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1 Programming of hypothalamic neuropeptide gene expression in rats by maternal
2 dietary protein content during pregnancy and lactation.

3 R.L.Cripps¹, M.S. Martin-Gronert¹, Z.A.Archer², C.N.Hales^{1*}, J.G.Mercer² and
4 S.E.Ozanne¹.

5 ¹Metabolic Research Laboratories, Level 4, Institute of Metabolic Science
6 Box 289, Addenbrooke's Hospital, Cambridge, CB2 0QQ, UK

7 ²University of Aberdeen, Rowett Institute of Nutrition and Health, Greenburn Road,
8 Bucksburn, Aberdeen, AB21 9SB, UK

9 * Deceased

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11 Corresponding author contact details

12 Email: rlc34@cam.ac.uk

13 Telephone: +441223 336784

14 Fax: +441223 330598

15

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21 **ABSTRACT**

22 Epidemiological studies show a link between low birthweight and increased obesity.
23 In contrast, slow growth during the lactation period reduces obesity risk. This study
24 investigates potential underlying mechanisms. Rats were established as follows: (a)
25 controls (offspring of control dams fed a 20% protein diet), (b) recuperated animals
26 (offspring of dams fed an isocaloric low-protein (8%) diet during pregnancy, nursed
27 by control dams) and (c) postnatal low-protein animals (offspring of control dams
28 nursed by low-protein dams). Serum and brains were collected from fed and fasted
29 animals at weaning. Expression of hypothalamic energy balance genes was assessed
30 using *in situ* hybridisation. Recuperated pups were smaller at birth, but caught up with
31 controls by day 21 and gained more weight than controls between weaning and 12
32 weeks of age ($p < 0.05$). At 21 days they were hypoleptinemic compared to controls in
33 the fed state with generally comparable hypothalamic gene expression. Postnatal low-
34 protein offspring had significantly lower body weight than controls at weaning and 12
35 weeks of age ($p < 0.001$). At 21 days they were hypoglycemic, hypoinsulinemic and
36 hypoleptinemic. Leptin receptor gene expression in the arcuate nucleus was increased
37 in postnatal low-protein animals compared to controls. Consistent with the
38 hypoleptinemia, hypothalamic gene expression for orexigenic neuropeptides,
39 neuropeptide Y and, agouti-related peptide, was increased, and that for anorexigenic
40 neuropeptides, proopiomelanocortin and cocaine-and amphetamine-regulated
41 transcript was decreased. These results suggest that the early nutritional environment
42 can affect the development of energy balance circuits and consequently obesity risk.

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43 INTRODUCTION

44 Over recent decades, there has been a large increase in the proportion of both
45 adults and children that are either overweight or obese in developed countries. In the
46 United States, 64.5% of adults had a body mass index (BMI) greater than 25kg m^{-2} in
47 2000, increased from 55.9% in 1988-94, while the proportion of adults that had a BMI
48 over 30 kg m^{-2} had increased from 22.9% in 1988-94 to 30.5% in 2000 [1]. More
49 worrying is the increase in childhood obesity with 22 million children under the age
50 of 5 overweight and 10% of US preschool children obese [2]. The consequences of
51 being overweight or obese are far reaching in terms of mortality and morbidity as well
52 as escalating healthcare costs. Obesity increases the risk of type 2 diabetes,
53 dyslipidaemia, hypertension and certain types of cancer such as those of the breast,
54 colon and pancreas [3].

55 Many of the diseases where obesity is a risk factor have been linked by
56 numerous epidemiological studies with low birth weight [4, 5]. These relationships
57 have been found in populations worldwide but the mechanisms underlying this link
58 are still unclear. However there is compelling evidence to suggest that the early
59 environment and in particular early nutrition plays a key role. Studies of adults who
60 were *in utero* during the 'Dutch Hunger Winter' provide direct evidence in humans
61 for the importance of maternal nutrition on offspring's health. Men exposed to famine
62 in early gestation had increased rates of obesity at 19 years of age while those exposed
63 during late gestation and in the first months of life showed reduced rates of obesity
64 [6]. Breast feeding provides infants with a lower plane of neonatal nutrition than
65 formula feeding, and a meta analysis of studies investigating the effect of breast
66 feeding showed that breast fed infants have a reduced risk of obesity compared to
67 formula fed infants [7]. A variety of protocols have been established to investigate the
68 effects of maternal diet on obesity risk in the offspring. The offspring of severely
69 undernourished rat dams are hyperphagic and obese [8]. Further evidence for the
70 importance of the early postnatal period can be found in cross fostering studies. In
71 mice, offspring of dams fed a reduced protein diet, cross fostered in small litters to
72 control fed dams are born small, undergo catch up growth and are more susceptible to
73 diet induced obesity. In contrast, offspring of control dams cross fostered onto a dam
74 fed a reduced protein diet during lactation are resistant to the obesity inducing effects
75 of a highly palatable diet [9, 10]. These studies show the long-term detrimental effects
76 of "catch up" growth in early life while slow growth during this period appears to
77 confer a protective effect, a programmed reduction in voluntary food intake.

