

1 **Title- Postprandial Regulation of Prouroguanylin in humans of a Healthy Weight and those who**
2 **are Overweight or with Obesity**

3

4 **Authors- Michael Patterson¹, Hannah Ward¹, Delaram Halvai^{1*}, Heidi Anett Holm Nilsen^{1*}and**
5 **Sue Reeves¹**

6 ¹ Department of Life Sciences, University of Roehampton, London, UK

7 * These authors contributed equally to the study

8

9 Corresponding Author

10 Dr Michael Patterson, Department of Life Sciences, Faculty of Health Sciences Research
11 Centre, University of Roehampton, London SW15 4JD, UK Tel: +44 (0)20 8392 3189;
12 Email: michael.patterson@roehampton.ac.uk.

13 **Running Title - Postprandial Regulation of Prouroguanylin**

14 **Keywords Words**

15 **Obesity, Food Control, Gastrointestinal Tract**

16

17 **Highlights**

- 18 • Mixed macronutrient meals high in fat or carbohydrates cause a delayed increase in
19 prouroguanylin concentrations
- 20 • Fasting concentrations of prouroguanylin are suppressed in those who are overweight/ with
21 obesity
- 22 • People who are overweight /with obesity remain sensitive to the effects of meals on
23 prouroguanylin

24

25

26 **Abstract**

27 Uroguanylin is a peptide gut hormone proposed to have a role in signalling post meal satiety.
28 Uroguanylin circulates as its pro-hormone, prouroguanylin. There has been limited investigation of the
29 regulation of prouroguanylin by food; therefore we investigated prouroguanylin regulation following
30 meals. In separate experiments we investigated the effects of high calorie (1451 kcal) and medium
31 calorie (725 kcal), high fat meals, on plasma prouroguanylin concentrations. We then examined the
32 effect of a 722.5 kcal high carbohydrate breakfast on prouroguanylin concentrations, comparing the
33 response in healthy weight adults versus those who are overweight/ with obesity. The 1451 kcal meal
34 increased prouroguanylin concentrations, versus fasting at 60 (P<0.05), 90 (P<0.01) and 120 (P<0.001)
35 minutes. After the 725 kcal meal hormone concentrations rose more slowly and were significant versus
36 fasting concentrations at 120 minutes (P<0.01). The high carbohydrate breakfast 722.5 kcal, led to an
37 initial suppression of hormone concentrations at 30 mins post meal (P<0.05) followed by an increase
38 in concentrations until they were significant versus fasting at 120 mins (P<0.01). Participants
39 overweight/ with obesity had lower fasting prouroguanylin concentrations (P<0.05), but post meal
40 concentrations did not differ between the groups. Our results suggest there is a delayed increase in
41 prouroguanylin concentrations following, large and regular sized mixed macronutrient meals rich in fat
42 or carbohydrate. Fasting levels are suppressed in people who are overweight/ with obesity, but the post
43 meal response remains intact. There may be potential to target post meal release of prouroguanylin in
44 obesity.

45

46

47

48

49

50

51 **1. Introduction**

52 Hormones secreted from the gastrointestinal system (gut hormones) are essential regulators of appetite
53 and satiety (1). Pharmacological (2) and dietary (3)(4) innovations are targeting the release of these
54 hormones for the treatment of obesity.

55 Uroguanylin is a peptide ligand of the Guanylyl cyclase C receptor (GUCY2C). It is highly expressed
56 in the proximal intestine, and in particular the enterochromaffin cells (5). However, in humans there is
57 some debate over the cell type expressing uroguanylin; one study found expression in the human
58 duodenum, but suggested the cells expressing uroguanylin were not enterochromaffin cells (6). In
59 addition to the small intestine uroguanylin is also expressed in the colon (6)(7). Within the gut
60 uroguanylin has paracrine actions, activating GUCY2C to regulate electrolyte and fluid balance (8), and
61 uroguanylin and GUCY2C have been targeted to treat gastrointestinal disorders including, constipation
62 (9) and irritable bowel syndrome (10). There is also evidence uroguanylin acts outside the gut to regulate
63 electrolyte and fluid balance (8). More recently uroguanylin has been proposed as a novel appetite and
64 body mass regulating gut hormone (11). Valentino et al.'s (11) studies suggested prouroguanylin
65 (precursor of uroguanylin) is secreted into circulation after a meal and is processed to active uroguanylin
66 in the hypothalamus where it signals satiety (11). In support of this hypothesis, in their study transgenic
67 mice lacking GUCY2C receptor are hyperphagic and developed obesity (11). Subsequent studies in
68 mice have supported some aspects of Valentino's work and challenged others (12)(13)(14)(15)(16). A
69 study using knockout models found loss of uroguanylin, but not GUCY2C, led to increased body mass
70 and adiposity, and central administration of GUCYC agonists had no affect on feeding (12). While
71 another study reported chronic central infusion of uroguanylin led to increased body mass and adiposity,
72 but this effect was not mediated by chronic increased feeding (13). However, they did observe an acute
73 effect of urogunaylin on feeding in the first few hours after injection. In contrast a very recent study
74 found neither administration or upregulating the expression of either uroguanylin or prouroguanylin
75 had any effect on feeding or glucose homeostasis (16). Yet two other studies have reported uroguanylin
76 levels are effected by diet, leptin and obesity(14)(15). In summary, most, but not all rodent studies do

77 support a role for uroguanylin in the regulation of body mass, but effects on feeding and glucose
78 homeostasis remain controversial.

79 There have been limited further studies of uroguanylin regulation in humans. Of note, a recent study
80 (17) suggested fasting prouroguanylin concentrations are lower in people with obesity and rise
81 following Roux-en-Y gastric bypass (RYGB), a pattern similar to is observed with glucagon like
82 peptide -1 (GLP-1) and peptide YY (PYY) (18). In support of this another study reported an
83 upregulation of uroguanylin mRNA following RYGB (16). A further recent study also reported female
84 adolescents with obesity had lower fasting concentrations of prouroguanylin than those who did not
85 have obesity, but post meal changes in prouroguanylin were similar in both groups (19). However, there
86 has been limited work to elucidate the factors influencing the release of prouroguanylin as a post meal
87 signal of satiety in adults. Valentino (11) and colleagues only investigated prouroguanylin release
88 following a single large (1460 kcal) mixed macronutrient meal in healthy weight adult male volunteers,
89 while Di Guglielmo et al. (19) studied prouroguanylin following a single meal in adolescents. To begin
90 to understand prouroguanylin release in response to a meal we aimed to determine the effects of smaller
91 meals more typical of the energy intake of real life single meals on circulating prouroguanylin
92 concentrations, and to see if fasting and post meal concentrations of prouroguanylin differed between
93 adults of a healthy weight and those who are overweight/ with obesity.

