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Differences in circulating appetite-related hormone concentrations between younger and older adults: a systematic review and meta-analysis

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Abstract

Ageing is associated with reduced appetite and energy intakes. However, the mechanisms underlying this phenomenon are not fully understood. This systematic review and meta-analysis quantified differences in circulating concentrations of appetite-related hormones between healthy older and younger adults.

Six databases were searched through 12th June 2018 for studies that compared appetite-related hormone concentrations between older and younger adults. Data were pooled using random-effects meta-analysis and are presented as standardised mean difference (Hedges' *g*) with 95% confidence intervals (95% CI).

Thirty five studies were included involving 710 older adults (mean±SD; age: 73±5 years) and 713 younger adults (age: 28±7 years). Compared with younger adults, older adults exhibited higher fasted and postprandial concentrations of the anorectic hormones cholecystokinin (Fasted: SMD 0.41 (95%CI 0.24, 0.57); *p*<0.001. Postprandial: SMD 0.41 (0.20, 0.62); *p*<0.001), leptin (Fasted: SMD 1.23 (0.15, 2.30); *p*=0.025. Postprandial: SMD 0.62 (0.23, 1.01); *p*=0.002) and insulin (Fasted: SMD 0.24 (-0.02, 0.50); *p*=0.073. Postprandial: SMD 0.16 (0.01, 0.32); *p*=0.043). Higher postprandial concentrations of peptide-YY were also observed in older adults compared with younger adults (SMD 0.31 (-0.03, 0.65); *p*=0.075). Compared with younger adults, older adults had lower energy intakes (SMD -0.98 (-1.74, -0.22); *p*=0.011), and lower hunger perceptions in the fasted (SMD -1.00 (-1.54, -0.46); *p*<0.001) and postprandial state (SMD -0.31, (-0.64, 0.02); *p*=0.064).

Higher circulating concentrations of insulin, leptin, cholecystokinin and peptide-YY accord with reduced appetite and energy intakes in healthy older adults. Interventions to reduce circulating levels of these hormones may be beneficial for combatting the anorexia of ageing.

PROSPERO registration number: CRD42017083747.

Introduction

Currently 962 million people worldwide are over the age of 60 years [1] and it is predicted that 22% of the global population will be 60 years and older by 2050 [2]. A high proportion of health care is spent on older adults and this is set to increase given the projected rise in this population. Therefore, improving health in older adults is a crucial public health priority.

The “anorexia of ageing” describes a reduction in appetite and energy intake in older adults. Current observations suggest that this phenomenon has a prevalence of 15-30% in independently-living older adults, with higher incidence occurring in hospital and nursing home settings [3]. The anorexia of ageing represents an independent risk factor for frailty, incident disability, morbidity and mortality [4,5,3]. Consequently, developing a better understanding of the mechanisms underlying the anorexia of ageing is important in designing interventions to prevent further deterioration in quality of life.

Appetite is regulated physiologically by the neuroendocrine system [6], with multiple hormones implicated as mediators of hunger and satiety. Several hormones have been identified to exert appetite-suppressing effects, including peptide YY (PYY), glucagon-like-peptide-1 (GLP-1), oxytomodulin, pancreatic polypeptide (PP), cholecystokinin (CCK), gastric inhibitory peptide (GIP), insulin and leptin. Alternatively, ghrelin represents the only peripheral peptide known to exert an orexigenic effect [7,6]. Current research suggests that changes in appetite-related hormones may mediate the reductions in appetite and energy intake observed in older adults. However, conclusions are yet to be drawn due to inconsistent findings between studies, likely due to differences in methodologies and the hormones measured. For example, results from Giezenaar *et al.* [8] demonstrated higher plasma concentrations of CCK in older adults compared with younger adults, however, these concentrations did not differ between older and younger adults in the study of Winkels *et al.* [9].

A systematic evaluation of the literature is required to gain a clear understanding of which hormones exhibit altered concentrations between older and younger adults, as well as the direction and size of any effects. Thus, the purpose of this research was to provide a systematic review and meta-analysis of studies which compared circulating appetite-related hormone concentrations between metabolically-healthy older and younger adults in the fasted and/or postprandial state, with no known co-morbidities or pre-existing medical conditions. Comparisons of appetite perceptions and energy intake between the groups were also included where available. Understanding these effects contributes to the identification of potential mechanistic targets for future interventions to reduce the anorexia of ageing.

Methods

This systematic review and meta-analysis was performed in accordance with the PRISMA (Preferred Reporting Items for Systematic Review and Meta-analyses) guidelines [10] and was prospectively registered with the PROSPERO database (CRD42017083747).

Literature Search

PubMed and The Cochrane Library as well as MEDLINE, SPORTDiscus, PsycINFO and CINAHL, via EBSCOhost, were searched in December 2017, with an updated search on 12th June 2018. These databases were used to provide a thorough approach for searches to obtain the maximum number of relevant studies, including relevant data from studies in which exercise or psychological interventions were implemented. Keyword searches were based on the population (i.e., older adults) in combination with appetite-related hormones, and appetite perceptions and/or energy intake. The specific keywords and the full search strategy can be found in supplementary material 1. The search strategy provided a total of 328 combinations. Reference lists of eligible studies and review articles were also hand searched. If only the

abstract or partial data were published, then the author was contacted for the full data set. No language or date of publication restrictions were applied during the searches.

Inclusion Criteria

For inclusion, studies were required to meet the following criteria; participants in the study were not noted to be smokers, pregnant, or have a history of diabetes, gastrointestinal, inflammatory, metabolic, cardiovascular, neurological or psychological disease(s). Studies were excluded if there was evidence to show the implementation of a diet to induce energy imbalance, the administration of a supplement or taking medication as this is shown to affect appetite. Studies which investigated participants under the age of 18 were excluded. Studies were included if they were published in peer-review journals, or were available as published conference proceedings, theses or dissertations to minimise the effect of any potential publication bias.

All studies were required to contain at least one of the following measures of circulating appetite-related hormone concentrations; leptin, acylated ghrelin, total ghrelin, oxyntomodulin, PYY, GLP-1, PP, GIP, CCK and/or insulin, in distinct groups of older and younger adults within the same study. Any postprandial comparisons of appetite-related hormone concentrations where <125kJ was provided during feeding were excluded from the analysis. Studies which involved the infusion of a nutrient as the feeding intervention were included, however studies involving the infusion of pharmacological products were excluded. In addition to these outcomes, appetite perceptions and/or energy intake data were extracted if provided in the included studies.

Two researchers (KJ and OS) independently assessed titles and abstracts of studies for inclusion and later compared notes to reach a mutual consensus. Researchers were not blinded to the articles. However, research has shown that blinding during study selection and data

extraction had neither a clinically nor a statistically significant effect on the summary outcome [11]. Disagreements about the eligibility of any particular studies were resolved by a third reviewer (KD). Potential studies that could be included based on their title or abstract were retrieved in full-text and reviewed against the inclusion/exclusion criteria independently by two researchers (KJ and OS) with a third researcher (KD) used to settle any disputes. In total 35 studies met the inclusion criteria and were included in this meta-analysis (see flow chart in supplementary figure 1). For a variable to be included in the meta-analysis, a minimum of three studies measuring the respective variable were required to meet the inclusion criteria.

Data Abstraction

Data were extracted independently by two separate researchers (KJ and OS) into a standardised spreadsheet which included (i) characteristics of articles valid for review; (ii) the critical appraisal tool to assess the quality of cross-sectional studies (AXIS) (iii) outcome data suitable for successive analysis based on mean, SD and sample size. Researchers were not blinded to articles during this process, however risk of bias can be effectively conducted under blinded conditions [12]. Additional data were collected for study design, participant characteristics, the duration of observations and number of blood samples taken, hormone analytical method, as well as the assessment method of energy intake.

Where values were only presented in figure form authors were contacted to provide the full data set. If the dataset could not be retrieved ($n = 17$) the figure was digitised using graph digitiser software (DigitizeIt, Germany) and the means and SD/SEM were measured manually at the pixel level to the scale provided on the figure. Digitizeit has been shown to provide valid and reliable results which provide good agreement with raw data values[13]. If area under the curve values were reported rather than the mean values, the mean values were calculated by dividing the area under the curve scores by time duration.