78 The energy balance system is regulated by feedback from peripheral hormonal
79 and metabolic signals onto central integratory circuits, which are concentrated in the
80 hypothalamus. Within the hypothalamus, neuropeptides and their receptors are
81 prominent. Leptin is a major energy balance signal derived from adipose tissue that
82 relays long-term information to the brain about the size of body fat stores and shorter
83 term information about the state of energy flux in adipose tissue. Leptin receptors are
84 heavily expressed in the arcuate nucleus of the hypothalamus and colocalise with both
85 the orexigenic (NPY/AgRP) and anorexigenic pathways (CART/POMC). The arcuate
86 nucleus is a site of leptin feedback into the brain. Consequently, the leptin signal and
87 its integration into the hypothalamic network are likely to be involved in the
88 programming of energy balance systems by early life nutrition. A number of strands
89 of evidence support this suggestion. *Ob/ob* mice, which lack leptin, show reduced
90 neuronal outgrowth from the arcuate nucleus. Furthermore leptin caused neurite

91 growth from neonatal arcuate explants but not adult ones [11]. During the lactation
92 period it is thought that leptin is playing a developmental rather than an energy
93 balance role. There is a leptin surge during this period [12] and this surge is earlier in
94 undernourished mouse pups [13]. If control pups are given exogenous leptin, to mimic
95 the premature leptin surge seen in the undernourished offspring, they show
96 accelerated weight gain when placed on a high fat diet [13]. However in rats, if the
97 offspring of undernourished dams are given exogenous leptin postnatally then the
98 detrimental metabolic phenotype seen in adulthood can be reversed [14]. Alterations
99 in neonatal leptin concentrations induced by changes in maternal nutrition could
100 therefore affect hypothalamic development of energy balance circuits.

101 The aim of this study was therefore to investigate the effect of fetal growth
102 restriction followed by catch up growth, or normal fetal growth followed by slowed
103 growth during neonatal life on anatomically resolved expression of a panel of
104 neuropeptide and receptor genes known to be involved in energy balance regulation.
105 An early time point was chosen allowing the elucidation of possible mechanisms
106 rather than later life consequences.

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107 **METHODS**

108 *Animals and experimental protocol*

109 All procedures involving animals were carried out under the UK Home Office
 110 Animals (Scientific Procedures) Act, 1986. Virgin female Wistar rats weighing 240-
 111 260g were housed individually and maintained at 22°C on a 12h light-dark cycle
 112 (lights on 0700-1900). They were mated and day 1 of gestation was taken as the day
 113 on which vaginal plugs were expelled. Rats were then fed either a control (20%
 114 protein) diet or an isocaloric low protein (8%) diet. These were purchased from Arie
 115 Blok (Woerden, Netherlands) and their composition has been described previously
 116 [15]. The diet has no effect on litter size (control 13 ± 0.3 vs low protein 13 ± 0.4 ,
 117 $p=0.64$), sex ratio (Control 48 ± 2.3 % males, Low Protein 53 ± 4.4 % males, $p=0.33$)
 118 or pup survival. Maternal weight and food intake were measured daily until weaning.
 119 After spontaneous birth on day 21, the control dams were either continued on the
 120 control diet with their own litter culled to 8 offspring ('control') or received and
 121 suckled 4 randomly selected offspring from the litter of a low protein dam
 122 ('recuperated'). The culling of this litter to only 4 served to maximise the plane of
 123 nutrition received by these pups during suckling and also the rate of catch up growth.
 124 Low protein dams that had donated their pups to a control dam received that dam's
 125 litter (both male and female pups) uncultured ('postnatal low protein') (litter size
 126 13 ± 0.41). This litter was left uncultured to minimise the amount of nutrition during
 127 suckling and therefore the rate of growth. For the pups killed on postnatal day 22,
 128 weights were taken on postnatal days 3,7,14 and 21.

129 Pups were weaned at 21 days. One cohort of male animals was immediately
 130 killed (at 0900) and a separate group of male animals were starved overnight and
 131 killed on day 22 (at 0900). This enabled study of the animals in both fed and fasted
 132 states. No more than 2 pups were taken from each litter and each cohort of animals
 133 studied used separate groups of dams giving 4-8 dams used for each group of
 134 offspring studied at each timepoint. On the day of the experiment, animals were
 135 weighed and then killed by CO₂ inhalation and exsanguination. Blood serum was
 136 collected. Brains were removed, immediately frozen on dry ice and then stored at -
 137 80°C for *in situ* hybridisation. Retroperitoneal and mesenteric fat pads were weighed.
 138 An additional cohort of animals were studied up to 12 weeks of age after weaning
 139 onto a standard laboratory chow diet at 21 days of age (LAD1, SDS, Witham, UK).
 140 Body weights and food intakes were measured weekly. At 12 weeks of age this cohort
 141 was killed by CO₂ inhalation and exsanguinations. The fat pads were dissected and
 142 weighed.

143

144 *Circulating hormones and metabolites.*

145 Blood glucose was measured (5µl) using a blood glucose analyser (Hemocue
 146 AB, Ängelholm, Sweden). Leptin concentrations in serum were measured using an
 147 ELISA assay from CrystalChem (IL, USA), the sensitivity of the assay is reported as
 148 12.5 nmoles/litre and the intra-assay coefficient was 4.9%. Serum insulin
 149 concentrations were measured using an ultra sensitive rat/mouse ELISA assay from
 150 Mercodia (Uppsala, Sweden). The sensitivity of the assay is reported as 3.5
 151 pmoles/litre and the intra-assay coefficient was 1.7%.

