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2 **Gastrointestinal capacity, gut hormones and appetite change during rat**
3 **pregnancy and lactation**

4

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14 Short title: Gut changes during rat pregnancy and lactation

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16

17 **Abstract**

18 Pregnancy and lactation increase maternal appetite and adiposity, which in humans
19 can lead to long-term body mass retention. Previous rat reproduction studies
20 suggest that appetite-inhibiting gut hormone, peptide-YY (PYY), is elevated, despite
21 hyperphagia; also that gastrointestinal size increases. The present study
22 characterised changes in orexigenic (appetite-stimulating) ghrelin and anorexigenic
23 (appetite-inhibiting) PYY and glucagon-like peptide-1 (GLP-1), and gastrointestinal
24 architecture during pregnancy and lactation, in matched fed and fasted plasma and
25 gut tissue samples taken during the dark phase. Enteroendocrine cells were
26 immunolabelled, and gut masses and lengths measured. Fasted plasma ghrelin
27 reduced during pregnancy: it was lowest by day 18, recovered to control values at
28 parturition, then increased by the end of lactation. Ghrelin-immunoreactive stomach
29 cells and stomach ghrelin concentrations were highest at birth, prior to the onset of
30 lactation-associated hyperphagia. Plasma fed GLP-1 concentrations were elevated
31 during pregnancy; and together with higher colon concentrations of PYY and GLP-1
32 during early lactation, they were associated with gastrointestinal tissue expansion,
33 not satiety. Body mass increased during lactation, whereas white adipose tissue
34 depots depleted. Extensive gut remodelling coincided with elevated colon
35 concentrations of PYY and GLP-1. Modifications included: stomach and caecum
36 expansion, and duodenal, ascending and descending colon circumference
37 increases, all peaking by day 10 of lactation; increased intestinal masses and
38 lengths peaking at lactation day 10 for small intestine and lactation day 25 for large
39 intestine. If these physical tissue increases persist post-partum, they could

40 accelerate future nutrient assimilation and storage in dams, and may contribute to
41 increased obesity risk.

42 **Introduction**

43 Pregnancy and lactation involve extensive maternal adaptation for foetal and
44 neonatal growth, and to build and replace maternal energy reserves. In rats, dam
45 body mass increases start soon after conception (Cripps & Williams, 1975), partly by
46 adipose tissue accumulation (Lopez-Luna *et al.*, 1991, Pujol *et al.*, 2006). Post-
47 partum, body mass increases again, to above that of non-pregnant controls, despite
48 ~60% loss of adiposity by day 16 lactation (Naismith *et al.*, 1982). Thus maternal
49 body composition changes, although has not been extensively studied.

50 Hyperphagia during rodent early pregnancy increases food intake by 20% (Crean &
51 Rumsey, 1971, Trujillo *et al.*, 2011), with peaks of 50-60% (Cripps & Williams, 1975)
52 compared to nulliparous controls. Maternal intake during lactation peaks during week
53 3 lactation to ~250-300% of controls (Crean & Rumsey, 1971, Cripps & Williams,
54 1975, Denis *et al.*, 2004). Hyperphagia during lactation is supported by reduced
55 leptin and decreased adiposity (Woodside *et al.*, 2000) and although appetite
56 increases occur, studies of changes in gut appetite-regulating hormones have so far
57 produced conflicting results.

58 Ghrelin increases with fasting, elevating prior to feeding (Nakazato *et al.*, 2001) and
59 is suppressed by increased leptin in males (Ueno *et al.*, 2004). In *ad lib* fed pregnant
60 rats, Taylor *et al.* (2009) found total ghrelin in plasma and some gut tissues was not
61 suppressed, despite increased leptin mid-pregnancy, whereas another study found
62 ghrelin decreased (Shibata *et al.*, 2004). During lactation in rats, Taylor *et al.* (2009)
63 reported no difference in either plasma or tissue total ghrelin, whereas Shibata *et al.*

64 (2004) found lower ghrelin and hypothalamic mRNA during lactation than late
65 pregnancy, suggesting possible systemic reductions after birth. Inconsistencies may
66 be due to non-standardised and/or physiologically inappropriate sampling times,
67 such as during light periods for feeding studies, as rodents consume most food
68 during the active dark phase. This study addressed these issues with dark phase
69 sampling and looking closely at ghrelin-secreting cell location and abundance, to
70 address previously conflicting findings.

71 Satiety hormones PYY and GLP-1 have received less research interest than ghrelin.
72 GLP-1 may have differential abilities to regulate food intake based on nutritional
73 status, with limited effects when fasted (Ronveaux *et al.*, 2014). Also, GLP-1
74 treatment during proestrus was detrimental to early pregnancy events (Outeirino-
75 Iglesias *et al.*, 2015). During rat pregnancy, plasma total PYY increased (Tovar *et al.*,
76 2004, Taylor *et al.*, 2009) and gradual increases were documented in descending
77 colon and rectum (Taylor *et al.*, 2009). Taylor *et al.* (2009) reported increased
78 plasma PYY at day 5 lactation in rats, paralleled by increases in DC and rectum; also
79 hypothalamic PYY mRNA (days 5 + 15, Suzuki *et al.*, 2014). It appears plausible that
80 elevated PYY has an important role in rat lactation. Why a purported satiety hormone
81 was elevated, despite pregnancy and lactation-associated hyperphagia, remains to
82 be established. No studies have explored circulating or gut tissue GLP-1 during
83 lactation, hence the current focus.

84 Maternal adaptation during pregnancy and lactation in many animals involves GI tract
85 structural changes (Speakman, 2008, Reiff *et al.*, 2015) to accommodate large food
86 intake increases, although the mechanisms involved are poorly understood. Stomach
87 tissue mass has been documented to increase during pregnancy and peak by late
88 lactation in rats (Cripps & Williams, 1975, Taylor *et al.*, 2009) and mice (Campbell &

89 Fell, 1964). The small intestine is most extensively studied (Cripps & Williams, 1975,
90 Burdett & Reek, 1979; Datta *et al.* 1995) with documented increases of: 27% length
91 by late lactation, 140-150% mucosal epithelium mass; also surface area (Boyne *et al.*,
92 1966, Penzes & Regius, 1985). Colon length increases with pregnancy, and mass with
93 lactation (Cripps and Williams, 1975; Taylor *et al.*, 2009). PYY, GLP-1 and GLP-2 are
94 co-secreted from gut L-cells (Mojsov *et al.*, 1986) and have been linked with gut growth
95 (Drucker *et al.*, 1996) and increased capacity for nutrient absorption (Brubaker *et al.*,
96 1997; Ghatei *et al.*, 2001), although not yet in reproduction. In adult female mice, PYY
97 stimulated growth of both the small and large intestines in a dose-dependent manner;
98 whereas colon size only increased at higher doses (Gomez *et al.*, 1995).

99 The aim of this study was therefore to clarify and elucidate roles of ghrelin, PYY and
100 GLP-1 during maternal adaptation. Samples of fed and fasted plasma were obtained
101 from the same animals, taken in the dark phase; also from matched gut tissue, with
102 PYY and GLP-1 concentrations measured for the first time during different
103 reproductive stages. Comprehensive measurements have explored gut capacity and
104 expansion in detail, thus this study advances and updates the current knowledge of
105 gut growth during pregnancy and lactation and allows investigation into potential
106 relationships between gut peptides and intestinal remodelling.

107 **Materials and Methods**

108 **Animals**

109 This work was licensed under the Home Office Animals (Scientific Procedures) Act
110 1986 and had approval from The Open University Ethics Committee. Rats were
111 chosen for this study to obtain an adequate volume of blood for matched fed and fasted
112 circulating hormone analysis, with matched tissue peptide comparisons. Female

113 Wistar rats (Harlan, Bicester, UK; n=49) were housed in groups of three or four, with
114 free access to standard rodent breeding diet (801730, Special Diets Service Essex,
115 UK), water and bedding material. The animals were adjusted to 12-hour reverse
116 lighting conditions (lights off between 11:00 and 23:00 hr) for a minimum of two weeks
117 before study start, then permanently housed under these conditions. All procedures
118 were carried out during the dark phase, in contrast to most prior studies, so that
119 samples were obtained when most physiologically relevant for natural feeding
120 behaviour. There were seven experimental groups: days 4, 12 and 18 of pregnancy
121 and days 0, 5, 10 and 25 of lactation (n=7 per group). The time points used for sample
122 groups were optimised based on the findings of Taylor *et al.* (2009).

123 The oestrus dance (Feder, 1981) was used to accurately time-mate females for
124 pregnancy time-points, and for some lactation time-points when appropriate females
125 presented with the dance (Stramek *et al.*, 2018). Dams for the lactation time-points
126 had their litters standardised to 8 ± 1 each by day two postpartum. Pups remained
127 with the dams throughout the study and had free access to the cage diet when they
128 were able to reach it, from approximately 16 days of age. Dams used on the day of
129 birth had their litters standardised shortly after birth, when a nest had been
130 established, and were sacrificed approximately 4-5 hours after birth. Pregnant dams
131 for lactation time-points were separated into their own cage with nesting material
132 between days 18 and 20 of pregnancy.