Study Appraisal and Quality Assessment

The critical appraisal tool to assess the quality of cross-sectional studies (AXIS) [14] was independently assessed by two reviewers (KJ and OS). Each included study was systematically evaluated against a 17-item checklist to judge study quality and the possibility of bias. Reviewers answered “yes” or “no” to each question within the checklist. When insufficient detail was reported the reviewers answered the question with “do not know”. Disagreements were initially resolved via discussion between two independent reviewers (KJ and OS) however a third reviewer (KD) was consulted for any necessary dispute resolution. Due to the cross-sectional nature of study designs within this meta-analysis, questions 7, 13 and 14 were removed in regards to addressing non-responders. Studies were not excluded based on the results of the quality assessment, however for transparency the quality of included studies has been presented in supplementary table 26.

Statistical Analysis

Missing standard deviations were calculated from standard errors or confidence intervals (CI). Outcome measures were converted into the standardised mean difference (SMD) and expressed as Hedges’ *g* with 95% CI which were used as the summary statistic. Correction using Hedges’ *g* is believed to yield an unbiased estimate of effect size [15]. The SMD represents the size of the difference between groups relative to the variability observed in the groups. A random-effects meta-analysis was performed by KJ and KD using Comprehensive Meta-Analysis Software (version 3, Biostat, Englewood, NJ, USA). A random effects model was employed for all analyses based on the assumption that heterogeneity would exist between included studies due to the variability in study designs [16]. The inputted data included sample sizes and outcome measures with their respective standard deviations.

Interpretation of SMD values was as follows: <0.20 as trivial, 0.20-0.39 as small, 0.40-0.80 as moderate and >0.80 as large [17]. A positive ES value indicated that hormone concentrations

were higher in older adults compared with younger adults. Heterogeneity between trials was assessed using the I-squared statistic, where 0-40% suggests heterogeneity might not be important, 30-60% may represent moderate heterogeneity, 50-90% may represent substantial heterogeneity and 75-100% represent substantial heterogeneity [18]. This measure of heterogeneity was complimented by also reporting the Tau-squared statistic and the Chi-squared statistic. To examine whether any conclusions were dependent on a single study, sensitivity analyses were employed for each variable by repeating the analysis with each study omitted in turn.

Where data were available, subgroup meta-analysis was performed for feeding method (infusion versus eating), hormone analytical method (RIA versus ELISA) and energy intake assessment method (researcher-measured versus self-reported). Each subgroup was required to have a minimum of three studies.

Exploration of small study effects

Small study effects were explored with funnel plots of SMD versus standard errors [19] and by quantifying Egger's linear regression intercept. A large and statistically significant Egger's statistic indicates the presence of small study effects.

Results

Overview

Supplementary figure 1 outlines the flowchart of study selection. In total 35 studies met the inclusion criteria for the meta-analysis. All included studies had been published (or accepted for publication) in peer-review scientific journals at the time of inclusion (see supplementary material 2 for article references). Within the 35 studies, there were 31 fasted and 25 postprandial comparisons that were included between older and younger adults. Comparisons were segregated into fasted (see supplementary table 1) and postprandial (see supplementary

table 2) responses to differentiate between findings during these two distinct periods of appetite regulation. Fasted comparisons represent single time points obtained after an overnight fast, whereas postprandial comparisons represent means of multiple time points after feeding.

There were four studies that involved more than two age groups; consequently, the youngest and oldest age groups were extracted for these studies. Out of the 35 studies which assessed circulating appetite-related hormone concentrations, 11 compared energy intake between older and younger adults (supplementary table 3). One study obtained energy intake by the 24-hour recall method, six studies by *ad libitum* meals and four through food diaries lasting three to five days. All studies which reported postprandial comparisons provided standardised meals or infusions to participants with an energy content of 502-4064 kJ (mean: 2454 kJ) and had hormone observation periods of 0.5 - 24 hours (mean: 4.41 hours). All studies involving postprandial comparisons provided mixed macronutrient meals, with 19 studies providing it in solid form, six studies involving the infusion of a nutrient, two studies providing it in liquid form and one study comparing both liquid and solid form. Three studies manipulated the macronutrient composition of the mixed meals to identify if this dictated postprandial outcomes. If a study contained more than one postprandial comparison for each age group, then the results were combined with the study being the unit for analysis. The effects of macronutrient manipulation were conflicting between studies, therefore, it is difficult to draw conclusions on the effects of macronutrient distribution on differences in appetite-related hormone concentrations between older and younger adults.

Hunger was assessed alongside appetite-related hormone concentrations in 13 out of the 35 studies, with seven studies evaluating both fasted and postprandial responses, five studies reporting only fasted responses and one study only presenting postprandial hunger perceptions between older and younger adults (supplementary material 4).

Participant demographics

A total of 713 younger adults and 710 older adults were included in this meta-analysis. Gender was reported in 31 out of 35 studies (532 men and 573 women; 48.1% men). Mean age ranged from 19 to 50 (mean: 28) years for younger adults. For older adults all studies ranged from 65 to 85 (mean: 73) years. Mean BMI was reported in 25 out of 35 studies and ranged from 21.2 to 28.1 (mean: 23.6) kg·m⁻² for younger adults and 20.5 to 26.9 (mean: 24.8) kg·m⁻² for older adults, therefore falling in the healthy weight category for both groups.

Meta-analysis

Individual study statistics and results for each outcome variable are summarised in supplementary tables 5 - 22.

Standardised mean difference for acylated ghrelin concentrations

Only one study which met the inclusion criteria included the measurement of circulating acylated ghrelin concentrations in the fasted state. Consequently, this could not be included in the meta-analysis.

Postprandial acylated ghrelin concentrations were lower in older adults than younger adults, with a small difference between groups (SMD: -0.21, 95% CI: -0.76 to 0.35; $n = 5$; $p = 0.466$; supplementary figure 2). The degree of heterogeneity may be moderate between studies ($I^2 = 52.6\%$; $Q = 8.4$, $\tau^2 = 0.208$, $df = 4$).

Standardised mean difference for total ghrelin concentrations

There was little difference in fasted total ghrelin concentrations when comparing older adults with younger adults (SMD: 0.13, 95% CI: -0.12 to 0.39; $n = 6$; $p = 0.312$; supplementary figure 3a). The degree of heterogeneity was found to be low between studies ($I^2 = 0.00\%$; $Q = 5.0$, $\tau^2 < 0.0005$, $df = 5$).

There was little difference in postprandial total ghrelin concentrations when comparing older adults with younger adults (SMD: 0.17, 95% CI: -0.06 to 0.40; $n = 8$; $p = 0.146$; supplementary figure 3b). The degree of heterogeneity was found to be low between studies ($I^2 = 0.0\%$, $Q = 2.8$, $\tau^2 < 0.0005$, $d_f = 7$).

Standardised mean difference for CCK concentrations

Fasted CCK concentrations were higher in older adults than younger adults with a moderate difference between groups (SMD: 0.41, 95% CI: 0.24 to 0.57; $n = 9$; $p < 0.0005$; figure 1a). The degree of heterogeneity was found to be low between studies ($I^2 = 0.0\%$, $Q = 7.6$, $\tau^2 < 0.0005$, $d_f = 8$).

Postprandial CCK concentrations were higher in older adults than younger adults with a moderate difference between groups (SMD: 0.41, 95% CI: 0.20 to 0.62, $n = 8$; $p < 0.0005$; figure 1b). The degree of heterogeneity was found to be low between studies ($I^2 = 7.2\%$, $Q = 7.5$, $\tau^2 = 0.085$, $d_f = 7$).

INSERT FIGURE 1 NEAR HERE

Standardised mean difference and moderator variables for GLP-1 concentrations

There was little difference in fasted GLP-1 concentrations when comparing older adults with younger adults (SMD: -0.02, 95% CI: -0.44 to 0.40; $n = 7$; $p = 0.942$; supplementary figure 4a). The degree of heterogeneity may be substantial between studies ($I^2 = 75.8\%$; $Q = 24.8$, $\tau^2 = 0.235$ and $d_f = 6$).

There was little difference in postprandial GLP-1 concentrations when comparing older adults with younger adults (SMD: 0.11, 95% CI: -0.19 to 0.41; $n = 7$; $p = 0.474$; supplementary figure

4b). The degree of heterogeneity was found to be moderate between studies ($I^2 = 48.7\%$, $Q = 11.7$, $\tau^2 = 0.078$, $df = 6$).

Standardised mean difference for leptin concentrations

Fasted leptin concentrations were higher in older adults than younger adults with a large difference between groups (SMD: 1.23, 95% CI: 0.15 to 2.30; $n = 9$; $p = 0.025$; figure 2a). The degree of heterogeneity was found to be considerable between studies ($I^2 = 96.7\%$, $Q = 238.9$, $\tau^2 = 2.545$, $df = 8$).