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153 **Hypothalamic gene expression**

154 Hypothalamic gene expression for a number of energy balance related
155 neuropeptides and receptors was quantified using *in situ* hybridization techniques,
156 which are described in detail elsewhere [16]. Riboprobes complementary to partial
157 fragments of Agouti related protein (AgRP), pro-opiomelanocortin (POMC), cocaine-
158 and amphetamine-regulated transcript (CART), melanocortin-4 receptor (Mc4R) and
159 cannabinoid receptor 1 (CBR1) were generated from cloned cDNAs as previously
160 described [17-19]. Neuropeptide Y (NPY) riboprobes were generated using a partial
161 rat cDNA sequence [20]. Riboprobes for suppressor of cytokine signaling-3 (mSOCS-
162 3) and leptin receptor (OB-Rb, long form) were generated using partial mouse and rat
163 cDNA sequences, respectively [21, 22].

164 Briefly, hypothalamic sections were collected from the very caudal extent of
165 the arcuate nucleus (ARC) through to the rostral extent of the paraventricular nucleus
166 (PVN) onto 2 sets of 10 slides spanning the full extent of the ARC, approximating –
167 4.52 to –2.30 mm, relative to Bregma, according to the atlas of the rat brain [23], and
168 continuing through to –1.40 mm relative to Bregma and including both rostral and
169 caudal extents of the PVN. Sections were fixed, acetylated, and hybridized overnight
170 at 58°C using ³⁵S-labeled antisense riboprobes (1–1.5 × 10¹⁰ dpm/L). Slides were
171 treated with RNase A to remove unhybridized probe and then desalted with a final
172 high-stringency wash in 0.1X SSC at 60°C for 30 min. The slides were air dried and
173 exposed to Kodak BioMax MR autoradiography film (Amersham Pharmacia Biotech
174 UK). Autoradiographic images were quantified using the Image-Pro Plus system
175 (Media Cybernetics). This determined the intensity and area of the hybridization
176 signal on the basis of set parameters; the integrated intensity was then computed using
177 standard curves generated from ¹⁴C autoradiographic microscales (Amersham UK).
178 Depending on the probe used, image analysis was performed on up to 5 sections
179 spanning the ARC and 2 or 3 sections from the PVN, dorsomedial nucleus (DMH)
180 and ventromedial nucleus (VMH).

181 This experiment was initially conducted on overnight fasted animals at 22
182 days of age. However to remove the possibly confounding effect of differential
183 susceptibility to fasting or weaning stress between the groups it was then repeated on
184 *ad libitum* fed animals at 21 days of age. As these experiments were not conducted at
185 the same time, it is not possible to directly compare animals in the fed and fasted
186 state.

187

188 **Statistical Analysis**

189 Data are expressed as mean ± SEM except for serum insulin, which is shown
190 as geometric means (95% confidence intervals). Statistical differences between means
191 were assessed using one-way ANOVA with maternal diet as the independent variable
192 followed by Duncans post hoc test where appropriate. Serum insulin data was log
193 transformed prior to testing as it was not normally distributed, and so is presented as
194 geometric means (95% confidence intervals). Differences between means were
195 considered significant at values of p<0.05.

196 **RESULTS**197 ***Maternal Weight and Food Intake***

198 Low protein dams were smaller than control fed dams by the end of gestation
199 ($388.9 \pm 10.4\text{g}$ vs. $426.0 \pm 4.8\text{g}$, $p < 0.01$). Total maternal food intake during gestation
200 was not significantly different (Low protein dam intake $525.9 \pm 24.2\text{g}$ vs. control dam
201 intake $561.5 \pm 13.0\text{g}$). After cross fostering, there are 3 experimental groups of dams.
202 During lactation, the weight of the low protein dams decreased further so by the end
203 of lactation they were 30% smaller than control dams ($254.3 \pm 5.1\text{g}$ vs. $355.6 \pm 5.7\text{g}$,
204 $p < 0.001$) while the control dams nursing either control or recuperated litters
205 maintained their weight.

206
207 ***Growth of pups until weaning***

208 Data are shown in Figure 1. At birth, pups whose mothers were fed the low
209 protein diet were significantly smaller than the control pups ($p < 0.001$). This is a mild
210 intervention and did not significantly affect litter size (Control 13.0 ± 0.3 , Low
211 Protein 13.0 ± 0.4 , $p = 0.64$) or the sex ratio of the offspring (Control $48\% \pm 2.3$ males,
212 Low Protein $53\% \pm 4.4$ males). Recuperated pups caught up with control pups by day
213 14 and postnatal low protein pups were smaller than control pups by day 7 ($p < 0.001$).
214 Postnatal low protein pups remained smaller throughout lactation and weighed less
215 than 50% of control pups on day 21 ($p < 0.001$).

216
217 ***Organ weights of fasted 22 day old offspring***

218 The weight of the brain ($p < 0.01$), retroperitoneal ($p < 0.001$) and mesenteric fat
219 pads ($p < 0.001$) were all significantly lower in the postnatal low protein animals
220 compared to controls (Table 1). However the reduction in brain weight and the fat pad
221 weights were not in proportion with the reduction in body weight (Table 1). The
222 growth of the brain ($p < 0.001$) was relatively spared whereas the reduction in fat
223 masses (both $p < 0.001$) were greater than overall body weight (Table 1).

