

2016-10-01

Increased peptide YY blood concentrations, not decreased acyl-ghrelin, are associated with reduced hunger and food intake in healthy older women: Preliminary evidence.

Hickson, Mary

<http://hdl.handle.net/10026.1/4955>

10.1016/j.appet.2016.06.002

Appetite

Elsevier

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

1 **This is an Accepted Manuscript of an article published by Elsevier in Appetite**
2 **available at: DOI: [10.1016/j.appet.2016.06.002](https://doi.org/10.1016/j.appet.2016.06.002)**

3 **Increased Peptide YY blood concentrations, not decreased acyl-**
4 **ghrelin, are associated with reduced hunger and food intake in**
5 **healthy older women: preliminary evidence.**

6 Professor Mary Hickson^{1,2}, Dr Charlotte Moss², Professor Waljit S Dhillon³, Jeanne Bottin²,
7 Professor Gary Frost²

8 ¹Plymouth University, School of Health Professions, Plymouth, United Kingdom

9 ²Imperial College London, Nutrition and Dietetic Research Group, Section of Investigative
10 Medicine, London, United Kingdom

11 ³Imperial College London, Section of Investigative Medicine, London, United Kingdom

12 **Corresponding author**

13 Professor Mary Hickson
14 Peninsula Allied Health Centre
15 School of Health Professions
16 Faculty of Health and Human Sciences
17 Plymouth University
18 Derriford Road
19 Plymouth
20 PL6 8BH
21 Tel: +44 1752 587542
22 Email: mary.hickson@plymouth.ac.uk

23

24

25

26 **Abstract (245 words)**

27 With ageing there is frequently a loss of appetite, termed anorexia of ageing, which can
28 result in under-nutrition. We do not know how appetite control alters with ageing. The
29 objective of this study was to investigate whether differences in the release of, and
30 response to, gastrointestinal appetite hormones is altered in young compared to old healthy
31 volunteers. We hypothesised that an increase in PYY and GLP-1 or a decrease ghrelin may
32 result in a decreased appetite. A comparative experimental design, using a cross-sectional
33 sample of ages from a healthy population, matched for sex and BMI was used. The study
34 compared total ghrelin, acyl-ghrelin, PYY, GLP-1 and subjective appetite responses to
35 ingestion of a standardised 2781kj (660kcal) test meal. 31 female volunteers aged between
36 21-92yrs took part. Multiple linear regression showed that both age and sex had an
37 independent effect on energy intake. Subjective appetite scores showed that hunger,
38 pleasantness to eat, and prospective food intake were significantly lower in the older age
39 groups. PYY incremental area under the curve (IAUC) was greater in the oldest old
40 compared to younger ages $f(3,27)=2.9$, $p=0.05$. No differences in GLP-1, ghrelin or acyl-
41 ghrelin were observed in the older compared to younger age groups. Our data suggest that
42 there may be increases in postprandial PYY(3-36) levels in female octogenarians, potentially
43 resulting in reduced appetite. There does not appear to be any change in ghrelin or acyl-
44 ghrelin concentrations with ageing.

45 **Key words**

46 Ageing, appetite, ghrelin, PYY, anorexia.

47

48 **Introduction**

49 The ageing population is increasing and with ageing there is frequently a loss of appetite,
50 occurring even in the absence of disease, termed 'anorexia of ageing', which can result in
51 under-nutrition (Morley & Silver 1988). Under-nutrition results in a decline in functional
52 status, impaired muscle function, poor wound healing, higher hospital admission rates, and
53 increased mortality, and contributes to increasing healthcare costs (Norman et al. 2008).
54 Current consensus suggests that under-nutrition is an under diagnosed problem and this is
55 reflected in national and international guidance about nutritional screening (Kondrup et al.
56 2003). In the UK, prevalence is estimated to be three million, based on prevalence data from
57 the community, nursing homes, residential homes, and hospitals (BAPEN Malnutrition
58 Advisory Group 2009). Centres for Disease Control and Prevention in USA estimate that up
59 to 3000 people die each year from malnutrition related causes (Lee & Berthelot 2010). In
60 2006, the rate of malnutrition mortality for all adults was 0.8 per 100,000 people, but for
61 75-84 years it was 5.2, and for over 85 years it was 20.9 (Lee & Berthelot 2010).

62 Under-nutrition is normally treated using nutritional supplements, which are effective but
63 not universally (Milne et al. 2009). Studies have also looked at additional food and eating
64 assistance, but these also have mixed outcomes (Wade & Flett 2012). Poor outcome of
65 nutritional interventions appear to be mediated by appetite; if appetite is suppressed it will
66 limit the consumption of enough food and/or supplements.

67 Evidence exists to suggest a dysregulation of appetite control with ageing; older adults have
68 been shown to have an inability up-regulate appetite after periods of under-nutrition
69 compared to younger. This was related to differences in the sensations of hunger and
70 fullness and resulted in a failure to regain lost weight (Moriguti et al. 2000).

71 Appetite is controlled by hormonal and neural factors communicating between the gut and
72 brain (Hameed S, 2009). Briefly, there are several hypothesised satiety hormones including
73 cholecystinin, peptide tyrosine tyrosine (PYY₃₋₃₆) and glucagon-like-peptide-1 (GLP-1), and
74 there is only one gastrointestinal hormone which increases food intake, ghrelin. Our team
75 has demonstrated that the appetite loss in elderly people with fractured neck of femur is
76 associated with dysregulation of PYY and ghrelin (Nematy et al. 2006b). However, changes
77 seen during illness may be different to those related only to ageing. Therefore, there is a
78 need to investigate whether there are changes in appetite control that occur in healthy
79 older adults, before overt under-nutrition or illness is evident, that may precede these
80 conditions and place the older person at greater risk of developing these conditions. In this
81 study we carefully recruited individuals with no diagnosed health conditions.

82 Our study hypothesis was that appetite would be suppressed leading to lower energy
83 intakes in healthy older people, compared with young, and this would be related to either a
84 decrease in ghrelin or an increase in PYY and GLP-1.

85

86 **Methods**

87 The Riverside Research Ethics Committee granted ethical approval for the study (REC No
88 08/H0706/128). All volunteers gave written informed consent. The study was carried out
89 between 2009 and 2011, at John McMichael Clinical Research Facility, Imperial College
90 Healthcare NHS Trust.

91 Our aim was to investigate the effects of ageing alone on appetite regulation therefore we
92 recruited healthy subjects from the local community (via posters, adverts in local
93 organisational newsletters, and through the local General Practitioner research network),
94 ensuring equal numbers in each age range: 20-39; 40-59; 60-79, 80+ years, and matched

95 groups for sex and BMI (within $\pm 3\text{kg/m}^2$). We recognise that healthy subjects over 60 years
 96 are not necessarily representative of their population due to the lack of ill health, but
 97 excluding illness was necessary to test whether ageing per se is associated with changes in
 98 appetite control. The inclusion criteria were no diagnosed acute disease and no chronic
 99 disease or medication known to interfere with gut peptide secretion or appetite (this
 100 excludes virtually all chronic diseases; stable hypertension controlled by medication was the
 101 most common condition allowed). The main exclusion criteria were: history of alcoholism or
 102 substance abuse; raised blood pressure ($>90/140\text{mmHg}$); pregnant or breastfeeding
 103 women; recent treatment with an investigational drug; recent blood donation; current
 104 smokers; scoring 15 or more in restraint section of the Three Factors Eating Questionnaire.
 105 The primary outcome was total ghrelin concentration. Secondary outcomes included acyl-
 106 ghrelin, PYY and GLP-1 concentration, insulin and glucose levels, appetite visual analogue
 107 scores, food intake at the *ad libitum* meal, and gastric emptying.

108 [Volunteers were invited to a screening visit where their body mass index \(BMI: \$\text{kg/m}^2\$ \), and body fat percentage \(multi-](#)
 109 [frequency segmental bioelectrical impedance analysis; Tanita Body Composition Analyser, Amsterdam, Netherlands\)](#)
 110 [were calculated. They were screened for blood abnormalities \(full blood count, urea and electrolytes and liver function](#)
 111 [tests\) and asked about any current medications and illnesses. Participants then completed the restraint section of the](#)
 112 [three factors eating questionnaire \(Stunkard & Messick 1985\) to exclude people with high eating restraint scores that](#)
 113 [may affect food intake at the test meal. Finally, subjects chose which *ad libitum* meal they would prefer. These meals](#)
 114 [were manufactured composite meals chosen for their similar macronutrient profiles \(see](#)

115
 116 Table 1) and homogenous consistency.

117
 118

119 **Table 1: Nutritional Composition of ad libitum meals**

Values/100g	Bolognese bake	Chicken Tikka Masala	Macaroni Cheese
Energy KJ (kcal)	665 (160)	665 (160)	631 (151)
Protein (g)	8.5	7.3	7.0
Carbohydrate (g)	15.6	16.6	14.0
of which sugars (g)	1.5	3.6	1.0
Fat (g)	6.8	6.9	7.4
of which saturates (g)	3.5	2.4	4.8

Mono-unsaturates (g)	2.7	3.0	2.3
Poly-unsaturates (g)	0.3	1.1	0.3

120

121 Subjects then attended for two ½ day visits, arriving at 08.30 fasted (no food and only water
122 from 9pm the night before) and having avoided alcohol and excessive exercise prior to the
123 visit. The first visit was a sham visit, mimicking the process for the true test meal except that
124 minimal blood was taken at each sampling point. Gut hormones are influenced by stress and
125 therefore a sham visit was necessary to accustom volunteers to the environment and
126 protocol. (The process for the study is shown in Figure 1S, supplementary data). The second
127 visit followed this process and the required blood sample volumes were taken.

128 The standard meal was consumed immediately after the first fasting blood sample and
129 consisted of 600ml nutritionally complete supplement drink (Ensure plus, Abbott, USA). This
130 is routinely used in our laboratory as it provides a fixed known amount of macro-nutrients
131 and energy that can be consumed quickly, ensuring a standardised stimulus for gut
132 hormone release. Subjects consumed all of the drink over a 15 minutes period
133 (2781kj/660kcal, 27.5g protein, 89g CHO, 21.6g fat). Directly after the test meal the subjects
134 took 1.5g of soluble paracetamol to measure gastric emptying. Blood samples and visual
135 analogue scales were then completed at regular intervals for 3 hours. After 180 minutes the
136 participants were offered a pre-weighed excess of the chosen meal and asked to eat until
137 comfortably full. The remaining food was weighed to calculate the energy and nutrient
138 intake at the meal. Between one and three volunteers were studied at any given time and
139 no contact was allowed between participants.