94

95

96

97

98

99

100

101 **2. Methods**

102 **2.1 Ethics and Recruitment**

103 All studies were performed according to the principles of the Declaration of Helsinki and were approved
104 by the local research ethics committee at the University of Roehampton. Participants were recruited via
105 posters displayed in the University of Roehampton. For all studies we recruited healthy volunteers over
106 the age of 18. Participants with Diabetes, gastrointestinal conditions/diseases or food allergies were
107 excluded. For studies 1 and 2 we recruited males with a BMI between 18 and 30 kg/m² to allow
108 comparisons to the previous study (11). For study 3 we recruited both sexes with a minimum BMI of
109 18 kg/m² and aimed to have a similar number of participants with BMIs under and over 25 kg/m².
110 Females who were pregnant, lactating or having given birth in the past year were excluded.

111 **2.2 Study 1- The effect of a large 1451 kcal meal on plasma Prouroguanylin concentrations in**
112 **males**

113 Seven healthy male participants fasted overnight (12hours) then consumed a 1451 kcal meal of similar
114 composition to sausage and egg breakfast meal given by Valentino et al., 2011 (11). Finger prick blood
115 samples were taken and plasma extracted for prouroguanylin measurement (as described below) fifteen
116 minutes before the breakfast (time 0 minutes) and at intervals up to 120 minutes after the meal (15
117 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes). Hunger levels were measured at all time
118 points using a visual analogue scale.

119

120 **2.3 Study 2- The effect of a 725 kcal meal on plasma Prouroguanylin concentrations in males**

121 Seven healthy male participants fasted overnight (12 hours) then consumed a 725 kcal meal with the
122 same components as study 2, but half the portions. Finger prick blood samples were taken at the same
123 timepoints and plasma extracted for prouroguanylin measurement.

124 **2.4 Study 3- The effect of a 722.5 kcal carbohydrate rich breakfast on plasma Prouroguanylin**
125 **concentrations in males and females of a healthy weight and those who are overweight/ with**
126 **obesity.**

127 Eighteen participants (10 male, 8 female) fasted overnight (12hours) then consumed a 722.5 kcal meal.
128 Nine of the participants (3 female and 5 male) had a BMI over 25 kg/m² and 9 under 25 kg/m². Finger
129 prick blood samples were taken and plasma extracted for prouroguanylin measurement as described for
130 study 1 except blood samples were only taken fifteen minutes before the breakfast (time 0 minutes) and
131 30 minutes, 60 minutes and 120 minutes post breakfast. These time points were chosen as the most
132 important based on the results from studies 1 and 2. A decision was made to limit the time points for
133 this study to minimise finger prick samples and therefore any discomfort to participants. This was both
134 from an ethical point of view and to enhance recruitment.

135 **2.5 Composition of meals**

136 Contents of the meals were determined from food packaging if stated and estimated using Dietplan 7
137 (Forestfield Software) when not stated. Full meal contents can be found in Table 1 and 2. For Study 1
138 our target was to design a high calorie, unhealthy meal similar to the one used by Valentino et al.(11).
139 However, some changes were made to accommodate for foods easily available, and regularly consumed
140 for breakfast in the UK. The mixed macronutrient meal contained 1451 kcal and was 132 g carbohydrate
141 (36.3% of the calories), 57 g protein (15.7% of the calories), 75 g fat (48% of the calories). The meal
142 used in study 2 was identical to the meal in study 1 except all portions were half the size (725 kcal,
143 36.3%-66 g carbohydrate, 15.7%-29 g protein, 48%-38 g fat).

144 The high carbohydrate breakfast used in study 3 was designed to be closer to what may have been
145 considered traditionally to be a healthy breakfast in the UK (20), while keeping the calorie content
146 similar to study 2. The main components were fruit (apple, banana, dried apricot), muesli and orange
147 juice. It contained 722.5 kcal and was 134.7 g (73.9% of the calories) carbohydrate, 24.5 g protein
148 (13.4% of the calories), 10.3 g (12.7% of the calories) fat. The main difference compared to study 2
149 was this meal had a much higher carbohydrate content and lower fat content. Given uroguanylin's

150 reported role in regulating fluid and salt balance (21)(22)(8), it is also important to note the high
151 carbohydrate breakfast had a much lower sodium content (0.4 g versus study 1, 4.3 g and study 2, 2.15
152 g).

153

154 **2.6 Sample collection and Prouroguanylin measurement**

155 Blood samples were collected using the finger prick method into Ethylenediaminetetraacetic acid
156 (EDTA) tubes, gently mixed and put on ice. The finger tip was cleaned using an alcohol swab then skin
157 punctured using a safety lance (Sarstedt, Germany), the finger was lightly pressed to start blood flow
158 (if required), then up to 0.5ml blood (per sample) was collected using a capillary EDTA tube. Before
159 processing the samples they were centrifuged at 3000g and plasma separated and stored at -20°C.
160 Samples were stored at -20°C for no longer than 8 weeks before measurement. Prouroguanylin
161 concentrations were measured using an ELISA kit from BioVendor (Human Prouroguanylin ELISA,
162 Cat. No. RD191069200R). The assay was performed according to the manufacturer's instructions. This
163 assay has previously been validated for measurement of prouroguanylin (23). Samples from each
164 participant were measured consecutively and on the same ELISA plate. The limit of detection was 86
165 pg/ml and intra and inter- assay co-efficient of variation were 2.3% and 6.4% respectively. For study 3
166 samples from participants with healthy weights and the participants who were overweight/with obesity
167 were assayed in random order to minimise any effect of variation within the assay.

168 **2.7 Data Analysis**

169 For studies 1 and 2 a Repeated Measures ANOVA was used with post hoc Dunnetts test to see if there
170 was a difference between pre meal concentrations (time 0 minutes) and time points. To compare post
171 meal changes from the meals given in study 1 and 2, percentage change from baseline (time 0 minutes)
172 was calculated for each time point and comparisons made using Repeated Measures Two way ANOVA.
173 For study 3 data from all participants were analysed by Repeated Measures ANOVA post hoc Dunnetts
174 test. When data were split into two groups, data were analysed by Repeated Measures Two way
175 ANOVA with post hoc Bonferroni test. To compare data from studies 2 and 3; males from study 3 with

176 a BMI < 26 kg/m² (the maximum BMI of any participant in study 2) were selected, and for each study
177 percentage change from baseline (time 0 minutes) was calculated for each post meal time point and
178 comparisons made using Repeated Measures Two way ANOVA with post hoc Bonferroni test. Fasting
179 and peak prouroguanylin concentrations from the 3 studies were analysed by independent t-test where
180 applicable. All analysis was performed using Graphpad, Prism 6 (GraphPad Software, San Diego, CA)
181 software. In all cases P<0.05 was considered significant.

