, 24.4±2.0 $kg \cdot m^{-2}$) completed the study. Healthy appetite and low appetite were determined based on BMI, habitual energy intake, self-reported appetite, and laboratory-assessed *ad libitum* lunch intake. Participants provided a fasted measure of subjective appetite and blood sample (0 minutes) before consuming a standardised breakfast (450 kcal). Appetite was measured and blood samples were drawn throughout a 240-minute rest period. At 240 minutes, an *ad libitum* lunch meal was consumed. Relative intake at lunch (expressed as percentage of estimated total energy requirement) was lower for OA-LA (19.8±7.7%) than YA (41.5±9.2%, $p<0.001$) and OA-HA (37.3±10.0%, $p<0.001$). Ghrelin suppression was greater for OA-LA (net AUC, -78719±74788 $pg \cdot mL^{-1} \cdot 240min^{-1}$) than both YA (-23899±27733 $pg \cdot mL^{-1} \cdot 240min^{-1}$, $p=0.016$) and OA-HA (-21144±31161 $pg \cdot mL^{-1} \cdot 240min^{-1}$, $p=0.009$). There were trends for higher GLP-1 concentrations in OA-LA compared with YA at 90 minutes (8.85±10.4 pM vs. 1.88±4.63 pM, $p=0.073$) and 180 minutes (5.00±4.71 pM vs. 1.07±2.83 pM, $p=0.065$). There was a trend for a greater PYY response for OA-LA compared with OA-HA (net AUC $p=0.062$). "Anorexigenic response score" – a composite score of gut hormone responses to feeding – showed greater anorexigenic response in OA-LA, compared with YA and OA-HA. No differences were seen in subjective appetite. These observations suggest augmented anorexigenic responses of gut hormones to feeding may be causal mechanisms of anorexia of ageing.

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KEY WORDS

Anorexia of ageing, hunger, satiety, ageing, malnutrition

ABBREVIATIONS

AEBSF – 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride

ANOVA – Analysis of variance

nAUC – Net area under the curve

CCK - Cholecystokinin

EDTA – Ethylenediaminetetraacetic acid

ELISA – Enzyme-linked immunosorbent assay

HA-OA – Healthy appetite older adults

IPAQ – International Physical Activity Questionnaire

LA-OA – Low appetite older adults

METs – Metabolic equivalents

OA – Older adults

PP – Pancreatic polypeptide

PYY – Peptide tyrosine-tyrosine

SNAQ – Simplified Nutritional Appetite Questionnaire

TER – Total energy requirements

VAS – Visual analogue scale

YA – Young adults

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INTRODUCTION

Anorexia of ageing describes the age-related decline in appetite and food intake experienced in later life (Morley, 1997). A loss of appetite affects over 30% of community dwelling older adults (van den Broeke et al., 2018) and up to 60% of older adult hospital patients (Cox et al., 2020; Ray et al., 2014). Anorexia of ageing has been strongly implicated in malnutrition (Dent et al., 2019), which is associated with sarcopenia, frailty (Ligthart-Melis et al., 2020), and mortality (Söderström et al., 2017). The subsequent increased healthcare utilisation is substantial. Annual health and social care costs are estimated to be 3 times greater for undernourished older adults, compared with those adequately nourished (Russell & Elia, 2014). With an ageing global population, malnutrition in later life is an imposing challenge for current and future healthcare provisions.

The causes of anorexia of ageing are yet to be conclusively determined. It is likely a multifaceted phenomenon, including age-related changes in physiological and hedonic control, and societal factors (Cox et al., 2020). One proposed mechanism is a change in appetite-associated gut hormone secretion with age. A meta-analysis by Johnson et al. (2020) showed elevated concentrations of the anorexigenic hormones leptin, CKK, and PYY in older adults compared with younger adults. However, the effect of ageing on concentrations of other appetite-associated hormones, such as ghrelin and GLP-1 were less clear.

A potential reason for the remaining contention regarding age-related changes in gut hormones – and regarding other mechanisms of anorexia of ageing – relates to the common design of studies in this field. Typically, studies compare mechanisms of interest, such as gut hormone responses to feeding, between younger adults and older adults, with little consideration of the heterogeneity of the older adult cohort. Heterogeneity in eating behaviour (ter Borg et al., 2015) and nutritional needs of older adults (Krondl et al., 2008) is well-established, and has been identified as a challenge when attempting to identify relationships between participant characteristics and eating patterns or weight status (Hsiao et al., 2011). In addition, variance in gut hormone responses to feeding in older adults is often considerable (Johnson et al., 2020). Indeed, with the prevalence of anorexia of ageing in community dwelling older adults being around 30%, it is likely that study cohorts of older adults consist of some with impaired appetite and some with unimpaired, healthy appetite. Pooling both in the same study cohort likely masks some responses that are not a function of ageing but are exclusive to those with suppressed appetite. Consequently, hormonal dysregulation that may be causal of anorexia of ageing could be overlooked.

Identifying those with low appetite is challenging. The limitations of free-living, self-reported measures of habitual dietary intake are well-known (Ravelli & Schoeller, 2020; Saravia et al., 2022), especially in older adults where recall bias may be increased (Freedman et al., 2014; Park et al., 2018; Rhodes et al., 2019) and adherence to food diaries has been shown to be low (Rowland et al., 2018).

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Changes in body mass, indicating inadequate energy intake, are not always detected as only around 50% of people self-weigh regularly (Gavin et al., 2015; VanWormer et al., 2012) and access to weighing scales is limited for some cohorts of the population (Bramante et al., 2020). Questionnaires have been developed for assessing appetite, such as the Simplified Nutritional Appetite Questionnaire (SNAQ). This tool has proved a quick and simple way to identify individuals at risk of undernutrition, with validity having been shown in community-dwelling (Lau et al., 2020) and hospitalised patients (Kruizenga et al., 2005). However, there is contention over cut-off points for identifying low-appetite (Wilson et al., 2005; Lau et al., 2020) and conformation of criterion validity against an objective measure of eating behaviour or appetite is lacking.

Recently, we used a multi-criteria approach, including an objective, laboratory-measured assessment of energy intake at an *ad libitum* test meal, to identify older adults with low appetite. This model enabled us to observe differences in ghrelin metabolism between healthy-appetite older adults and low-appetite older adults (Holliday et al., 2024). Phenotyping older adults exhibiting anorexia of ageing in this way should facilitate the exploration of the mechanisms underpinning why some older adults experience low appetite and some do not.

The aim of this study was to determine gut hormone response to feeding in older adults with apparent healthy appetite and older adults exhibiting low appetite. We aimed to confirm our recent findings of differences in ghrelin response in a slightly extended sample, in combination with determining responses of anorexigenic hormones PYY and GLP-1. Comparing gut hormone responses to feeding between younger adults, older adults with a healthy appetite, and older adults with low appetite will shed light on changes in gut hormones that reflect normal ageing and those which may underpin the age-related decline in appetite and energy intake characteristic of anorexia of ageing. A secondary aim was to assess the appropriateness of our four-criteria method of phenotyping older adults with low appetite.

METHOD

Study Design

In a cross-sectional, observational study, responses of ghrelin, PYY, and GLP-1 to feeding were compared between younger adults (YA, aged 18 – 29 years), older adults (aged ≥ 65 years) with a healthy appetite (OA-HA), and older adults with low appetite (OA-LA). The study adhered to the ethical guidelines as stated in the Declaration of Helsinki. Ethical approval was granted by the Newcastle University Faculty of Medical Sciences Research Ethics Committee (LREC #: 2146/13433/2020).