140 All plasma peptide hormones and insulin were measured in duplicate in a single assay. Total
141 ghrelin (Patterson et al. 2005), total PYY (Adrian et al. 1985b, 1987), GLP-1(1-36) (Kreymann
142 et al. 1987), and insulin by radioimmunoassay; glucose using hexokinase and G-6-PDH with

143 Abbott ci8200 analysers (Abbott Diagnostics, Maidenhead, UK); acyl-ghrelin using sandwich
144 ELISA kit (Millipore, USA) specific for human acyl-ghrelin; and paracetamol using
145 Enzymatic/Colorimetric method with Abbott ci800 analysers (Abbott Diagnostics,
146 Maidenhead, UK). The time to peak concentration for paracetamol was used as a
147 measurement of gastric emptying (Heading et al. 1973). An index of fasting insulin
148 resistance was measured (HOMA) (Matthews et al. 1985).

149 Appetite visual analogue scales (VAS) include five scales to assess hunger, nausea,
150 pleasantness to eat, prospective desire to eat, and fullness (Stubbs et al. 2000). Each scale is
151 a 100mm line which is marked by the participant and measured to the mark, providing a
152 score out of 100 assuming 1mm equals a score of one.

153 Gut hormone concentration and VAS scores are measured over time and thus the area
154 under the curve (AUC) and the incremental area under the curve (IAUC) were used to
155 quantify these measures over the whole test meal period. The IAUC includes the area
156 between the plasma level curve and the fasting concentration (T=0), with any area beneath
157 fasting being ignored. It is calculated using the sum of the areas of the triangles and
158 rectangles making up the area, calculated geometrically by applying the trapezoid rule
159 (Wolever & Jenkins 1986). The IAUC is the preferred method to determine changes over
160 time since it takes into account the baseline value, however, it could mask the overall effect
161 of ageing. For example, hunger levels may not decrease significantly more in ageing in
162 response to a test meal, but overall hunger levels may be consistently lower, demonstrating
163 an overall effect of ageing rather than just a response to a test meal, therefore AUC was also
164 used.

165 For a power of 90% and $\alpha=0.05$, with a $sd=256.6$ (Neary et al. 2004) and to detect a
166 difference of 100pmol/l (difference in total ghrelin levels seen in our previous work and

167 previously shown to result in altered food intake) 72 patients were needed in total (18 in
168 each age group).

169 The sample was grouped by age as follows: 20-39, 40-59, 60-79 and 80 + years. Age groups
170 were compared either using a one-way ANOVA followed by a Hochberg post-hoc test (for
171 data judged to be normally distributed using Kolmogorov-Smirnov test) or a Kruskal Wallis
172 test followed by a Mann Whitney U test (for data judged to be non-normal).

173 Multiple regression analysis was also used to explore the association of age with hormone
174 concentration, and the factors potentially affecting total energy intake. Exploratory analyses
175 were performed with composite variables constructed from the main endpoints, and the
176 relationship of these composite variables with age was tested with a linear regression
177 analysis. To construct the composite variables, they were first standardised to a common
178 scale by dividing each endpoint by its standard deviation, and subtracting the mean value.
179 Residuals from the regression analysis testing the composite variables were tested for
180 normality using the Shapiro-Wilk statistic and no statistically significant deviations from
181 normality were detected.

182 **Results**

183 58 volunteers were recruited (details are shown in figure 2S, supplementary data). All age
184 group recruitment targets were met except those over 80 years. This proved an extremely
185 challenging group to recruit, and despite accessing several General Practitioner patient
186 databases, inviting hundreds of potential patients and screening many, we were only able to
187 recruit 6 females eligible for the study. Most individuals were not suitable due to
188 comorbidities and/or current medications. Therefore we have presented here the data for
189 females only due to the absence of males in the oldest age group (data for the whole group
190 is in supplementary information).

191 Demographic details are shown in Table 2. As expected body composition of the older
 192 participants was different to the younger; fat percentage increased and lean percentage
 193 decreased with increasing age. Matching for weight and BMI was effective with no
 194 significant differences between the age groups.

195 **Table 2: Female Participants' Characteristics**

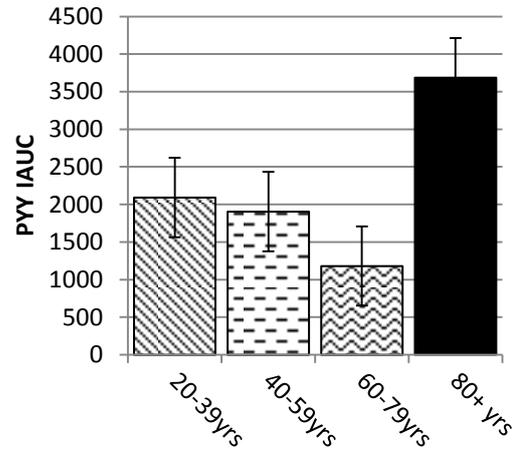
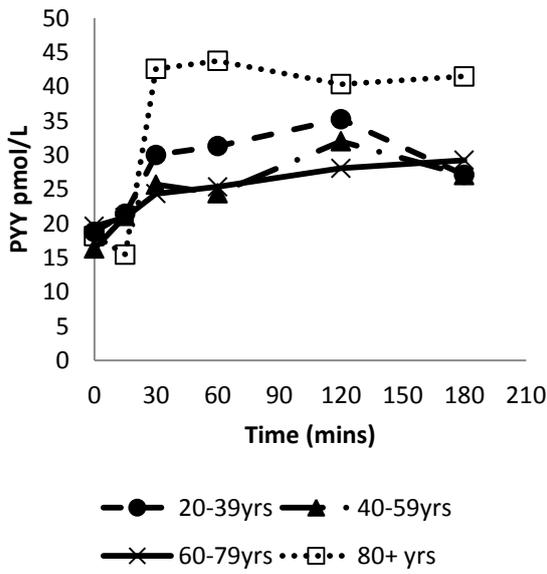
	Mean (SD)				
	Total N=31	20-39 years N=9	40-59 years N=9	60-79 years N=7	80+ years N=6
Age (years)	54.0 (21.8)	27.2 (5.0)	51.2 (7.3)	66.4 (4.8)	85.0 (4.8)
BMI (kg/m²)	22.9 (1.8)	22.2 (1.4)	23.2 (1.7)	23.4 (1.6)	23.5 (2.7)
Body Fat (%)	31.9 (7.9) ^a	28.4 (3.2)	33.5 (13.0)	32.5 (5.4) ^a	34.0 (4.2)
Lean Mass (%)	18.5 (1.3) ^a	19.0 (1.1)	19.0 (1.3)	17.7 (1.1) ^a	17.7 (1.1)
Weight (kg)	63.2 (8.1)	61.6 (6.4)	65.1 (6.7)	64.0 (12.2)	61.8 (8.1)

196 ^a=1 missing value; BMI=Body Mass Index.

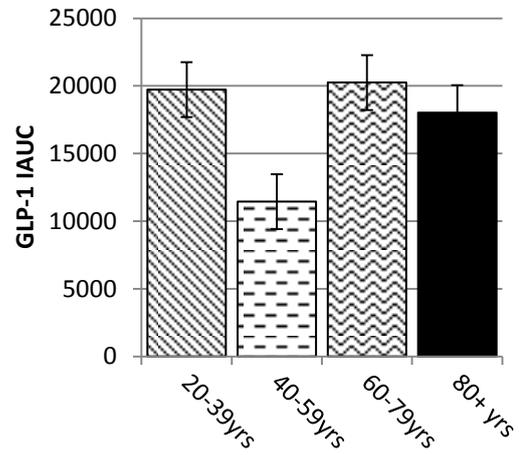
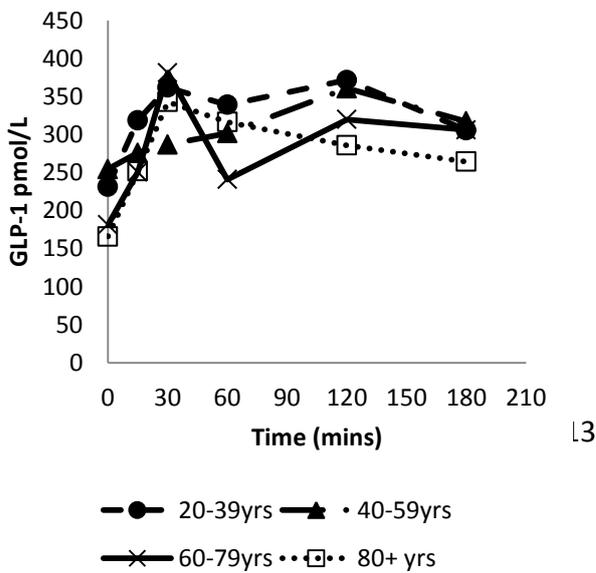
197

198 Plasma hormone analysis can be seen in Figure 1 and shows statistically significant
 199 differences between the female age groups for PYY IAUC ($f(3,27)=2.9$, $p=0.05$). Post hoc
 200 analysis using Hochberg test showed that the 80+ age group had a greater IAUC compared
 201 to the 60-79 at the 0.05 level. All other comparisons were not significant. No significant
 202 differences in GLP-1, acyl or total ghrelin were observed. The regression analysis found no
 203 associations with age for either PYY, GLP-1 or ghrelin. Further post hoc tests exploring
 204 different age cut offs for the oldest group (either 75+ or 70+) did not change the pattern of
 205 results and only the *a priori* planned analyses are presented.

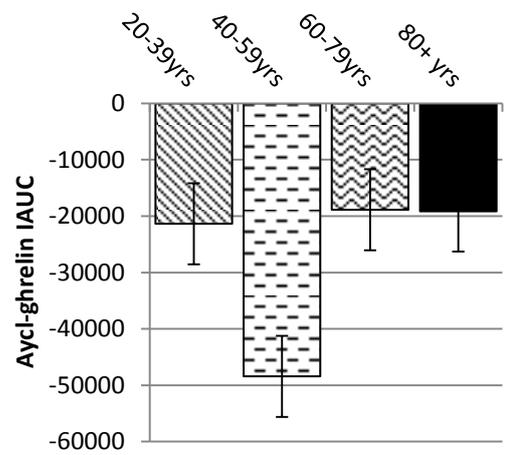
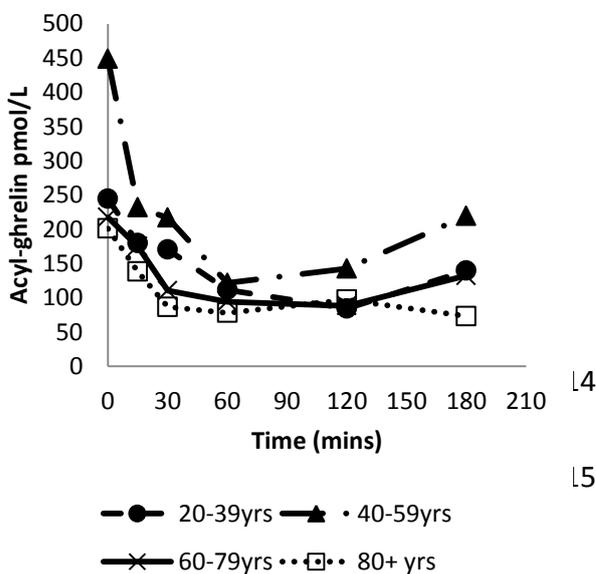
206 **Figure 1: Difference in PYY, GLP-1 and Acyl-ghrelin** 211
 207 **blood concentrations between age groups**



208



209



210

216 There was a decline in energy intake at the *ad libitum* meal from the young to the old
217 group (ANOVA: $f(3,27)=2.9$, $p=0.05$) as shown in Table 3.