Postprandial leptin concentrations were higher in older adults than younger adults with a moderate difference between groups (SMD: 0.62, 95% CI: 0.23 to 1.01; $n = 7$; $p = 0.002$; figure 2b). The degree of heterogeneity was found to be moderate between studies ($I^2 = 51.5\%$, $Q = 12.4$, $\tau^2 = 0.138$, $df = 6$).

INSERT FIGURE 2 NEAR HERE

Standardised mean difference and moderator variables for insulin concentrations

Fasted insulin concentrations were higher in older adults than younger adults with a small difference between groups (SMD: 0.24, 95% CI: -0.02 to 0.50; $n = 19$; $p = 0.073$; figure 3a). The degree of heterogeneity may be substantial between studies ($I^2 = 74.3\%$, $Q = 70.1$, $\tau^2 = 0.225$, $df = 18$). Subgroup analysis revealed no significant effect of the hormone analytical method on outcomes ($p = 0.99$; Table 1).

There was a trivial but significant difference in postprandial insulin concentrations between younger and older adults (SMD: 0.16, 95% CI: 0.01 to 0.32, $n = 17$; $p = 0.043$; figure 3b). The degree of heterogeneity was found to be low between studies ($I^2 = 10.5\%$, $Q = 17.9$, $\tau^2 = 0.001$,

df = 17). Subgroup analysis revealed no significant effect of the hormone analytical method or feeding method on outcomes (both $p > 0.42$; Table 1).

INSERT FIGURE 3 NEAR HERE

Standardised mean difference for PYY concentrations

Fasted PYY concentrations were lower in older adults than younger adults with a small difference between groups (SMD: -0.35, 95% CI: -1.10 to 0.40, $n = 4$; $p = 0.357$; figure 4a).

The degree of heterogeneity may be substantial between studies ($I^2 = 77.7\%$, $Q = 13.4$, $\tau^2 = 0.445$, $df = 3$).

Postprandial PYY concentrations were higher in older adults than younger adults with a small difference between groups (SMD: 0.31, 95% CI: -0.03 to 0.65; $n = 4$; $p = 0.075$; figure 4b).

The degree of heterogeneity was found to be low between studies ($I^2 = 0.0\%$, $Q = 2.0$, $\tau^2 < 0.0005$, $df = 3$).

INSERT FIGURE 4 NEAR HERE

Standardised mean difference and moderator variables for GIP concentrations

Fasted GIP concentrations were higher in older adults than younger adults with a small difference between groups (SMD: 0.16, 95% CI: -0.23 to 0.54, $n = 4$; $p = 0.334$; supplementary figure 5a). The degree of heterogeneity was found to be substantial between studies ($I^2 = 63.8\%$, $Q = 8.3$, $\tau^2 = 0.102$, $df = 3$).

Postprandial GIP concentrations were higher in older adults than younger adults with a small difference between groups (SMD: 0.25, 95% CI: -0.19 to 0.70; $n = 4$; $p = 0.263$; supplementary figure 5b). The degree of heterogeneity was found to be substantial between studies ($I^2 = 68.3\%$, $Q = 9.5$, $\tau^2 = 0.140$, $df = 3$).

Standardised mean difference and moderator variables for energy intake

Energy intake was lower in older adults than younger adults with a large difference between groups (SMD: -0.98, 95% CI: -1.74 to -0.22; $n = 11$; $p = 0.011$; figure 5). The degree of heterogeneity is substantial between studies ($I^2 = 94.6\%$; $Q = 186.1$, $\tau^2 = 1.455$, $df = 10$). Subgroup analysis revealed a difference in energy intake between assessment types ($p = 0.015$), with older adults only showing a significantly lower energy intake compared with younger adults when assessed via self-report methods (table 1).

INSERT FIGURE 5 NEAR HERE

Standardised mean difference for hunger score

Fasted hunger scores were lower in older adults than younger adults with a moderate difference between groups (SMD: -1.00, 95% CI: -1.54 to -0.46; $n = 12$; $p = 0.0003$; figure 6a). The degree of heterogeneity may be substantial between studies ($I^2 = 76.1\%$; $Q = 46.1$, $\tau^2 = 0.671$, $df = 11$).

Postprandial hunger scores were lower in older adults than younger adults with a small difference between groups (SMD: -0.31, 95% CI: -0.64 to 0.02; $n = 8$; $p = 0.064$; figure 6b). The degree of heterogeneity may be moderate between studies ($I^2 = 37.7\%$; $Q = 11.2$, $\tau^2 = 0.082$, $df = 7$).

INSERT FIGURE 6 NEAR HERE

INSERT TABLE 1 NEAR HERE

Sensitivity Analysis

Out of the 18 variables assessed in the meta-analysis, 11 variables revealed only minor changes when one study was omitted in turn from each analysis. The remaining seven variables revealed the removal of one or more comparison caused significant changes to the SMD (supplementary table 23-25).

Egger's Regression

Five out of the 18 variables assessed in the meta-analysis demonstrated some evidence of small study effects after inspection of the funnel plot and Egger's Regression Intercept (see supplementary tables 23-25). The remainder of studies provided little evidence of small study effects.

Quality assessment

The quality of studies appeared to vary. Most studies met more than 8 of the quality criteria items out of a possible 17 (mean (SD): 11 (2)). All studies used the appropriate measurements to conduct assessment on appetite-related hormone concentrations. This included ELISA, RIA and standard chemiluminescent immunometric assays. The majority of studies included information regarding attaining ethical approval. However, the majority of studies failed to justify the sample size used or provide evidence for a power calculation. Some studies also lacked information on sources of recruitment and whether the sample represented the population investigated (table 2). Reviewer evaluations matched on 88-100% of questions for

each study. Since studies compared the difference between older and younger adults, the criteria regarding non-responders were not relevant (supplementary table 26).

INSERT TABLE 2 NEAR HERE

Discussion

Understanding differences in appetite-related hormones concentrations between older and younger adults provides mechanistic insight into the effect of ageing on the appetite-regulatory system. This systematic review and meta-analysis revealed higher fasted and postprandial circulating concentrations of CCK, insulin and leptin in older adults. We also observed higher postprandial concentrations of PYY in older versus younger adults. These findings were concomitant with reduced hunger and energy intake in older adults, which accords with the anorectic effects of these hormones [6]. Alternatively, there were no clear differences between older and younger adults for circulating concentrations of GLP-1, GIP, total ghrelin and acylated ghrelin. The results from the present study suggests even without the presence of known metabolic diseases or co-morbidities, older adults experience alterations in appetite regulation.

The increases in CCK observed in the present review supports previous speculation that CCK signalling is increased during ageing. Indeed, the anorectic effects from elevated concentrations of CCK in the fasted and postprandial state may even be enhanced in older adults due to increased sensitivity to the actions of this hormone [21] via CCK1 receptors on the nucleus of the solitary tract and vagal signalling [22]. In the postprandial state CCK is released from the intestine in response to nutrients in the intestinal lumen and appears to be, at least partly, responsible for meal termination [23]. Additional to increased fasted satiating signals to the

vagal nerve, increased circulating CCK also delays the rate of gastric emptying which is commonly seen in older adults. It has been suggested that delays in gastric emptying in older adults could reflect increased small intestinal feedback as a result of increased sensitivity and/or exposure of small intestine receptors to CCK [24]. Elevated postprandial concentrations and signalling of CCK may play a further role in enhanced satiation in combination with reductions in the gastric emptying rates of older adults.

The most robust differences between older and younger adults in the current review were observed for circulating concentrations of leptin, which were higher in older adults in both the fasted and postprandial state, with a large and moderate effect size, respectively. Leptin is an anorectic hormone secreted by adipose tissue [25] and therefore circulates in proportion to body fat stores as a tonic regulator of energy balance [26]. Consequently, the higher levels of leptin in older adults may be due to greater adiposity. However, adiposity is unlikely to be the sole mechanism for these elevations in leptin as Zamboni et al. [27] demonstrated that after the adjustment for body mass, fasted levels of plasma leptin increased with age in a population of older women aged 67-78 years. It remains unclear whether older adults experience leptin resistance in association with increased circulating concentrations, as experienced by obese populations [28]. Consequently, understanding leptin sensitivity in older adults is important to appreciate whether elevated concentrations of this hormone exert an anorectic effect and whether leptin represents a therapeutic target for improving appetite outcomes in this population.