133 Pregnant rats had an age range of 24-38 weeks and lactating rats 18-37 weeks by
134 the end of the study. Data from a group of 6 proestrus nulliparous females (aged 34-
135 37 weeks) were included as a reference point for the study but the focus of this study
136 was on the differences between the groups progressing through pregnancy and
137 through lactation.

138 Body mass and food intake

139 Live body mass was recorded prior to tail bleed (below) and once again after the
140 fasting period, before sacrifice. Food intake was monitored in singly housed females
141 by weighing their food hopper in the days immediately prior to birth and into lactation
142 before the pups started eating solid food.

143 Blood collection and preparation

144 Fed blood samples were taken from a tail vein, between 12:00 and 13:00 hr, under
145 anaesthesia (isoflurane; IsoFlo, Abbott Laboratories, Maidenhead, UK) to minimise
146 stress during collection, as optimised from an earlier study (Johnson *et al.*, 2016).
147 Lubrithal (VetXX Ltd, Stoke-on-Trent, UK) was applied to the eyes of the rats whilst
148 under anaesthetic to prevent them from drying out and a spray-on dressing (OpSite,
149 Smith & Nephew Medical Ltd, Watford, UK) was applied to the tail tip after sample
150 collection. Once conscious, females were immediately returned to their home cage in
151 their established social groups (pregnancy) or with their pups (lactating).
152 Cage food was removed (access to water maintained) at 08:00 the following day
153 prior to sacrifice between 12:00 and 16:00 hr. Rats were fully anaesthetised and
154 decapitated, and a fasted blood sample was obtained from trunk blood. All blood was
155 collected into EDTA coated tubes with additional protease inhibitor (aprotinin;
156 Trasylol, Bayer plc, Reading, UK). All fed and 1 ml of fasted blood samples were
157 immediately acidified by dilution at 1:10 in buffer (0.1 M ammonium acetate, 0.5 M
158 NaCl, pH 3.6) as recommended for optimal peptide preservation and recovery
159 (Stengel *et al.*, 2009).

160 Gastrointestinal tissue measurements

161 The gastrointestinal (GI) tract was measured and sampled in several locations:

162 stomach, small intestine (SI), caecum, large intestine (LI; ascending and descending
163 colon). Measurements of gastrointestinal length were made by emptying the gut of
164 contents and free-floating the tissue in PBS, taking care not to stretch the tissue.

165 Masses were recorded after emptying the gut and blotting the tissue dry on tissue
166 paper. Due to differences in early sample collection, small intestine wet weight has
167 been excluded from analysis for the day 25 lactating dam group and the proestrus
168 controls.

169 Stomachs were removed and mass was recorded after opening along the greater
170 curvature and rinsing in PBS. Once weighed, the stomach incision was extended to
171 cut the stomach in half; one half was snap frozen for peptide extraction (see below)
172 and the body of the other half was fixed in 4% paraformaldehyde overnight, then
173 rinsed in 3 x 10 minutes PBS and placed in 30% sucrose at 4°C for a minimum of 48
174 hours until frozen in OCT for cryosectioning transversely at 10 µm. The small and
175 large intestines were removed whole, emptied, and wet weight and length recorded.

176 The caecum was removed and treated similarly to the stomach (see above).

177 Approximately 1 cm of mid duodenum, proximal ascending colon and proximal
178 descending colon was removed for circumference measurements. Circumference
179 measurements were standardised by measurement after 20 minute incubation in
180 PBS containing 10^{-6} M nicardipine hydrochloride to maximally relax the smooth
181 muscle. Nicardipine could not be used to standardise gut length measurements due
182 to the possibility of it interfering with other methodologies (e.g. peptide extraction)
183 that the tissue subsequently underwent; circumference measurements were taken

184 from one small piece of tissue. In addition to gut measurements, all of the white
185 adipose tissue (WAT) in the abdominal cavity was carefully removed and weighed.

186 Gastrointestinal tissue preparation for radioimmunoassay

187 Half of each stomach, and ~2 cm portion of mid-duodenum, mid-ascending colon
188 and mid-descending colon were collected and immediately frozen on dry ice. Tissue
189 samples were extracted in 1 ml of 0.5 M glacial acetic acid per 100 g of tissue
190 collected, and boiled in a 100 °C water bath for 20 minutes. The liquid portion of the
191 boiled samples was stored at -20 °C until assayed.

192 Radioimmunoassay

193 As all fed plasma collected was acidified (thereby diluted), this was analysed for each
194 peptide. Fasted acidified plasma was used in ghrelin assays as recommended to
195 stabilise the acyl peptide form, and for PYY and GLP-1 assays, neat un-
196 acidified/undiluted fasted plasma was used because concentrations of both of these
197 peptides were expected to be lowest in the fasted state. All samples were analysed in
198 duplicate and according to the manufacturer's protocol for total ghrelin, total PYY and
199 total GLP-1 concentration using radioimmunoassay kits (Millipore, Watford, UK). An
200 Excel spreadsheet was used to calculate sample concentrations from the standard
201 curve, adjusted for sample dilution, with internal controls (provided with the kit)
202 confirming optimal assay performance. All samples were added to assays based on
203 sample type, not by pregnancy or lactation stage.

204 Total ghrelin

205 Preliminary testing measured high concentrations of ghrelin in all samples, so they
206 were diluted ten times by a reduction in sample volume in the assay tubes. Due to
207 cost, fed plasma ghrelin was not measured as a previous study (Johnson *et al.*, 2016)

208 found no difference between fed and fasted concentrations. Stomach tissue extracts
209 underwent an additional 1:250 dilution for pregnant and 1:400 for lactating dams. The
210 mean intra-assay variation was 4.4% for plasma and 10.1% for tissue and the mean
211 inter-assay variation was 2.07% for plasma and 9.95% for tissue.

212 Total PYY

213 Fasted plasma samples were added to the kits as suggested by the manufacturers'
214 guidelines. Although fed plasma samples were analysed, the dilution effect of
215 acidifying the samples caused peptide recovery issues so these data are not
216 presented – see Discussion for why the interpretation of fasted PYY sample
217 concentrations are as equally valid as fed samples during pregnancy. For both
218 pregnant and lactating dams, ascending and descending colon extracts were diluted
219 1:80 prior to addition to the assays. The mean intra-assay variation was 4.4% for
220 plasma and 6.2% for tissue and the mean inter-assay variation was 2.9% for plasma
221 and 7.71% for tissue.

222 Total GLP-1

223 Fasted non-acidified plasma samples were extracted and added to each kit as outlined
224 in the assay protocol. Double the volume of fed acidified plasma was required to
225 undergo the kit extraction protocol in order to bring these samples onto the linear part
226 of the standard curve. Ascending colon samples were diluted 1:80 and descending
227 colon samples were diluted 1:70. The mean intra-assay variation was 4.5% for plasma
228 and 6% for tissue and the mean inter-assay variation was 9.4% for plasma and 3.4%
229 for tissue.

230 Immunofluorescence of stomach tissue

231 Stomach sections were stained for total ghrelin peptide using a standard
232 immunofluorescence protocol, with incubations carried out at room temperature.
233 Briefly, slides were incubated with normal horse serum (10%) for 90 minutes,
234 washed in PBS, and incubated overnight with 1:800 goat anti-ghrelin antibody (Santa
235 Cruz Biotechnology, Dallas, USA). After 3 x 10 minute washes in PBS, a biotinylated
236 horse anti-goat IgG (Vector) at 6 µg/ml was applied for 120 minutes, followed by a
237 further wash step. The slides were then incubated with streptavidin fluorescein (4
238 µg/ml) for 120 minutes before a final wash step, and were then cover-slipped using
239 Citifluor (Agar Scientific, Stanstead, UK). Negative controls were: antibody-dilution
240 solution only, primary antibody only, secondary antibody only and streptavidin
241 fluorescein only.

242 Quantification of immunolabelled cell numbers

243 Images were obtained using an Olympus BX fluorescence microscope. In order to
244 perform a manual cell count of each immunoreactive (IR) cell, serial images were
245 taken of the entirety of each section of tissue stained using a x10 objective lens and
246 all IR cells were counted from these images. The programme ImageJ was used to aid
247 manual cell counting, using the cell counter plugin to mark each IR cell in each image.
248 In order to count these images blind, an online list randomiser
249 (<http://www.random.org/lists/>) was used to assign a random number to each animal
250 number. Each image was then renamed using this random number and cell counting
251 was completed before counts were un-blinded for statistical analysis.