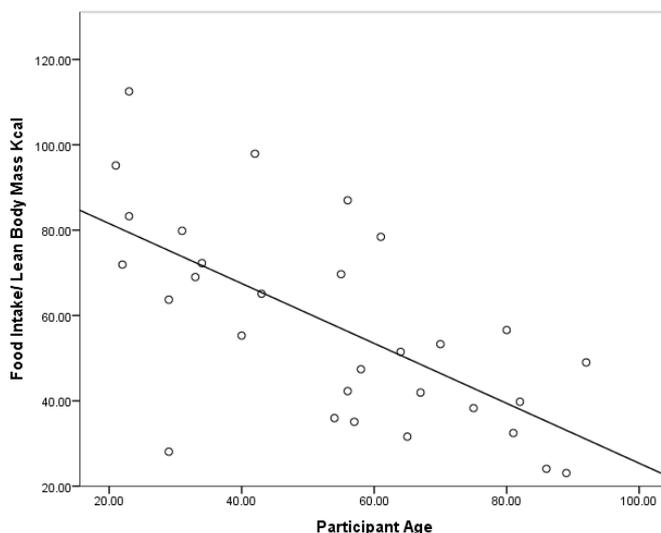
218 **Table 3: Energy intake from ad libitum meal (women only)**

Age group	Mean energy (kcal) intake at <i>ad libitum</i> meal (\pm sem)
20-39 yrs	885.5 (\pm 107.0)
40-59 yrs	744.0 (\pm 105.3)
60-79 yrs	684.0 (\pm 130.4)
80+ yrs	415.6(\pm 72.4)

219

220 Post hoc testing (Hochberg) revealed significant differences between youngest compared
221 to oldest age group at the 0.05 level. All other comparisons were not significant. These
222 differences were maintained when lean body mass was controlled for, showing a negative
223 correlation between energy intake (kcal)/lean body mass (kg) ($p<0.001$, $r=-0.66$) (see
224 Figure 2).

225 **Figure 2: Association between age and energy intake (kcal)/kg lean mass from the *ad libitum* meal.**



226

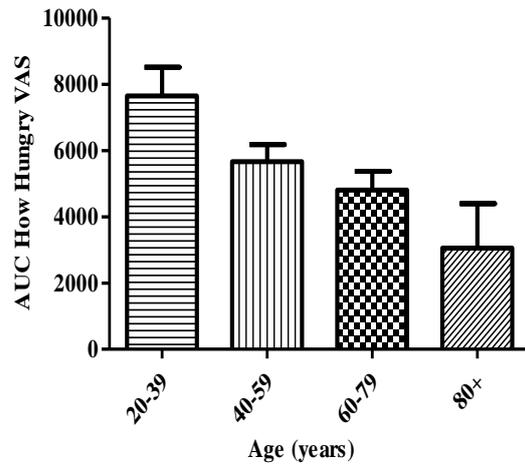
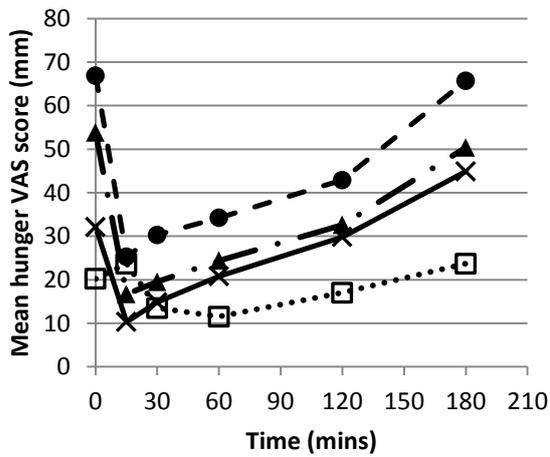
227 A multiple linear regression analysis (including men and women) showed that only age
228 ($p<0.001$) and sex ($p=0.03$) had an independent effect on energy intake at this meal, and

229 energy expenditure, lean mass and water consumption were all excluded from the final
230 model.

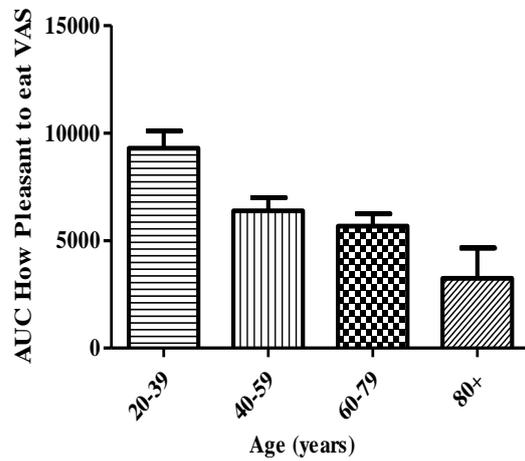
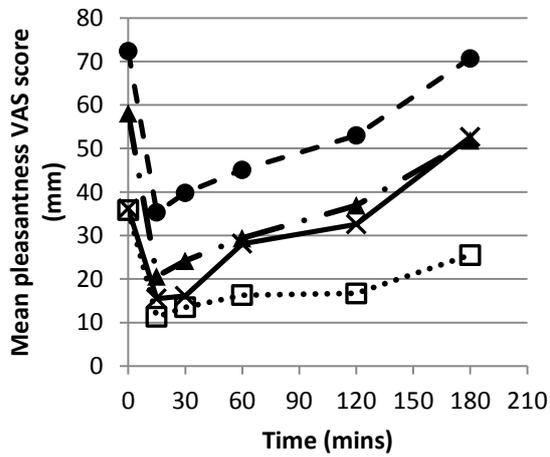
231 We also explored the VAS results in the whole cohort, men and women; the Kruskal-
232 Wallis test showed that hunger ($H(3)=10.8$, $p=0.013$), pleasantness to eat ($H(3)=16.6$,
233 $p=0.001$), and prospective desire to eat ($H(3)=18.4$, $p=0.001$) scores were significantly
234 affected by age. Pairwise comparisons with adjusted p values showed that hunger was
235 significantly lower in the oldest compared to the youngest ($p=0.016$); pleasantness to eat
236 was reduced for 80+ ($p=0.001$) and 60-79yrs ($p=0.023$) compared to the youngest; and
237 similarly prospective desire to eat was reduced for 80+ ($p=0.001$) and 60-79yrs ($p=0.011$)
238 compared to the youngest. (See Figure 3). Fullness and nausea scores were not different
239 (data not shown).

240

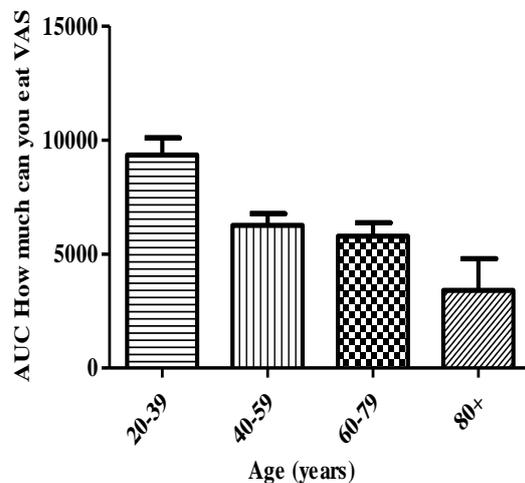
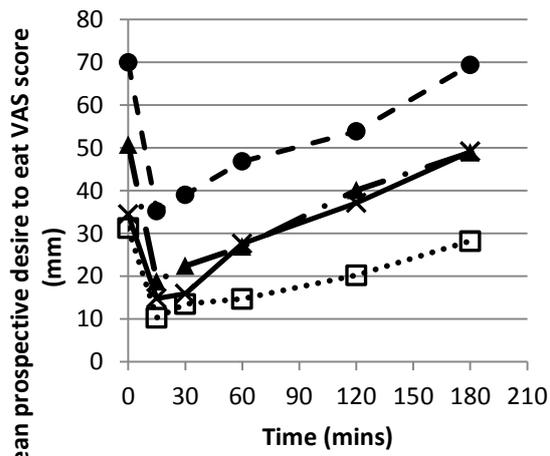
241 **Figure 3: Mean VAS score over time and AUC for hunger, pleasantness to eat, and prospective desire to eat.**



242



243



244

-●- 20-39yrs -▲- 40-59yrs
 -×- 60-79yrs -□- 80+ yrs

245 Insulin, glucose and insulin resistance were all raised in the older age groups compared to
246 the younger group, as expected from previous research data (Fraze et al. 1987). Peak
247 paracetamol concentrations did not change with age, indicating no difference in gastric
248 emptying between the four age groups. Further details of this data can be found in the
249 supplementary data.

250

251 Discussion

252 This data confirms that healthy older adults have lower energy intake at the *ad libitum*
253 meal and lower daily energy expenditure, with age as an independent factor, when
254 controlled for body composition. The subjective appetite data (VAS) shows a
255 corresponding picture of significantly less hunger and desire to eat in older subjects
256 explaining lower intakes.

257 We hypothesised that a difference in the pattern of concentration of gut hormones would
258 account for appetite suppression in older healthy people. The results are inconsistent.
259 These data suggest that the age related difference in energy intake in those aged over 80
260 years may result from higher blood concentrations of PYY but not from an increase in GLP-
261 1 or a reduction in ghrelin concentration. The regression analysis using age as a
262 continuous variable did not support this finding and found no relationship between age
263 and any hormone blood concentration.

264 We also found higher insulin, glucose and insulin resistance in the oldest age group as
265 would be expected, and this may be explained to some extent by the differences in body
266 fat mass (Paolisso et al. 1999). We found no difference in gastric emptying between
267 young and old, thus eliminating this factor as contributing to the reduced food intake.