182

183

184

185

186

187

188

189

190

191

192

193

194

195

196

197 **3. Results**

198 ***3.1 Study 1- The effect of a large 1451 kcal meal on plasma Prouroguanylin concentrations in males***

199 Seven males completed this study. The mean BMI was $23.4 \text{ kg/m}^2 \pm 3.8$ with a range between 19.8 and
200 24.5 kg/m^2 . The mean age was 23.5 years with a range between 21-35 years. Following the meal there
201 was a significant increase in mean plasma prouroguanylin concentrations at 60, 90 and 120 minutes
202 ($P < 0.05$, 0 minutes versus 60 minutes; $P < 0.01$, 0 minutes versus 90 minutes; $P < 0.001$, 0 minutes versus
203 120 minutes) (Figure 1 A). Prouroguanylin concentrations at 15 and 30 minutes post meal were not
204 significantly increased versus baseline. In fact concentrations at 30 minutes appeared slightly decreased,
205 but this change was not significant. Feelings of hunger subsided after the meal and participants were
206 least hungry at 15 minutes post meal before steadily feeling hungrier with time (Figure 1 B). When
207 comparing the pattern of prouroguanylin concentrations and hunger levels there was no indication that
208 prouroguanylin concentrations increased as hunger subsided and there was not a significant correlation
209 between hunger and prouroguanylin concentrations.

210 ***3.2 Study 2- The effect of a 725 kcal meal on plasma Prouroguanylin concentrations in males***

211 Seven males completed this study. The mean BMI was $22.6 \text{ kg/m}^2 \pm 2.0$ with a range between 20.3 and
212 25.1 kg/m^2 . The mean age was $28.9 \text{ years} \pm 11.2$ with a range between 21- 51 years. Following the meal
213 there was a significant increase in mean plasma Prouroguanylin concentrations at 120 minutes ($P < 0.01$,
214 0 versus 120 minutes) (Figure 2 A). There were no significant changes at any other time point. When
215 percentage change from baseline (0 minutes) was calculated for each time point and compared to the
216 response seen in study 1 (1451kcal meal) (Figure 2 B), there was a significant interaction between time
217 and meal ($P < 0.05$), but examining individual time points and peak level, the difference between the two
218 meals fell just short of being significant (Peaks, 1451 kcal $172.4\% \pm 49.7$ versus 725 kcal, $129.2\% \pm$
219 20.6 ; $P = 0.055$).

220 ***3.3 Study 3- The effect of a 722.5 kcal carbohydrate rich breakfast on plasma Prouroguanylin***
221 ***concentrations in males and females of a healthy weight and those who are overweight/ with obesity.***

222 10 males and 8 females were recruited for this study. The mean BMI was $25.1 \text{ kg/m}^2 \pm 4.3$ with a range
223 between 20.5 and 39.8 kg/m^2 . The mean age was $35.9 \text{ years} \pm 17.7$. When data were split into BMI <
224 25 kg/m^2 (healthy weight) versus BMI $>25 \text{ kg/m}^2$ (overweight and with obesity) the mean BMIs were
225 $22.4 \text{ kg/m}^2 \pm 1.2$ and $28.5 \text{ kg/m}^2 \pm 4.5$ respectively. The mean ages in each group were almost identical
226 (healthy weight 36.1 ± 19.3 years and overweight and with obesity 35.6 ± 15.4 years).

227 When all data from participants were analysed as a single group plasma prouroguanylin concentrations
228 were significantly decreased versus fasting (0 minutes) at 30 minutes post meal, ($P < 0.05$, 0 versus 30
229 minutes) and increased at 120 minutes after the meal ($P < 0.01$, 0 versus 120 minutes) (Figure 3 A).
230 There were no significant changes 0 versus 60 minutes. When data were split by BMI $< 25 \text{ kg/m}^2$
231 (healthy weight) versus BMI $>25 \text{ kg/m}^2$ (overweight and with obesity), fasting concentrations (time 0)
232 were significantly lower in the group who were overweight/with obesity ($P < 0.05$) (Figure 3 B), but
233 there were no differences at post meal time points. In fact when analysing the percentage change from
234 pre meal fasting concentrations (time 0) to peak concentrations the change was similar between groups.
235 When data were split by sex there was no significant difference in prouroguanylin concentrations,
236 however groups were not perfectly matched with males having a higher mean BMI (26.1 kg/m^2 versus
237 24.0 kg/m^2).

238 To compare to study 2 we then selected males only with a BMI under 26 kg/m^2 . The mean BMI was
239 $24.1 \text{ kg/m}^2 \pm 2.0$ with a range between 22.2 and 25.8 kg/m^2 ($n = 9$). There was no significant difference
240 found when comparing the magnitude and profile of post meal changes (Figure 3 C).

241

242

243

244

245

246 4. Discussion

247 We have demonstrated that prouroguanylin concentrations are significantly increased after regular size
248 meals (725kcal) as well as following very large meals (1451 kcal). However, we found the rise is
249 delayed and does not follow the same pattern as self-reported hunger levels; prouroguanylin
250 concentrations remain constant or are slightly decreased up to 30 minutes after a meal, then steadily
251 rise, remaining at, or close to peak concentrations 120 minutes after a meal. Fasting prouroguanylin
252 concentrations are lower in the group who were overweight/ with obesity versus those of a healthy
253 weight, but post meal changes in hormone concentrations are similar in each group. Finally, changing
254 the carbohydrate, fat or salt content of the meal did not appear to affect the post meal increase in
255 hormone concentrations.

256 Prouroguanylin, is the prohormone and circulating form of the gut hormone uroguanylin (24)(25)(11).
257 When we started this study a single previous study had reported prouroguanylin concentrations rise
258 after a large meal peaking at 45 minutes post meal in healthy adult males (11). In contrast we found
259 following a fairly similar meal concentrations did not significantly change from fasting concentrations
260 until 60 minutes after a meal, peaking at 90 minutes and remaining at similar concentrations by 120
261 minutes, our final time point. The reasons for the difference in findings are currently unclear. An
262 obvious difference between the two studies is the immunoassays used to measure prouroguanylin. We
263 used a commercially available two site ELISA produced by Biovendor. We chose this assay as it had
264 been previously assessed and validated for the measurement of prouroguanylin (23). Valentino et al.
265 (11) used a one site ELISA they developed that is not commercially available. There has been limited
266 characterisation of prouroguanylin and uroguanylin in circulation. There may be breakdown
267 products/inactive forms of prouroguanylin or indeed multiple active forms of the hormone as is the case
268 for several other gut hormones (26)(27)(28). Immunoassay's can often detect multiple forms of peptide
269 hormones, including breakdown products that have only lost one or two amino acids compare to the
270 full hormone (28)(26)(29)(30). Thus, while we know both ELISAs detect intact prouroguanylin, we do
271 not know if either assay is detecting other as yet unknown forms of prouroguanylin or breakdown

272 products. A better understanding of circulating prouroguanylin and uroguanylin and their metabolites
273 would aid further investigation in this area.

274 Recently a study in adolescents (aged 14-17 y) has examined prouroguanylin concentrations after meals
275 (800-1100 kcal) (19). Prouroguanylin concentrations were assayed using the same Biovendor ELISA
276 used in our studies. The post meal changes in prouroguanylin observed were similar to our study; they
277 reported a decrease immediately after a meal, with concentrations then rising up to their final time point,
278 90 minutes post meal. This suggests regulation of prouroguanylin in adolescents and adults is similar,
279 and that the different prouroguanylin assays used are the most likely cause of the discrepancy between
280 our study and Valentino et al.'s study (11).