252 Statistical analysis

253 Values represent mean \pm S.E.M. Statistical analysis was initially carried out using a
254 one-way ANOVA with a Tukey post-hoc test on normally distributed data (shown on
255 figures with asterisks e.g. * $P < 0.05$). Data not normally distributed were normalised
256 by log transformation (e.g. GLP-1 AC, DC; caecum and large intestine wet masses).
257 When data were not normally distributed and could not be normalised, a Kruskal-
258 Wallis test was used, with subsequent pairwise comparisons (Mann-Whitney), with
259 Bonferroni correction. A paired-samples t -test was used to compare plasma peptides
260 in the fed and the fasted states, and to compare peptide concentrations in different
261 areas/regions of the colon. As the experimental design included multiple factors
262 including fed and fasted status, different regions of the gut and different time-points
263 during pregnancy and lactation, as well as a proestrus control, GLM univariate
264 analysis was used to test for any interactions. Tukey post-hoc tests were used to
265 investigate the sources of interaction and are shown on Figures 1, 4, 5, 8, 9, 10 with
266 e.g. $a > b$, $P < 0.001$ etc to indicate significance between different pregnancy and
267 lactation charts. Italicised $a > b$ are also used to differentiate from other significant
268 interactions. All statistical tests were performed using IBM SPSS Statistics 24.
269 $P < 0.05$ was considered statistically significant.

270 Results

271 Ghrelin concentrations were suppressed during pregnancy and elevated
272 by the end of lactation

273 All stomach tissue samples had higher total ghrelin concentrations than plasma
274 samples ($P < 0.001$; Figure 1C $>$ 1A; Figure 1D $>$ 1B). GLM analysis found significant

275 main (direct) effects of sample type (plasma/stomach; $F(7,112)=2.470$, $P=0.023$) and
276 stage (proestrus, pregnancy or lactation time-points; $F(1,112)=643.676$, $P=0.000$)
277 and a significant interaction (joint effect) of sample type with reproductive stage
278 ($F(7,112)=2.269$, $P=0.035$).

279 Plasma

280 Fasted plasma total ghrelin during pregnancy remained consistently reduced ($F(7,$
281 $49)=27.751$, $P=0.001$; Figure 1A) compared with proestrus controls and all stages of
282 lactation (Figure 1B). During pregnancy, ghrelin was significantly ($F(2, 18)=3.767$,
283 $P=0.043$) less concentrated in day 18 pregnant (d18P) dams compared with day 4
284 pregnant dams (d4P). By the day of birth (d0L), fasted plasma ghrelin concentrations
285 had increased back to control amounts, and by day 25 of lactation (d25L) had
286 significantly increased ($F(3, 24)=4.546$, $P=0.012$) further compared with d0L
287 ($P=0.023$) and day 5 lactating (d5L; $P=0.017$) dams (Figure 1B).

288 Stomach tissue

289 During pregnancy, the amounts of ghrelin in stomach tissue varied within each dam
290 group, but were reduced in comparison with proestrus controls ($P<0.014$; Figure 1C),
291 in d12P ($P=0.035$). Stomach tissue ghrelin concentration was significantly (Kruskal-
292 Wallis, $\chi^2=10.057$, 3 df, $P=0.018$) increased by d0L compared with d12P ($P=0.001$)
293 and d18P ($P=0.036$) dams, with a later, significant decrease by d25L ($P=0.025$)
294 (Figure 1D). Although d0L dams had the highest concentration of ghrelin in their
295 stomach tissue out of all of the lactating and pregnant dams, this concentration was
296 similar to that found in the proestrus controls.

297

298

299 **Figure 1. Concentrations of ghrelin in fasted plasma and stomach tissue**
300 **throughout pregnancy and lactation**

301 Ghrelin-immunoreactive (IR) cells in stomach tissue were found throughout the
302 mucosa, predominantly so towards the mucosal-submucosal border, in all groups
303 quantified: proestrus, day 12 pregnant (d12P) and d0L (Figure 2A, B, C). There was
304 no difference in the mucosal area of the stomach, nor in the mean maximum
305 thickness of the mucosa of muscle layers. However, the ghrelin-IR cell density was
306 significantly different between the sample groups ($F(2, 17)=29.735$, $P<0.001$) and
307 increased significantly from the proestrus controls to d12P, and dams in the
308 transition stage of parturition (d0L) had a significantly higher ghrelin cell density than
309 in both the other sample groups (Figure 2D).

310 **Figure 2. Representative images of stomach tissue (A-C) showing the**
311 **distribution of ghrelin immunoreactive staining and (D) quantification of**
312 **stomach tissue ghrelin cell density at proestrus, d12P and day of birth (d0L)**

313

314 **Peptide-YY (PYY) concentrations were increased during lactation**

315 All colon tissue samples had higher total PYY concentrations than plasma samples
316 ($P<0.001$; Figure 3C, D, E, F > Figure 3A, B) and GLM analysis of colon region
317 (ascending/descending) found significant main (direct) effects on colon tissue PYY of
318 stage (proestrus, pregnancy or lactation time-points; $F(7,101)=4.701$, $P<0.001$;
319 Figure 3C, D, E, F with peak PYY concentrations at day 5 of lactation for ascending
320 colon ($P=0.021$; Figure 3D) and descending ($P=0.024$; Figure 3F) colon compared
321 with proestrus DC concentrations (Figure 3F).

322 Plasma

323 Both the pregnant and lactating dams had similar fasted plasma total PYY
324 concentrations, although they were elevated compared with proestrus controls in
325 d10L dams ($F(4,35)=4.683$, $P=0.004$; Figure 3A, B), with a tendency ($P=0.067$, n.s.)
326 in d12P dams.

327 Colon tissue

328 Ascending and descending colon PYY concentrations varied considerably within
329 each pregnancy group and were not significantly different from each other, although
330 there was a numerical increase, especially in ascending colon, and PYY in the d18P
331 dams in descending colon was two times higher than the proestrus controls (no
332 ascending colon data), suggesting that PYY gradually elevated during pregnancy.

333 PYY concentrations in the ascending colon were significantly ($F(6, 46)=3.215$,
334 $P=0.011$) highest in day 5 lactating (d5L) dams - more than double - compared with
335 the start of pregnancy (d4P, $P=0.025$) and with the end of lactation (d25L, $P=0.021$).
336 Descending colon PYY concentrations were over three times higher in d5L dams
337 than the proestrus controls (Kruskal-Wallis, $\chi^2=16.955$, 7 df, $P=0.018$).

338 **Figure 3. Concentrations of PYY in fasted plasma, ascending and descending**
339 **colon tissues throughout pregnancy and lactation**

340

341 Glucagon-like peptide-1 peptide concentrations were suppressed in
342 pregnancy when fasted and elevated when fed in pregnancy and
343 lactation

344 All colon tissue samples had higher total GLP-1 concentrations than plasma samples
345 ($P < 0.001$; Figure 5 *cf* Figure 4). GLM analysis found significant main (direct) effects
346 on plasma GLP-1 of fed/fasted status $F(7,109) = 5.208$, $P = 0.000$) and stage
347 (proestrus, pregnancy or lactation time-points; $F(1,109) = 110.679$, $P = 0.000$) and a
348 significant interaction (joint effect) of fed/fasted status with reproductive stage
349 ($F(7,109) = 5.648$, $P = 0.000$).

350 Plasma

351 There was a large magnitude of GLP-1 differences between fed and fasted, as well
352 as between pregnant, lactating and proestrus controls. Fed proestrus rats had twice
353 the amount of circulating GLP-1 than fasted proestrus animals, but fed values were
354 highly variable (56 ± 25.7 *cf* 28 ± 6.5 pg/ml; $P = 0.368$, n.s.). During pregnancy, GLP-1
355 concentrations were significantly - approximately 28 times - higher ($t(20) = 7.463$,
356 $P < 0.001$) in fed plasma (165 ± 21.4 pg/ml) than in paired fasted plasma (6 ± 1.1
357 pg/ml) (Figure 4A *cf* 4C). For the whole lactating group, GLP-1 was 5 fold more
358 concentrated ($t(24) = 5.502$, $P < 0.001$) in the fed state (249 ± 25.8 pg/ml) than in the
359 fasted state (52 ± 17.8 pg/ml) (Figure 4B *cf* 4D).

360 Fed plasma GLP-1 in pregnant dams did not significantly differ ($P = 0.30$, n.s)
361 between groups, but was elevated 2 to 4 times during pregnancy by d12 ($P = 0.024$)
362 compared to proestrus fed nulliparous controls $F(3,23) = 3.24$, $P = 0.041$; Figure 4A). In
363 contrast, fasted plasma GLP-1 concentrations, were significantly decreased at each
364 stage of pregnancy ($F(3, 25) = 8.613$, $P < 0.001$) compared with proestrus fasted

365 controls, starting at approximately three times lower in early pregnancy at d4P until
366 values were seven times lower by d18P (Figure 4C) towards the end of pregnancy.
367 Fasted plasma GLP-1 was significantly decreased during pregnancy ($F(2,18)=3.664$,
368 $P=0.046$), with a trend ($P=0.057$, n.s.) for d18P dams to have less fasted plasma
369 GLP-1 than d4P dams.
370 Dams at the beginning of lactation had even higher ($F(4,26)=9.532$, $P<0.001$) fed
371 plasma GLP-1 than during pregnancy, that was approximately six fold more than
372 proestrus controls (Figure 4B). Early lactation dams (d0L and d5L) had higher fed
373 GLP-1 values than in all the fasted and fed pregnant (except d12P fed, the third
374 highest time point) and other lactating dams (Figure 4A, C, D). There were no
375 significant differences in fasted plasma GLP-1 during lactation and amounts were
376 closer to proestrus controls (Figure 4D).