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Participants

Fifteen non-obese, low-to-moderately active YA; and thirty non-obese, low-to-moderately active OA were recruited. Participants were recruited via Facebook interest groups local to Newcastle and the surrounding areas, and through the public engagement platform VOICE Global. Inclusion criteria were habitual early-to-mid morning (07:00 – 10:00) breakfast consumer, a score of < 3000 MET mins · week^{-1} on the International Physical Activity Questionnaire (IPAQ; Craig et al., 2003), body mass index (BMI) of < 30 kg·m^{-2} for YA and < 33 kg·m^{-2} for OA (such a BMI value is associated with increased risk of mortality in older adults (Winter et al., 2014)), non-smoker, not attempting to intentionally change bodyweight or composition, not taking medication likely to impact on appetite, and free from metabolic disease. Those aged 30 – 64 years were excluded as ghrelin concentration has been shown to increase during the menopause (Sowers et al., 2008). OA were categorised as either exhibiting a healthy appetite (OA-HA) or exhibiting signs of low appetite (OA-LA). Low appetite was identified if two of four *a priori* criteria were met (Holliday et al., 2024). These were:

1) Low BMI (< 23 kg·m^{-2} (such a BMI value is associated with increased risk of mortality in older adults (Winter et al., 2014)).
2) Low habitual energy intake (<75% estimated total energy requirement (TER), as identified by the World Health Organisation as indicative of undernutrition) as measured by 24-hour dietary recall.
3) Low score (< 15) on the Simplified Nutritional Appetite Questionnaire (SNAQ; Lau et al., 2020).
4) A laboratory-measured *ad libitum* lunch intake of < 25% of estimated TER (based on a typical lunch energy intake of ~27% of total energy intake in UK mid-life adults (Pot et al., 2014)). Details of the lunch meal are provided in the "*Ad libitum* food intake" subsection, below.

Younger adults who met two of these four criteria (low BMI cut off of < 18.5 kg·m^{-2}) were excluded.

Enrolment and Familiarisation

Participants attended the Human Nutrition Suite at Newcastle University for a single enrolment and familiarisation session. Informed written consent was obtained after the study procedures had been explained verbally and after any questions had been addressed. Height and weight were recorded, and habitual physical activity (IPAQ) and appetite (SNAQ) were assessed. An assessment of habitual daily food intake was obtained using the computerised, multiple-pass, 24-hour dietary recall system, Intake24 (Foster et al., 2019). Total daily energy requirement was estimated using the Mifflin-St Joer equation (Mifflin et al., 1990).

Participants were then familiarised with the test meals to be consumed on the trial visit. The breakfast meal was provided in full to ensure all participants could finish the entire portion in the standardised time of between five and six minutes. Those unable to consume the entire portion were excluded from the study. Palatability of the lunch meal was qualitatively confirmed by providing a small sample to taste. Participants were asked to confirm that the meal was "palatable", and that they would be able to eat until "satisfyingly full" during the experimental trial and not stop eating before reaching fullness due to disliking the food. All screened participants rated the meal as "palatable".

Experimental Procedures

Participants returned to the Human Nutrition Suite at Newcastle University within two weeks of the enrolment visit for the experimental trial. Participants were instructed to abstain from exercise, caffeine, and alcohol on the day before the experimental visit, and to consume a standardised, nutrient-balanced evening meal of beef hash, yoghurt and orange juice (691 kcal; 47% energy from carbohydrate, 29% fat, 23% protein) a minimum of 12 hours prior to arrival at the laboratory the following day. Participants arrived at the laboratory between 08:00 and 09:00, fasted but having drunk 300mL of water upon waking. Adherence to dietary and exercise controls were confirmed. Subjective appetite was then assessed using the visual analogue scale (VAS) method immediately prior to the insertion of a cannula into the antecubital vein of the arm (time: t=0 minutes, see Figure 1). Ten minutes after cannulation (t=10 minutes), a fasted blood sample was obtained. At t=15 minutes, participants consumed a standardised breakfast test meal of porridge (made with whole milk) with natural yoghurt and honey (450kcal, with a macronutrient balance representative of UK dietary recommendations: 50% carbohydrate, 15% protein, 35% fat). The breakfast was standardised in absolute (kcal), rather than relative ($kcal \cdot kg^{-1}$, or percentage of total energy requirements), terms to ensure the same nutrient consumption and same nutrient challenge for all participants. The meal represented a substantive, mixed nutrient challenge to elicit gut hormone response, while also proving manageable for low-appetite older adults to consume (as identified through pilot testing). Preparation of the porridge breakfast meal, including cooling time, was identical for all participants. To standardise the rate of eating, all participants consumed the meal in between five and six minutes (identified as the mean time for consuming the meal in pilot testing with young and older adults).

At t=30 minutes, subjective appetite was measured and a second blood sample was obtained. Participants then rested for a further 210 minutes, with appetite measured every 30 minutes and blood samples obtained at t=60, 90, 120, 180 and 240 minutes (see Figure 1). Participants remained seated and were free to read, watch television, or use a laptop computer. Activity was monitored to

ensure the avoidance of food cues in reading and viewing material. On occasions when more than one participant was present in the laboratory, they rested in separate sectioned areas. When interacting with other participants, participants were politely asked to avoid discussions relating to food or to the measurements being recorded.

At t=240 minutes, the cannula was removed and participants were provided with an *ad libitum* pasta-based lunch meal. Completion of the meal represented the end of the trial.

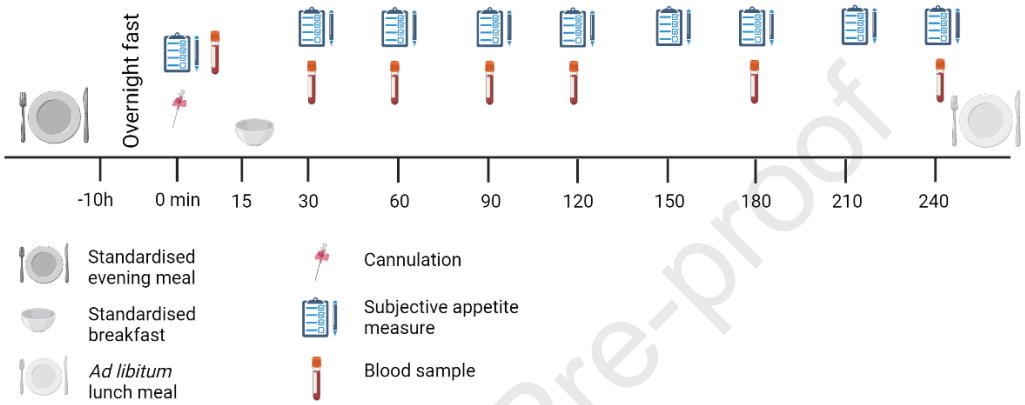


Figure 1. Study protocol

Outcome Measures

Plasma concentration of ghrelin, PYY and GLP-1

Blood was collected in EDTA-treated blood collection tubes for the measure of total PYY and GLP-1. Blood obtained for the measure of ghrelin was collected in EDTA tubes pre-treated with AEBSF protease inhibitor (1g·mL^{-1} of whole blood (Deschaine & Leggio, 2020)). Whole blood was centrifuged at 2000g and 4^{o}C for 15 minutes to separate plasma from cellular material. Plasma was aliquoted (0.5mL per aliqout) and stored at -80^{o}C for batch analysis after all data was collected. Plasma aliquots for the measure of ghrelin were treated with 0.02mL of 1M hydrochloric acid.

Total ghrelin was measured by enzyme-link immunosorbent assay (ELISA). Ghrelin was measured using commercially available kits (Human Ghrelin (total) ELISA kit, Merck Millipore). Sensitivity was 156 pg·mL^{-1}. Coefficients of variation (CV) was 6.38%. Samples from 35 participants (11 YA, 11 OA-HA, 13 OA-LA) were measured for total GLP-1 and total PYY using in-house established radioimmunoassay (RIA) at Imperial College London (Kreymann et al., 1987, Adrian et al., 1985). Sensitivity and CV of RIA were 0.36 pg.mL^{-1}, 4.43% and 2.885 pg.mL^{-1}, 3.97% for GLP-1 and PYY respectively. Samples from 5 participants (1 YA, 2 OA-HA, 2 OA-LA) were measured by

ELISA using commercially available kits (Human PYY (Total) ELISA kit and Multi Species GLP-1 Total ELISA, Merck Millipore) due to the unavailability of RIA labels. Sensitivity and CV were 1.5 pM, 6.95% and 1.4 $pg.mL^{-1}$, 3.28% for GLP-1 and PYY respectively.