268 To date most research to explore appetite control and ageing has focussed on ghrelin
269 concentration (Moss et al. 2012), testing the hypothesis that a reduction in this appetite
270 stimulating hormone may explain the decrease in appetite seen with ageing (Moss et al.
271 2012). The results from these studies are mixed. Part of the reason for this is because
272 older studies did not measure the two forms of ghrelin; desacyl (inactive) and acyl
273 (biologically active), but only total ghrelin. Only four of the nine published studies
274 investigating ageing and ghrelin measured acyl ghrelin (Bauer et al. 2010; Di Francesco et
275 al. 2008; Nass et al. 2013; Schneider et al. 2008) but even these have used different
276 methods for acyl-ghrelin analysis (RIA and Sandwich ELISA) which could in part explain the
277 inconsistent results found in these studies (Prudom et al. 2010). One study compared
278 ghrelin concentration over 24hr in young and old subjects (Nass et al. 2013) and found
279 lower levels of ghrelin concentration during the night but not post-prandially. This
280 supports our results in part, but it is problematic to compare this study to ours as the
281 purpose and methodologies are different. Only one other study shows a lower fasting and
282 post-prandial ghrelin concentrations in older adults (Di Francesco et al. 2008), supporting
283 the theory that a reduction in ghrelin concentration causes a reduced appetite with
284 ageing. This study uses the most robust methodology in terms of the calorie content of
285 the study meals provided, time of day, and the matching of groups on age range, body
286 mass index and sex. In contrast our study, also carefully designed to limit many of these
287 methodological problems, does not support the theory that ghrelin concentration is
288 reduced with ageing. It should be noted that our study was powered to detect a
289 100pmol/l difference between groups and the sample size was too low in the oldest age
290 group.

291 Many studies have demonstrated that PYY₃₋₃₆ exerts an anorectic effect in humans
292 (Batterham et al. 2003; Degen et al. 2005; Sloth et al. 2006). PYY concentrations increase

293 significantly after a meal in proportion to the amount of calories consumed reaching a
294 plateau after approximately 1 h and remains at the plateau for up to 6h (Adrian et al.
295 1985a). We and others have demonstrated that in a number of chronic conditions and in
296 critical care, appetite dysregulation is related to increased PYY and/or decreased ghrelin
297 (le Roux et al. 2005; Nematy et al. 2006a; Nematy et al. 2006b; Oner-Iyidogan et al. 2011).
298 Increased circulating PYY concentrations, early PYY production post-prandially or
299 increased sensitivity to PYY in ageing could all result in elevated feelings of fullness,
300 leading to the cessation of food intake, and may explain the changes in appetite seen
301 during ageing. Studies exploring the sensitivity to PYY in young healthy adults have shown
302 ambiguous results and that even near physiological doses can elicit nausea and vomiting
303 (Batterham et al. 2003; Degen et al. 2005; Sloth et al. 2006), thus such studies in older
304 adults are challenging to carry out.

305 There are currently only two studies that have explored the effect of ageing on PYY
306 production (Di Francesco et al. 2005; MacIntosh et al. 1999). MacIntosh et al (MacIntosh
307 et al. 1999) found no effect of age when comparing PYY concentrations in young (20-
308 34yrs, n=7) and older (65-80yrs, n=8) male adults (healthy and matched for BMI) during an
309 infusion of lipid or glucose directly into the duodenum. These researchers aimed to study
310 small intestine nutrient-mediated feedback, making the method of nutrient delivery
311 different to normal eating, where the effects of food consumption, nutrients in the
312 stomach and gastric emptying play a role. It is therefore difficult to compare this evidence
313 with the current study, but in fact show data in line with those reported here, since the
314 age of the older group was less than 80 years. The other study aimed, like ours, to
315 replicate normal eating, albeit in a controlled manner and environment. Di Francesco et al
316 (Di Francesco et al. 2005) used a study protocol involving an overnight fast followed by a
317 3347kJ (800kcal; 15% protein, 45% fat, 40% carbohydrates) solid meal with water,

318 comparing young (25-53yrs, n=9) and older (72-82yrs, n=10) men and women (M:F=10:9),
319 all healthy and matched for BMI. Their study demonstrated that older subjects had no
320 difference in fasting PYY or PYY concentrations up to 120 minutes post-prandially, but a
321 significantly higher concentration at 180 and 240 minutes. An absence of an altered GLP-1,
322 total ghrelin, acyl ghrelin and gastric emptying with ageing was also observed in this
323 investigation. These data are in agreement with our findings and are consistent with the
324 post-prandial satiety effects of PYY which appears to be released in greater quantity in
325 response to food intake in older individuals. In addition, our data suggest that these
326 effects are only clearly apparent in the very old healthy population. This information
327 supports the hypothesis that ageing is associated with increased PYY production, resulting
328 in satiety, and offering some explanation for the anorexia of ageing. Our data also showed
329 that in the older group there appears to be a suppression of PYY at 15 minutes following
330 the meal. The physiological relevance of this is not known. However there is no
331 suggestion from the VAS that this cause a decrease in satiety at this time point. It is also
332 worth noting that both these studies measured cholecystokinin (CCK) (not done in this
333 study) and found higher concentrations in older subjects both fasting and post prandially.
334 Another group has also reported elevated CCK (Sturm, 2003) but Serra-Prat et al was
335 unable to reproduce this observation (Serra-Prat et al 2009).

336 The main limitation of this study is the low number of healthy participants over 80yrs
337 (n=6) and that they were all female, thus it is possible that these results are a type 1
338 statistical error or due to the sex of the subjects. We have presented the female only
339 analysis but the analysis for all data is shown in the supplementary information,
340 suggesting few differences between the sexes.. There is little published data to suggest an
341 effect of sex on gut hormone concentration and appetite control and this evidence relates
342 to phases of the menstrual cycle (Brennan et al. 2009). Larger studies, ideally with both

343 men and women, using similar standardised protocols are required to confirm our results.

344 Another limitation is that total PYY was measured not PYY₃₋₃₆ only.

345 It is difficult to establish from the published literature exactly what age anorexia may start;

346 it appears to be a gradual process but is most common along-side chronic disease

347 (Visvanathan, 2015). Our data shows that food intake decreases even with no ill health

348 continuing up to 92 years.

349 We also did not identify an incremental change in PYY concentration in the 60-79yr age

350 group, as would be expected assuming a gradual alteration in gut hormone concentration

351 with ageing. However, this is not necessarily a sound assumption as anorexia of ageing

352 effects the very old to a greater extent (Malafarina et al. 2013). It may be that these

353 changes in PYY concentration are limited to the oldest age groups. However, the VAS data

354 do show a linear reduction of appetite with age, suggesting other factors may be at play.

355 Appetite remains under the influence of many external factors, such as lifestyle

356 (retirement), psychological (bereavement, depression) or hormonal (menopause) changes.

357 These changes may precede later physiological changes suggested from our results.

358 Although subjects were matched for BMI, percentage body fat was found to be higher in

359 the oldest age group compared to the youngest and so could be a possible confounder.

360 However, there is scant evidence that PYY is affected by BMI or fat mass. One study

361 (Batterham et al. 2003) found lower PYY levels in obese compared to lean young adults,

362 and a negative relationship between BMI and PYY, suggesting that PYY would be lower

363 with higher fat mass. Therefore, the difference in fat mass is unlikely to be a significant

364 factor in the finding that PYY was increased in the oldest age group.

365 Gastric emptying was also not different between age groups eliminating this factor as one

366 which contributed to the reduction in food intake seen in our aged study sample. In

367 theory; increased time to empty the stomach could reduce appetite and speed up satiety.
368 The use of a small volume meal may have ensured that gastric emptying was not affected
369 allowing us to conclude that the reduction in food intake is due to the changes in PYY
370 concentration.

371 Our data suggest that there may be an increase in the concentration of PYY after meals
372 with healthy ageing in females, potentially resulting in a reduced appetite. There does not
373 appear to be any change in ghrelin or acyl-ghrelin concentration with ageing.

374 **Acknowledgements**

375 Our thanks go to the GPs who were part of the North West London Research Network for
376 assistance with recruiting our older volunteers. MH, GF, WD, CM designed research;
377 statistical advice regarding the power calculation was received from Joseph Eliahoo,
378 Statistical Advice Service, Imperial College, London; advice on the regression analysis was
379 received from Les Huson; CM, JB conducted research; MG provided essential reagents or
380 provided essential materials; MH, CM, WD & GF analyzed data; MH wrote paper, all
381 authors reviewed the final manuscript and MH has primary responsibility for final content.

382 This work was supported by an Imperial College Healthcare Charity PhD fellowship for CM;
383 WD is funded by a National Institute of Health Research (NIHR) Career Development
384 Fellowship. The Department is funded by an Integrative Mammalian Biology (IMB)
385 Capacity Building Award, a FP7-HEALTH-2009-241592 EurOCHIP grant, and funding from
386 the NIHR Biomedical Research Centre Funding Scheme. The research was carried out at
387 the NIHR Wellcome Trust Clinical Research Facility at Imperial College Healthcare NHS
388 Trust. The views expressed are those of the authors and not necessarily those of the
389 Charity, NHS, NIHR or the Department of Health.

390 **Conflict of Interest**

391 All authors declare no conflicts of interest in relation to this paper.

392

393 **Reference List**

394 Adrian TE, Savage AP, Fuessl HS, Wolfe K, Besterman HS, & Bloom SR (1987) Release of
395 peptide YY (PYY) after resection of small bowel, colon, or pancreas in man. *Surgery*
396 101:715-719

397 Adrian TE, Savage AP, Sagor GR, Allen JM, Bacarese-Hamilton AJ, Tatemoto K, Polak JM, &
398 Bloom SR (1985a) Effect of peptide YY on gastric, pancreatic, and biliary function in
399 humans. *Gastroenterology* 89:494-499

400 Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR (1985b) Human
401 distribution and release of a putative new gut hormone, peptide YY.
402 *Gastroenterology* 89(5):1070-7.

403 BAPEN Malnutrition Advisory Group (2009) Combating Malnutrition: Recommendations
404 For Action.

405 Batterham RL, Cohen MA, Ellis SM, le Roux CW, Withers DJ, Frost GS, Ghatei MA, & Bloom
406 SR (2003) Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J*
407 *Med* 349:941-948

408 Bauer JM, Haack A, Winning K, Wirth R, Fischer B, Uter W, Erdmann J, Schusdziarra V, &
409 Sieber CC (2010) Impaired postprandial response of active ghrelin and prolonged
410 suppression of hunger sensation in the elderly. *J Gerontol A Biol Sci Med Sci*
411 65:307-311

412 Brennan IM, Feltrin KL, Nair NS, Hausken T, Little TJ, Gentilcore D, Wishart JM, Jones KL,
413 Horowitz M, & Feinle-Bisset C (2009) Effects of the phases of the menstrual cycle
414 on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in
415 healthy lean women. *Am J Physiol Gastrointest Liver Physiol* 297:G602–G610

416 Degen L, Oesch S, Casanova M, Graf S, Ketterer S, Drewe J, & Beglinger C (2005) Effect of
417 Peptide YY3–36 on Food Intake in Humans. *Gastroenterology* 129:1430–1436

418 Di Francesco V, Fantin F, Residori L, Bissoli L, Micciolo R, Zivelonghi A, Zoico E, Omizzolo F,
419 Bosello O, & Zamboni M (2008) Effect of age on the dynamics of acylated ghrelin in
420 fasting conditions and in response to a meal. *J Am Geriatr Soc* 56:1369-1370