224
225 ***Characteristics of offspring blood/serum***

226 There was a significant overall effect of maternal diet ($p < 0.001$) on blood
227 glucose concentrations. Postnatal low protein animals had lower glucose
228 concentrations compared to controls ($p < 0.05$ for fed animals, $p < 0.001$ for fasted rats)
229 (Table 2). There was a significant overall effect of maternal diet manipulation
230 ($p < 0.001$) on serum leptin concentrations. Leptin concentrations were lower in
231 postnatal low protein rats compared to controls ($p < 0.001$ for fed animals, $p < 0.05$ for
232 fasted rats). In fed rats, recuperated animals had lower serum leptin concentrations
233 compared to controls ($p < 0.001$). However in the fasted state, recuperated male
234 animals had higher serum leptin concentrations compared to controls ($p < 0.05$) (Table
235 2). Maternal diet also altered insulin concentrations ($p < 0.001$). Insulin concentrations
236 being lower in postnatal low protein rats compared to controls ($p < 0.001$ for fed
237 animals, $p < 0.05$ for fasted rats) (Table 2).

238
239 ***Hypothalamic gene expression***240 **Receptors and SOCS-3**

241 Data are shown in Figure 2. There was a significant overall effect of maternal diet on
242 OB-Rb gene expression in the ARC of *ad libitum* fed rats ($p < 0.001$) and overnight
243 fasted rats ($p < 0.01$). In each case, ARC OB-Rb gene expression was elevated in
244 postnatal low protein rats compared to controls, but this difference was more

245 substantial in fed rats than in fasted ones. In the VMH, there were no effects on OB-
246 Rb gene expression in *ad libitum* fed rats but there was a significant overall effect of
247 maternal diet ($p < 0.001$) in overnight fasted animals. OB-Rb gene expression being
248 high in the VMH of postnatal low protein animals compared to control animals in the
249 fasted state.

250 There was a significant overall effect of maternal diet ($p < 0.001$) on SOCS3 gene
251 expression in the ARC of *ad libitum* fed animals. Both recuperated and postnatal low
252 protein animals had reduced SOCS3 expression compared to control animals in the
253 fed state. There was also an effect of maternal diet ($p < 0.01$) on SOCS3 expression in
254 overnight fasted animals. However, the effect of maternal diet was only observed in
255 postnatal low protein animals, which had lower SOCS3 gene expression compared to
256 control animals.

257 There was an overall effect of maternal diet ($p < 0.01$) on MC4R gene expression in the
258 PVN in *ad libitum* fed animals, with postnatal low protein animals having lower
259 MC4R gene expression. However this effect was not observed in fasted animals.

260

261 **Orexigenic peptides**

262 Data are shown in Figure 3. There was a significant effect of maternal diet ($p < 0.001$),
263 on NPY gene expression in the ARC of both fed ($p < 0.001$) and fasted rats ($p < 0.05$).
264 In each case, ARC NPY gene expression was elevated in postnatal low protein rats
265 compared to controls. This difference was more marked in the fed rats than in the
266 fasted animals. In the DMH there was no effect of maternal diet on NPY gene
267 expression in either fed or fasted conditions. There was a significant effect of
268 maternal diet on AgRP gene expression in the ARC of both fed ($p < 0.001$) and fasted
269 ($p < 0.01$) rats. In each case, ARC AgRP gene expression was elevated in postnatal low
270 protein rats compared to controls. This difference was again more marked in fed rats
271 than in fasted.

272

273 **Anorexigenic peptides**

274 Data are shown in Figure 4. There was a significant effect of maternal diet on POMC
275 gene expression in the ARC of fed ($p < 0.001$) and fasted ($p < 0.01$) rats. POMC gene
276 expression was lower in postnatal low protein rats compared to controls. The down
277 regulation of POMC in the postnatal low protein group was more substantial in fed
278 rats than in fasted.

279 There was a significant effect of maternal diet on CART gene expression in the ARC
280 of fed ($p < 0.001$) and fasted ($p < 0.01$) rats, with CART gene expression being lower in
281 postnatal low protein rats compared to controls. This effect was not as substantial as
282 that observed in fed rats.

283

284 **Growth and energy intake post weaning**

285 There was a significant effect of maternal diet on body weight gain from weaning to
286 12 weeks of age ($p < 0.001$). Recuperated animals gained more weight than controls
287 ($p < 0.05$), while postnatal low protein animals were gained less weight ($p < 0.001$)
288 (Table 3). This led to recuperated animals being heavier than control animals ($p < 0.05$)
289 at 12 weeks of age and postnatal low protein animals were lighter ($p < 0.001$) (Table
290 3). There was a significant effect of maternal diet on cumulative energy intakes during
291 this period with the same pattern, recuperated animals consumed more energy
292 ($p < 0.05$) compared to control animals while postnatal low protein animals consumed
293 less energy ($p < 0.001$) (Table 3).

294 The weights of both the retroperitoneal and epididymal fat pads were affected
295 significantly by maternal diet in adult rats ($p < 0.001$ for both). Postnatal low protein
296 animals had smaller fat pads than control animals while there was a tendency ($p = 0.06$
297 for both) for the recuperated animals to have heavier fat pads than control animals
298 (Table 3). When expressed relative to body weight, there was still an overall effect of
299 maternal diet ($p < 0.001$ for both fat pads) with postnatal low protein animals having
300 lower relative fat masses ($p < 0.001$ for both) but the recuperated animals relative fat
301 pad weights were not significantly different from control animals (Table 3).