In accordance with the higher levels of leptin in older adults, fasted and postprandial insulin concentrations were also elevated. Circulating levels of insulin are determined primarily by peripheral insulin sensitivity [29] which alongside leptin correlate with adiposity [30] and deliver information on peripheral energy stores to the central nervous system. Although the primary role of insulin relates to metabolic regulation, it has also been shown to exert anorectic

effects by acting on the arcuate nucleus of the hypothalamus to signal satiety and reduce food intake [31]. The observed increases in insulin may therefore contribute towards the attenuation of hunger in older adults observed in this review. However, as with leptin, insulin resistance of the hypothalamus may occur in older adults [32], thereby hindering the anorectic effects of this peptide. The relative importance of ageing and altered body composition as mediators of the increased insulin concentrations is unclear. The mean BMI value for young ($23.6 \text{ kg}\cdot\text{m}^{-2}$) and older adults ($24.8 \text{ kg}\cdot\text{m}^{-2}$) were similar, suggesting that differences observed in insulin concentration are not a result of differences in weight-status (as a proxy of body composition). In addition, despite differences in fasted insulin concentrations between older and younger adults, mean fasted plasma glucose concentrations were similar at $5.3 \text{ mmol}\cdot\text{L}^{-1}$ and $5.0 \text{ mmol}\cdot\text{L}^{-1}$, respectively, and suggested a healthy glucose tolerance in both groups. Additionally, mean HOMA-IR values for participants in the included studies were 1.67 for older adults and 1.57 for younger adults. Regardless, these findings highlight the need for interventions to reduce circulating concentrations of insulin from both an appetite and metabolic health perspective.

The PYY findings from this meta-analysis demonstrated a strong trend towards statistical significance for higher postprandial concentrations in older versus younger adults. This effect was observed despite the inclusion of only four studies and the subsequent likelihood of the analysis being underpowered. Interestingly, out of the four included studies in the present review, Moss [33] and Di Francesco et al. [34] both showed significantly higher circulating concentrations of PYY in older adults following the ingestion of a test meal. However, following intraduodenal infusion of lipid and glucose [35] and whey-protein [36], circulating postprandial PYY concentrations were comparable between younger and older adults. Consequently, the effects of age on PYY concentrations appear to be most prominent when nutrients are naturally ingested, thereby including mastication and swallowing, rather than

directly reaching the distal gastrointestinal tract [37]. It is possible that the observed results in the present review support previous speculations that bypassing of the stomach during intraduodenal infusion may attenuate PYY release, thereby diminishing any potential appetite-regulatory effects [7]. Critically, the ingestion of food as standardised test-meals reflects typical human eating behaviours which suggests that increased PYY concentrations are likely to be experienced by older adults in response to habitual feeding. Unlike leptin and insulin, obesity does not seem to cause a peripheral resistance to PYY [38-40], suggesting that the observed increases in older adults are likely to exert anorectic effects. The increased circulating PYY concentrations may further promote satiety by also contributing to reductions in gastric emptying rates in older adults [24].

Surprisingly, acylated ghrelin, total ghrelin, GLP-1 and GIP concentrations were not significantly different between older and younger adults in the present review. Alternatively, the differences in leptin, CCK, PYY and insulin appear to align with the reductions in hunger and energy intake in older adults observed in this meta-analysis. Consequently, it seems most beneficial for future interventions to target CCK, leptin, PYY and insulin when investigating methods to augment energy intake to reduce the anorexia of ageing from an endocrine perspective.

As part of the present review, we extracted and analysed data for hunger perceptions and energy intake when measured alongside appetite-related hormones in the included studies. These findings mirror those of the recent meta-analysis by Giezenaar et al. [41] by demonstrating that energy intake was significantly lower in older adults than younger adults. However, in the present review, energy intake appeared to be moderated by the method of dietary assessment. Specifically, the results revealed that a difference in energy intake was only observed when self-report methods were used and not through researcher-assessed methods. It is apparent within the research literature that there is generalised underreporting when using self-report

methods to assess energy intake. Therefore, our findings should be considered with caution as it is clear that limitations are present in self-report methods [42]. Nonetheless the results indicate large decreases in fasted hunger in older adults compared with younger adults which is a known predictor for energy intake [43]. Postprandial hunger perceptions also tended to be lower in older adults than younger adults but with only a small effect size. In combination with differences in appetite-related hormone concentrations, these effects may also be mediated by diminished homeostatic regulation of physiological functions in older adults. For example, older adults have been shown to experience delayed gastric emptying rate, altered pyloric motility and increased antral area [44,45,35,34].

In the current review, the hormones investigated which aligned with observed reductions in hunger and energy intake in older adults were CCK, leptin, insulin and PYY. It must be stressed that hormones involved in appetite regulation are only one aspect of a complex system that regulates feeding behaviour and that there may be other factors interacting which could also contribute to the changes in hunger and energy intake observed. Nevertheless, interventions to reduce circulating concentrations of these anorectic hormones may be useful to oppose the anorexia of ageing.

Despite the important findings in the current meta-analysis, some notable limitations must be acknowledged. First the hormones PP, oxyntomodulin and fasted acylated ghrelin are known to be mediators of appetite regulation. However, these hormones could not be included in the analysis due to only one study meeting the criteria for fasted acylated ghrelin and the absence of any studies measuring oxyntomodulin or PP concentrations. The present study explored differences in appetite regulation in healthy older adults, and it is therefore appreciated that the findings cannot be generalised to older adults who possess underlying co-morbidities. It must also be noted that the presence of co-morbidities and of the taking of medication amongst participants was reported in only 22 studies, respectively. Consequently, the authors cannot be

certain that all participants were free of underlying co-morbidities and were not taking medication. Additionally, despite our extensive search retrieving 3103 records, we cannot guarantee that our search was completely exhaustive of the relevant literature. However, having hand searched the reference lists of all included studies and review articles we are confident that we have included all available relevant studies.

Conclusions

This meta-analysis reveals that circulating concentrations of insulin, leptin, CCK and PYY are increased in older adults. Given the anorectic effect of these appetite-related hormones, such changes in older adults provide an underlying mechanism which may contribute towards the anorexia of ageing. Interventions to reduce circulating levels of these hormones may be beneficial for increasing appetite in older adults to attenuate, delay, or prevent the anorexia of ageing.

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Table 1. Summary of moderator variable analysis for energy intake, GLP-1 and insulin by subgroup.

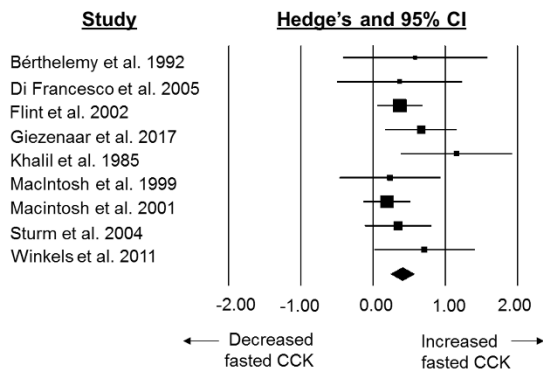
Moderator Variable	<i>p</i> value	Comparison
Hormone Analytical Method		
Fasted Insulin	0.985	RIA (n = 10; SMD 0.26, 95% CI -0.03 to 0.55)
		ELISA (n =7; SMD 0.22, 95% CI -0.35 to 0.80)
Postprandial Insulin	0.438	RIA (n = 7; SMD 0.34, 95% CI 0.07 to 0.60)
		ELISA (n = 7; SMD 0.06, 95% CI -0.19 to 0.31)
Type of feeding		
Postprandial Insulin	0.427	Infusion (n = 3; SMD 0.01, 95% CI -0.41 to 0.44)
		Feeding (n = 14; SMD 0.20, 95% CI 0.03 to 0.37)
Postprandial GLP-1	0.296	Feeding (n = 3; SMD 0.30; 95% CI -0.12 to 0.73)
		Infusion (n = 4; SMD -0.01, 95% CI -0.41 to 0.39)
Energy intake assessment method		
	0.015	Researcher measured (n = 7; SMD -0.10, 95% CI -0.88 to 0.68)
		Self-report (n = 4; SMD -2.89, 95% CI -4.99 to -0.78)

Table 2. Criteria for quality assessment and number (%) of studies scoring points for each criterion