421 Di Francesco V, Zamboni M, Dioli A, Zoico E, Mazzali G, Omizzolo F, Bissoli L, Solerte SB,
422 Benini L, & Bosello O (2005) Delayed postprandial gastric emptying and impaired
423 gallbladder contraction together with elevated cholecystokinin and peptide YY
424 serum levels sustain satiety and inhibit hunger in healthy elderly persons. *J*
425 *Gerontol A Biol Sci Med Sci* 60:1581-1585

- 426 Frazee E, Chiou YA, Chen YD, & Reaven GM (1987) Age-related changes in postprandial
427 plasma glucose, insulin, and free fatty acid concentrations in nondiabetic
428 individuals. *J Am Geriatr Soc* 35:224-228
- 429 Hameed S, Dhillon WS, & Bloom SR (2009) Gut hormones and appetite control. *Oral Dis*
430 15:18-26
- 431 Heading RC, Nimmo J, Prescott LF, & Tothill P (1973) The dependence of paracetamol
432 absorption on the rate of gastric emptying. *Br J Pharmacol* 47:415-421
- 433 Kondrup J, Allison SP, Elia M, Vellas B, & Plauth M (2003) ESPEN guidelines for nutrition
434 screening 2002. *Clin Nutr* 22:415-421
- 435 Kreymann B, Williams G, Ghatei MA, & Bloom SR (1987) Glucagon-like peptide-1 7-36: a
436 physiological incretin in man. *Lancet* 2:1300-1304
- 437 le Roux CW, Ghatei MA, Gibbs JS, & Bloom SR (2005) The putative satiety hormone PYY is
438 raised in cardiac cachexia associated with primary pulmonary hypertension. *Heart*
439 91:241-242
- 440 Lee MR & Berthelot ER (2010) Community covariates of malnutrition based mortality
441 among older adults. *Ann Epidemiol* 20:371-379
- 442 MacIntosh CG, Andrews JM, Jones KL, Wishart JM, Morris HA, Jansen JB, Morley JE,
443 Horowitz M, & Chapman IM (1999) Effects of age on concentrations of plasma
444 cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to
445 appetite and pyloric motility. *Am J Clin Nutr* 69:999-1006
- 446 Malafarina V, Uriz-Otanoa F, Gil-Guerrero L, & Iniesta R (2013) The anorexia of ageing:
447 Physiopathology, prevalence, associated comorbidity and mortality. A systematic
448 review. *Maturitas* 74:293-302
- 449 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, & Turner RC (1985)
450 Homeostasis model assessment: insulin resistance and beta-cell function from
451 fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419
- 452 Milne AC, Potter J, Vivanti A, & Avenell A (2009) Protein and energy supplementation in
453 elderly people at risk from malnutrition. *Cochrane Database Syst Rev* CD003288-
- 454 Moriguti JC, Das SK, Saltzman E, Corrales A, McCrory MA, Greenberg AS, & Roberts SB
455 (2000) Effects of a 6-week hypocaloric diet on changes in body composition,
456 hunger, and subsequent weight regain in healthy young and older adults. *J*
457 *Gerontol A Biol Sci Med Sci* 55:B580-B587
- 458 Morley JE & Silver AJ (1988) Anorexia in the elderly. *Neurobiol Aging* 9:9-16
- 459 Moss C, Dhillon WS, Frost G, & Hickson M (2012) Gastrointestinal hormones: the regulation
460 of appetite and the anorexia of ageing. *J Hum Nutr Diet* 25:3-15
- 461 Nass R, Farhy LS, Liu J, Pezzoli SS, Johnson ML, Gaylinn BD, & Thorner MO (2013) Age-
462 dependent decline in acyl-ghrelin concentrations and reduced association of acyl-
463 ghrelin and growth hormone in healthy older adults. *J Clin Endocrinol Metab*

- 464 Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA,
465 Coombes RC, & Bloom SR (2004) Ghrelin increases energy intake in cancer patients
466 with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin*
467 *Endocrinol Metab* 89:2832-2836
- 468 Nematy M, O'Flynn J, Wandrag L, Brynes A, Brett S, Patterson M, Ghatei MA, Bloom SR, &
469 Frost GS (2006a) Changes in appetite related gut hormones in Intensive Care Unit
470 patients: a pilot cohort study. *Critical Care* 10:R10-
- 471 Nematy M, Powell CA, Brynes AE, Pearse M, Patterson M, Ghatei MA, Bloom SR, & Frost
472 GS (2006b) Peptide YY (PYY) is increased in elderly patients with femoral neck
473 fractures: a prospective cohort study. *JPEN J Parenter Enteral Nutr* 30:530-531
- 474 Norman K, Pichard C, Lochs H, & Pirlich M (2008) Prognostic impact of disease-related
475 malnutrition. *Clin Nutr* 27:5-15
- 476 Oner-Iyidogan Y, Gurdol F, Kocak H, Oner P, Cetinalp-Demircan P, Caliskan Y, Kocak T, &
477 Turkmen A (2011) Appetite-regulating hormones in chronic kidney disease
478 patients. *J Ren Nutr* 21:316-321
- 479 Paolisso G, Tagliamonte MR, Rizzo MR, & Giugliano D (1999) Advancing age and insulin
480 resistance: new facts about an ancient history. *Eur J Clin Invest* 29:758-769
- 481 Patterson M, Murphy KG, le Roux CW, Ghatei MA, & Bloom SR (2005) Characterization of
482 ghrelin-like immunoreactivity in human plasma. *J Clin Endocrinol Metab* 90:2205-
483 2211
- 484 Prudom C, Liu J, Patrie J, Gaylinn BD, Foster-Schubert KE, Cummings DE, Thorner MO, &
485 Geysen HM (2010) Comparison of competitive radioimmunoassays and two-site
486 sandwich assays for the measurement and interpretation of plasma ghrelin levels. *J*
487 *Clin Endocrinol Metab* 95:2351-2358
- 488 Schneider SM, Al-Jaouni R, Caruba C, Giudicelli J, Arab K, Suavet F, Ferrari P, Mothe-Satney
489 I, Van OE, & Hebuterne X (2008) Effects of age, malnutrition and refeeding on the
490 expression and secretion of ghrelin. *Clin Nutr* 27:724-731
- 491 Serra-Prat M, Palomera E, Clave P, Puig-Domingo M. (2008) Effect of age and frailty on
492 ghrelin and cholecystokinin responses to a meal test. *Am J Clin Nutr.* 89(5):1410-7
- 493 Sloth B, Holst JJ, Flint A, Gregersen NT, & Astrup A (2007) Effects of PYY1-36 and PYY3-36
494 on appetite, energy intake, energy expenditure, glucose and fat metabolism in
495 obese and lean subjects. *Am J Physiol Endocrinol Metab* 292:E1062-E1068
- 496 Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M, Stratton R, Delargy H, King
497 N, & Blundell JE (2000) The use of visual analogue scales to assess motivation to
498 eat in human subjects: a review of their reliability and validity with an evaluation of
499 new hand-held computerized systems for temporal tracking of appetite ratings. *Br*
500 *J Nutr* 84:405-415
- 501 Stunkard AJ & Messick S (1985) The three-factor eating questionnaire to measure dietary
502 restraint, disinhibition and hunger. *J Psychosom Res* 29:71-83

- 503 Sturm K, MacIntosh CG, Parker BA, Wishart J, Horowitz M, Chapman IM. (2003) Appetite,
504 food intake, and plasma concentrations of cholecystokinin, ghrelin, and other
505 gastrointestinal hormones in undernourished older women and well-nourished
506 young and older women. *J Clin Endocrinol Metab* 88(8):3747-55.
- 507 Visvanathan R (2015) Anorexia of aging. *Clin Geriatr Med*. 31(3):417-27.
- 508 Wade K & Flett M (2012) Which 'nutritional models-of-care' improve energy and protein
509 intake, clinical outcomes and malnutrition in hospitalised patients? *Nutrition and*
510 *Dietetics* 70:7-15
- 511 Wolever TM & Jenkins DJ (1986) The use of the glycemic index in predicting the blood
512 glucose response to mixed meals. *Am J Clin Nutr* 43:167-172
513
- 514

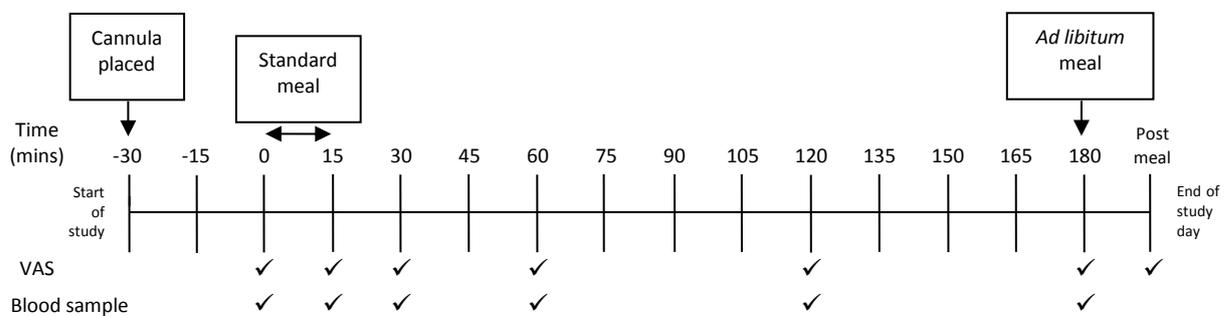
515 **Supplementary data**

516 **Title: Increased Peptide YY blood concentrations, not decreased acyl-**
517 **ghrelin, are associated with reduced hunger and food intake in healthy**
518 **older women: preliminary evidence**

519 Included here is additional information to supplement the methods and results sections of
520 the main manuscript, including an analysis of the whole sample (men and women), the
521 results for glucose, insulin, insulin resistance and gastric emptying times between the four
522 age groups and details of the female only analysis.
523

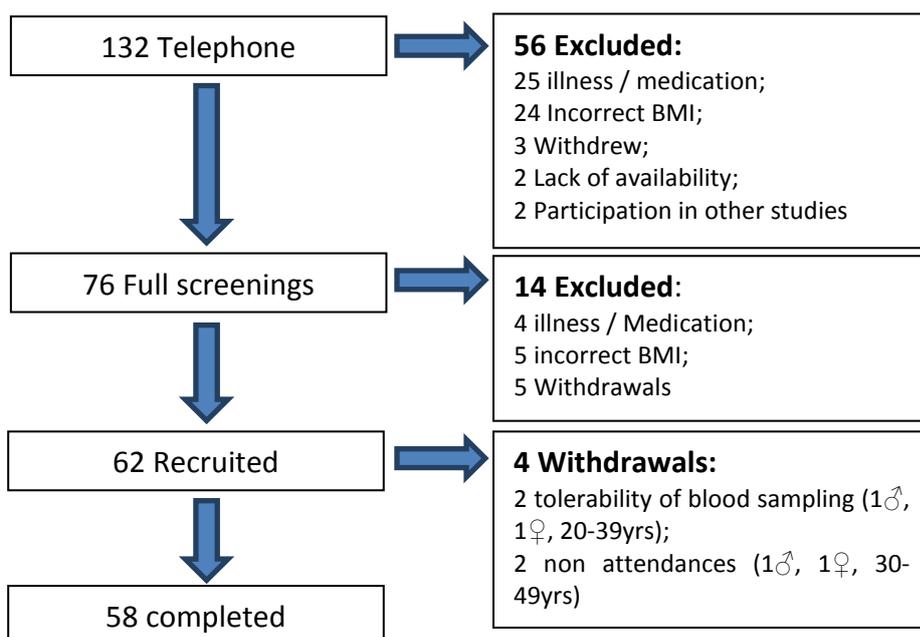
524 **Methods**

525 **Figure 4S: Overview of the test meal process, including all blood sampling points**