Subjective appetite

Subjective appetite perceptions were measured using the 4-item VAS method, assessing hunger ("How hungry are you?"), fulness ("How full are you?"), desire to eat ("How strong is your desire to eat?") and expected consumption ("How much would you expect to eat right now?") (Flint et al., 2000). Participants answered each item by placing a vertical mark on an ungraded, 100mm horizontal line anchored at each end with extreme response. The distance from the left-hand anchor to the participant's mark was measured to obtain a score for each item. A composite score was calculated from the four items as: (hunger score + (100-fullnessscore) + desire to eat score + expected intake score) / 4 (Holliday & Blannin, 2017).

Ad libitum food intake

To measure food intake, a homogeneous pasta-based *ad libitum* test meal was used (Deighton et al., 2016). The meal was nutrient-balanced to align with UK dietary recommendations and consisted of pasta, Bolognese sauce and grated cheese, with added olive oil (energy density = 1.79 $kcal \cdot g^{-1}$. 50% energy from carbohydrate, 15% protein, 35% fat). Participants were made aware that food consumption would be measured, and were instructed to eat until they felt "satisfyingly full." To avoid a situation whereby an empty bowl provided a cue to stop eating prior to satiation, each bowl of pasta was replaced with a fresh, full bowl before the participant emptied the previous one. Food was consumed in isolation, with an avoidance of distractions and food cues, and with no time limit. The mass of each bowl was pre-weighed immediately before presenting to the participant and re-weighed after being replaced, with the difference in mass representing the mass of food consumed. After the meal, the table and surrounding area was checked for food spillage. Any spillage was weighed and subtracted from the calculated mass of food consumed. Energy intake was calculated from the mass of food consumed and the known energy density of the meal.

Statistical Analyses

Values are presented and mean ± SD (mean ± SEM in figures). Fasted ghrelin, PYY, and GLP-1 concentrations and *ad libitum* lunch intake (expressed as absolute energy intake and intake as a percentage of estimated TER) were compared between YA and all OA, and between YA, OA-HA, and OA-LA by two-way analysis of variance (ANOVA), with sex included as fixed factor. This was done to account for uneven sex distribution across group. Sex main effects and group x sex interactions are identified and stated only where present but were not explored further as the study

was not powered to determine sex differences or effects. Subjective appetite, ghrelin, PYY, and GLP-1 response to feeding was presented as change-from-baseline. Differences between groups (between-subject factor) and over the trial period (within-subject factor) were assessed using a mixed-design analysis of variance (ANOVA), with sex included as a second between-subject factor. Net area-under-the-curve (nAUC) was calculated for each of these variables using the trapezium method. We also calculated an overall "anorexigenic response score". For this, z scores for AUC were calculated for ghrelin, PYY and GLP-1. The ghrelin Z score was inverted and a mean Z score for all three hormones was calculated, with a higher value representing a more anorexigenic response. Differences in nAUC and in anorexigenic response score between YA and all OA and between YA, OA-HA, and OA-LA were assessed by two-way ANOVA with sex included as a fixed factor.

Throughout, significant interactions and main effects were explored further using Bonferroni-corrected pairwise comparisons. Eta squared (η^2) and partial η^2 (η^2_p) effect sizes were calculated for main effects and interactions, respectively, while Cohen's *d* effect sizes were calculated for pairwise comparisons. Statistical significance was determined at an alpha level of 0.05. Probability (*p*) values of < 0.1 are described as a trend. Missing data were assessed by the multiple imputation method, with the mean value calculated from five iterations. This was the case for four data points for ghrelin and PYY (four participants, 2 x OA-HA, 2 x OA-LA) and 6 data points for GLP-1 (six participants, 1 x YA, 3 x OA-HA, 2 x OA-LA).

Associations between gut hormone response (represented by the anorexigenic response score), subjective appetite response (nAUC for subjective appetite response), and *ad libitum* lunch intake were assessed by Pearson's correlation. Significant associations were explored further by linear regression. These analyses were conducted for all participants, and separately for OA only.

Predictors of anorexigenic responses of gut hormones to feeding were also assessed. Backward elimination linear regression was conducted with anorexigenic response score as the outcome variable and the four variables included in the criteria to identify low appetite (BMI, SNAQ score, daily EI as percentage of TER, and *ad libitum* lunch intake) as predictors. At each step, the least significant variable (above a *p*-value threshold of 0.1) was eliminated from the model until remaining variables contributed independently to variance in the outcome measure. Principle component analysis for BMI, SNAQ score, daily EI as percentage of TER, and *ad libitum* lunch intake was attempted but aborted due to violations of sampling adequacy (Kaiser-Meyer-Olkin test = 0.414). All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS, Version 29.0.1.0).

An *a priori* power calculation was conducted to power the study to detect changes in line with previous studies which had observed differences in PYY and GLP-1 concentration between older

and younger adults (Geizenaar et al., 2018; Geizenaar et al., 2017). With statistical power of 0.8 and an alpha value of 0.05, a sample of at least 12 participants per group was required to detect a large difference (d = 0.8).

RESULTS

Participant characteristics

The characteristics of all participants included in analyses, as grouped by age and appetite are shown in Table 1. Two younger adults were excluded as they met two of the four criteria for identifying low appetite and one younger adult withdrew due to lack of time. One older adult withdrew due to lack of time, and one was excluded due to difficulty with phlebotomy procedures. The older and young adult cohorts did not differ by BMI, weight, or physical activity, but SNAQ score was significantly lower for older adults (p = 0.009, d = 0.953). The breakfast test meal constituted a greater relative energy intake, as percentage of TER, for older adults than younger adults (p < 0.001, d = 1.995). The mean breakfast energy content in the OA cohort of 23.9% of estimated TER was comparable with the typical energy intake at breakfast of OA in the UK (22% of daily energy intake (Gaal et al., 2018)).

When comparing YA, OA-HA, and OA-LA, no significant differences were seen in body mass, BMI or physical activity (all p > 0.1). A group main effect for SNAQ score (p < 0.001, η^2 = 0.405) was observed, with score being significantly lower in OA-LA compared with both YA (p < 0.001) and OA-HA (p = 0.001), but there was no difference between OA-HA and YA. Daily EI was lower in OA-LA compared with OA-HA (p = 0.046, d = 0.909), as was EI as percentage of TER (p = 0.041, d = 1.242). The breakfast test meal was a greater percentage of TER for YA compared with both OA-HA (p < 0.001, d = 2.113) and OA-LA (p < 0.001, d = 1.861). Age did not differ between OA-HA and OA-LA (p = 0.323).

Table 1 – Participant characteristics for younger adults, all older adults, older adults with healthy appetite, and older adults with low appetite

	Younger adults	**Older adults**		
		Total	**Healthy appetite**	**Low appetite**
N	12	28	13	15
Sex	6f, (50%) 6m (50%)	18f (64%), 10m (36%)	8f (62%), 5m (38%)	10f (67%), 5m (33%)
Age (years)	22 ± 2	73 ± 7 ***	75 ± 7 *	72 ± 7 *
BMI (kg · m^{-2})	24.4 ± 2.0	24.7 ± 3.5	26.0 ± 3.2	23.6 ± 3.1
Body mass (kg)	75.0 ± 11.0	68.2 ± 11.5	71.1 ± 11.4 *	65.7 ± 11.5 *
Physical activity (METs · day^{-1})	1916 ± 1272	1453 ± 1124	1300 ± 1162	1606 ± 1109
SNAQ score	16.8 ± 1.4	14.8 ± 2.3 **	16.2 ± 0.9	13.5 ± 2.5**$^{\#\#\#}$
Daily EI (kcal) **%TER**			2007 ± 893 110 ± 48	1358 ± 522 $^{\#}$ 72 ± 35 $^{\#}$
Breakfast test meal as %TER	16.8 ± 1.8	23.9 ± 4.7 ***	24.6 ± 4.9 ***	23.3 ± 4.6 ***

BMI, body mass index; METs, metabolic equivalents; SNAQ, Simplified Nutritional Appetite Questionnaire, EI, energy intake; TER, total energy requirements.