281

282 Although the choice of ELISA is the most logical explanation of differences between results in our
283 study and Valentino et al.'s, there were small differences in the meals given. Meals in both studies were
284 high calorie, high fat and sugar, unhealthy breakfasts, but the macronutrient content did differ; our study
285 meal had higher fat (48% versus 35%) and lower carbohydrate (36% versus 54%). It may be that this
286 difference affected the post meal response. However, given the higher carbohydrate and lower fat
287 content of our meal in study 3 did not lead to an earlier peak in prouroguanylin concentrations we feel
288 it is unlikely this accounts for the difference.

289 A final possible but less likely explanation for the differences between the two studies is the
290 demographic of the participants. The mean BMI of the participants was almost identical between the
291 two studies (23.2 versus, 23.4 kg/m²). The participants in Valentino et al.'s study were older; 31.7 years
292 versus 23.5 in our study 1 (1451 kcal). However, in our study 2 the mean age was 28.9 years, yet the
293 pattern of prouroguanylin concentrations we observed was similar, suggesting this magnitude of
294 difference in age is unlikely to affect prouroguanylin concentrations.

295 A 1451 kcal meal is large and represents 58% of the recommended daily calories for men and 72.5 %
296 for women. Before targeting the uroguanylin system for weight loss through diet or pharmacology it is
297 important to know whether smaller meals, more typical of real life, cause release of this hormone. We

298 went on to show for the first time in adults that smaller 722.5-725 kcal meals lead to a significant
299 increase in Prouroguaylin concentrations 120 minutes post meal. In study 2 (725 kcal) the meal was
300 identical to study 1 (1451 kcal) except all portions were halved in size and both studies just included
301 males. A similar pattern of post meal release was observed except changes did not become significant
302 until the 120 minute time point. When comparing the percentage change from baseline concentrations
303 in each study there was a significant interaction between time and meal with there appearing to be a
304 greater postprandial rise following the larger meal. However, there was not a significant effect at any
305 individual time point, and peak concentrations fell just short of being significantly different ($p=0.055$).
306 Therefore, while our results lead us to speculate that prouroguanylin concentrations, like other gut
307 hormones (29)(31), rise or fall in proportion to the calories consumed, we cannot confirm this pattern.
308 We acknowledge that as our two studies were separate experiments with different participants they may
309 not be ideal in terms of design to answer this question. Instead a paired or repeated measures design
310 where the same participants are measured following each meal is needed.

311 At present we do not know whether the post meal changes in prouroguanylin concentrations we
312 observed in study one or study two are sufficient to have any physiological effect. The reported EC50
313 for uroguanylin at GUCY2C is 500nM (32). The mean post meal changes in concentrations we report
314 in study one were approximately 1000 pg/ml which equates to 83 pM. This is some way below the
315 EC50 and casts doubt on whether changes would be sufficient to affect GUCYC signalling. However,
316 it is hypothesised prouroguanylin is processed to active uroguanylin in tissues such as the hypothalamus
317 (11) and we do not know the uroguanylin concentrations that may accumulate in these tissues.
318 Furthermore, circulating post meal changes of other gut hormones such as PYY (33) fall along way
319 below the reported EC50 (34), yet they are thought to play a role in the regulation of feeding. To help
320 us understand the significance of post meal changes in prouroguanylin, it important for future studies
321 to establish the circulating concentrations required to elicits any potential effects on body mass or
322 feeding.

323 Our final study recruited both sexes and examined prouroguanylin release following a mixed
324 macronutrient high carbohydrate meal (722.5 kcals). This meal contained cereal and fruit, and although

325 still relatively high energy for a breakfast could be consider closer to a traditional view of a healthy
326 breakfast (20). In accord with the first two studies prouroguanylin concentrations were increased versus
327 baseline 120 minutes post meal. However, in this study hormone concentrations were suppressed versus
328 baseline 30 minutes after the meal. This result was not entirely unexpected as in study 1 a similar pattern
329 was observed but the reduction at 30 minutes was smaller and non significant. While generally in accord
330 with the pattern observed in the first two studies, this is a clear difference to the pattern observed by
331 Valentino et al. However, results were similar to those observed by Di Guglielmo et al. (19) in
332 adolescents. This early post meal change could relate to uroguaylin's role within the gut regulating
333 fluid and electrolyte balance (8)(35) or gut motility. Based on evidence from the guanylate cyclase
334 agonist, Linaclotide, unlike other gut hormones such as PYY (36)(37), uroguanylin is likely to speed
335 up rather than slow down gastrointestinal transit (38).

336 Study 3 also examined the effect of BMI on prouroguanylin concentrations. In accord with Rodríguez
337 et al. (17), the group who were overweight/ with obesity had lower fasting prouroguanylin
338 concentrations. However, there was not a significant difference between the groups at later time points.
339 In fact, in contrast to other gut hormones such as PYY (39)(29), post meal changes in prouroguanylin
340 were at least equal in the group who were overweight/ with obesity, with a trend towards a greater rise
341 in concentrations (0 versus 120 minutes) in the group who were overweight/ with obesity. This finding
342 is interesting as the reduced post prandial release of PYY and suppression of ghrelin in those with
343 obesity has been hypothesised to be important in post meal satiety (29)(31). Based on our results the
344 same hypothesis could not be related to prouroguanylin. Furthermore, given some rodent studies report
345 uroguanylin administration suppresses feeding (13)(11), the robust post prandial response observed in
346 our group who were overweight/with obesity may suggest potential in targeting prouroguanylin,
347 through diet or pharmacological activators of nutrient receptors to treat obesity. However, we are
348 currently still some way from knowing whether prouroguanylin is a suitable target for obesity therapies.
349 For example, our study suggests prouroguanylin is not involved in the initial suppression of hunger
350 after a meal, but it still could be involved in later feeling of satiety and this needs to be investigated.
351 Our results examining the effect of BMI on prouroguanylin are again comparable to those recently

352 reported by Di Guglielmo et al. in adolescents (19). These results are at odds with a study in rodents
353 that suggested diet induced obesity suppressed post prandial uroguanylin secretion in mice (14). Further
354 investigation is needed to see if there is a species difference or perhaps whether only specific diets or
355 more severe obesity affect post meal secretion. Given its proposed role in body mass regulation it is
356 possible the decreased fasting concentrations of prouroguanylin observed in obesity may make it harder
357 for people to lose weight. The data from rodents (14) suggests obesity leads to low prouroguanylin
358 concentrations, rather than being the initial cause.

359 Rodents studies have demonstrated uroguanylin secretion and expression are regulated by leptin (15);
360 with uroguanylin lower following leptin administration or fasting. We would predict our healthy BMI
361 group would have lower leptin concentrations than the group who were overweight/ with obesity and
362 this may contribute to the difference in the fasting prouroguanylin concentrations. However, higher
363 leptin or possible leptin resistance in the adolescents and adults who were overweight/with obesity,
364 studied by Di Guglielmo et al. (19) and us does not appear to affect post meal changes.