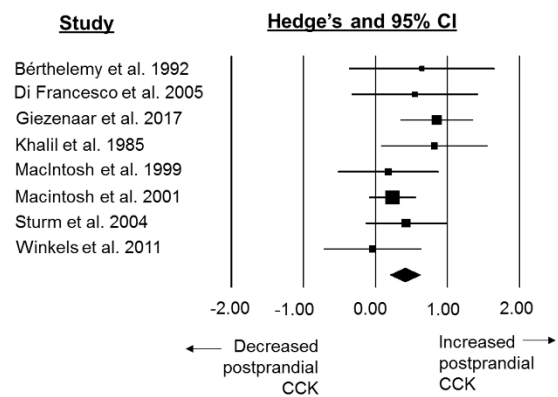
		Studies fulfilling the criteria n (%)		
		Yes	No	Don't Know
Criteria				
1	Were the aims/objectives of the study clear?	21 (52.5)	19 (47.5)	0 (0.0)
Methods				
2	Was the study design appropriate for the stated aim(s)?	34 (85.0)	5 (12.5)	1 (2.5)
3	Was the sample size justified?	4 (10.0)	36 (90.0)	0 (0.0)
4	Was the target/reference population clearly defined? (Is it clear who the research was about?)	26 (65.0)	12 (30.0)	2 (5.0)
5	Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?	12 (30.0)	3 (7.5)	25 (62.5)
6	Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?	9 (22.5)	5 (5.0)	26 (65.0)
7	Were measures undertaken to address and categorise non-responders?	-	-	-
8	Were the risk factor and outcome variables measured appropriate to the aims of the study?	37 (92.5)	3 (7.5)	0 (0.0)
9	Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?	40 (100.0)	0 (0.0)	0 (0.0)
10	Is it clear what was used to determined statistical significance and/or precision estimates? (e.g. p-values, confidence intervals)	27 (67.5)	13 (32.5)	0 (0.0)

11	Were the methods (including statistical methods) sufficiently described to enable them to be repeated?	20 (50.0)	20 (50.0)	0 (0.0)
Results				
12	Were the basic data adequately described?	23 (57.5)	17 (42.5)	0 (0.0)
13	Does the response rate raise concerns about non-response bias?	-	-	-
14	If appropriate, was information about non-responders described?	-	-	-
15	Were the results internally consistent?	32 (80.0)	7 (17.5)	1 (2.5)
16	Were the results presented for all the analyses described in the methods?	30 (75.0)	10 (25.0)	0 (0.0)
Discussion				
17	Were the authors' discussions and conclusions justified by the results?	29 (72.5)	11 (27.5)	0 (0.0)
18	Were the limitations of the study discussed?	21 (52.5)	19 (47.5)	0 (0.0)
Other				
19	Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results?	27 (67.5)	3 (7.5)	10 (25.0)
20	Was ethical approval or consent of participants attained?	38 (95.0)	0 (0.0)	2 (5.0)

A)



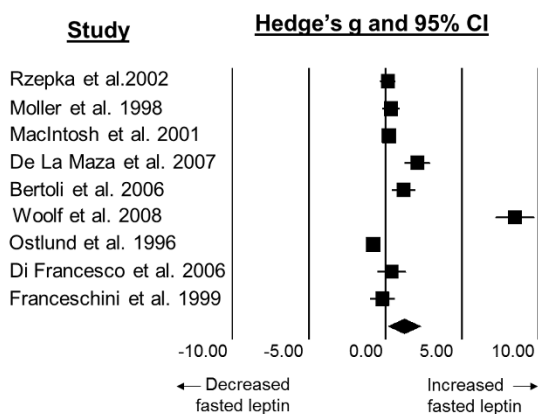
B)



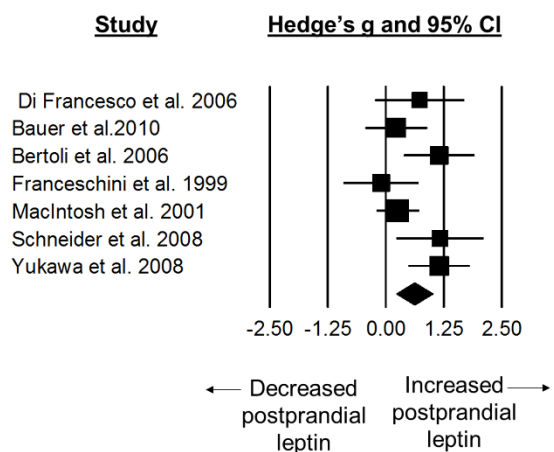
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1 **Fig 1.** Forest plot of standardised mean differences (means \pm 95% confidence intervals [CIs]
 2 for studies evaluating the differences in fasted CCK concentrations (a) and postprandial CCK
 3 concentrations (b) in older versus younger adults. The size of each square represents the
 4 relative weight of each comparison. The diamond represents the SMD (mean \pm 95% CI) for
 5 the model.

A)

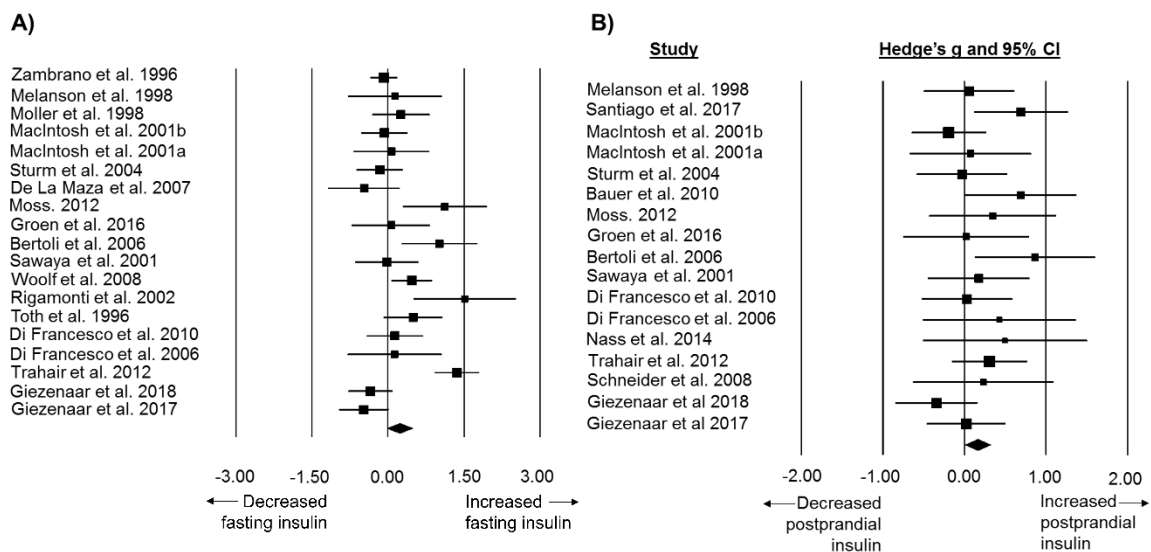


B)



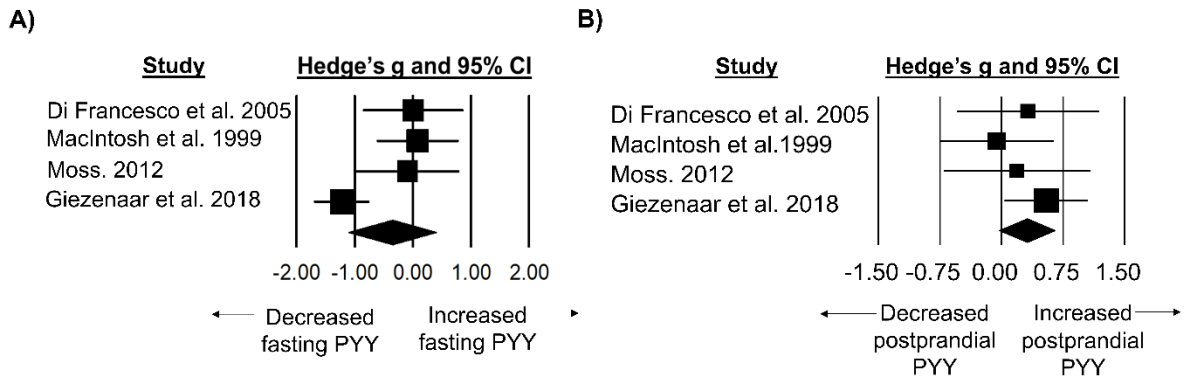
6

7 **Fig 2.** Forest plot of standardised mean differences (means \pm 95% confidence intervals [CIs]
 8 for studies evaluating the differences in fasted leptin concentrations (a) and postprandial
 9 leptin concentrations (b) in older versus younger adults. The size of each square represents
 10 the relative weight of each comparison. The diamond represents the SMD (mean \pm 95% CI)
 11 for the model.