* = significantly different to younger adults ($p < 0.05$); ** = significantly different to younger adults ($p < 0.01$); *** = significantly different to younger adults ($p < 0.001$); # = significantly different to older adults with healthy appetite ($p < 0.05$).

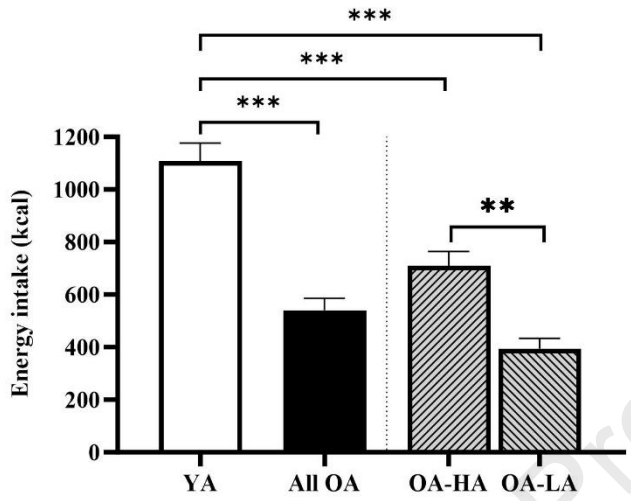
Energy intake

Absolute energy intake at the *ad libitum* lunch meal for YA, all OA, OA-HA and OA-LA is shown in Figures 2a. Energy intake was significantly greater for YA, compared with all OA (1108 ± 235 kcal vs. 532 ± 234 kcal, $p < 0.001$, $d = 2.456$). When comparing YA, OA-HA, and OA-LA, there was a significant group main effect ($p < 0.001$, $\eta^2 = 0.712$). Post hoc pairwise comparisons revealed intake was significantly greater for YA (1108 ± 235 kcal), compared with both OA-HA (705 ± 207 kcal, $p < 0.001$, d = 1.820) and OA-LA (395 ± 150 kcal, $p < 0.001$, $d = 3.617$). Intake was also greater for OA-HA than OA-LA ($p = 0.001$, $d = 1.713$).

When expressed relative to estimated TER (Figure 2b), energy intake as a percentage of TER was greater for YA compared with all OA (41.5 ± 9.2% vs. 27.6 ± 12.4%, $p < 0.001$, $d = 1.207$). When

comparing YA, OA-HA, and OA-LA, there was a significant group main effect ($p < 0.001$, $\eta^2 = 0.552$). Intake was lower for OA-LA (19.8 ± 7.7%) compared with both YA (41.5 ± 9.2%, $p < 0.001$, $d = 2.558$) and OA-HA (37.3 ± 10.0%, $p < 0.001$, $d = 1.961$). There was no difference between YA and OA-HA ($p = 0.781$).

a)



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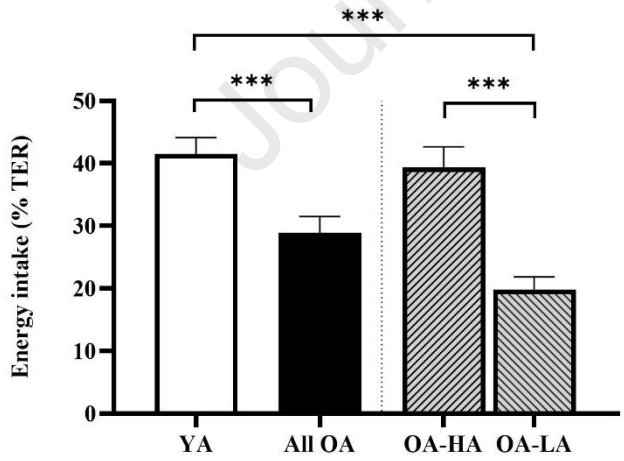

Figure 2. Mean ± SEM absolute *ad libitum* lunch intake (a) and lunch intake as a percentage of estimated TER (b) for YA, all OA, OA-HA, and OA-LA. *** = significant between-group difference, $p \leq 0.001$.

Fasted hormone concentrations

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Ghrelin

There was a trend for higher fasted plasma ghrelin concentration in OA compared with YA (1057 ± 621 pg·mL^{-1} vs. 636 ± 251 pg·mL^{-1}, $p = 0.056$, $d = 0.889$, Figure 3a). When comparing YA, OA-HA, and OA-LA, there was a significant group main effect ($p = 0.002$, $\eta^2 = 0.316$). Concentration was significantly higher in OA-LA (1328 ± 652 pg·mL^{-1}) compared with both YA (636 ± 251 pg·mL^{-1}, $p = 0.002$, $d = 1.315$) and OA-HA (744 ± 418 pg·mL^{-1}, $p = 0.007$, $d = 0.947$). There was also a sex main effect for fasted ghrelin concentration ($p = 0.036$, $\eta^2 = 0.123$).

PYY

Fasted plasma PYY concentration did not differ between YA and all OA (16.75 ± 7.80 pg·mL^{-1} vs. 24.18 ± 21.63 pg·mL^{-1}, $p = 0.264$, $d = 0.395$, Figure 3b), nor between YA, OA-HA, and OA-LA (16.75 ± 7.80 pg·mL^{-1} vs. 25.14 ± 20.87 pg·mL^{-1} vs. 23.29 ± 23.02 pg·mL^{-1}, $p = 0.408$, $\eta^2 = 0.050$).

GLP-1

Fasted plasma GLP-1 concentration did not differ between YA and all OA (2.93 ± 4.16 pM vs. 4.22 ± 3.93 pM, $p = 0.345$, $d = 0.324$, Figure 3c), nor between YA, OA-HA, and OA-LA (2.93 ± 4.16 pM vs. 3.57 ± 3.36 pM vs. 4.93 ± 4.50 pM, $p = 0.688$, $\eta^2 = 0.040$).

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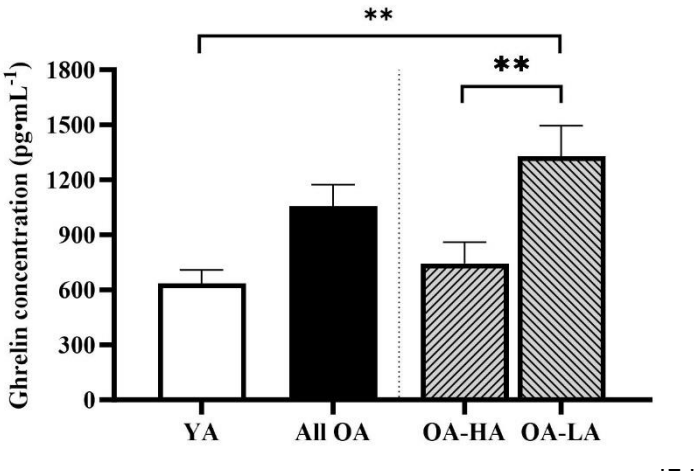
a)



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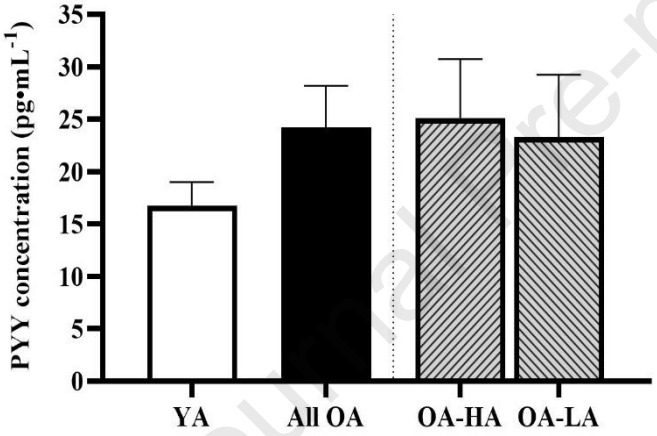