365 Some recent studies have suggested variation in the uroguanylin system between the sexes (19) (40).
366 One study reported a negative correlation between plasma fasting prouroguanylin concentration and
367 BMI in girls, but a positive correlation in boys (40). Another study found female but not male
368 adolescents with obesity had lower prouroguanylin concentrations (19). In our study numbers were too
369 small to examine the effect of BMI in each sex. Splitting our group from study 3 by sex alone suggested
370 there was no significant difference in prouroguanylin concentrations between males and females.
371 However, our groups weren't ideally matched as our male group had a higher mean BMI and this may
372 have affected the results of our comparison. It is also possible that in females prouroguanylin
373 concentrations vary across the menstrual cycle or are affected by contraception or childbirth. We
374 acknowledge that while we excluded those who were pregnant, breastfeeding or had recently given
375 birth, we did not record or control for other factors relating to the female reproductive system. Further
376 larger studies considering these factors are required to clarify whether there are differences in
377 prouroguanylin concentrations between adult males and females.

378 Finally, we carried out a post-hoc analysis comparing the percentage changes from baseline following
379 meals given in study 2 and study 3. For this we only included male subjects from study 3 within the
380 same BMI range as subjects from study 2. The meal given in study 2, versus study 3, had much higher
381 fat (48% versus 12.7%) and salt (2.1 grams versus 0.4 grams) content, and lower carbohydrate content
382 (36% versus 73.9%). Despite these differences there was no statistically significant difference in the
383 overall post meal pattern and the magnitude of the change from baseline to 120 minutes was very similar
384 (23% vs 28%). This suggests that variation in fat, carbohydrate and salt content of the meals did not
385 have a major influence on post prandial release. Given uroguanylin's role in salt regulation (24)(41), it
386 could be hypothesised, that dietary salt may affect circulating prouroguanylin concentrations. However,
387 our findings related to dietary salt are in accord with a recent publication demonstrating dietary salt
388 influenced uroguanylin RNA expression in the kidney (22), but not the proximal small intestine (the
389 predicted source of circulating prouroguanylin).

390 ***4.1 Conclusion***

391 This is the first study to examine prouroguanylin release in adults of a healthy weight and those who
392 are overweight/with obesity following large and medium sized meals. The results suggest that
393 immediately post meal prouroguanylin concentrations remain stable or decrease, then increase steadily
394 to above fasting concentrations, remaining at, or close to peak concentrations 120 minutes after
395 ingestion of a meal. Fasting concentrations are lower in those who are overweight/with obesity, but the
396 magnitude of the post meal rise of the hormone is similar. These results are in accord with recent
397 observations in healthy weight and adolescents with obesity (19). Our study suggests total calorie
398 content of a meal may influence prouroguanylin concentrations, but variation of fat, carbohydrate and
399 salt content do not appear to have a major effect. However, these were examined using a post-hoc
400 analysis of different experiments, so need to be confirmed by further studies. Overall our study has
401 increased understanding of the regulation of prouroguanylin and may help assess whether it is a viable
402 target for obesity therapies.

403 **Declaration of interests statement-** MP, HW, DH, HAHN and SR have no conflicts of interest to
404 declare

405 **Research Funding-** We thank the University of Roehampton for funding this project.

406 **Authors Contributions-** MP conceived study idea. All authors were involved in the design of the
407 studies. MP, HW, DH, HAHN performed the studies and analysed the data. MP and SR supervised the
408 studies and data analysis. All authors contributed to the writing of the manuscript.

409 **Acknowledgments-** We thank the University of Roehampton, Department of Life Sciences technical
410 team for their support.

411

412

413

414

415

416

417

418

419

420

421

422

423

424 **References**

- 425 1. Field BCT, Chaudhri OB, Bloom SR. Bowels control brain: Gut hormones and obesity. Vol. 6,
426 Nature Reviews Endocrinology. 2010. p. 444–53.
- 427 2. De Silva A, Bloom SR. Gut hormones and appetite control: A focus on PYY and GLP-1 as
428 therapeutic targets in obesity. Vol. 6, Gut and Liver. 2012. p. 10–20.
- 429 3. Trigueros L, Peña S, Ugidos A V, Sayas-Barberá E, Pérez-Álvarez JA, Sendra & E, et al.
430 Critical Reviews in Food Science and Nutrition Food Ingredients as Anti-Obesity Agents: A
431 Review Food Ingredients as Anti-Obesity Agents: A Review. Crit Rev Food Sci Nutr
432 [Internet]. 2013;53:929–42. Available from:
433 <http://www.tandfonline.com/loi/bfsn20%0Ahttp://dx.doi.org/10.1080/10408398.2011.574215>
434 [%0Ahttp://%0Awww.tandfonline.com/](http://www.tandfonline.com/)
- 435 4. Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK, et al.
436 Effects of targeted delivery of propionate to the human colon on appetite regulation, body
437 weight maintenance and adiposity in overweight adults. Gut. 2015;64(11):1744–54.
- 438 5. Perkins A, Goy MF, Li Z. Uroguanylin is expressed by enterochromaffin cells in the rat
439 gastrointestinal tract. Gastroenterology. 1997;113(3):1007–14.
- 440 6. Brenna O, Furnes MW, Munkvold B, Kidd M, Sandvik AK GB. Cellular localization of
441 guanylin and uroguanylin mRNAs in human and rat duodenal and colonic mucos. ell Tissue
442 Res. 2016;365:331–341.
- 443 7. Di Guglielmo MD, Perdue L, Adeyemi A, van Golen KL, Corao DU. Immunohistochemical
444 Staining for Uroguanylin, a Satiety Hormone, is Decreased in Intestinal Tissue Specimens
445 From Female Adolescents With Obesity. Pediatr Dev Pathol. 2018;21(3):285–95.
- 446 8. Rahbi H, Narayan H, Jones DJL, Ng LL. The uroguanylin system and human disease. Clin Sci
447 [Internet]. 2012;123(12):659–68. Available from:

448 <http://clinsci.org/lookup/doi/10.1042/CS20120021>

- 449 9. Miner Jr PB, Koltun WD, Wiener GJ, De La Portilla M, Prieto B, Shailubhai K, et al. A
450 Randomized Phase III Clinical Trial of Plecanatide, a Uroguanylin Analog, in Patients With
451 Chronic Idiopathic Constipation. *Am J Gastroenterol* [Internet]. 2017;112(4):613–21.
452 Available from: <http://www.nature.com/doi/10.1038/ajg.2016.611>
- 453 10. Wensel TM, Luthin DR. Linaclotide: a novel approach to the treatment of irritable bowel
454 syndrome. *Ann Pharmacother* [Internet]. 2011;45(12):1535–43. Available from:
455 <http://www.ncbi.nlm.nih.gov/pubmed/22045908>
- 456 11. Valentino MA, Lin JE, Snook AE, Li P, Kim GW, Marszalowicz G, et al. A uroguanylin-
457 GUCY2C endocrine axis regulates feeding in mice. *J Clin Invest*. 2011;121(9):3578–88.
- 458 12. Begg DP, Steinbrecher KA, Mul JD, Chambers AP, Kohli R, Haller A, et al. Effect of
459 guanylate cyclase-C activity on energy and glucose homeostasis. *Diabetes*. 2014;63(11):3798–
460 804.
- 461 13. Folgueira C, Beiroa D, Callon A, Al-Massadi O, Barja-Fernandez S, Senra A, et al.
462 Uroguanylin action in the brain reduces weight gain in obese mice via different efferent
463 autonomic pathways. *Diabetes*. 2016;65(2):421–32.
- 464 14. Kim GW, Lin JE, Snook AE, Aing AS, Merlino DJ, Li P, et al. Calorie-induced ER stress
465 suppresses uroguanylin satiety signaling in diet-induced obesity. *Nutr Diabetes*. 2016;6:e211.
- 466 15. Folgueira C, Sanchez-Rebordelo E, Barja-Fernandez S, Leis R, Tovar S, Casanueva FF, et al.
467 Uroguanylin levels in intestine and plasma are regulated by nutritional status in a leptin-
468 dependent manner. *Eur J Nutr*. 2016;55(2):529–36.
- 469 16. Fernandez-Cachon ML, Pedersen SL, Rigbolt KT, Zhang C, Fabricius K, Hansen HH, et al.
470 Guanylin and uroguanylin mRNA expression is increased following Roux-en-Y gastric
471 bypass, but guanylins do not play a significant role in body weight regulation and glycemic