377 **Figure 4. Concentrations of GLP-1 in fed and fasted plasma throughout**
378 **pregnancy and lactation.**

379

380 **Colon tissue**

381 GLM analysis found significant main (direct) effects on GLP-1 concentrations of
382 colon region (ascending/descending) $F(1,104)=43.446$, $P=0.000$) and stage
383 (proestrus, pregnancy or lactation time-points; $F(7,104)=7.551$, $P=0.000$) and a
384 significant interaction (joint effect) of colon region with reproductive stage
385 ($F(7,104)=2.637$, $P=0.016$).

386 A comparison between different areas of the colon found that ascending colon
387 concentrations of GLP-1 were significantly higher than in descending colon tissue
388 during all pregnancy time points (Figure 5A, 5C; d4P $t(6)=5.224$, $P=0.002$; d12P

389 $t(6)=4.466$, $P=0.004$; d18P $t(6)=4.234$, $P=0.005$). During lactation, ascending colon
390 GLP-1 was higher than descending colon (Figure 5C, 5D) at d0L ($t(6)=3.651$,
391 $P=0.01$) and d10L ($t(6)=4.369$, $P=0.005$), therefore GLP-1 concentrations in different
392 regions of the colon were only similar at d5L, due to a possible transient rise in
393 descending colon; and they were also equivalent at d25L when GLP-1 in both tissue
394 regions had reduced to close to control values.

395 Within ascending colon, GLP-1 concentrations were significantly different between
396 the pregnant dams ($F(2, 18)=3.919$, $P=0.039$; Figure 5A) and d18P dams had twice
397 the concentration of ascending colon GLP-1 than day 12 pregnant (d12P) dams,
398 although this did not reach significance with ANOVA post hoc tests ($P=0.051$, n.s.).
399 Descending colon tissue concentrations of GLP-1 were not significantly different with
400 pregnancy stage, but were approximately two to four times higher than in proestrus
401 controls (Figure 5C).

402 Ascending colon GLP-1 concentrations decreased as lactation progressed and were
403 significantly ($F(3, 22)=4.164$, $P=0.018$) higher in d0L dams than in d25L dams
404 ($P=0.016$; Figure 5B). GLP-1 concentrations in descending colon tissue were
405 significantly different ($F(3, 22)=4.493$, $P=0.013$) between the dam groups, and were
406 highest at d0L and d5L before a sharp decrease by d10L ($P=0.020$), with a
407 significant decrease also found between d0L and d10L ($P=0.030$) dams (Figure 5D).

408 Descending colon GLP-1 concentrations were higher at d0L and d5L ($P=0.001$;
409 $P=0.036$) than in the proestrus controls.

410 GLM analysis confirmed that GLP-1 concentrations in late pregnancy (d18P)
411 ascending colon (Figure 5A) were higher than all other time-points in both regions of
412 colon ($P=0.027-0.000$; Figure 5B, C, D) and d10L DC concentrations (Figure 5D)

413 were lower than all the pregnant AC concentrations ($P=0.047-0.000$; Figure 5A), as
414 well as d0 and d5L AC ($P=0.016-0.000$; Figure 5B).

415

416 **Figure 5. Concentrations of GLP-1 in ascending and descending colon**
417 **throughout pregnancy and lactation**

418

419 **Peripartum food intake**

420 The four dams that could be monitored continuously for the most consecutive days
421 around birth, halved their food intake from day 3 to day 1 prior to birth (Figure 6).

422 Food intake rapidly increased into the lactation period, doubling from d1L to d2L.

423 Food intake continued to increase and was highest at d8L, when monitoring stopped.

424 The d8L dams consumed approximately 250% more than the mean daily food intake
425 of all of the normally cycling nulliparous females (15.5 ± 0.09 g, $n=43$) used in a prior
426 study (Johnson *et al.*, 2016).

427

428 **Figure 6. Food intake during the late peri- and early postpartum period**

429

430 **Changes in body and gastrointestinal size during pregnancy and**
431 **lactation**

432 As expected, body mass significantly increased ($F(2, 18)=12.565$, $P<0.001$) with the
433 advancing stages of pregnancy until birth (Figure 7A). Body mass gain continued

434 during lactation ($F(2, 18)=12.942$, $P<0.001$; analysis excluding d0L as fed mass

435 included gravid uterus; Figure 7B) and body mass exceeded that of proestrus control

436 rats ($F(7,47)=16.208$, $P<0.001$) by d10L ($P=0.038$). Mass of abdominal cavity white

437 adipose tissue (WAT) was significantly largest ($F(2, 18)=6.248$, $P=0.009$; Figure 7C)
438 by d18P, and showed a significant decline after d0L ($F(3, 24)=13.899$, $P<0.001$;
439 Figure 7D) until the end of lactation. Day 18 pregnant dams had higher abdominal
440 WAT values than proestrus nulliparous controls, while control rats had up to three
441 times more WAT ($F(7,49)=10.495$, $P<0.001$) than rats at day 10 and day 25 of
442 lactation ($P<0.009$; $P<0.001$; Figure 7C and D).

443

444 **Figure 7. Changes in body mass and abdominal white adipose tissue mass**
445 **throughout pregnancy and lactation**

446

447 Stomach and caecum masses only increased during lactation, peaking
448 d10L

449 Stomach tissue wet mass did not change throughout pregnancy and remained
450 similar to the controls (Figure 8A). During lactation, stomach mass significantly
451 increased (Kruskal Wallis, $\chi^2=15.015$, 3 df, $P=0.002$), becoming heavier than early
452 pregnancy time points and control values by d10L, and d25L dams had significantly
453 heavier stomachs than d0L ($P=0.018$) and d5L ($P=0.002$) dams (Figure 8b) and all
454 pregnant groups ($F(7,48)=12.648$, $P<0.001$).

455 Similarly, caecum wet mass did not change significantly during pregnancy (Figure
456 8C), but was significantly ($F(3, 24)=40.888$, $P<0.001$) heavier in both the d10L and
457 d25L dams than in the d0L ($P<0.001$) and d5L ($P<0.001$) dams (Figure 8D) and all
458 pregnant groups ($F(7,48)=12.648$, $P<0.001$). Additionally, d5L dam caecum mass
459 was heavier than in d4P animals ($P=0.013$).

460 **Figure 8. Stomach mass and caecum mass throughout pregnancy and**
461 **lactation**

462

463 Small intestine mass and length increased during pregnancy and
464 lactation, peaking by d10L

465 Late pregnant, d18P dams had significantly heavier ($F(2, 18)=4.782, P=0.022$;
466 Figure 9A) and longer ($F(2, 18)=5.365, P=0.015$; Figure 9C) small intestines than
467 d4P dams. Further growth continued during lactation: d5L dams had significantly
468 heavier small intestines than d0L ($P=0.022$) dams, and in mid-lactation, d10L dams
469 had heavier ($F(2,18)=39.220, P<0.001$) small intestines than both d0L ($P<0.001$) and
470 d5L ($P<0.001$) dams (Figure 9B); there were no data for d25L (see methods). The
471 small intestine also significantly increased in length in later lactation ($F(3,$
472 $24)=17.944, P<0.001$; Figure 9D), being significantly longer in both d10L dams
473 (where peak growth had been reached) and d25L dams, than in d0L ($P<0.001$) and
474 d5L ($P<0.001$) dams. Day 10 and 25 lactation dams had approximately 48% longer
475 small intestines than proestrus controls, d0L and d5L dams and all pregnant groups
476 ($F(7,49)=20.546, P<0.001$).

477 Large intestine mass increased during pregnancy and lactation, with
478 length only increased by late lactation, peaking d25L

479 Large intestines of d18P dams were significantly heavier ($F(2, 18)=7.931, P=0.003$;
480 Figure 9E) than the d4P ($P=0.003$) and d12P ($P=0.046$) groups, although they were
481 significantly ($F(2, 18)=5.506, P=0.014$) shorter in d12P dams than in d4P ($P=0.044$)
482 and d18P ($P=0.017$) dams (Figure 9G). Dams at the end of the lactation period (d25L)
483 had significantly ($F(3, 19)=19.322, P<0.001$) heavier large intestine tissue than d0L

484 ($P<0.001$), d5L ($P<0.001$) and d10L ($P=0.001$) dams (Figure 9F) and all pregnant dam
485 groups ($F(7,47)=11.104$, $P<0.001$). Dams also had significantly shorter ($F(3,$
486 $24)=15.519$, $P<0.001$) large intestines at d0L ($P<0.001$) and d5L ($P=0.001$) compared
487 to d25L dams, with d0L dams also having shorter large intestines than d10L ($P=0.004$)
488 dams (Figure 9H). Both the pregnant dams and d5L dams had similar large intestine
489 lengths to the proestrus controls (Figure 9G, H).