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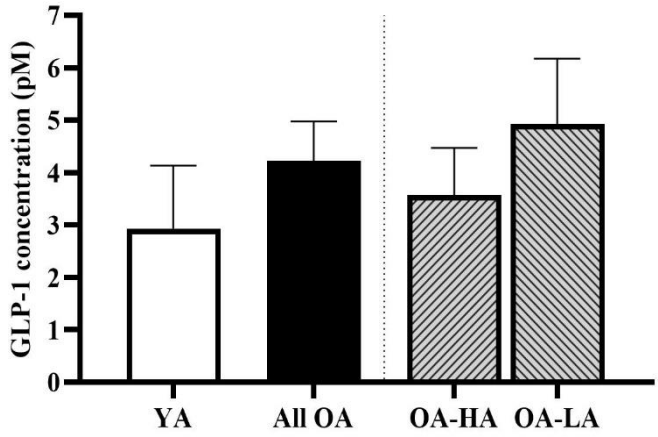

Figure 3. Mean ± SEM fasted concentrations of ghrelin (a), PYY (b), and GLP-1 (c). * = significantly different, $p < 0.05$. ** = significantly different, $p < 0.01$.

Hormone response to feeding

Ghrelin

The plasma ghrelin concentrations in response to the standardised breakfast meal are shown in Figure 4a and 4b. There was no significant group x time interaction when comparing YA and all OA ($p = 0.211$, $\eta^2 = 0.042$, Figure 4a), nor was there a group main effect ($p = 0.325$, $\eta^2 = 0.027$). nAUC did not differ between YA and all OA (-23899 ± 27733 pg·mL^{-1}·240min^{-1} vs -51988 ± 64705 pg·mL^{-1}·240min^{-1}, $p = 0.275$, $\eta^2 = 0.033$).

When comparing YA, OA-HA, and OA-LA, there was a significant group x time interaction ($p = 0.033$, $\eta^2_p = 0.128$, Figure 4b). Ghrelin concentration was lower in OA-LA compared with YA at 60 (-481 ± 426 pg·mL^{-1} vs. -163 ± 155 pg·mL^{-1}, $p = 0.038$, $d = 0.992$), 90 (-533 ± 449 pg·mL^{-1} vs. -174 ± 182 pg·mL^{-1}, $p = 0.033$, $d = 1.048$), and 180 min (-365 ± 386 pg·mL^{-1} vs. -48.0 ± 195 pg·mL^{-1}, $p = 0.028$, $d = 1.037$), with a trend for a difference at 120 min (-526 ± 443 pg·mL^{-1} vs. -208 ± 202 pg·mL^{-1}, $p = 0.066$, $d = 0.924$). Ghrelin concentration was significantly lower in OA-LA than OA-HA at 60 (-481 ± 426 pg·mL^{-1} vs. -147 ± 163 pg·mL^{-1}, $p = 0.014$, $d = 1.036$), 90 (-533 ± 449 pg·mL^{-1} vs. -161 ± 202 pg·mL^{-1}, $p = 0.007$, $d = 1.069$), 120 (-526 ± 443 pg·mL^{-1} vs. -176 ± 222 pg·mL^{-1}, $p = 0.013$, $d = 0.999$) and 180 min (-365 ± 386 pg·mL^{-1} vs. -107 ± 187 pg·mL^{-1}, $p = 0.048$, $d = 0.851$). There was also a group main ($p = 0.009$, $\eta^2 = 0.244$), with significant differences between OA-LA and YA ($p = 0.023$), and OA-LA and OA-HA ($p = 0.009$). There was a significant group main effect for nAUC ($p = 0.008$, $\eta^2 = 0.250$). Post hoc pairwise comparisons showed a more negative nAUC in OA-LA compared with both YA (-78719 ± 74788 pg·mL^{-1}·240min^{-1} vs -23899 ± 27733 pg·mL^{-1}·240min^{-1}, $p = 0.016$, $d = 0.972$) and OA-HA (-78719 ± 74788 pg·mL^{-1}·240min^{-1} vs -21144 ± 31161 pg·mL^{-1}·240min^{-1}, $p = 0.009$, $d = 1.005$). There were also sex main effects for ghrelin response to feeding ($p = 0.028$) and nAUC ($p = 0.022$).

PYY

The plasma PYY concentrations in response to the standardised breakfast meal are shown in Figure 4c and 4d. There was no significant group x time interaction when comparing YA with all OA ($p = 0.474$, $\eta^2_p = 0.021$, Figure 4c). There was also no group main effect ($p = 0.473$, $\eta^2 = 0.014$) and no difference in nAUC between YA and OA (2097 ± 2314 pg·mL^{-1}·240min^{-1} vs 2930 ± 3749 pg·mL^{-1}·240min^{-1}, $p = 0.449$, $d = 0.244$).

When comparing YA, OA-HA, and OA-LA, there was no significant group x time interaction ($p = 0.383$, $\eta^2_p = 0.057$, Figure 4d). The was a trend for a group main effect ($p = 0.066$, $\eta^2 = 0.144$), with a trend for a difference between OA-LA and OA-HA ($p = 0.068$). There was also a trend for a group main effect for nAUC ($p = 0.058$, $\eta^2 = 0.150$), with a trend for a greater nAUC in OA-LA

compared with OA-HA (4357 ± 4662 pg·mL^{-1}·240min^{-1} vs 1400 ± 1416 pg·mL^{-1}·240min^{-1}, p = 0.062, d = 0.858).

GLP-1

The plasma GLP-1 concentrations in response to the standardised breakfast meal are shown in Figure 4e and 4f. There was a significant group x time interaction when comparing YA and all OA (p = 0.006, η^2 = 0.098, Figure 4e). There was a more immediate increase in GLP-1 at 30 mins in YA compared with OA (7.55 ± 9.24 pM vs. 2.64 ± 4.08 pM, p = 0.036, d = 0.687). However, GLP-1 remained elevated in OA, with a trend for a higher concentration at 120 min (4.51 ± 5.09 pM vs. 1.38 ± 2.30 pM, p = 0.072, d = 0.792). Net AUC was not significantly different between YA and OA (576 ± 663 pM·240min^{-1} vs. 987 ± 1012 pM·240min^{-1}, p = 0.231, d = 0.446).

When comparing YA, OA-HA, and OA-LA, there was a significant group x time interaction (p = 0.037, η^2_p = 0.115, Figure 4f). There were trends for higher GLP-1 concentrations in OA-LA compared with YA at 90 (8.85 ± 10.4 pM vs. 1.88 ± 4.63 pM, p = 0.073, d = 0.866) and 180 mins (5.00 ± 4.71 pM vs. 1.07 ± 2.83 pM, p = 0.065, d = 1.011). There was no difference in nAUC (p = 0.129, η^2_p = 0.117).