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302 **DISCUSSION**

303 Alterations in maternal dietary protein content during gestation and lactation
304 have permanent effects on susceptibility to weight gain and longevity in rodents [9,
305 10, 24], but the molecular mechanisms that underlie these programming events remain
306 to be resolved. The current study examined alterations in leptin and the expression of
307 neuropeptides and receptors implicated in energy balance control after manipulation
308 of maternal diet protein content and the plane of nutrition received during lactation. In
309 addition, the effect of fasting on these young animals and their energy balance related
310 hypothalamic gene expression was examined.

311 Long term effects on weight gain could be mediated by changes in energy
312 intake and/or energy expenditure. In this model, recuperated animals consumed more
313 energy than control animals and this was accompanied by a larger increase in body
314 weight while postnatal low protein animals consumed less energy and so gained less
315 weight in adulthood. It is therefore clear that simple alterations in early life nutrition
316 had long-term effects on energy balance via energy intake. Currently it is unknown
317 whether there are similar effects on energy expenditure.

318 In this study we addressed mechanisms by which these programmed changes
319 in energy intake could occur by studying animals at weaning. By choosing an early
320 time point, programmed changes that could lead to the development of the phenotype
321 rather than changes as a consequence of the phenotype can be examined. At this age
322 the recuperated animals did not differ from the control animals in terms of body
323 weight. We demonstrated that the serum concentration of leptin was altered by
324 manipulation of maternal protein intake. Leptin during the lactation period is a
325 candidate for programming of the appetite system [11, 25, 26]. Postnatal low protein
326 animals had substantially reduced blood leptin concentration compared to control
327 animals under both fed and fasted conditions. This reduction is likely to be a
328 consequence of the dramatically reduced adiposity in this group. Recuperated animals
329 had lower leptin concentration than controls in the fed state, despite similar body
330 weights, suggesting a possible alteration in the control of leptin secretion. This could
331 lead to an increased drive to eat and could increase their susceptibility to obesity, as
332 seen by their higher cumulative energy intakes and body weights from weaning to 12
333 weeks of age. In addition there was a tendency for increased fat pad mass in the
334 recuperated animals at 12 weeks of age when compared to control animals although
335 this was in proportion with the increased body weight. After an overnight fast, the
336 leptin concentration in recuperated animals did not show the expected large drop seen
337 in both control and postnatal low protein animals. This resulted in recuperated animals
338 having an elevated leptin concentration compared to the control animals in the fasted
339 state. Recuperated animals at this age therefore seem to have a defect in their ability
340 to alter their plasma leptin concentration in response to feeding status.

341 Differences in long term susceptibility to weight gain may be a consequence
342 of changes in central leptin sensitivity. The mRNA of the signalling form of the leptin
343 receptor was more highly expressed in the arcuate nucleus of postnatal low protein
344 animals compared to control animals in both fed and fasting conditions. This,
345 combined with the lower expression of the negative regulator of leptin signalling,
346 SOCS3, may increase leptin sensitivity in these animals, which may contribute to
347 their long term reduction in weight gain compared to control animals. It has been
348 shown previously that low leptin concentrations can lead to upregulation of the leptin
349 receptor in the ARC [27]. The fed recuperated animals had low concentrations of
350 circulating leptin compared to the control animals, however, this hypoleptinemia was
351 not accompanied by an increase in the expression of the leptin receptor in the ARC.

352 This contrasts with the postnatal low protein animals and suggests there is a difference
353 in the response to low leptin levels between these two groups of animals. High levels
354 of leptin are presumed to signal positive energy balance and counteract this state
355 through two pathways. The expression of anorexigenic genes such as CART and
356 POMC are increased in response to leptin while the expression of orexigenic genes
357 such as NPY and AgRP are decreased [28]. In the fed state, expression of the mRNAs
358 for the orexigenic peptides, NPY and AgRP, in the ARC was higher in the postnatal
359 low protein animals and expression of the mRNAs for the anorexigenic peptides,
360 CART and POMC, was lower compared to control animals. These changes are
361 appropriate for the low serum leptin concentration in this group. This suggests that
362 these animals register the negative energy balance they are in i.e. their low body
363 weight compared with developmentally appropriate controls. However these postnatal
364 low protein animals remain small even when fed *ad libitum*, and so do not respond
365 appropriately to this multi-component signal. This suggests that these animals have a
366 fault downstream of leptin signalling.

367 This study reinforces the suggestion, which is growing in the literature, that
368 fetal and early life nutrition affects energy balance systems. Small litter size animals
369 that go on to become obese show reduced arcuate nucleus gene expression of OB-Rb
370 and increased arcuate mRNA expression of the orexigenic peptides NPY and AgRP
371 post weaning [29]. Neonatally overnourished rats at weaning have been shown to
372 have alterations in the paraventricular nucleus of the hypothalamus with decreased
373 numbers of CCK-positive neurons [30] and increased numbers of galanin positive
374 neurons [31]. There is also evidence for a change to the response of the ventromedial
375 hypothalamic nucleus to leptin. In neonatally normally nourished rats, leptin increases
376 firing from ventromedial hypothalamic neurons while in neonatally overnourished
377 animals, leptin inhibits firing [32]. Other models of altered early nutrition have shown
378 altered expression of energy balance neuropeptides in the hypothalamus; maternal
379 high fat feeding leads to increased expression of galanin in the paraventricular
380 hypothalamic nucleus and orexin in the lateral hypothalamus [33]. While the offspring
381 of food restricted rat dams have a lower level of hypothalamic POMC expression
382 [34]. This could cause an increase drive to eat in these offspring. Maternal obesity, as
383 a consequence of the genetic background of the dam, during lactation of the pups has
384 been shown to influence expression of energy balance neuropeptides in adult
385 offspring [35].