- 472 control. *Peptides*. 2018;101:32–43.
- 473 17. Rodríguez A, Gómez-Ambrosi J, Catalán V, Ezquerro S, Méndez-Giménez L, Becerril S, et al.
474 Guanylin and uroguanylin stimulate lipolysis in human visceral adipocytes. *Int J Obes*.
475 2016;40(9):1405–15.
- 476 18. Le Roux CW, Welbourn R, Werling M, Osborne A, Kokkinos A, Laurenus A, et al. Gut
477 hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. *Ann Surg*.
478 2007;246(5):780–5.
- 479 19. Di Guglielmo MD, Tonb D, He Z, Adeyemi A, Van Golen KL. Pilot Study Measuring the
480 Novel Satiety Hormone, Pro-Uroguanylin, in Adolescents with and Without Obesity. *J Pediatr*
481 *Gastroenterol Nutr*. 2018;66(3):489–95.
- 482 20. Reeves S, Halsey LG, McMeel Y, Huber JW. Breakfast habits, beliefs and measures of health
483 and wellbeing in a nationally representative UK sample. *Appetite*. 2013;60(1):51–7.
- 484 21. Potthast R, Ehler E, Scheving L a, Sindic a, Schlatter E, Kuhn M. High salt intake increases
485 uroguanylin expression in mouse kidney. *Endocrinology* [Internet]. 2001;142(7):3087–97.
486 Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11416031>
- 487 22. Fellner RC, Moss NG, Goy MF. Dietary salt regulates uroguanylin expression and signaling
488 activity in the kidney, but not in the intestine. *Physiol Rep*. 2016;4(9):1–16.
- 489 23. Kaar G, Dieplinger B, Gabriel C, Haltmayer M, Mueller T. Proguanylin and prouroguanylin -
490 Assay evaluation and clinical analyte characterization. *Clin Chim Acta*. 2011;412(23–
491 24):2277–83.
- 492 24. Moss NG, Fellner RC, Qian X, Yu SJ, Li Z, Nakazato M, et al. Uroguanylin, an intestinal
493 natriuretic peptide, is delivered to the kidney as an unprocessed propeptide. *Endocrinology*.
494 2008;149(9):4486–98.
- 495 25. Qian X, Moss NG, Fellner RC, Goy MF. Circulating prouroguanylin is processed to its active

- 496 natriuretic form exclusively within the renal tubules. *Endocrinology*. 2008;149(9):4499–509.
- 497 26. Holst J. The physiology of glucagon-like peptide 1. *Physiol Rev* [Internet].
498 2007;87(225):1409–39. Available from:
499 <http://physrev.physiology.org/content/87/4/1409?rss=1&ssource=mfr>
- 500 27. Kojima M, Kangawa K. Ghrelin discovery: A decade after. In: *The Ghrelin System*. 2013. p.
501 1–4.
- 502 28. Grandt D, Schimiczek M, Beglinger C, Layer P, Goebell H, Eysselein VE, et al. Two
503 molecular forms of Peptide YY (PYY) are abundant in human blood: characterization of a
504 radioimmunoassay recognizing PYY 1-36 and PYY 3-36. *Regul Pept*. 1994;51(2):151–9.
- 505 29. Le Roux CW, Batterham RL, Aylwin SJB, Patterson M, Borg CM, Wynne KJ, et al.
506 Attenuated peptide YY release in obese subjects is associated with reduced satiety.
507 *Endocrinology*. 2006;147(1):3–8.
- 508 30. Patterson M, Murphy KG, Le Roux CW, Ghatei MA, Bloom SR. Characterization of ghrelin-
509 like immunoreactivity in human plasma. *J Clin Endocrinol Metab*. 2005;90(4):2205–11.
- 510 31. Le Roux CW, Patterson M, Vincent RP, Hunt C, Ghatei MA, Bloom SR. Postprandial plasma
511 ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese
512 subjects. *J Clin Endocrinol Metab*. 2005;90(2):1068–71.
- 513 32. Müller T, Rasool I, Heinz-Erian P, Mildenerger E, Hülstrunk C, Müller A, et al. Congenital
514 secretory diarrhoea caused by activating germline mutations in *GUCY2C*. *Gut*.
515 2016;65(8):1306–13.
- 516 33. Chandarana K, Drew ME, Emmanuel J, Karra E, Gelegen C, Chan P, et al. Subject
517 Standardization, Acclimatization, and Sample Processing Affect Gut Hormone Levels and
518 Appetite in Humans. *Gastroenterology*. 2009;136(7):2115–26.
- 519 34. Henry KE, Elfers CT, Burke RM, Chepurny OG, Holz GG, Blevins JE, et al. Vitamin B12 in

- 520 conjugation of peptide-YY 3-36 decreases food intake compared to native peptide-YY 3-36
521 upon subcutaneous administration in male rats. *Endocrinology*. 2015;156(5):1739–49.
- 522 35. Vaandrager AB, Bot AGM, De Jonge HR. Guanosine 3',5'-cyclic monophosphate-dependent
523 protein kinase ii mediates heat-stable enterotoxin-provoked chloride secretion in rat intestine.
524 *Gastroenterology*. 1997;112(2):437–43.
- 525 36. Savage AP, Adrian TE, Carolan G, Chatterjee VK, Bloom SR. Effects of peptide YY (PYY)
526 on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy
527 volunteers. *Gut*. 1987;28(2):166–70.
- 528 37. Lin HC, Neevel C, Chen JH. Slowing intestinal transit by PYY depends on serotonergic and
529 opioid pathways. *Am J Physiol Liver Physiol* [Internet]. 2004;286(4):G558–63. Available
530 from: <http://www.physiology.org/doi/10.1152/ajpgi.00278.2003>
- 531 38. Busby RW, Bryant AP, Bartolini WP, Cordero EA, Hannig G, Kessler MM, et al. Linaclotide,
532 through activation of guanylate cyclase C, acts locally in the gastrointestinal tract to elicit
533 enhanced intestinal secretion and transit. *Eur J Pharmacol*. 2010;649(1–3):328–35.
- 534 39. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, et al. Inhibition of
535 Food Intake in Obese Subjects by Peptide YY 3–36. *N Engl J Med* [Internet].
536 2003;349(10):941–8. Available from: <http://www.nejm.org/doi/abs/10.1056/NEJMoa030204>
- 537 40. Folgueira C, Barja-Fernández S, Gonzalez-Saenz P, Castelao C, Vázquez-Cobela R, Pena-
538 Leon V, et al. Circulating Pro-Uroguanylin Levels In Children And Their Relation To Obesity,
539 Sex And Puberty. *Sci Rep*. 2018;8(1).
- 540 41. Potthast R. High Salt Intake Increases Uroguanylin Expression in Mouse Kidney.
541 *Endocrinology* [Internet]. 2001;142(7):3087–97. Available from:
542 <https://academic.oup.com/endo/article-lookup/doi/10.1210/en.142.7.3087>