Anorexigenic response score

Anorexigenic response score did not differ between YA and all OA (-0.27 ± 0.38 vs. 0.10 ± 0.83, p = 0.189). When comparing YA, OA-HA, and OA-LA, there was significant condition effect (p = 0.005, η^2_p = 0.259), with anorexigenic response score significantly greater in OA-LA (0.49 ± 0.98) than both YA (-0.27 ± 0.34, p = 0.015, d = 1.032) and OA-HA (-0.32 ± 0.30, p = 0.007, d = 1.121)

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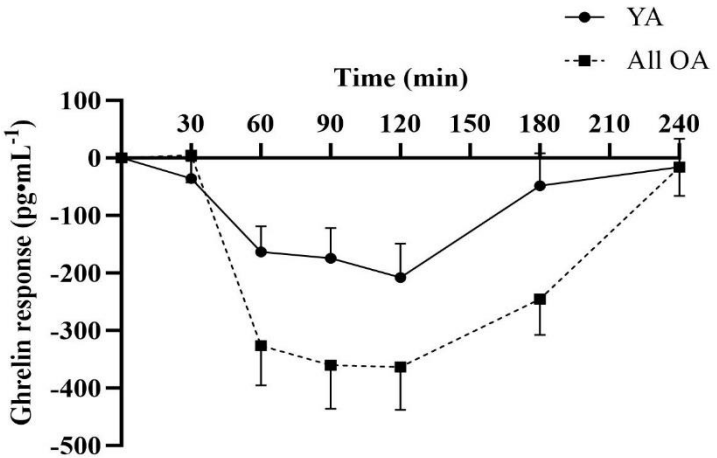

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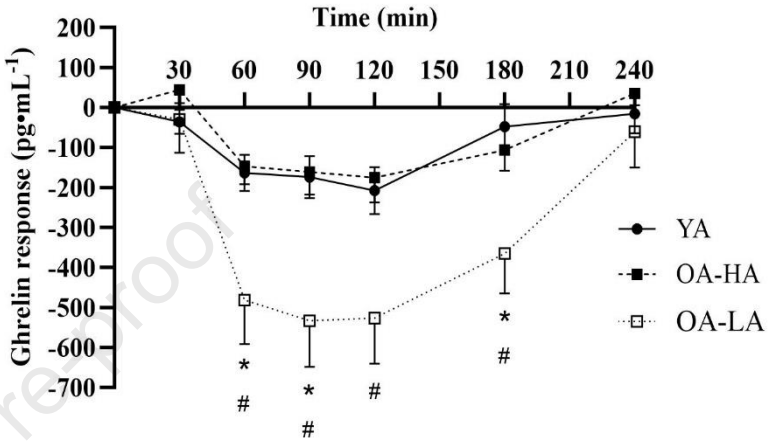

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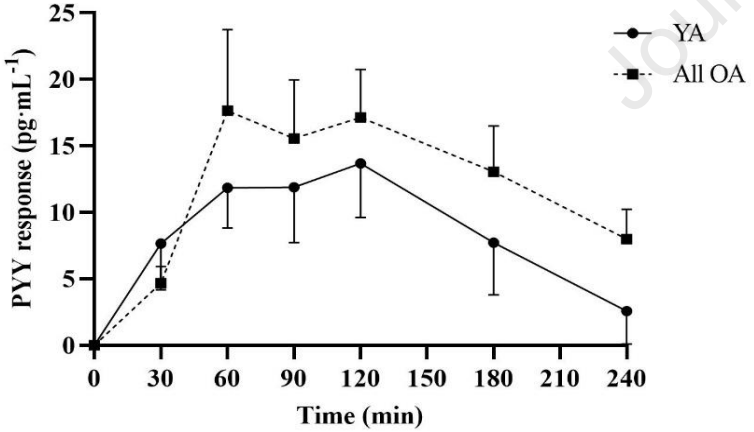

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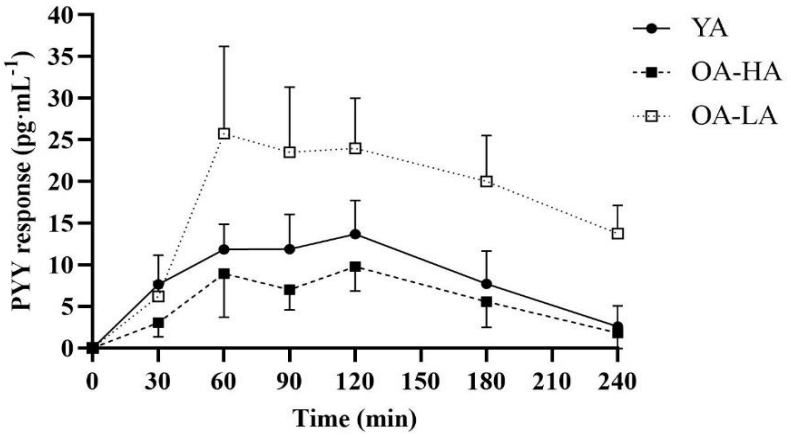

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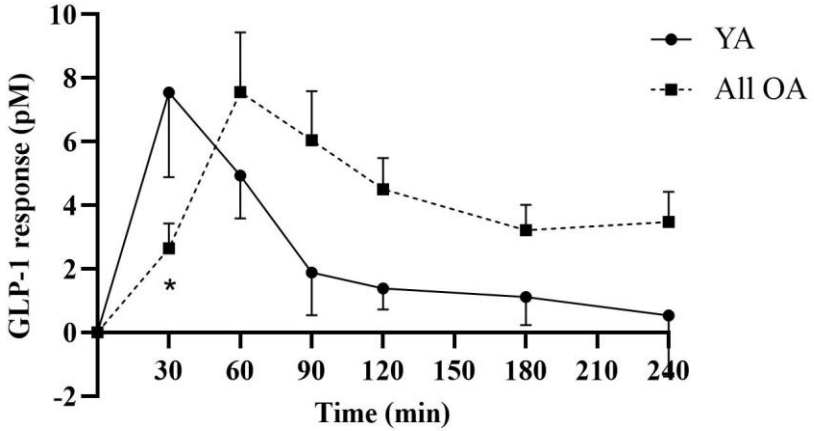
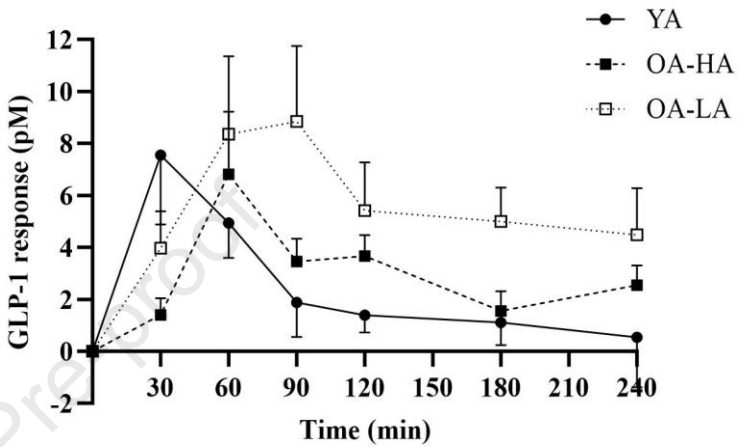
518 e)



f)



527 **Figure 4.** Mean ± SEM ghrelin (a and b), PYY (c and d), and GLP-1 (e and f) responses to feeding for YA (●, solid line) and all OA (■, dashed line)
528 (figures a, c, e) and for YA (●, solid line), OA-HA (■, dashed line), and OA-LA (□, dotted line) (figures b, d, f). * = significantly different to YA, $p < 0.05$.
529 # = significantly different to OA-HA, $p < 0.05$.

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Subjective appetite

When comparing YA with all OA, there was no difference in baseline subjective appetite score (67.4 ± 16.2mm vs. 60.2 ± 17.7mm, p = 0.256, d = 0.417. Figure 5a). When assessing appetite response to the standardised breakfast, as change-from-baseline, there was no significant group x time interaction (p = 0.102, η^2_p = 0.058), nor group main effect (p = 0.576; η^2 = 0.009) for subjective appetite across the trial period. Net AUC did not differ between groups (p = 0.522, d = 0.181).

When comparing YA, OA-HA, and OA-LA, there was no significant difference in baseline subjective appetite score (YA = 67.4 ± 16.2mm, OA-HA = 62.8 ± 14.0mm, OA-LA = 58.0 ± 20.6mm; p = 0.355, η^2_p = 0.059. Figure 5b). There was also no significant group x time interaction (p = 0.182, η^2_p = 0.085) nor group main effect (p = 0.843, η^2 = 0.010) for subjective appetite response to the standardised breakfast. Net AUC did not differ between groups (p = 0.802, η^2 = 0.013).