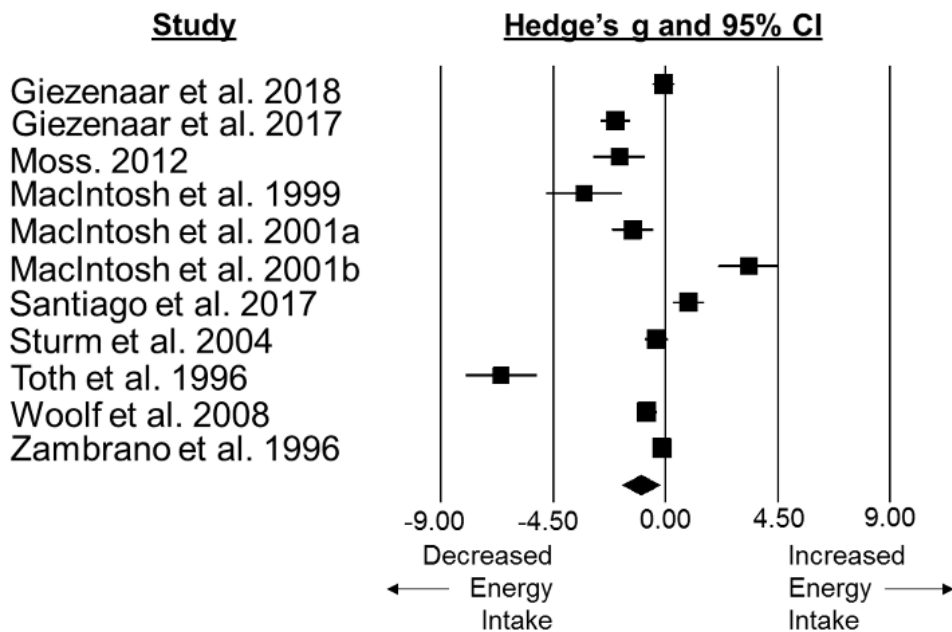
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13 **Fig 3.** Forest plot of standardised mean differences (means \pm 95% confidence intervals [CIs]
 14 for studies evaluating the differences in fasted insulin concentrations (a) and postprandial
 15 insulin concentrations (b) in older versus younger adults. The size of each square represents
 16 the relative weight of each comparison. The diamond represents the SMD (mean \pm 95% CI)
 17 for the model.



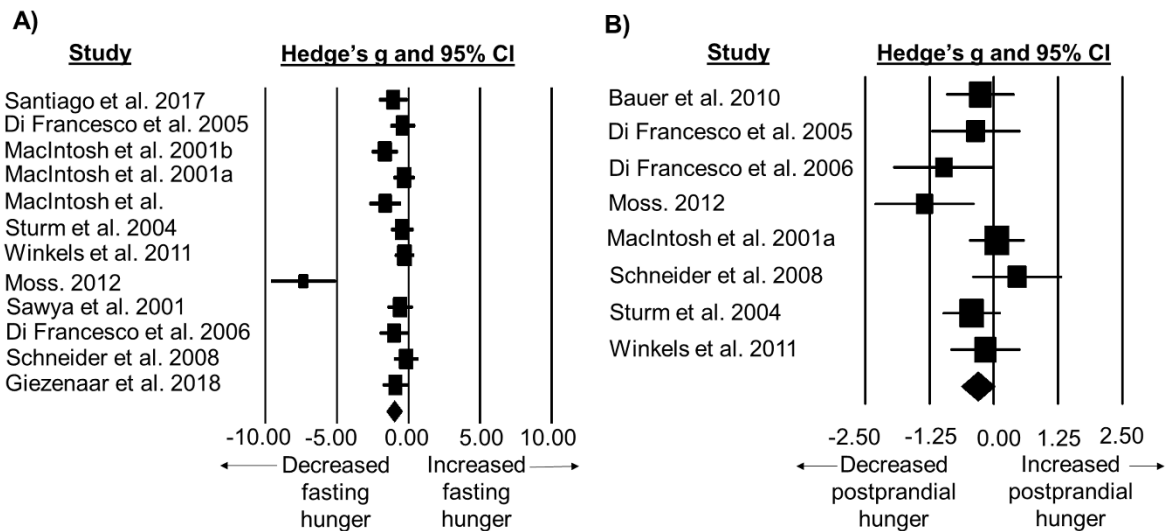
18

19 **Fig 4.** Forest plot of standardised mean differences (means \pm 95% confidence intervals [CIs]
 20 for studies evaluating the differences in fasted PYY concentrations (a) and postprandial PYY
 21 concentrations (b) in older versus younger adults. The size of each square represents the
 22 relative weight of each comparison. The diamond represents the SMD (mean \pm 95% CI) for
 23 the model.



24

25 **Fig 5.** Forest plot of standardised mean differences (means \pm 95% confidence intervals [CIs]
 26 for studies evaluating the differences in energy intake in older versus younger adults. The
 27 size of each square represents the relative weight of each comparison. The diamond
 28 represents the SMD (mean \pm 95% CI) for the model.



29
 30 **Fig 6.** Forest plot of standardised mean differences (means \pm 95% confidence intervals [CIs]
 31 for studies evaluating the differences in fasted hunger (a) and postprandial hunger (b) in older
 32 versus younger adults. The size of each square represents the relative weight of each
 33 comparison. The diamond represents the SMD (mean \pm 95% CI) for the model.

34 **Article Title:** Differences in circulating appetite-related hormone concentrations between
 35 older and younger adults: a systematic review and meta-analysis

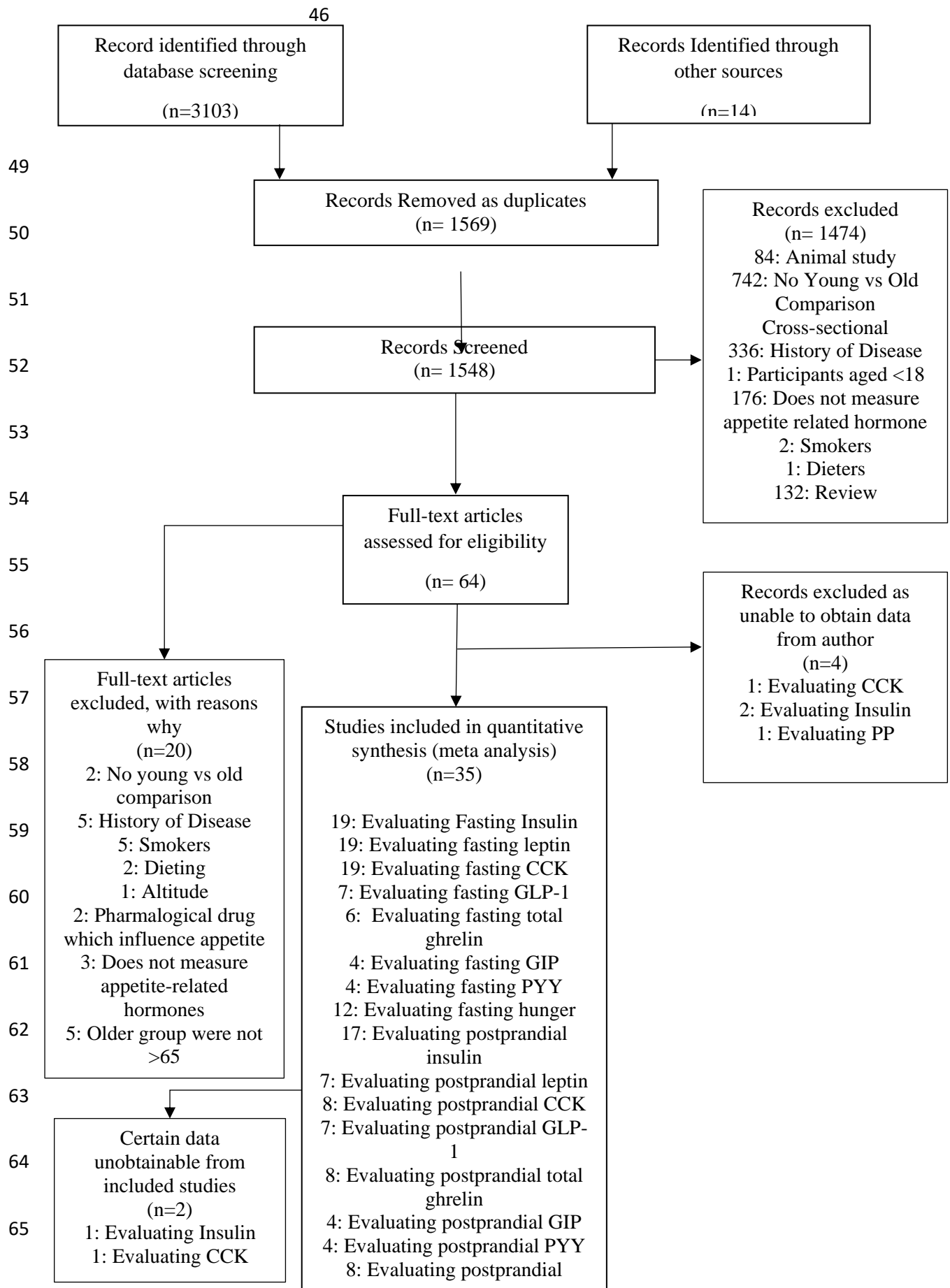
36 **Journal:** Aging Clinical and Experimental Research