386 This study has shown that hypothalamic mRNA expression of regulatory
387 neuropeptides and signalling proteins of energy balance systems are altered by
388 maternal dietary protein manipulations during pregnancy and lactation. Those animals
389 that grew slowly during lactation show evidence of changes in expression of
390 neuropeptides that should signal that they are in a state of negative energy balance.
391 However, the anticipated physiological response to these signals, namely behavioural
392 and energetic compensation does not develop, and the animals stay small during
393 adolescence and adult life when fed *ad libitum* with lower energy intakes over this
394 period than control animals. This could be a result of the action of the neuropeptides
395 being blocked further downstream or a complete alteration of the wiring of the
396 system. These animals that remain lean show changes in expression of leptin
397 signalling molecules that could indicate increased sensitivity to this important
398 regulator of energy balance. Animals that underwent *in utero* growth restriction
399 followed by catch up growth showed similar expression of examined neuropeptides to
400 control animals but reduced leptin in the fed state compared to control animals. This
401 could provide an increased drive to eat, even when fed, thus providing a possible

402 mechanism for their increased weight gain post weaning and indicates a potential
403 peripheral defect in these animals. This study provides new insight into the
404 mechanistic basis that underlies the link between low birth weight and obesity and
405 slow growth and leanness in adulthood.

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TABLE 1. Absolute and relative weights of fat pads and brain in 22 day old male rat pups

	CONTROL	RECUPERATED	POSTNATAL LOW PROTEIN
Absolute retroperitoneal fat pad weight (g)	0.20 ± 0.01	0.20 ± 0.03	0.03 ± 0.01 ***
Absolute mesenteric fat pad weight (g)	0.18 ± 0.01	0.20 ± 0.03	0.05 ± 0.01 ***
Absolute brain weight (g)	1.50 ± 0.02	1.47 ± 0.03	1.35 ± 0.05 **
Relative retroperitoneal fat pad weight (%)	0.37 ± 0.02	0.40 ± 0.05	0.14 ± 0.02 ***
Relative mesenteric fat pad weight (%)	0.35 ± 0.2	0.41 ± 0.05	0.20 ± 0.02 ***
Relative brain weight (%)	2.86 ± 0.07	3.0 ± 0.14	6.14 ± 0.2 ***

TABLE 2 Blood/serum data at 21 days (fed) and 22 days (fasted) of age

	Control	Recuperated	Postnatal low protein
Fed blood glucose (mM)	9.1 ± 0.6	10.0 ± 0.6	6.2 ± 0.8 *
Fasted blood glucose (mM)	5.0 ± 0.1	5.3 ± 0.1	4.3 ± 0.1 ***
Fed serum leptin (µM)	0.23 ± 0.021	0.11 ± 0.014 ***	0.09 ± 0.013 ***
Fasted serum leptin (µM)	0.06 ± 0.004	0.09 ± 0.009 *	0.03 ± 0.007 *
Fed serum insulin (pM)	95.8 (73.5-131.7)	65.7 (42.3-117.9)	3.9 (3.2-4.9) ***
Fasted serum insulin (pM)	20.0 (9.4-36.3)	10.4 (6.4-21.5)	7.7 (4.0-16.7) *

TABLE 3 Body weight and energy intakes at 12 weeks of age

	Control	Recuperated	Postnatal low protein
Body weight gain from weaning to 12 weeks (g)	322.2 ± 6.6	347.3 ± 8.4 *	282.3 ± 5.9 ***
Body weight at 12 weeks	403.2 ± 7.5	425.8 ± 9.4 *	322.0 ± 5.1 ***
Cumulative energy intake from weaning to 12 weeks (kJ)	16641 ± 224	17237 ± 467 *	13853 ± 258 ***
Absolute epididymal fat pad weight (g)	5.33 ± 0.32	6.20 ± 0.44 p=0.06	3.08 ± 0.18 ***
Absolute retroperitoneal fat pad weight (g)	6.56 ± 0.42	7.81 ± 0.54	3.27 ± 0.35 ***
Relative epididymal fat pad weight (%)	1.34 ± 0.08	1.48 ± 0.07 p=0.06	0.95 ± 0.06 ***
Relative retroperitoneal fat pad weight (%)	1.65 ± 0.11	1.88 ± 0.10	1.01 ± 0.11 ***

LEGENDS

TABLE 1

Tissue weights shown as absolute weight (g) and weight relative to body weight (%) collected from overnight fasted animals. n=8 per group. Data expressed as mean ± SEM and examined using a one-way ANOVA with appropriate Duncan's post hoc tests. p values shown are compared to control animals. ** p<0.01 and *** p<0.001 when compared to control animals.

TABLE 2

Serum and plasma characteristics of males pups at both 21 days of age (fed state) and 22 days of age (fasted state). n=8 per group. Data expressed as mean ± SEM and examined using a one-way ANOVA with appropriate Duncan's post hoc tests. p values shown are compared to control animals. Insulin data was log transformed prior to examination and is shown as geometric mean (confidence intervals). * p<0.05 and *** p<0.001 when compared to control animals.