544

545

546

547

548

549

550

551

552

553

554

555

556

557

558

559

560

561

562

563

564

565

566 **Figure Legends**

567 **Figure 1.** Plasma prouroguanylin concentrations A, and visual analogue hunger scores, B, from healthy
568 males (n=7) pre (0 minutes) and post a 1451 kcal. Data presented are the mean with error bar
569 representing the standard deviations. Repeated ANOVA was used with post hoc ,Dunnett's test to see
570 if there was a difference between individual time points (* =P < 0.05 versus pre meal, 0 minutes, ** =
571 P<0.01 versus pre meal, 0 minutes.)

572 **Figure 2. A.** Plasma prouroguanylin concentrations from healthy males (n=7) pre and post a 725 kcal.
573 Data presented are the mean with error bar representing the standard deviations. Repeated ANOVA was
574 used with post hoc Dunnett's test to see if there was a difference between individual time points (** =
575 P<0.01 versus 0 minutes.). **B.** Comparison of the percentage change from fasting (Time =0) at each post
576 meal time point, following the 725 kcal meal versus the 1451 kcal meal (n=7 for each study).

577 **Figure 3.** Plasma prouroguanylin concentrations following a 722.5 kcal high carbohydrate meal, from
578 a mixed sex group including, 9 healthy weight adults and 9 overweight or with obesity. Data presented
579 are the mean with error bar representing the standard deviations. **A** Includes all participants as a single
580 group. Repeated ANOVA was used with post hoc Dunnett's test to see if there was a difference between
581 individual time points (* =P < 0.05 versus 0 minutes, ** = P<0.01 versus 0 minutes.). **B** Shows the
582 data split into the participants with a healthy BMI (n=9) and those who were overweight or with obesity
583 (n=9). Fasting concentrations alone were significantly different between groups (* =P<0.05). But there
584 was no overall effect of BMI group when data were analysed by two way ANOVA of repeated
585 measures. **C** Study 2 versus Study 3, Males BMI under 26 kg /m² only. Comparison of the percentage
586 change from fasting (Time =0) at each post meal time point, following the 722.5 kcal high carbohydrate
587 meal (study 3) versus the 725 kcal high fat meal (study 2) (n=7-9).