526

527 **Figure 5S: Flow chart of screening process.**



528

529

530 **Results**

531 The characteristics of all subjects (both men and women) are shown in Table 4S.

532 Table 5S shows the AUC and IAUC for each hormone, and

533 Figure 6S shows the differences between age groups.

534 **Table 4S: Participants' Characteristics (men and women)**

	Total N=58	20-39 years N=18	40-59 years N=18	60-79 years N=16	80+ years N=6
Sex (male:female)	27:31 (47%:53%)	9:9	9:9	9:7	0:6
Mean (SD)					
BMI (kg/m²)	23.5 (1.9)	22.6 (1.6)	23.7 (1.7)	24.1 (2.0)	23.5 (2.7)
Lean Mass (%)[*]	20.1 (2.4) ^b	21.4 (2.8) ^a	20.1 (1.7)	19.7 (2.0) ^a	17.7 (1.1)
Median (Inter-quartile range)					
Weight (kg)	70.3 (61.8-77.6)	66.6 (59.4-72.7)	73.1 (64.2-81.3)	75.3 (61.4-80.2)	61.8 (57.2-65.9)
Body Fat (%)[§]	25.3 (17.1-31.3) ^a	20.2 (9.15-28.27)	23.3 (18.4-30.4)	25.3 (20.2-30.8) ^a	33.9 (31.4-37.0)
Age (years)	54 (29.8-65.5)	25 (22.8-29.3)	53 (43.8-56.0)	66 (61.5-71.5)	84 (80.8-89.8)

535 ^a=1 missing value; ^b=2 missing values; BMI=Body Mass Index.

536 * There was significant effect of age on lean mass percentage $F(3,52)=4.84$, $p=0.005$. Post
537 hoc analysis using Hochberg test showed that the oldest group lower % lean mass
538 compared to the youngest group at the 0.05 level.

539 [§]Body fat percentage was significantly different between age groups, $H(3)=12.7$, $p=0.005$.
540 Jonckheere's test revealed a significant trend as expected; with increasing age the median
541 fat % increased $J=816.5$, $z=3.34$, $p=0.001$.

542

543
544

Table 5S: Mean area under the curve (AUC) and incremental AUC (IAUC) values for the plasma analysis (whole sample – men and women)

<i>Mean (sd)</i>	<i>Total N=58</i>	<i>20-39 years N=18</i>	<i>40-59 years N=18</i>	<i>60-79 years N=16</i>	<i>80+ years N=6</i>	<i>P value</i>
Total Ghrelin	82007.0	68315.5	93323.3	75895.4	105430.5	0.12
AUC	(44799)	(32696)	(46464)	(50931)	(46995)	
IAUC	-39409.0	-29995.4	-48104.9	-35836.2	-51089.8	0.35
	(46134)	(25917)	(44918)	(64145)	(44490)	
Acyl Ghrelin	21011.6	19875.4	23294.5	21194.6	17083.0	0.92
AUC	(15336)	(13150)	(19472)	(14485)	(11526)	
IAUC	-25835.7	-29727.1	-29681.5	-19646.7	-19127.7	0.83
	(33667)	(31040)	(45281)	(26751)	(15596)	
PYY AUC	5547.3	5228.9	5612.0	5302.7	6960.7	0.40
	(2532)	(2093)	(1783)	(3333)	(3334)	
IAUC	1940.3	1667.2	1967.4	1562.4	3686.8	0.02[§]
	(1969)	(1689)	(1127)	(2775)	(1637)	
*GLP-1 AUC	53936.0	54163.7	54682.2	55141.4	47924.0	0.85
	(22849)	(23336)	(25970)	(19432)	(25586)	
IAUC	16109.5	15389.9	13876.9	18571.5	18029.0	0.74
	(16334)	(12567)	(23558)	(9008)	(19723)	

545 *1 value missing (40-59yrs group). IAUC measured in pmol/l/min.

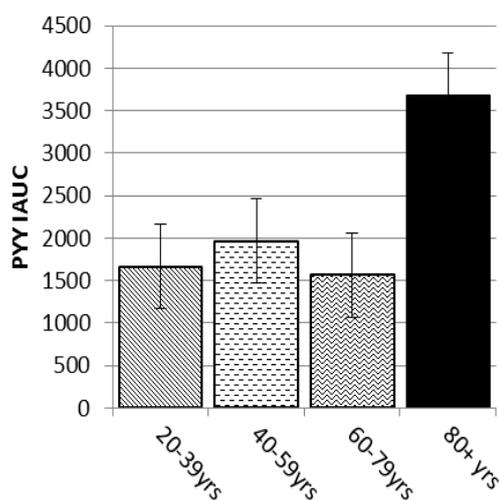
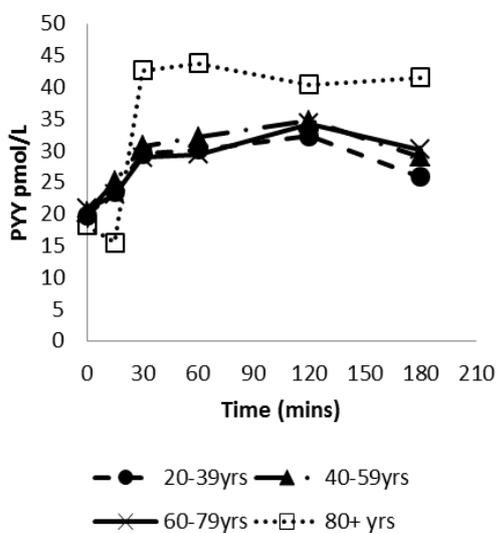
546 [§]PYY IAUC was significantly different between age groups, H(3)=9.69, p=0.012 (tested
547 using Kruskal-Wallis). Step-down follow-up analysis showed that the oldest age group had
548 significantly higher IAUC compared to the youngest; however, there were no differences
549 between the three youngest age groups, p=0.2.

550 Data were not distributed consistently, some were skewed, some parametric. All are
551 presented as mean and standard deviation and the appropriate comparative tests used.

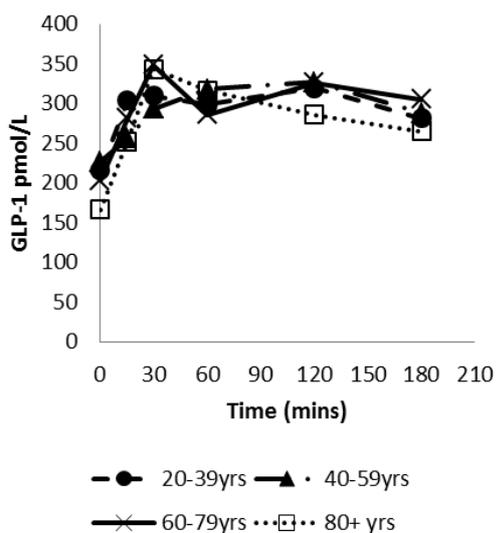
552

553 **Figure 6S: Difference in PYY, GLP-1 and Acyl-ghrelin blood concentrations between oldest and younger age groups.**

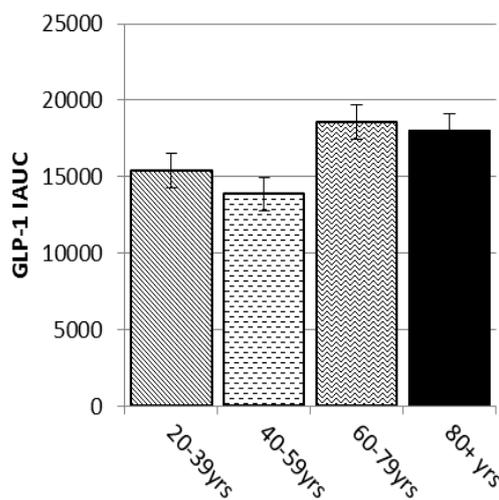
554



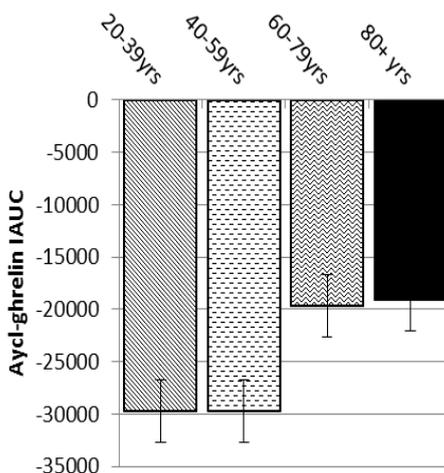
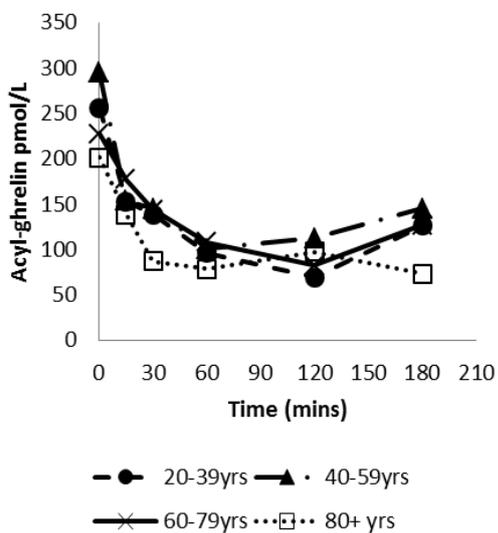
555



556



557



558

559

563 **Insulin, glucose and gastric emptying measurements**

564

565 ***Plasma insulin***

566 Table 6S shows the data for insulin levels, each age group, both men and women (3Sa), and
 567 females only (3Sb).

568 **Table 6Sa: Plasma insulin AUC and IAUC, in the 20-39, 40-59, 60-79 and 80+ age groups, for all patients.**

Age group*	Plasma Insulin IAUC Mean (SD)	Plasma Insulin AUC Mean (SD)
20-39 (n=11)	4312.5 (1100.9)	6042.3 (1200.5)
40-59 (n=11)	4462.7 (1391.6)	5912.2 (1675.2)
60-79 (n=12)	4943.4 (2559.8)	6775.1 (2819.5)
80+ (n=4)	5496.8 (1691.6)	8386.2 (1885.6)