TABLE 3

Bodyweight (g) at 12 weeks, bodyweight gain (g) from weaning until 12 weeks, cumulative energy intake over this period and absolute and relative fat pad weights at 12 weeks in male rats. n=10 per group. Data expressed as mean ± SEM and examined using a one-way ANOVA with appropriate Duncan's post hoc tests. * p<0.05 and *** p<0.001 when compared to control animals.

FIGURE 1

Growth curves of male rats during lactation. n=10 per group. Data expressed as mean ± SEM and examined using one-way ANOVA with appropriate Duncan's post hoc tests. *** p<0.001 when compared to control animals of the same age.

■ CONTROL, ■ RECUPERATED and □ POSTNATAL LOW PROTEIN

FIGURE 2

Gene expression of OB-Rb, SOCS3 and MC4R in indicated regions of the hypothalamus in fed 21 day old males (A) and fasted 22 day old males (B). n=8 per group. Data expressed as % of control and shown as mean ± SEM and examined using a one-way ANOVA with appropriate Duncan's post hoc tests. * p<0.05 ** p<0.01 and *** p<0.001 when compared to control animals.

■ CONTROL, ■ RECUPERATED and □ POSTNATAL LOW PROTEIN

FIGURE 3

Gene expression of NPY and AgRP in indicated regions of the hypothalamus in fed 21 day old males (A) and fasted 22 day old males (B). n=8 per group. Data expressed as % of control and shown as mean ± SEM and examined using a one-way ANOVA

with appropriate Duncan's post hoc tests. * $p < 0.05$ and *** $p < 0.001$ when compared to control animals.

■ CONTROL, ■ RECUPERATED and □ POSTNATAL LOW PROTEIN

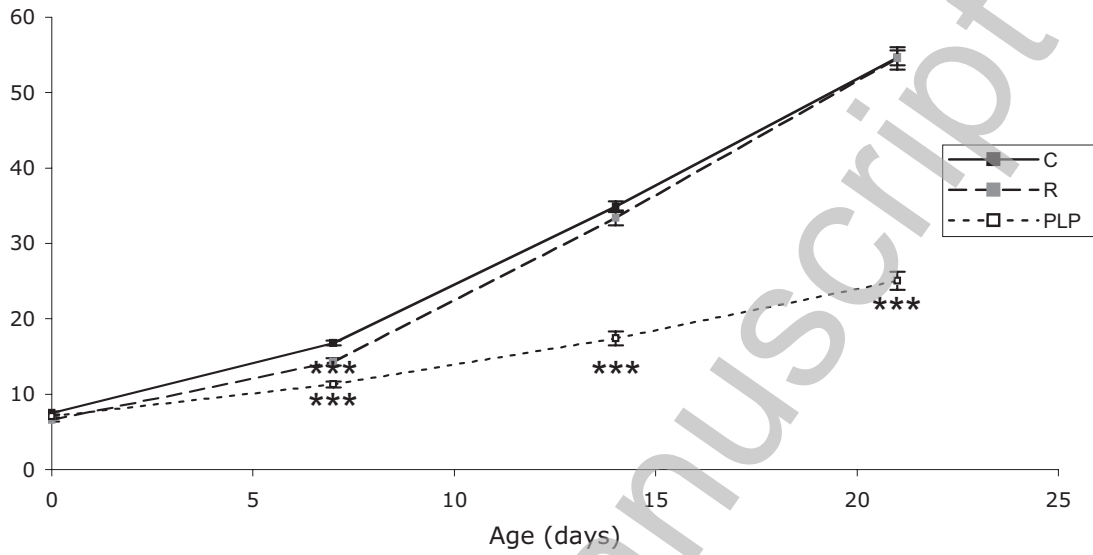
FIGURE 4

Gene expression of POMC and CART in the arcuate nucleus of the hypothalamus in fed 21 day old males (A) and fasted 22 day old males (B). $n=8$ per group. Data expressed as % of control and shown as mean \pm SEM and examined using a one-way ANOVA with appropriate Duncan's post hoc tests. ** $p < 0.01$ and *** $p < 0.001$ when compared to control animals.

■ CONTROL, ■ RECUPERATED and □ POSTNATAL LOW PROTEIN

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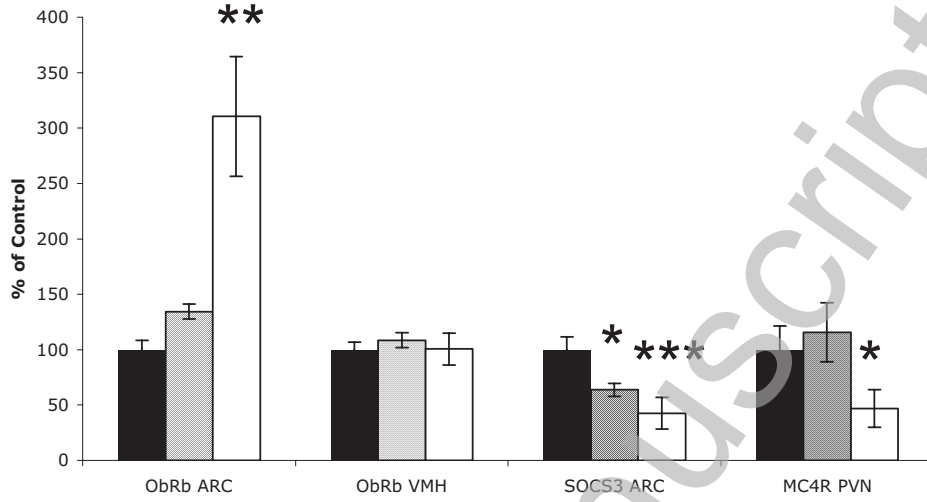
Figure 1