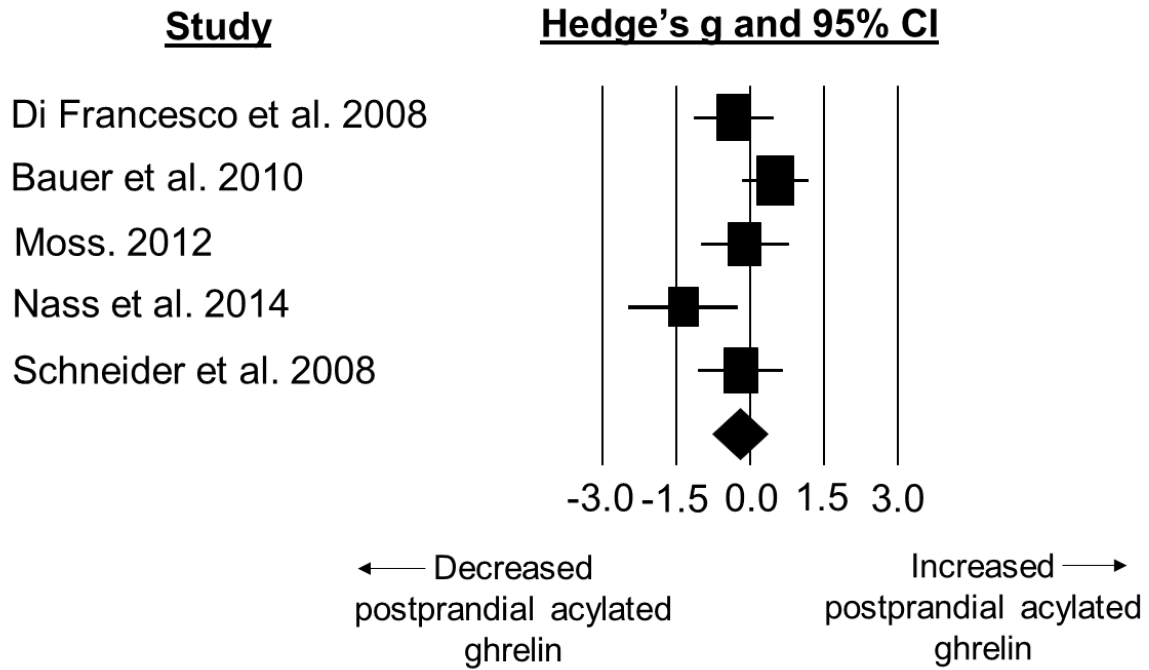
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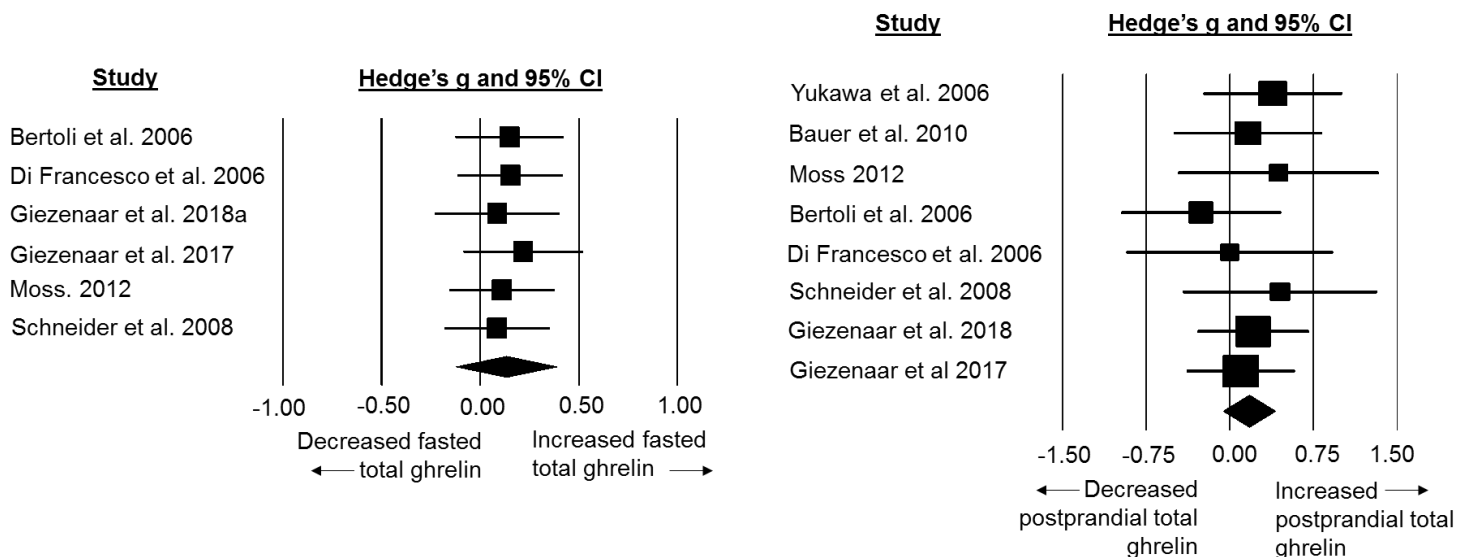
43 **Supplementary Figure 1.** Flowchart of study selection. CCK: cholecystokinin; PP:
 44 pancreatic polypeptide; GLP-1: glucagon-like-peptide 1; GIP: gastric inhibitory peptide;
 45 PYY: peptide YY





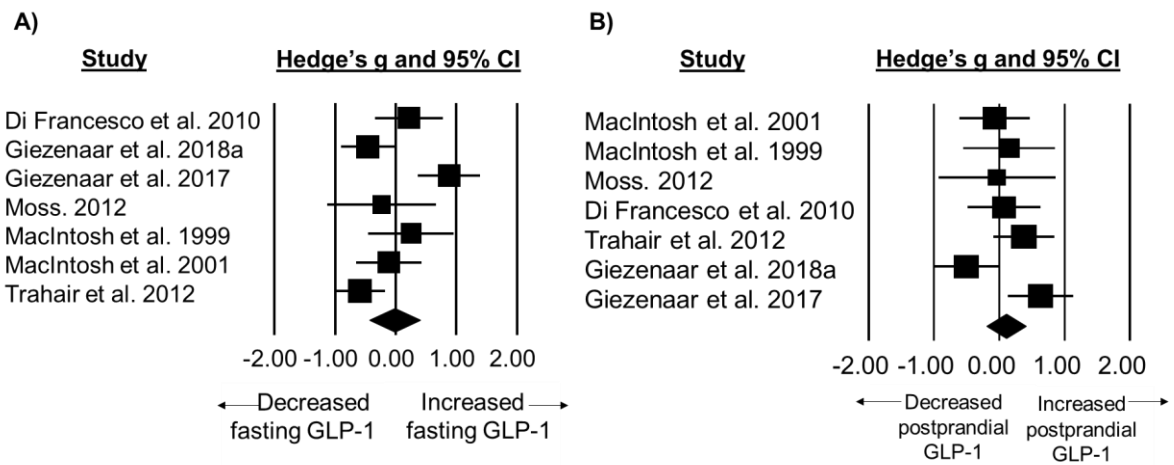
66

67 **Supplementary Figure 2.** Forest plot of standardised mean differences (means \pm 95%
68 confidence intervals [CIs]) for studies evaluating postprandial acylated ghrelin
69 concentrations in older versus younger adults. The size of each square represents the
70 relative weight of each comparison. The diamond represents the standardised mean
71 difference (mean \pm 95% CI) for the model.
72