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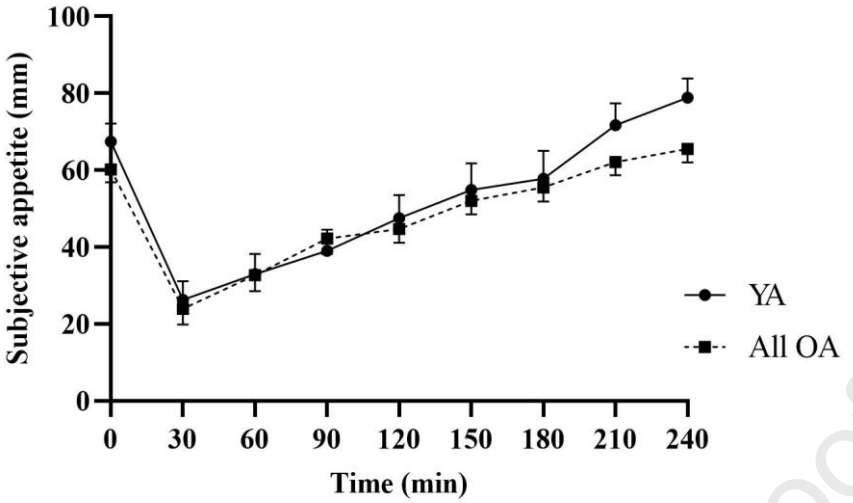
a)



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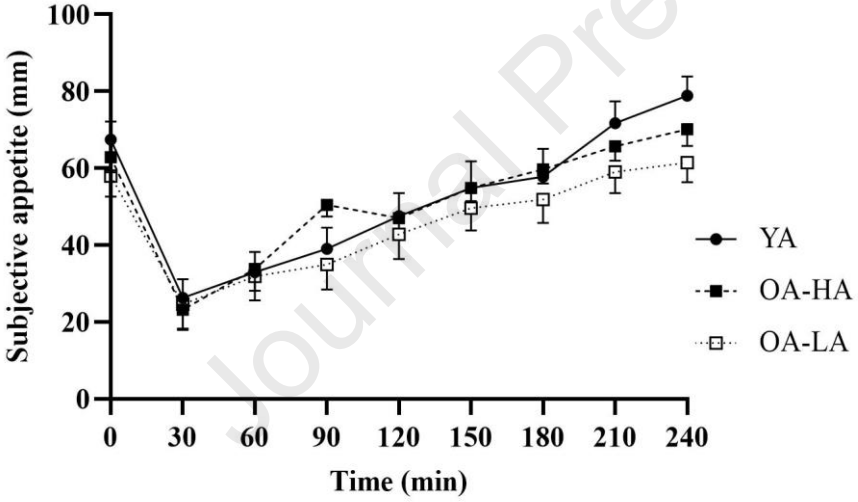

Figure 5. Mean ± SEM subjective appetite for YA vs. all OA (a), and YA vs. OA-HA vs. OA-LA (b).

Correlation and Regression analysis

The correlation matrix for associations between anorexigenic response, subjective appetite response, subjective appetite at 240 minutes, and *ad libitum* lunch intake is shown in Table 2. For all participants, anorexigenic response score was negatively associated with *ad libitum* lunch intake (p = 0.008), explaining 17.6% of the variance (R^2 = 0.176, β = -196, p = 0.008). However, anorexigenic response score was not associated with subjective appetite response (p = 0.970), nor with subjective appetite at 240 minutes (p = 0.723). Subjective appetite response was not associated

574 with *ad libitum* lunch intake ($p = 0.538$), but subjective appetite score at 240 minutes was positively
575 associated with *ad libitum* lunch intake ($p = 0.010$, $R^2 = 0.165$, $\beta = 7.90$).

576 For OA only, anorexigenic response score remained negatively associated with *ad libitum* lunch
577 intake ($p = 0.031$), explaining 17.3% of the variance ($R^2 = 0.173$, $\beta = -114$). There was no
578 association between anorexigenic response score and subjective appetite response ($p = 0.990$) or
579 subjective appetite at 240 min ($p = 0.912$). Subjective appetite response was also not associated
580 with *ad libitum* lunch intake ($p = 0.196$), but there was an association between subjective appetite
581 at 240 minutes and *ad libitum* lunch intake ($p = 0.033$, $R^2 = 0.169$, $\beta = 5.45$).

582

583 **Table 2**. Correlation matrix for the associations between anorexigenic response, subjective appetite
584 response, subjective appetite at 240min, and *ad libitum* lunch intake for all participants of the study
585 (All), and for older adults only (OA).

	Anorexigenic response score	Subjective appetite response	Subject appetite score at 240 min	*Ad libitum* lunch intake
Anorexigenic response score		All: -0.006 OA: -0.041	All: -0.058 OA: 0.001	All: -0.420 ** OA: -0.416 *
Subjective appetite response			All: 0.303 OA: 0.447 *	All: -0.102 OA: 0.257
Subject appetite score at 240 min				All: 0.407 * OA: 0.412 *
Ad libitum lunch intake				

586

587 ** = $p < 0.01$, * = $p < 0.05$.

588

589 The correlation matrix for the four predictor variables (BMI, daily energy intake as a percentage of
590 TER, SNAQ score, and laboratory *ad libitum* lunch intake) and the outcome variable (anorexigenic
591 response score) is shown in Table 3. SNAQ score and *ad libitum* lunch intake were significantly,
592 negatively associated with anorexigenic response score. SNAQ score and *ad libitum* lunch intake
593 were significantly correlated with one another.

594 Regression analysis showed that a model containing all four predictor variables was a significant
595 predictor of gut hormone anorexigenic response, with variance in these variables explaining 48%
596 of variance in gut hormones response. In the model containing all four predictors, the only
597 significant predictor or gut hormone anorexigenic response was SNAQ score ($\beta = -0.555$, $p = 0.010$).

Three backward eliminations were performed, producing a total of four models (Table 4). The decrease in R^2 with each elimination was not significant. The model with the greatest predictive power, as denoted by the adjusted R^2 value, was the model containing SNAQ score, daily EI and *ad libitum* lunch intake (adjusted R^2 = 0.398).

Table 3. Correlation matrix for predictor variables BMI, daily EI as a percentage of TER, SNAQ score, and *ad libitum* lunch intake and outcome variable anorexigenic response score.

	Anorexigenic response score	BMI	Daily EI	SNAQ score	*Ad libitum* lunch intake
Anorexigenic response score		-0.182	-0.350	-0.634 ***	-0.416 *
BMI			-0.358	0.232	0.366
Daily EI				0.168	0.235
SNAQ score					0.342
Ad libitum lunch intake					

BMI, body mass index; EI, energy intake; SNAQ, Simplified Nutritional Appetite Questionnaire. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$.

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Table 4. Backward elimination regression analyses of SNAQ score, daily EI, *ad libitum* lunch intake, and BMI as predictors of anorexigenic response of gut hormones.

Model	R^2	Adj R^2	Predictors	β	*p*
1	0.481	0.351	SNAQ score	-0.555	**0.010**
			Daily EI	-0.235	0.286
			Ad libitum intake	-0.145	0.491
			BMI	-0.038	0.865
2	0.480	0.398	SNAQ score	-0.562	**0.007**
			Daily EI	-0.217	0.246
			Ad libitum intake	-0.159	0.404
3	0.457	0.397	SNAQ score	-0.599	**0.003**
			Daily EI	-0.249	0.171
4	0.396	0.363	SNAQ score	-0.629	**0.002**

SNAQ, Simplified Nutritional Appetite Questionnaire; BMI, body mass index; EI, energy intake; β, standardised β coefficient.