569

570 **Table 3Sb: Plasma insulin AUC and IAUC, in the 20-39, 40-59, 60-79 and 80+ age groups, for females only.**

Age group*	Plasma Insulin IAUC Mean (SD)	Plasma Insulin AUC Mean (SD)
20-39 (n=4)	4194.7 (1322.1)	6083.8 (1264.7)
40-59 (n=5)	4530.5 (955.9)	6023.5 (1123.0)
60-79 (n=4)	4770.9 (1012.5)	7074.9 (2376.6)
80+ (n=4)	5496.8 (1691.6)	8386.2 (1885.6)

*missing values due to haemolysed blood samples. Values (n=19) were imputed when there were no more than 2/6 values missing, the values were not from 0 or 180 time points, and the missing values were not consecutive. Values were imputed by calculating the rate of change between the two available values, multiplying by the time to the missing value, and adding on to the previous available value, using the following formula:

$Missing\ value = (difference\ between\ values\ immediately\ before\ and\ after) / (minutes\ between\ the\ two\ values) * (minutes\ of\ missing\ value\ from\ the\ last) + value\ before\ missing\ one$

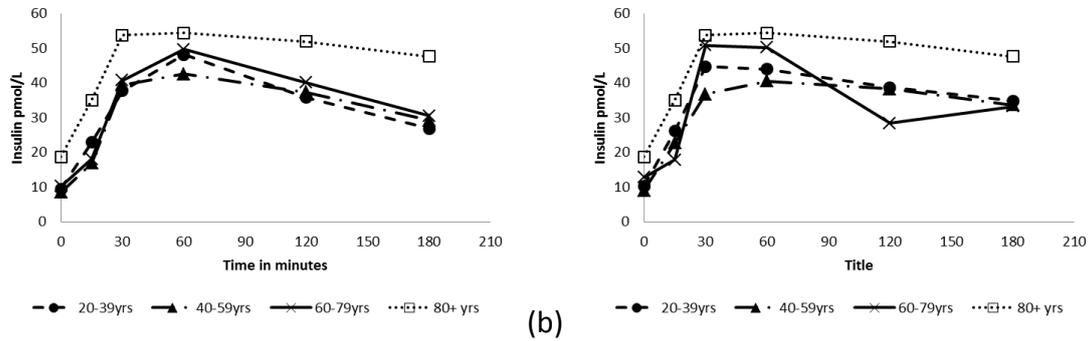
571

572 Plasma insulin concentrations at each time point can be seen in Figure 7S, and AUC and
 573 IAUC in Figure 8S ((a) all patients; (b) females only). AUC and IAUC scores for insulin
 574 increased with age but these differences did not achieve significance. AUC is reported as the
 575 baseline for the older age group is higher and consequently the IAUC may mask differences.
 576 Lack of significance may be due to low numbers because of missing values.

577

578

579 **Figure 7S: Plasma insulin concentrations over the 3 hour test meal in the 20-39, 40-59, 60-79 and 80+ age groups: (a) All**
 580 **patients; (b) Females only.**

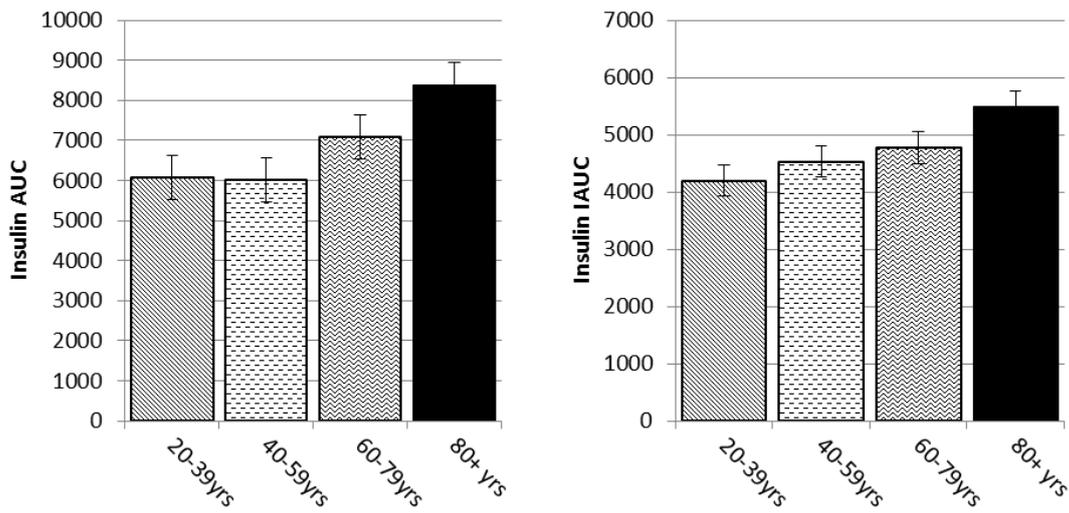


581 (a)

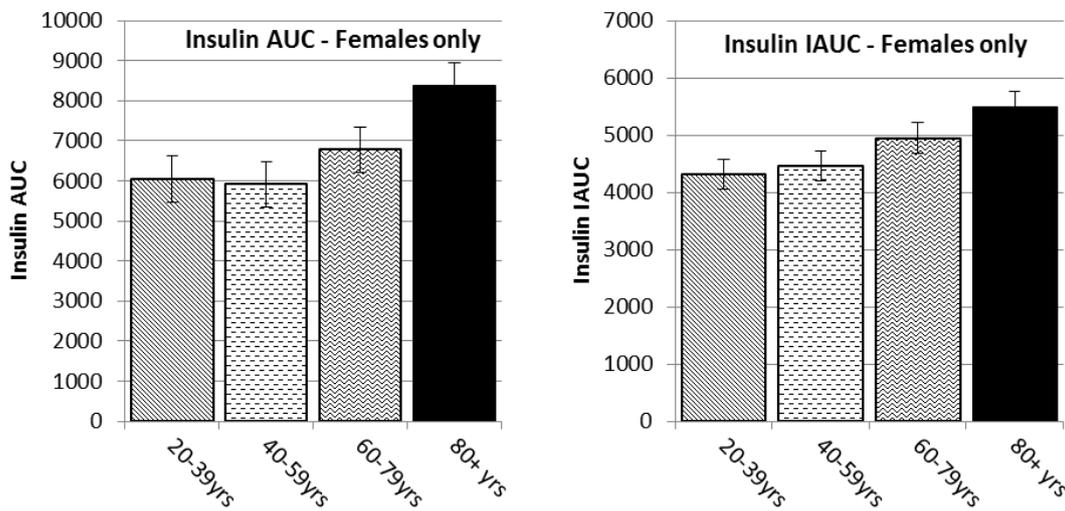
(b)

582

583 **Figure 8S: Plasma insulin AUC and IAUC in the 20-39, 40-59, 60-79 and 80+ age groups: (a) All patients; (b) Females only.**



584 (a)



585 (b)

586

587

588 **Plasma glucose**

589 Data on plasma glucose is shown in Table 7S (a&b), Figure 9S and Figure 10S. For the whole
 590 group glucose AUC was significantly different between age groups, $H(3)=9.75$, $p=0.021$
 591 (tested using Kruskal-Wallis). Pairwise comparisons with adjusted p values showed that the
 592 oldest group had significantly higher AUC compared to the youngest ($p=0.03$), and there
 593 were no other significant differences. IAUC scores were also the highest in the 80+ age
 594 group but this did not reach significance. For females only there were no significant
 595 differences between age categories for either IAUC or AUC glucose, possibly due to the
 596 reduced number of subjects.
 597

598 **Table 7Sa: Plasma glucose AUC and IAUC, in the 20-39, 40-59, 60-79 and 80+ age groups, for all patients.**

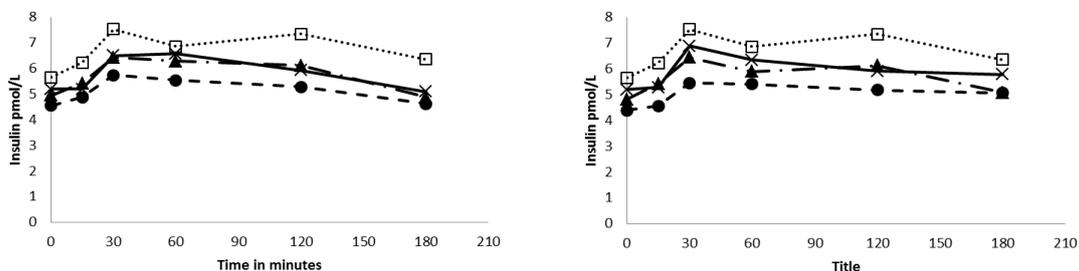
Age group	<i>Plasma Glucose IAUC</i> Mean (SD)	<i>Plasma Glucose AUC</i> Mean (SD)
20-39 (n=18)	121.1 (137.2)	939.6 (143.4)
40-59 (n=18)	166.8 (215.7)	1057.3 (214.6)
60-79 (n=16)	70.8 (237.9)	1077.7 (226.2)
80+ (n=6)	227.1 (113.8)	1244.1 (363.2)

599
 600 **Table 4Sb: Plasma glucose AUC and IAUC, in the 20-39, 40-59, 60-79 and 80+ age groups, for females only.**

Age group	<i>Plasma Glucose IAUC</i> Mean (SD)	<i>Plasma Glucose AUC</i> Mean (SD)
20-39 (n=9)	121.1 (137.2)	928.8 (150.4)
40-59 (n=9)	166.8 (215.7)	1046.5 (174.6)
60-79 (n=7)	70.8 (237.9)	1113.0 (311.1)
80+ (n=6)	227.1 (113.8)	1244.1 (363.2)

601

602 **Figure 9S: Plasma glucose concentrations over the 3 hour test meal in the 20-39, 40-59, 60-79 and 80+ age groups: (a) All**
 603 **patients; (b) Females only.**



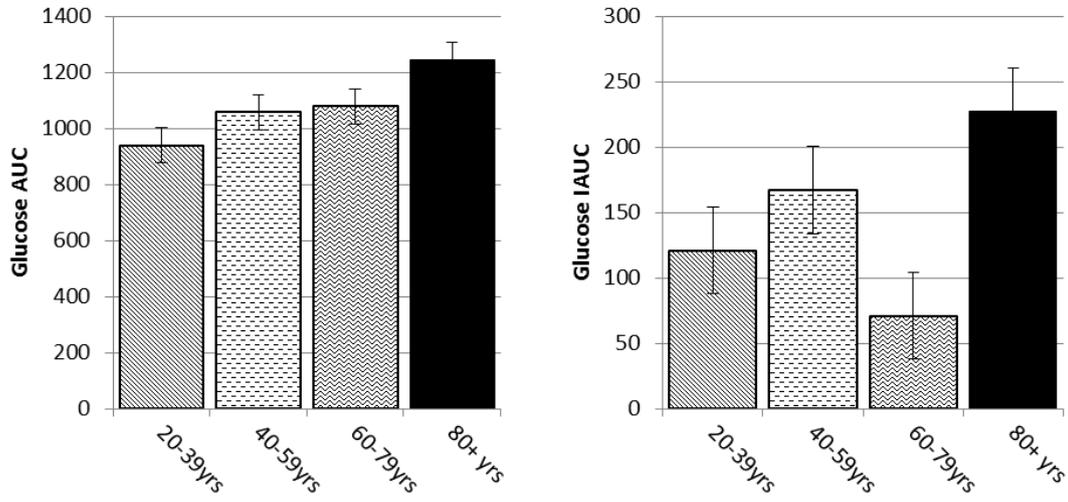
604 (a) ● 20-39yrs ▲ 40-59yrs × 60-79yrs ◻ 80+ yrs