490

491 **Figure 9. Changes in small and large intestine sizes throughout pregnancy and**
492 **lactation**

493

494 Gut circumferences were greater in pregnancy and lactation, peaking by
495 d10L

496 Gut circumference measurements for duodenum and ascending colon were greater in
497 the pregnant dams, significantly at d4P ($F(7,49)=4.108$; 5.906 , $P<0.001$) compared
498 with proestrus controls, with only descending colon values similar (Figure 10A).
499 Between the different pregnant groups, neither the duodenum nor descending colon
500 tissue circumferences differed, however, ascending colon circumference was
501 significantly wider ($F(2, 18)=3.953$, $P=0.038$) in early pregnant d4P dams compared
502 with d12P dams ($P=0.033$), which coincided with reduced large intestine length in
503 d12P rats. d4P and d18P rats also had wider AC values than d25L rats (Figure 10 A,
504 B).

505 Lactating dams at d10 had larger duodenum circumferences than proestrus controls
506 (Figure 10B), at the same time as the small and large intestine growth increases at

507 d10L (Figure 9). By late lactation/weaning duodenum circumference ($F(3, 24)=3.052$,
508 $P=0.048$) had significantly reduced by d25L from the earlier peak at d10L ($P=0.046$).
509 Ascending colon circumference ($F(3, 24)=6.506$, $P=0.002$) was also significantly
510 increased in early, compared to late lactation: d0L ($P=0.045$), d5L ($P=0.035$), and
511 peaked in d10L ($P=0.001$) dams compared to d25L dams and proestrus controls
512 (Figure 10B). The reduction in late lactation coincided with peak large intestine
513 increases (Figure 9).
514 Circumference of descending colon ($F(3, 24)=4.346$, $P=0.014$) was also significantly
515 smaller in d25L than in d10L ($P=0.012$) dams. Both ascending and descending
516 circumferences at d25L were reduced compared to earlier in lactation and were also
517 smaller than proestrus controls – this coincided with the large intestine peak mass and
518 length increases at d25L.

519 **Figure 10. Changes in gut circumference throughout pregnancy and lactation**

520

521 **Discussion**

522 This is the first study to analyse total orexigenic ghrelin, anorexigenic PYY and GLP-
523 1, in matched fed and fasted plasma and gut tissues, with samples taken during the
524 nocturnal, active phase. We provide new and updated information about organ
525 remodelling in dams, including changes to GI capacity and tissue sizes, matched
526 with peripherally circulating and tissue concentrations of ghrelin, PYY and GLP-1.
527 Ghrelin stimulates appetite, thus is expected to increase during pregnancy. However,
528 fasted plasma ghrelin was decreased between d4P and d18P, consistent with animal
529 (Shibata *et al.*, 2004) and human studies (Fuglsang *et al.*, 2005, Tham *et al.*, 2009).
530 Suppression of this appetite-stimulating signal occurred throughout pregnancy for

531 reasons unknown, and despite ghrelin-secreting cell increases by d12P compared
532 with proestrus controls that continued until the onset of lactation. Unknown
533 mechanisms underlie peripheral ghrelin suppression during early pregnancy,
534 although increasing leptin may be involved later. What initiates and supports early
535 pregnancy hyperphagia if the only peripheral orexigenic hormone is not involved?
536 These observations caution against endogenous appetite hormone use as body
537 mass control therapies during pregnancy until established that alterations are not
538 harmful to developing embryos.

539 Reduction in food intake before birth followed low systemic ghrelin at d18P, and the
540 100% increase post-partum occurred after peak ghrelin-IR cells and the highest
541 stomach ghrelin measured. Fasted plasma ghrelin increased from early to d25
542 lactation; dams had 20% higher than proestrus controls. Shibata *et al.* (2004)
543 reported no changes to fed plasma ghrelin in rats between d5L and d15L, and
544 Suzuki *et al.* (2014) likewise found no difference in fed acyl ghrelin. The plasma
545 differences we report are novel and arguably more reliable, as samples were both
546 fasted and taken in the more physiologically-relevant dark phase. Late lactation
547 ghrelin increases could be explained by decreased WAT/reduced leptin (Woodside
548 *et al.*, 2000, Taylor *et al.*, 2009), which in turn could unsuppress circulating ghrelin
549 (Ueno *et al.*, 2004). Dams still need to eat more to replenish energy reserves
550 following weaning (very low WAT masses d25L), despite body mass increases (see
551 later discussion). Dams had larger stomach masses by lactation end, with potentially
552 more ghrelin-secretory cells following the measured increase at parturition,
553 contributing to higher circulating ghrelin by d25L.

554 PYY was not altered during pregnancy, agreeing with Valsamakis *et al.* (2010),
555 although values were higher than non-pregnant. Fasted plasma PYY was 63%

556 higher than controls at d10L, demonstrating elevated peripheral concentrations,
557 following earlier peaks in d5L colon tissue. Other studies report plasma PYY fed and
558 fasted d5L peaks (Tovar *et al.*, 2004, Taylor *et al.*, 2009; Suzuki *et al.*, 2014).
559 Together, these studies indicate that fed/fasting status of *ad lib* fed dams does not
560 diminish observed PYY peaks. During lactation, AC and DC PYY peaked in d5L
561 dams, similar to d5L plasma peaks (Taylor *et al.*, 2009; Suzuki *et al.*, 2014). Despite
562 gut region/timing differences between studies, increases were consistent. It remains
563 to be established why a purported satiety hormone elevates during physiological
564 states of hyperphagia; some explanations relating to GI remodelling are explored
565 below.

566 GLP-1 is a satiety hormone and decreases in insulin-resistant states (Toft-Nielsen *et*
567 *al.*, 2001, Muscelli *et al.*, 2008, Lim *et al.*, 2009). Pregnancy-associated insulin
568 resistance could explain decreases in fasted plasma GLP-1 throughout pregnancy,
569 with lowest values by d18P. As with PYY, there were unexpected increases, as fed
570 GLP-1 was greatly elevated (25x) in pregnancy and highest in d18P AC tissue.
571 Likewise, fed GLP-1 was also very high with d0 and d5 peaks in lactation; the main
572 source of circulating GLP-1 was likely AC, as DC was very low after d5L. In contrast,
573 human studies found no GLP-1 lactational changes (Larson-Meyer *et al.*, 2016).

574 The current study found both PYY and GLP-1, so-called 'satiety' hormones, to be
575 increased, with high values persistent in lactation despite food intake increasing 199%
576 by d5L. High sustained PYY and GLP-1 may initiate the ileal brake mechanism (Lin *et*
577 *al.*, 1996; Maljaars *et al.*, 2008), against a low ghrelin background and reduced gastric
578 emptying (Levin *et al.*, 2006), thus slowing gut transit times to allow digestion and
579 nutrient extraction from increased feed. The currently accepted role of PYY and GLP-

581 reduction but the opposite occurred in pregnant and lactating dams, despite
582 hyperphagia (except briefly at parturition).

583 This study also investigated physical gut changes to help explain contradictory
584 'appetite' hormone observations. Following birth, dam body mass increased although
585 WAT reserves decreased, reflecting body composition changes. We explored GI tract
586 remodelling and have described a number of modifications that show how dam
587 physiology altered to accommodate lactational demands of eight growing pups.
588 Neither stomach nor caecum mass changed across pregnancy, but wet masses
589 increased by end of lactation, consistent with previous studies (Cripps & Williams.
590 1975; Taylor *et al.*, 2009). Small intestine wet weight and length increased between
591 d4P and d18P, by 20% and 15%. This increase in size and capacity could be an early,
592 rapid adaptation to increase absorption of nutrients from more food, to support the
593 production of reproductive tissues; also building reserves for lactation. Lactating dams
594 had further increased small intestine wet weight and length by d10L, 50% longer than
595 non-pregnant controls. Large intestine mass increased with pregnancy and was even
596 heavier by d25L, in agreement with Cripps & Williams (1975). Any variation in adaptive
597 changes between studies are likely due to differences in diet composition and food
598 quantities consumed. In our study, there was consistency of timing of SI and LI tissue
599 expansion with increased stomach and caecum masses, although SI increases
600 peaked earlier at d10 of lactation, whereas LI growth continued to d25L.

601 Gut circumferences provided further novel information. Although neither duodenum
602 nor DC were different between pregnancy stages, AC circumferences were. Changes
603 along the GI tract could be an additional mechanism to support pregnancy/lactation,
604 increasing surface area and gut capacity to process nutrients from more food, altering
605 transit times. Day 4P dams had largest AC circumferences, with higher values in

606 duodenum and DC, compared to controls. These data may reflect the earliest
607 pregnancy adaptation to rapidly increase capacity, especially of caecum to hold more
608 food (as microbiome composition changes: Mann *et al.*, 2018), prior to gut lengthening
609 later in pregnancy, further increasing surface area. In early pregnancy there are less
610 competing demands for space between gravid uterus and abdominal organs, making
611 temporary expansion possible.