DISCUSSION

Our primary aim was to determine the ghrelin, PYY and GLP-1 responses to feeding in both older adults with unimpaired, healthy appetite and older adults with low appetite. Our novel findings show augmented anorexigenic gut hormone responses to feeding in older adults identified as having low appetite, but not in older adults with a healthy appetite. Suppression of the hunger hormone ghrelin was greater in older adults with low appetite, compared with both younger adults and older adults with healthy appetite. Increases in postprandial plasma concentration of the satiety hormones PYY and GLP-1 were greater and more enduring in older adults with low appetite, compared with younger adults. This was not observed in older adults with healthy appetite. These gut hormone

responses were combined to calculate a composite "anorexigenic response score". Anorexigenic response score was also greater for older adults with low appetite compared with both younger adults and older adults with a healthy appetite. Therefore, we propose that augmented anorexigenic responses of gut hormones to feeding is not a function of ageing *per se*, but instead may be a causal mechanism of anorexia of ageing.

Our approach of identifying older adults with low appetite allowed for comparisons between all older adults and young adults, and between older adults with low appetite, older adults with healthy appetite, and younger adults. When making comparisons purely on age, we observed greater postprandial increases in GLP-1 in older adults than younger adults, and non-significant greater postprandial responses of ghrelin and PYY. When comparing older adults with low appetite, older adults with healthy appetite, and young adults, it was revealed that these apparent age-related differences in gut hormone concentrations were driven by responses exclusively seen in older adults with low appetite.

Previous studies had evidenced age-related differences in postprandial concentration of ghrelin (di Francesco et al., 2008; Nass et al., 2014), PYY (Giezenaar et al., 2018a), and GLP-1 (Giezenaar et al., 2018b; Giezenaar et al., 2020). Our data indicate that such differences are not functions of ageing *per se* but are unique and specific to those with impaired appetite. Other studies have shown no difference in postprandial ghrelin (Bauer et al., 2010; Bertoli et al., 2006; Giezenaar et al., 2018a; Giezenaar et al., 2018b), PYY (di Francesco et al., 2005; MacIntosh et al., 1999) and GLP-1 (MacIntosh et al., 1999; MacIntosh et al., 2001; Trahair et al., 2012; Herpich et al., 2022) concentrations between older and younger adults. It is possible that these studies failed to observed differences due to the older adult cohort largely consisting of non-appetite suppressed older adults. Recruiting older adult study cohorts heterogeneous in appetite regulation, perceptions and eating behaviour could mask dysregulation of gut hormone responses exclusive to those with low appetite.

An amplified response of gut hormones to feeding is indicative of hypersensitivity of the gut to nutrient delivery. Gut hormones are secreted from specialised enteroendocrine cells of the GI tract in response to the sensing of nutrients or to changes in nutrient status. PYY and GLP-1 are secreted from enteroendocrine L-cells of the small and large intestine (Eissele et al., 1992; Sjölund et al., 1983), while ghrelin is secreted from X/A cells in the epithelium of the stomach (Date et al., 2000). Secretion is regulated by the sensing of nutrients by nutrient receptors and transporters, and the subsequent activation of intracellular signalling pathways. A hypersecretory response to feeding, as observed in older adults with low appetite, would suggest upregulation, or dysregulation, of nutrient sensing or cellular signalling. As such, we further propose that augmented anorexigenic gut hormones response to feeding may be a result of hypersensitivity of the gut to nutrients, and this hypersensitivity may be a causal mechanism of anorexia of ageing.

669 In the present study, the gut hormone response to feeding proved a significant predictor of *ad*
670 *libitum* lunch intake. This was observed in all participants and when assessing OA alone, with gut
671 hormone response accounting for 18% of variance in lunch intake amongst older adults. This
672 indicates that changes in gut hormones concentration are likely to play a meaningful role in the
673 control of eating behaviour and food intake in older adults.

674 Subjective appetite response across the postprandial period was not a predictor of *ad libitum* lunch
675 intake, and neither was it associated with gut hormone response. Indeed, subjective appetite
676 response did not differ between groups, despite differences in gut hormone response and *ad libitum*
677 lunch intake. Disparity between gut hormone concentration and subjective appetite rating (Holliday
678 & Blannin, 2017; Smeets et al., 2008), and between subjective appetite ratings and subsequent
679 energy intake (Holt et al., 2017; Sadoul et al., 2014) have been seen previously. However,
680 subjective appetite score at 240 minutes was positively associated with *ad libitum* lunch intake.
681 Given that immediate pre-meal appetite perceptions and gut hormone response to feeding were
682 both associated with *ad libitum* intake, but not associated with one another, this would suggest that
683 gut hormones exert control over acute energy intake independent of immediate pre-meal appetite.

684 The secondary aim of this study was to assess the appropriateness of our approach to phenotyping
685 older adults with low appetite. This was required for the comparison of gut hormone responses
686 between older adults with a healthy appetite and older adults with low appetite in the present study,
687 and an effective approach to phenotyping those with anorexia of ageing could prove beneficial for
688 future research in this field. We adopted a four-criteria classification based on BMI, habitual daily
689 energy intake, SNAQ score, and an objective, laboratory-measured *ad libitum* lunch intake. This
690 approach has recently been deployed to determine differences in ghrelin metabolism (Holliday et
691 al., 2024). The regression analyses of the present study support the appropriateness of this
692 classification model for identifying those with low appetite and phenotyping anorexia of ageing.
693 Variance in the four criteria explained 48% of variance in gut hormone response in older adults.
694 SNAQ score was the strongest individual predictor of gut hormone response, which supports the
695 application of the SNAQ for identifying community-dwelling older adults with low appetite (Lau
696 et al., 2020). The model with the strongest predictive power, however, included SNAQ score,
697 habitual daily energy intake, and *ad libitum* lunch intake. This evidences the beneficial inclusion of
698 an objective energy intake measure for identifying appetite. The inclusion of BMI to the model
699 provided little additional predictive power. This is perhaps not surprising, as BMI appears not to be
700 associated with protein-energy malnutrition in older adults (van der Pols-Vijlbrief et al., 2014), and
701 between 20 and 35% of older adults with a BMI of greater than 25 $kg \cdot m^{-2}$ are at risk of
702 undernutrition (Klee Oehlschlaeger et al., 2014; Özkaya & Gürbüz, 2019; Sulmont-Rossé et al.,
703 2022).

A limitation of the present study is that the sex distribution differed between groups. As there is evidence for sex differences in gut hormone response to feeding (Giezenaar et al., 2018c), we accounted for sex in our analyses. Consequently, we can be confident the differences observed are true group differences and not due to uneven sex distribution across groups. We did not explore se effects in depth as the study was not powered for such analyses. However, sex effects were observed for ghrelin response. As such, it would be of interest for future studies to specifically determine any sex differences in gut hormone response to feeding in appetite-suppressed older adults.

Although the present study determined postprandial responses of ghrelin, PYY, and GLP-1, other gut hormones may be of interest. We did not measure cholecystokinin (CCK) or gastric inhibitory polypeptide (GIP). As there is evidence to suggest both hormones exhibit greater responses to feeding in older adults than younger adults (Giezenaar et al., 2018a; Giezenaar et al., 2018b; Johnson et al., 2020), it would have been interesting to confirm if such responses were also specific to those with low appetite. The effects of feeding on other gut hormones, such as pancreatic polypeptide (PP) and oxyntomodulin, have yet to be determined in older adults (Johnson et al., 2020). Further research is required to allow a more complete understanding of age-related changes in gut response to nutrients, and how such changes impact upon the appetite and eating behaviour of older adults.

CONCLUSION

This is the first study to demonstrate that augmented anorexigenic responses of gut hormones to feeding are observed in older adults with low appetite but not in older adults with a healthy appetite. This highlights two different phenotypes of appetite regulation and response in older adults. As such, we propose that amplified gut hormone response, resulting from gut hypersensitivity to nutrients, may be a causal mechanism of anorexia of ageing. Future research is warranted to explore the presence of nutrient sensing and signalling dysregulation in appetite-suppressed older adults.

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ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

A.H., A.D., B.C., and G.F. conceived the research question. A.H., D.R.C., designed the study. A.H. and J.W. collected data. A.H, K.S., and A.D. conducted data analyses. A.H. wrote the manuscript. A.D V.C., B.C., D.R.C., and G.F. edited the manuscript. All authors approved the final version.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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