(b) ● 20-39yrs ▲ 40-59yrs × 60-79yrs ◻ 80+ yrs

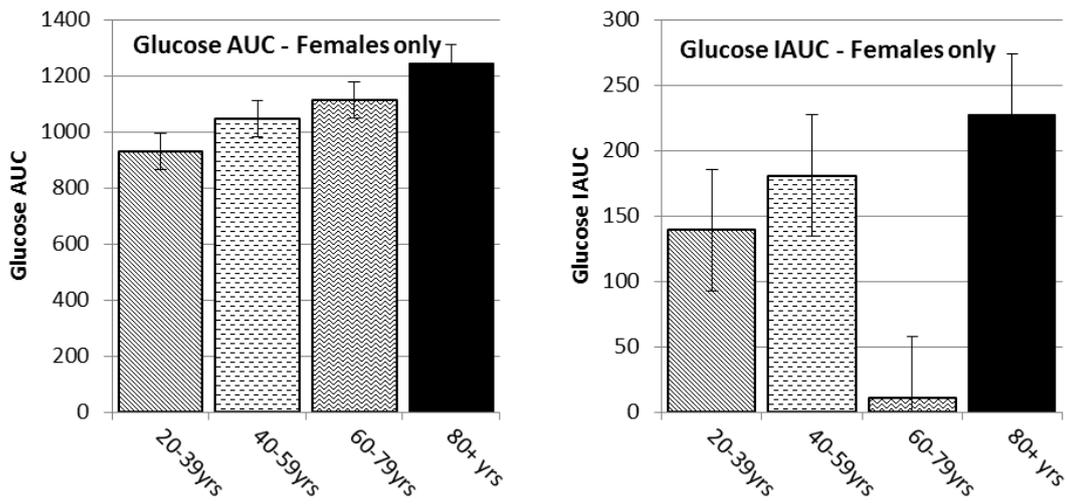
605

606

607 Figure 10S: Plasma glucose AUC and IAUC in the 20-39, 40-59, 60-79 and 80+ age groups: (a) All patients; (b) Females
608 only.



609 (a)



610 (b)

611

612

613 **Insulin resistance**

614 An index of fasting insulin resistance (HOMA) was measured using the following equation;

615
$$\text{HOMA} = (\text{glucose mmol/L} \times \text{Insulin mU/L}) / 22.5$$

616 [where mU/L = pmol/7.5 (Raben et al.,2001)]

617

618 Data is shown in Table 8S (a&b) and Figure 11S. The Kruskal-Wallis test showed that HOMA

619 was significantly affected by age ($H(3)=8.79$, $p=0.032$). Pairwise comparisons with adjusted p

620 values showed that HOMA was significantly higher in the oldest compared to the youngest

621 ($p=0.037$) and the 40-59yrs group ($p=0.033$). All other comparisons did not indicate a

622 difference. For females only the pattern of data reminded the same by differences were

623 not significant due to lower sample numbers.

624

625 **Table 8Sa: HOMA in the 20-39, 40-59, 60-79 and 80+ age groups, for all patients.**

Age group	HOMA Median (IQ Range)
20-39 (n=17)	0.20 (0.16 – 0.34)
40-59 (n=16)	0.23 (0.15 – 0.36)
60-79 (n=11)	0.20 (0.16 – 0.38)
80+ (n=6)	0.59 (0.46 – 0.80)

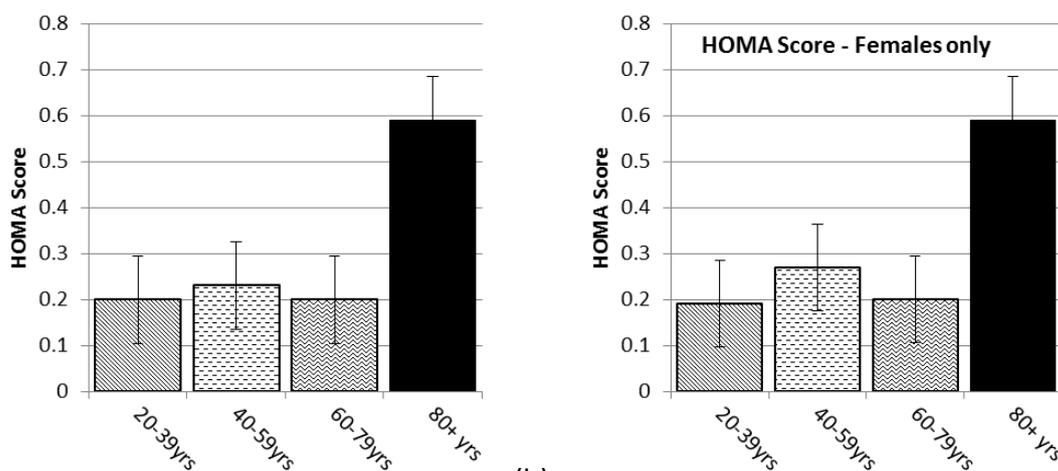
626

627 **Table 5Sb: HOMA in the 20-39, 40-59, 60-79 and 80+ age groups, for females only.**

Age group	HOMA Median (IQ Range)
20-39 (n=8)	0.19 (0.13-0.41)
40-59 (n=8)	0.27 (0.11-0.36)
60-79 (n=3)	0.20 (0.13-0.66)
80+ (n=6)	0.59 (0.46-0.80)

628

629 **Figure 11S: HOMA scores in the 20-39, 40-59, 60-79 and 80+ age groups: (a) All patients; (b) Females only.**



630 (a)

(b)

631

632

633 **Paracetamol method- gastric emptying**

634 The time to peak concentration for paracetamol was used as a measurement of gastric
 635 emptying. Data is shown in Table 9S (a&b). Peak paracetamol concentrations did not change
 636 with age, no significance was detected between the four age groups for peak paracetamol
 637 concentrations in either all patients or females only.

638 **Table 9Sa: Peak paracetamol in the 20-39, 40-59, 60-79 and 80+ age groups, for all patients.**

Age group	Time to peak paracetamol concentration Median (IQ Range)
20-39 (n=18)	150 (26.3 – 180.0)
40-59 (n=18)	180 (120.0 – 180.0)
60-79 (n=16)	180 (60.0 – 180.0)
80+ (n=6)	180 (97.5 – 180.0)

639

640 **Table 6Sb: Peak paracetamol in the 20-39, 40-59, 60-79 and 80+ age groups, for females only.**

Age group	Time to peak paracetamol concentration Median (IQ Range)
20-39 (n=9)	180 (75.0-180.0)
40-59 (n=9)	180 (150.0-180.0)
60-79 (n=7)	180 (120.0-180.0)
80+ (n=6)	180 (97.5-180.0)

641

642

643 **Energy intake from ad libitum meal**

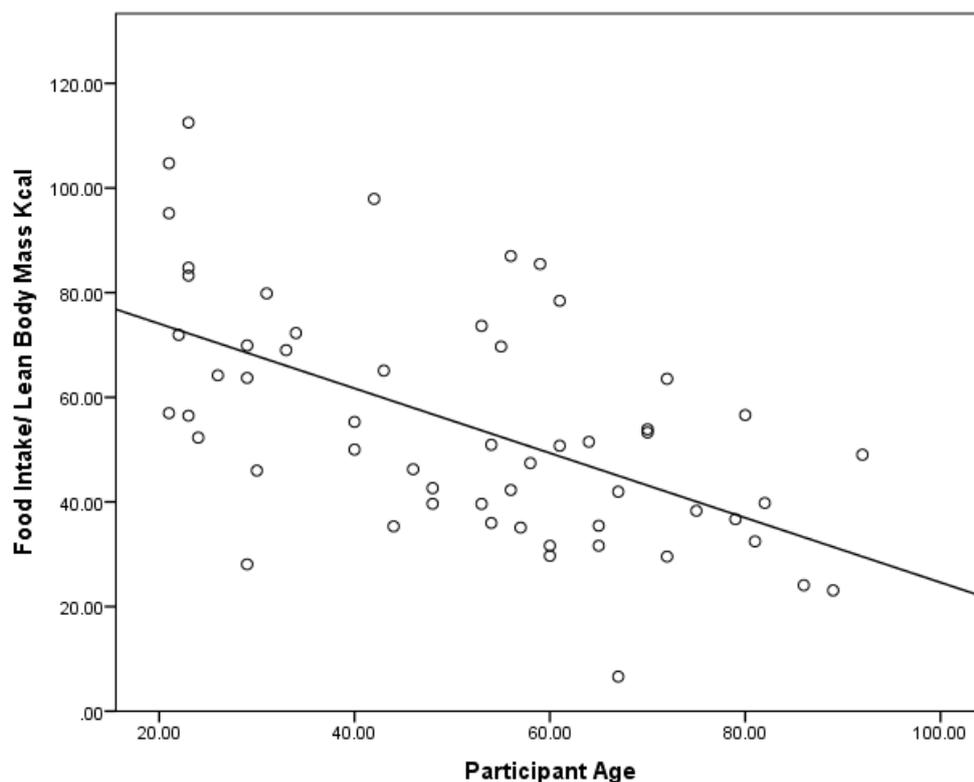
644 Table 10S shows the energy intake at the *ad libitum* meal by age group for all subjects (men
645 and women), which was significantly different ($p < 0.001$). Post hoc analysis (Hochberg)
646 showed differences at the 0.05 level between the youngest and two oldest age groups (20-
647 39yrs vs 60-79 yrs & 80+ yrs). Figure 12S shows the association with age when energy
648 intake is controlled for lean mass; showing a negative correlation between energy intake
649 (kcal)/lean body mass (kg) ($p < 0.001$, $r = -0.57$) and age.

650 **Table 10S: Energy intake from ad libitum meal (men and women)**

Age group	Energy intake (kcal) at <i>ad libitum</i> meal (sd)
20-39 yrs	1018.9 (352.3)
40-59 yrs	801.1 (275.3)
60-79 yrs	638.7 (302.2)
80+ yrs	415.62 (177.4)

651

652 **Figure 12S: Association between age and energy intake (kcal)/kg lean mass from the *ad libitum* meal (men and women).**



653
654