612 In lactating dams, duodenum, AC and DC had increased circumferences leading up
613 to d10L peaks, with decreases from d10L to d25L. Narrowing of colon circumferences
614 coincided with maximal tissue hypertrophy – increased lengths and masses – and
615 capacity, which may reflect final adjustments to maximal feed intakes during lactation.
616 Thus, in addition to later gut hypertrophy, early maternal adaptations to hyperphagia
617 included widening/dilation of specific portions of GI tract to accommodate greater
618 volumes of food and aid nutrient acquisition. In lactating rats on restricted diets,
619 Campbell and Fell (1964) found SI was similarly dilated, with only partial hypertrophy
620 compared to *ad lib* fed. That study also reported that the absorptive capacity of SI did
621 not differ between nulliparous and lactating rats with varying gut hypertrophy,
622 suggesting changes are proportionate to requirements. Datta *et al.* (1995) also
623 reported that food restriction prevented SI hypertrophy and Tovar *et al.* (2004) found
624 a 30% food restriction completely suppressed pregnancy-associated PYY rises.
625 Combined, these findings indicate both physical (food mass) and hormonal (PYY/GLP-
626 1) stimulation are needed to initiate and maintain gut growth, as has been expounded
627 in this study, where extensive organ remodelling, with increases in SI and LI masses
628 and lengths, occurred during later lactation, coincident with elevations of and following
629 PYY and GLP-1 peak concentrations that had occurred earlier in lactation.

630 PYY and GLP-1 are co-secreted, with GLP-2 (Mojsov *et al.*, 1986); and have
631 previously been linked with gut growth in adult female mice (PYY: Gomez *et al.*, 1995),
632 male mice (Drucker *et al.*, 1996) and rats (GLP-2 > GLP-1: Ghatei *et al.*, 2001). PYY
633 and GLP-2 cause substantial intestinal hypertrophy, with smaller GLP-1 increases.
634 Our findings of gut hypertrophy under conditions of high PYY and very high GLP-1
635 concentrations highlight that it is imperative to further elucidate the role of L-cells and
636 their secretory products after gut surgeries for body mass reduction, as these
637 techniques lead to rapidly increased concentrations of appetite hormones
638 (Chandarana *et al.*, 2011) and may be stimulating intestinal growth in attempts to
639 regenerate remaining gut tissues (le Roux *et al.*, 2010).

640 This study has revealed that when maternal nutritional requirements are highest,
641 additional and unexpected supportive changes to increased intake occur in 'appetite'
642 hormones PYY and GLP-1. These hormonal alterations are likely to be homeorhetic
643 adjustments (Bauman and Currie, 1980) to pregnancy, and especially lactation, that
644 have a more pronounced influence on GI remodelling, than satiety. The observed
645 structural changes contribute towards meeting dam enhanced energy requirements
646 and maximising nutrient recovery from the increased intake of pregnancy and more
647 extreme hyperphagia of lactation. They may aid feeding efficiency via altered
648 metabolism and nutrient uptake, slowing food passage rate (Hammond, 1997) and
649 facilitate caecum microbe diversity changes (Mann *et al.*, 2018). Our observations
650 show how gut adaptations continue until (and possibly persist beyond) weaning, in
651 rat dams that raise litters of 8 pups.

652 In conclusion, despite hyperphagia, fasted plasma ghrelin was suppressed in
653 pregnancy, although ghrelin-IR cells and stomach ghrelin were highest at birth,
654 supporting onset of lactation-associated hyperphagia. Plasma fed GLP-1 was

655 elevated during pregnancy, and increases of colon PYY and GLP-1 during early
656 lactation were associated with GI expansion, not satiety. All three 'appetite'
657 hormones were altered in unexpected ways, with important implications for any
658 surgical or pharmaceutical interventions designed to act as weight-control measures,
659 in reproductive age females. Extensive stomach, caecum and gut expansion and
660 remodelling coincided with PYY and GLP-1 peaks. Then increased intestinal
661 masses, lengths and circumferences followed, with peaks at d10L for SI and d25L
662 for LI. These modifications demonstrate how lactating rats process and assimilate
663 more food to support eight pups to weaning. More important questions arise,
664 including what implications may be for future maternal health if GI expansion
665 persists, as modifications could accelerate future nutrient assimilation and storage,
666 leading to long-term body mass retention. Also, whether the increased incidence of
667 obesity and insulin resistance in younger human populations may additionally
668 amplify maternal adaptations, thus influencing metabolic programming and future
669 health of any offspring.

670 **Declaration of interest**

671 VJT served on the Council of Management for the Society for Reproduction and
672 Fertility (SRF) 2013-16. MLJ was Postdoc representative on SRF Council of
673 Management 2017-19. MLJ was the winning recipient of the SRF Post Doctoral
674 Scientist Prize talk based on this work: Johnson ML, Saffrey MJ, Taylor VJ (2015)
675 *Hyperphagia of pregnancy and lactation is associated with changes in appetite-*
676 *regulating hormones and gastrointestinal modifications in Wistar rats.* Society for
677 Reproduction and Fertility Annual Conference 2015, 20-22 July 2015, St Catherine's
678 College, Oxford, UK. A repeat talk was given at the Annual Meetings of the

679 Endocrine Society of Australia and Society for Reproductive Biology (SRB) and
680 Australia and New Zealand Bone and Mineral Society 2016, 21-24 August 2016,
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692 **References**

- 693 Bauman DE & Currie WB 1980 Partitioning of nutrients during pregnancy and
694 lactation: a review of mechanisms involving homeostasis and homeorhesis.
695 *Journal of Dairy Science* **63** 1514-1529.
- 696 Boyne R, Fell BF & Robb I 1966 The surface area of the intestinal mucosa in the
697 lactating rat. *Journal of Physiology* **183** 570-575.
- 698 Brubaker PL, Izzo A, Hill M & Drucker DJ 1997 Intestinal function in mice with small
699 bowel growth induced by glucagon-like peptide-2. *American Journal of*
700 *Physiology* **272** E1050-1058.

- 701 Burdett K & Reek C 1979 Adaptation of the small intestine during pregnancy and
702 lactation in the rat. *Biochemistry Journal* **184** 245-251.
- 703 Campbell RM & Fell BF 1964 Gastro-Intestinal Hypertrophy in the Lactating Rat and
704 Its Relation to Food Intake. *Journal of Physiology* **171** 90-97.
- 705 Chandarana K, Gelegen C, Karra E, Choudhury AI, Drew ME, Fauveau V, Viollet B,
706 Andreelli F, Withers DJ & Batterham RL 2011 Diet and gastrointestinal
707 bypass–induced weight loss. *Diabetes* **60** 810-818.
- 708 Crean GP & Rumsey RD 1971 Hyperplasia of the gastric mucosa during pregnancy
709 and lactation in the rat. *Journal of Physiology* **215** 181-197.
- 710 Cripps AW & Williams VJ 1975 The effect of pregnancy and lactation on food intake,
711 gastrointestinal anatomy and the absorptive capacity of the small intestine in
712 the albino rat. *British Journal of Nutrition* **33** 17-32.
- 713 Datta UK, Datta AN & Mukherjee S 1995 Role of hyperphagia in structural changes
714 of small intestine during lactation. *Indian Journal of Physiology and*
715 *Pharmacology* **39** 259-262.
- 716 Denis RG, Bing C, Brocklehurst S, Harrold JA, Vernon RG & Williams G 2004
717 Diurnal changes in hypothalamic neuropeptide and SOCS-3 expression:
718 effects of lactation and relationship with serum leptin and food intake. *Journal*
719 *of Endocrinology* **183** 173-181.
- 720 Drucker DJ, Erlich P, Asa SL & Brubaker PL 1996 Induction of intestinal epithelial
721 proliferation by glucagon-like peptide 2. *Proceedings of the National Academy*
722 *of Sciences USA* **93** 7911-7916.
- 723 Feder HH 1981 Estrous Cyclicity in Mammals. In *Neuroendocrinology of*
724 *Reproduction: Physiology and Behavior*, pp. 279-348. ED Adler NT. Boston,
725 MA: Springer US.

726 Fuglsang J, Skjaerbaek C, Espelund U, Frystyk J, Fisker S, Flyvbjerg A & Ovesen P
727 2005 Ghrelin and its relationship to growth hormones during normal
728 pregnancy. *Clinical Endocrinology (Oxford)* **62** 554-559.

729 Ghatei MA, Goodlad RA, Taheri S, Mandir N, Brynes AE, Jordinson M & Bloom SR
730 2001 Proglucagon-derived peptides in intestinal epithelial proliferation:
731 glucagon-like peptide-2 is a major mediator of intestinal epithelial proliferation
732 in rats. *Digestive Diseases and Science* **46** 1255-1263.