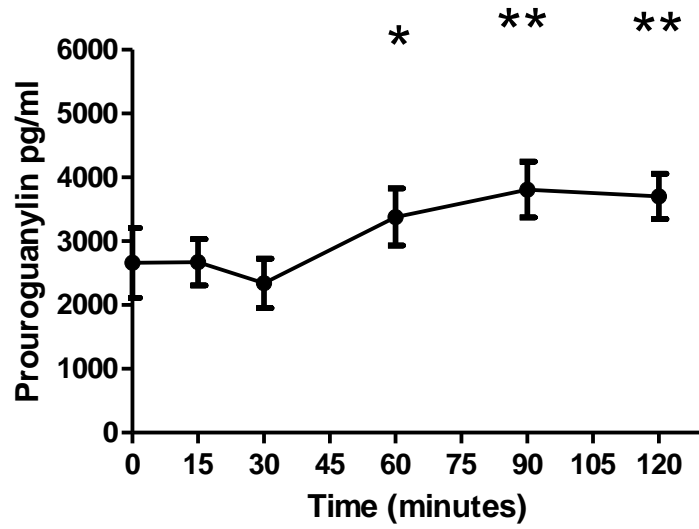
588

589 **Figure 1.**

590

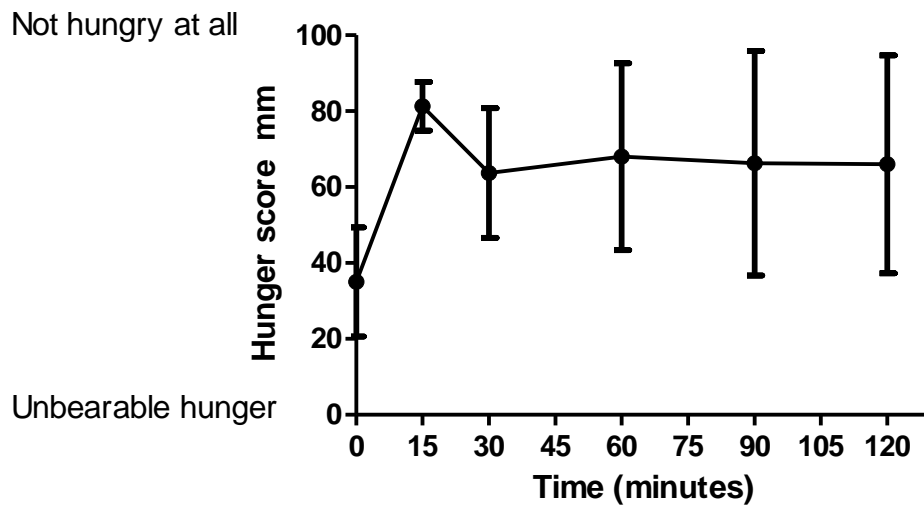
591

592 **A**



593

594 **B**



595

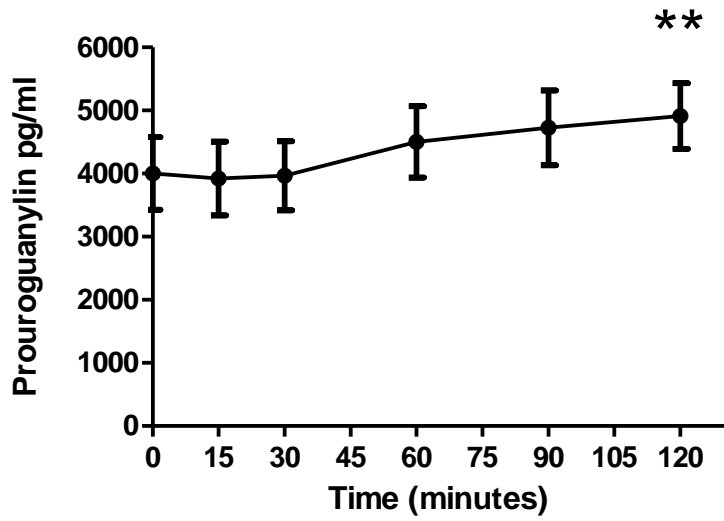
596

597

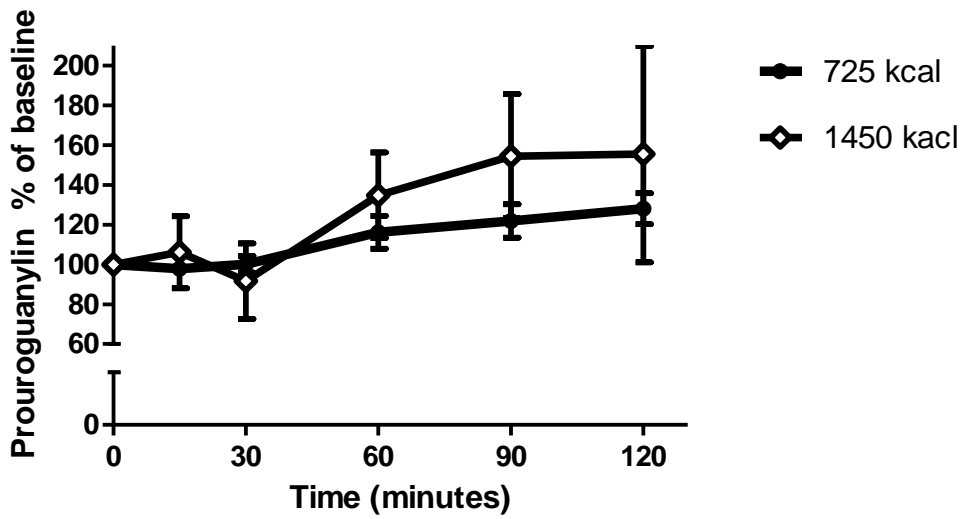
598 **Figure 2**

599

600 **A**



601 **B**



602

603

604

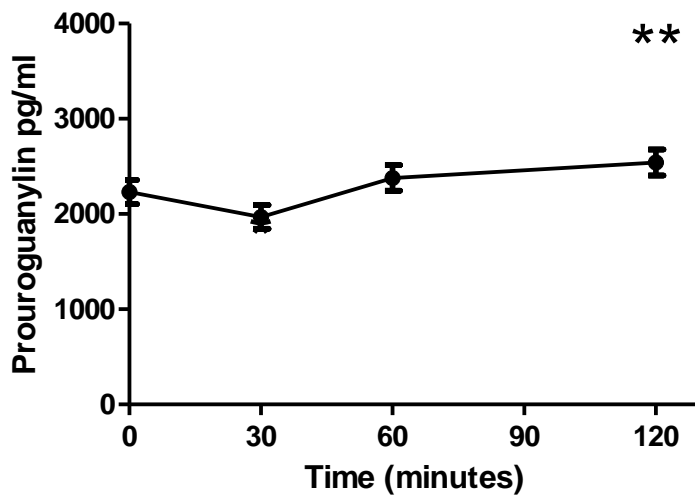
605

606 **Figure 3.**

607

608 **A**

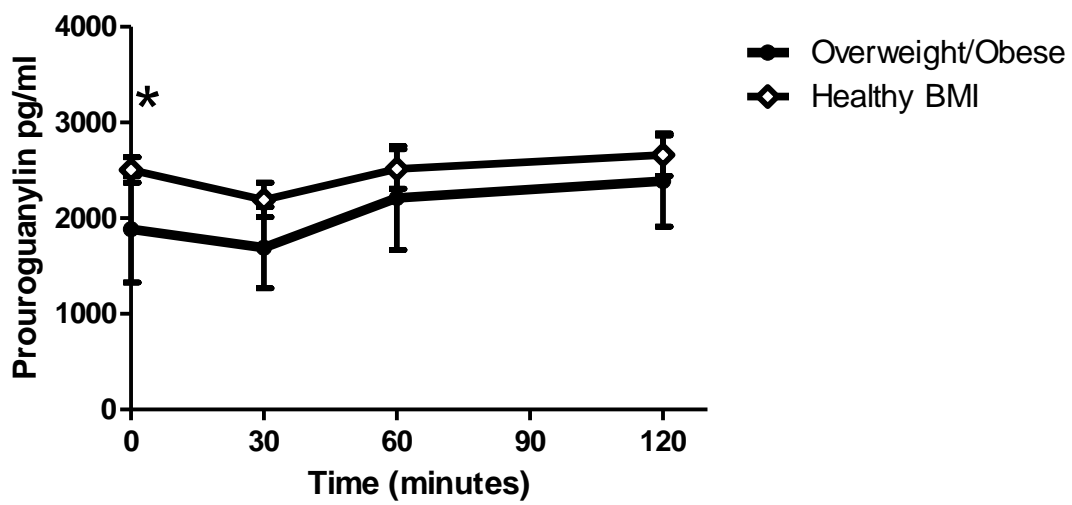
609



610

611

612 **B.**

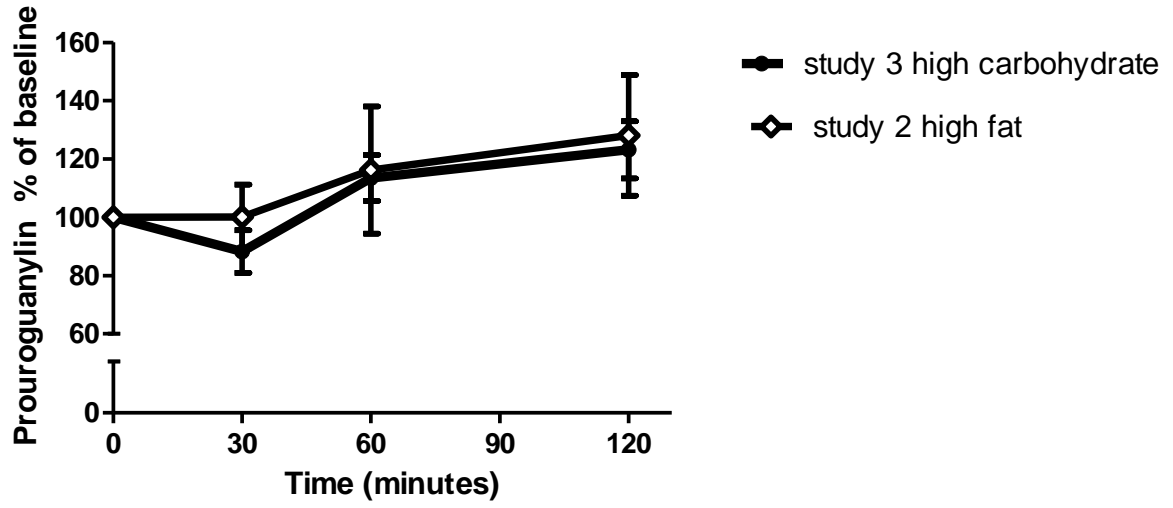


613

614

615

616 C.



617