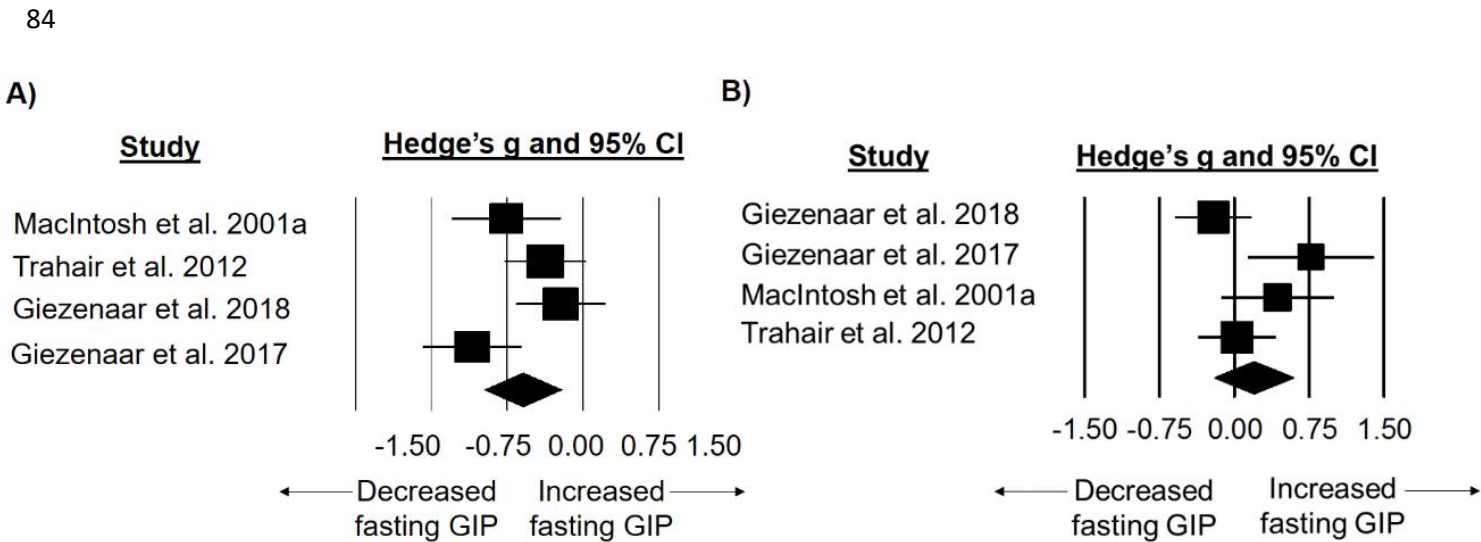


73 **Supplementary Figure 3.** Forest plot of standardised mean differences (means \pm 95%
74 confidence intervals [CIs]) for studies evaluating fasted total ghrelin concentrations (A) and
75 postprandial total ghrelin concentrations (B) in older versus younger adults. The size of each

76 square represents the relative weight of each comparison. The diamond represents the
 77 standardised mean difference (mean \pm 95% CI) for the model.



78
 79 **Supplementary Figure 4.** Forest plot of standardised mean differences (means \pm 95%
 80 confidence intervals [CIs]) for studies evaluating fasting GLP-1 concentrations (A) and (B)
 81 postprandial GLP-1 concentrations in older versus younger adults. The size of each square
 82 represents the relative weight of each comparison. The diamond represents the standardised
 83 mean difference (mean \pm 95% CI) for the model.



85 **Supplementary Figure 5.** Forest plot of standardised mean differences (means \pm 95%
 86 confidence intervals [CIs]) for studies evaluating fasting GIP concentrations (A) and
 87 postprandial GIP concentrations (B) in older versus younger adults. The size of each square

88 represents the relative weight of each comparison. The diamond represents the standardised
89 mean difference (mean \pm 95% CI) for the model.

90 **Supplementary Material 1**

91 Search Strategy

92 The following terms were searched for in titles/abstracts of each database, with MeSH terms
93 utilised where appropriate.

- 94 1. older people
- 95 2. older persons
- 96 3. older person
- 97 4. older individuals
- 98 5. older individual
- 99 6. older adults
- 100 7. older adult
- 101 8. aged
- 102 9. aged [MeSH Terms]
- 103 10. elderly
- 104 11. aging [MeSH Terms]
- 105 12. aging
- 106 13. ageing
- 107
- 108 14. appetite hormones
- 109 15. appetite hormone
- 110 16. appetite-related hormones
- 111 17. appetite-related hormone
- 112 18. appetite-regulating hormones
- 113 19. appetite-regulating hormone
- 114 20. gastrointestinal hormones
- 115 21. gastrointestinal hormone
- 116 22. GI hormones
- 117 23. GI hormone
- 118 24. Cholecystokinin
- 119 25. Cholecystokinin [MeSH Terms]
- 120 26. CCK
- 121 27. Peptide YY
- 122 28. PYY
- 123 29. Glucagon-like-peptide-1
- 124 30. Glucagon like peptide 1
- 125 31. Glucagon-like peptide 1 [MeSH Terms]
- 126 32. Glucagon-like peptide 1
- 127 33. Glucagon-like peptide 1
- 128 34. Glucagon-like-peptide 1
- 129 35. Glp 1
- 130 36. Glp-1

131 37. Leptin
132 38. Leptin [MeSH Terms]
133 39. Ghrelin
134 40. Ghrelin [MeSH Terms]
135 41. Pancreatic polypeptide
136 42. Gastric inhibitory peptide
137 43. Gastric-inhibitory-peptide
138 44. GIP
139 45. Oxyntomodulin
140 46. Oxyntomodulin [MeSH Terms]
141 47. OXM
142 48. Insulin
143 49. Insulin [MeSH Terms]
144
145 50. appetite
146 51. appetite [MeSH Terms]
147 52. hunger
148 53. hunger [MeSH Terms]
149 54. fullness
150 55. satiation
151 56. satiation [MeSH Terms]
152 57. satiety
153 58. desire to eat
154 59. prospective food consumption
155
156 60. energy intake
157 61. food intake
158 62. dietary intake
159 63. animals
160 64. humans

161

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Study Population	Appetite Related Hormones	Subjective Appetite Sensations	Ad-Lib intake
older people older persons older person older individuals older individual older adults older adult aged aged [MeSH Terms] elderly aging [MeSH Terms] aging ageing	appetite hormones appetite hormone appetite-related hormones appetite-related hormone appetite-regulating hormones appetite-regulating hormone gastrointestinal hormones gastrointestinal hormone GI hormones GI hormone Cholecystokinin Cholecystokinin [MeSH Terms] CCK Peptide YY PYY Glucagon-like-peptide-1 Glucagon like peptide 1 Glucagon-like peptide 1 [MeSH Terms] Glucagon-like peptide 1 Glucagon-like peptide 1 Glucagon-like-peptide 1 Glp 1 Glp-1 Leptin Leptin [MeSH Terms] Ghrelin Ghrelin [MeSH Terms] Pancreatic polypeptide Gastric inhibitory peptide Gastric-inhibitory-peptide GIP Oxyntomodulin Oxyntomodulin [MeSH Terms] OXM Insulin Insulin [MeSH Terms]	appetite appetite [MeSH Terms] hunger hunger [MeSH Terms] fullness satiation satiation [MeSH Terms] satiety desire to eat prospective food consumption	energy intake food intake dietary intake

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I. older people or older persons or older person or older individuals or older individual or older adults or older adult or aged[MeSH Terms] or elderly or aging[MeSH Terms] or aging or ageing

AND

II. appetite hormones or appetite hormone or appetite-related hormones or appetite-related hormone or appetite-regulating hormones or appetite-regulating hormone or gastrointestinal hormones or gastrointestinal hormone or GI hormones or GI hormone or Cholecystokinin or Cholecystokinin [MeSH Terms] or CCK or Peptide YY or PYY or Glucagon-like-peptide-1 or Glucagon like peptide 1 or Glucagon-like peptide 1 [MeSH Terms] or Glucagon-like peptide 1 or Glucagon-like peptide 1 or Glucagon-like-peptide 1 or Glp 1 or Glp-1 or Leptin or Leptin [MeSH Terms] or Ghrelin or Ghrelin [MeSH Terms] or Pancreatic polypeptide or Gastric inhibitory peptide or Gastric-inhibitory-peptide or GIP or Oxyntomodulin or Oxyntomodulin [MeSH Terms] or OXM or Insulin or Insulin [MeSH Terms]

AND

III. appetite or appetite [MeSH Terms] or hunger or hunger [MeSH Terms] or fullness or satiation or satiation [MeSH Terms] or satiety or desire to eat or prospective food consumption

OR

IV. energy intake or food intake or dietary intake

NOT

animals [MeSH Terms] NOT humans

Supplementary Material 2. Meta-Analysis References

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