733 Gomez G, Zhang T, Rajaraman S, Thakore KN, Yanaihara N, Townsend CM,
734 Thompson JC & Greeley GH 1995 Intestinal Peptide YY - Ontogeny of Gene-
735 Expression in Rat Bowel and Trophic Actions on Rat and Mouse Bowel.
736 *American Journal of Physiology-Gastrointestinal and Liver Physiology* **268**
737 G71-G81.

738 Hammond KA 1997 Adaptation of the maternal intestine during lactation. *Journal of*
739 *Mammary Gland Biology and Neoplasia* **2** 243-252.

740 Johnson ML, Saffrey MJ & Taylor VJ 2016 Plasma Ghrelin Concentrations Were
741 Altered with Oestrous Cycle Stage and Increasing Age in Reproductively
742 Competent Wistar Females. *PLOS ONE* **11** e0166229. doi:10.1371/journal.
743 pone.0166229

744 Larson-Meyer DE, Ravussin E, Heilbronn L & DeJonge L 2010 Ghrelin and peptide
745 YY in postpartum lactating and nonlactating women. *American Journal of*
746 *Clinical Nutrition* **91** 366-372.

747 Larson-Meyer DE, Schueler J, Kyle E, Austin KJ, Hart AM & Alexander BM 2016 Do
748 Lactation-Induced Changes in Ghrelin, Glucagon-Like Peptide-1, and Peptide
749 YY Influence Appetite and Body Weight Regulation during the First
750 Postpartum Year? *Journal of Obesity* **2016** 1-11.

751 le Roux CW, Borg C, Wallis K, Vincent RP, Bueter M, Goodlad R, Ghatei MA, Patel
752 A, Bloom SR & Aylwin SJB 2010 Gut Hypertrophy After Gastric Bypass Is
753 Associated With Increased Glucagon-Like Peptide 2 and Intestinal Crypt Cell
754 Proliferation. *Annals of Surgery* **252** 50-56.

755 Levin F, Edholm T, Schmidt PT, Grybäck P, Jacobsson H, Degerblad M, Höybye C,
756 Holst JJ, Rehfeld JF, Hellström PM *et al.* 2006 Ghrelin stimulates gastric
757 emptying and hunger in normal-weight humans. *Journal of Clinical*
758 *Endocrinology and Metabolism* **91** 3296-3302.

759 Lim GE, Huang GJ, Flora N, LeRoith D, Rhodes CJ & Brubaker PL 2009 Insulin
760 regulates glucagon-like peptide-1 secretion from the enteroendocrine L cell.
761 *Endocrinology* **150** 580-591.

762 Lin HC, Zhao XT, Wang L & Wong H 1996 Fat-induced ileal brake in the dog
763 depends on peptide YY. *Gastroenterology* **110** 1491-1495.

764 Lopez-Luna P, Maier I & Herrera E 1991 Carcass and tissue fat content in the
765 pregnant rat. *Biology of the Neonate* **60** 29-38.

766 Maljaars PWJ, Peters HPF, Mela DJ & Masclee AAM 2008 Ileal brake: A sensible
767 food target for appetite control. A review. *Physiology & Behavior* **95** 271-281.

768 Mann PE, Huynh K & Widmer G 2018 Maternal high fat diet and its consequence on
769 the gut microbiome: A rat model. *Gut Microbes* **9** 143-154.

770 Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L & Habener JF 1986
771 Preproglucagon gene expression in pancreas and intestine diversifies at the
772 level of post-translational processing. *Journal of Biological Chemistry* **261**
773 11880-11889.

774 Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, Holst JJ &
775 Ferrannini E 2008 Separate impact of obesity and glucose tolerance on the

776 incretin effect in normal subjects and type 2 diabetic patients. *Diabetes* **57**
777 1340-1348.

778 Naismith DJ, Richardson DP & Pritchard AE 1982 The utilization of protein and
779 energy during lactation in the rat, with particular regard to the use of fat
780 accumulated in pregnancy. *British Journal of Nutrition* **48** 433-441.

781 Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K & Matsukura S
782 2001 A role for ghrelin in the central regulation of feeding. *Nature* **409** 194-
783 198.

784 Outeirino-Iglesias V, Romani-Perez M, Gonzalez-Matias LC, Vigo E & Mallo F 2015
785 GLP-1 Increases Preovulatory LH Source and the Number of Mature Follicles,
786 As Well As Synchronizing the Onset of Puberty in Female Rats.
787 *Endocrinology* **156** 4226-4237.

788 Penzes L & Regius O 1985 Changes in the intestinal microvillous surface area
789 during reproduction and ageing in the female rat. *Journal of Anatomy* **140 (Pt**
790 **3)** 389-396.

791 Pujol E, Proenza AM, Roca P & Llado I 2006 Changes in mammary fat pad
792 composition and lipolytic capacity throughout pregnancy. *Cell Tissue*
793 *Research* **323** 505-511.

794 Reiff T, Jacobson J, Cognigni P, Antonello Z, Ballesta E, Tan KJ, Yew JY,
795 Dominguez M & Miguel-Aliaga I 2015 Endocrine remodelling of the adult
796 intestine sustains reproduction in *Drosophila*. *Elife* **4** e06930.

797 Ronveaux CC, de Lartigue G & Raybould HE 2014 Ability of GLP-1 to decrease food
798 intake is dependent on nutritional status. *Physiology & Behaviour* **135** 222-
799 229.

800 Shibata K, Hosoda H, Kojima M, Kangawa K, Makino Y, Makino I, Kawarabayashi T,
801 Futagami K & Gomita Y 2004 Regulation of ghrelin secretion during
802 pregnancy and lactation in the rat: possible involvement of hypothalamus.
803 *Peptides* **25** 279-287.

804 Speakman JR 2008 The physiological costs of reproduction in small mammals.
805 *Philosophical Transactions Royal Society London B Biological Sciences* **363**
806 375-398.

807 Stengel A, Keire D, Goebel M, Evilevitch L, Wiggins B, Tache Y & Reeve JR 2009
808 The RAPID Method for Blood Processing Yields New Insight in Plasma
809 Concentrations and Molecular Forms of Circulating Gut Peptides.
810 *Endocrinology* **150** 5113-5118.

811 Stramek A, Johnson ML, Taylor VJ 2018 Improved timed-mating, non-invasive
812 method using fewer unproven female rats with pregnancy validation via early
813 body mass increases. *Laboratory Animals*
814 <https://doi.org/10.1177/0023677218774076>.

815 Suzuki Y, Nakahara K, Maruyama K, Okame R, Ensho T, Inoue Y & Murakami N
816 2014 Changes in mRNA expression of arcuate nucleus appetite-regulating
817 peptides during lactation in rats. *Journal of Molecular Endocrinology* **52** 97-
818 109.

819 Taylor VJ, Patterson M, Ghatei MA, Bloom SR & Wilson CA 2009 Ghrelin and
820 peptide YY (PYY) profiles in gastrointestinal tissues and the circulation of the
821 rat during pregnancy and lactation. *Peptides* **30** 2213-2220.

822 Tham E, Liu JH, Innis S, Thompson D, Gaylinn BD, Bogarin R, Haim A, Thorner MO
823 & Chanoine JP 2009 Acylated ghrelin concentrations are markedly decreased
824 during pregnancy in mothers with and without gestational diabetes:

825 relationship with cholinesterase. *American Journal of Physiology-*
826 *Endocrinology and Metabolism* **296** E1093-E1100.

827 Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK &
828 Holst JJ 2001 Determinants of the impaired secretion of glucagon-like
829 peptide-1 in type 2 diabetic patients. *Journal of Clinical Endocrinology and*
830 *Metabolism* **86** 3717-3723.

831 Tovar SA, Seoane LM, Caminos JE, Nogueiras R, Casanueva FF & Dieguez C 2004
832 Regulation of peptide YY levels by age, hormonal, and nutritional status.
833 *Obesity Research* **12** 1944-1950.

834 Trujillo ML, Spuch C, Carro E & Senaris R 2011 Hyperphagia and central
835 mechanisms for leptin resistance during pregnancy. *Endocrinology* **152** 1355-
836 1365.

837 Ueno N, Dube MG, Inui A, Kalra PS & Kalra SP 2004 Leptin modulates orexigenic
838 effects of ghrelin and attenuates adiponectin and insulin levels and selectively
839 the dark-phase feeding as revealed by central leptin gene therapy.
840 *Endocrinology* **145** 4176-4184.

841 Valsamakis G, Margeli A, Vitoratos N, Boutsiadis A, Sakkas EG, Papadimitriou G,
842 Al-Daghri NM, Botsis D, Kumar S, Papassotiriou I, *et al.* 2010 The role of
843 maternal gut hormones in normal pregnancy: fasting plasma active glucagon-
844 like peptide 1 level is a negative predictor of fetal abdomen circumference and
845 maternal weight change. *European Journal of Endocrinology* **162** 897-903.

846 Woodside B, Abizaid A & Walker C 2000 Changes in leptin levels during lactation:
847 implications for lactational hyperphagia and anovulation. *Hormones and*
848 *Behavior* **37** 353-365.

849

850 **Figure 1. Concentrations of ghrelin in fasted plasma and stomach tissue**
851 **throughout pregnancy and lactation**

852 Fasted plasma ghrelin during (A) pregnancy (* $P<0.05$; $a>b$, *** $P<0.001$) and (B)
853 lactation (* $P<0.05$); GLM: ($a>b$, $P<0.001$); stomach tissue ghrelin during (C)
854 pregnancy ($c>d$, $P<0.05$) and (D) lactation (* $P<0.05$); GLM: ($a>b$, $P<0.001$, $c>d$,
855 $P<0.05$). (d4P, day 4 pregnant, $n=7$; d12P, day 12 pregnant, $n=7$; d18P, day 18
856 pregnant, $n=7$; d0L, day 0 of lactation, $n=7$; d5L, day 5 of lactation, plasma, $n=7$;
857 stomach tissue, $n=5$; d10L, day 10 of lactation, $n=7$; d25L, day 25 of lactation, $n=7$.
858 Dotted line represents mean of proestrus controls, $n=6$).

859

860 **Figure 2. Representative images of stomach tissue (A-C) showing the**
861 **distribution of ghrelin immunoreactive staining and (D) quantification of**
862 **stomach tissue ghrelin cell density at proestrus, d12P and day of birth (d0L)**

863 L, luminal/mucosal surface; M, mucosal-submucosal border (proestrus, $n=6$; d12P,
864 day 12 of pregnancy, $n=7$; d0L, day 0 of lactation, $n=7$. ** $P<0.01$, *** $P<0.001$).

865

866 **Figure 3. Concentrations of PYY in fasted plasma, ascending and descending**
867 **colon tissues throughout pregnancy and lactation**

868 Fasted plasma PYY in (A) pregnancy; and (B) lactation ($a>b$, $P<0.005$); ascending
869 colon PYY during (C) pregnancy; and (D) lactation (* $P<0.05$); GLM: ($c>d$, $P<0.025$);
870 descending colon PYY during (E) pregnancy; and (F) lactation ($c>d$, $P<0.025$). GLM:
871 ($c>d$, $P<0.025$). (d4P, day 4 pregnant, $n=7$; d12P, day 12 pregnant, $n=7$; d18P, day
872 18 pregnant, $n=7$; d0L, day 0 of lactation, $n=7$; d5L, day 5 of lactation, $n=7$; d10L,
873 day 10 of lactation, $n=7$; d25L, day 25 of lactation, fed plasma, $n=6$; fasted plasma,
874 $n=7$. Dotted line represents mean of proestrus controls, $n=6$; no control data for AC).

875

876 **Figure 4. Concentrations of GLP-1 in fed and fasted plasma throughout**
877 **pregnancy and lactation.**

878 GLP-1 in fed plasma during (A) pregnancy, ($c>d$, $P<0.025$); and (B) lactation (*
879 $P<0.05$); GLM: ($a>b$, $P<0.001$; $e>f$, $P<0.05$); and GLP-1 in fasted plasma during (C)
880 pregnancy ($a>b$, $P<0.001$); and (D) lactation; GLM: ($a>b$, $P<0.001$; $c>d$, $P<0.025$);
881 note y axes differences between Figures (C) and (A), (B) and (D). (d4P, day 4
882 pregnant, $n=7$; d12P, day 12 pregnant, $n=7$; d18P, day 18 pregnant, $n=7$; d0L, day 0
883 of lactation, $n=7$; d5L, day 5 of lactation, $n=5$; d10L, day 10 of lactation, $n=7$; d25L,
884 day 25 of lactation, fed plasma, $n=6$; fasted plasma, $n=7$. Dotted line represents
885 mean of proestrus controls, $n=6$.)

886

887 **Figure 5. Concentrations of GLP-1 in ascending and descending colon**
888 **throughout pregnancy and lactation**

889 Ascending colon GLP-1 concentrations during (A) pregnancy and (B) lactation (*
890 $P<0.05$); descending colon GLP-1 during (C) pregnancy; (D) lactation (* $P<0.05$, $e>f$,
891 $P<0.05$); t -tests: pregnancy ($a>b$, $P<0.01$); lactation ($a>b$, $P<0.01$); GLM: ($c>d$,
892 $P<0.03$; $e>f$, $P<0.05$); (d4P, day 4 pregnant, $n=7$; d12P, day 12 pregnant, $n=7$; d18P,
893 day 18 pregnant, $n=7$; d0L, day 0 of lactation, $n=7$; d5L, day 5 of lactation, $n=5$;
894 d10L, day 10 of lactation, $n=7$; d25L, day 25 of lactation, $n=7$. Dotted line represents
895 mean of proestrus controls, $n=6$.)

896

897 **Figure 6. Food intake during the late peri- and early postpartum period**

898 Dam food intake from 3 days PP until day 8 of lactation (d3PP-d1PP, days 3-1
899 peripartum; d0L-d8L, days 0 to 8 of lactation; n=4. Dotted line represents mean of
900 proestrus controls, n=6).

901

902 **Figure 7. Changes in body mass and abdominal white adipose tissue mass**
903 **throughout pregnancy and lactation**

904 Changes in fed body mass during (A) pregnancy (** $P<0.01$, *** $P<0.001$) and (B)
905 lactation (** $P<0.01$, *** $P<0.001$; e>f, $P<0.05$); (fed state is shown to avoid effects of
906 short-term fast and d0L fed masses not shown as they include gravid uterus) and
907 dissected WAT mass during (C) pregnancy (* $P<0.05$; a>b, $P<0.001$) and (D)
908 lactation (** $P<0.01$, *** $P<0.001$; a>b, $P<0.001$; c>d, $P<0.01$). (d4P, day 4 pregnant,
909 n=7; d12P, day 12 pregnant, n=7; d18P, day 18 pregnant, n=7; d0L, day 0 of
910 lactation, n=7; d5L, day 5 of lactation, n=7; d10L, day 10 of lactation, n=7; d25L, day
911 25 of lactation, n=7. Dotted line represents mean of proestrus controls, n=6).

912

913 **Figure 8. Stomach mass and caecum mass throughout pregnancy and**
914 **lactation**

915 Changes in empty stomach mass during (A) pregnancy and (B) lactation (** $P<0.01$;
916 * $P<0.05$; a>b, $P<0.001$); a>b, $P<0.001$, c>d, $P<0.01$, e>f, $P<0.05$; and empty
917 caecum mass during (C) pregnancy and (D) lactation (*** $P<0.001$); a>b, $P<0.001$,
918 c>d, $P<0.02$. (d4P, day 4 pregnant, n=7; d12P, day 12 pregnant, n=7; d18P, day 18
919 pregnant, n=7; d0L, day 0 of lactation, n=7; d5L, day 5 of lactation, n=7; d10L, day
920 10 of lactation, n=7; d25L, day 25 of lactation, n=7. Dotted line represents mean of
921 proestrus controls, n=6; no data for caecum: (C), (D)).

922

923 **Figure 9. Changes in small and large intestine sizes throughout pregnancy and**
924 **lactation**

925 The mass (A, B, E, F) and length (C, D, G, H) of the small (A, B, C, D) and large (E,
926 F, G, H) intestines during pregnancy and lactation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$;
927 $a > b$, $P < 0.001$, $c > d$, $c > d$, $P < 0.01$, $e > f$, $P < 0.05$. There were no data for small intestine
928 masses at d25L or proestrus controls for small and large intestine masses. (d4P, day
929 4 pregnant, $n = 7$; d12P, day 12 pregnant, $n = 7$; d18P, day 18 pregnant, $n = 7$; d0L, day
930 0 of lactation, mass, $n = 4$; length, $n = 7$; d5L, day 5 of lactation, $n = 7$; d10L, day 10 of
931 lactation, $n = 7$; d25L, day 25 of lactation, mass, $n = 5$; length, $n = 7$. Dotted line
932 represents mean of proestrus controls, $n = 6$).

933

934 **Figure 10. Changes in gut circumference throughout pregnancy and lactation**

935 Changes in duodenum (D), ascending colon (AC) and descending colon (DC)
936 circumferences during (A) pregnancy and (B) lactation. * $P < 0.05$, ** $P < 0.01$; $a > b$, $a > b$,
937 $P < 0.001$, $c > d$, $P < 0.01$, $e > f$, $e > f$, $P < 0.05$. (d4P, day 4 pregnant, $n = 7$; d12P, day 12
938 pregnant, $n = 7$; d18P, day 18 pregnant, $n = 7$; d0L, day 0 of lactation, $n = 7$; d5L, day 5
939 of lactation, $n = 7$; d10L, day 10 of lactation, $n = 7$; d25L, day 25 of lactation, $n = 7$. Dotted
940 line represents mean of proestrus controls, $n = 6$).