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Figure 2

A



B

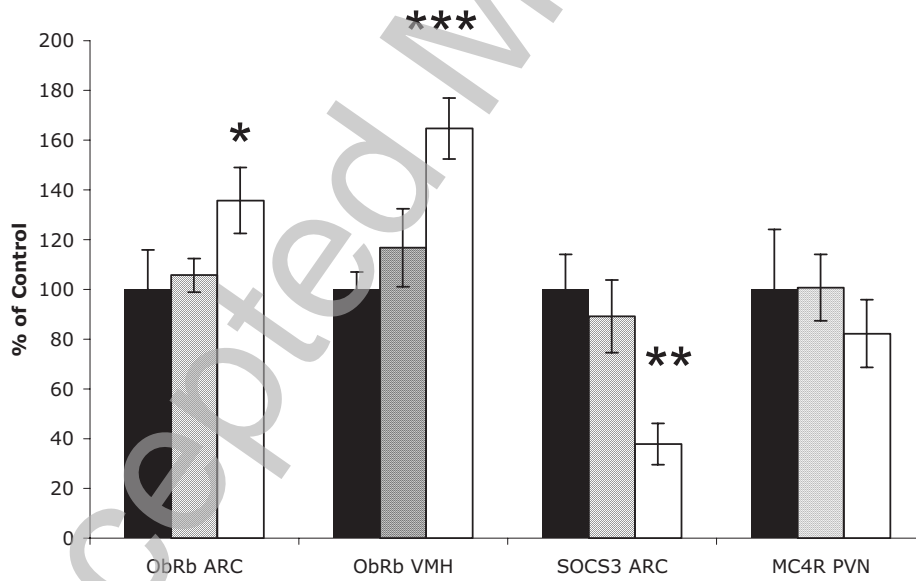
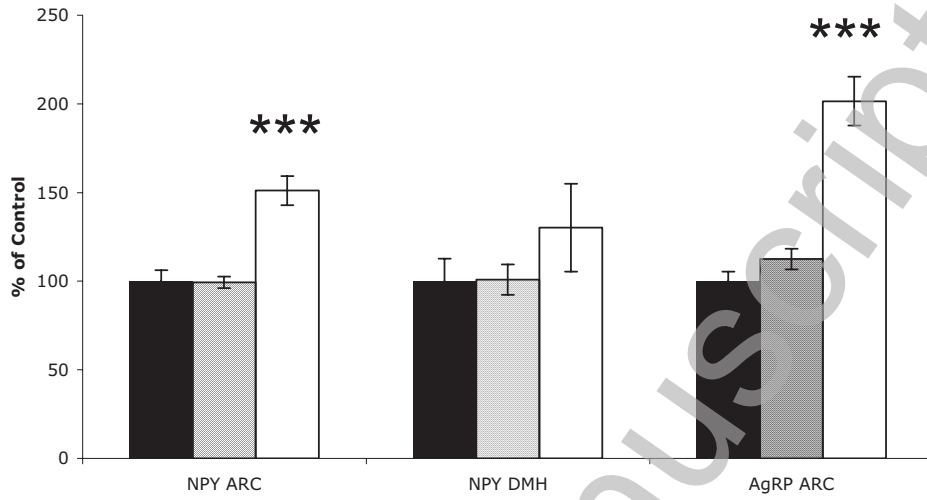


Figure 3

A



B

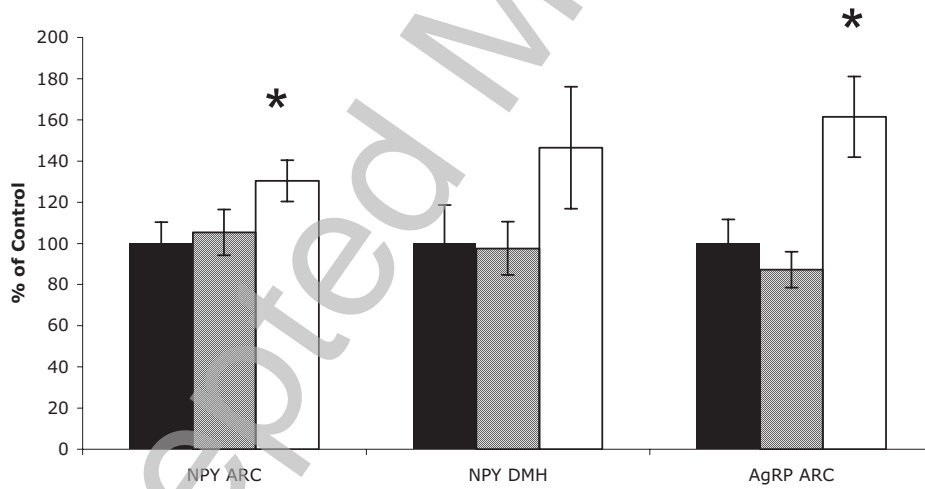
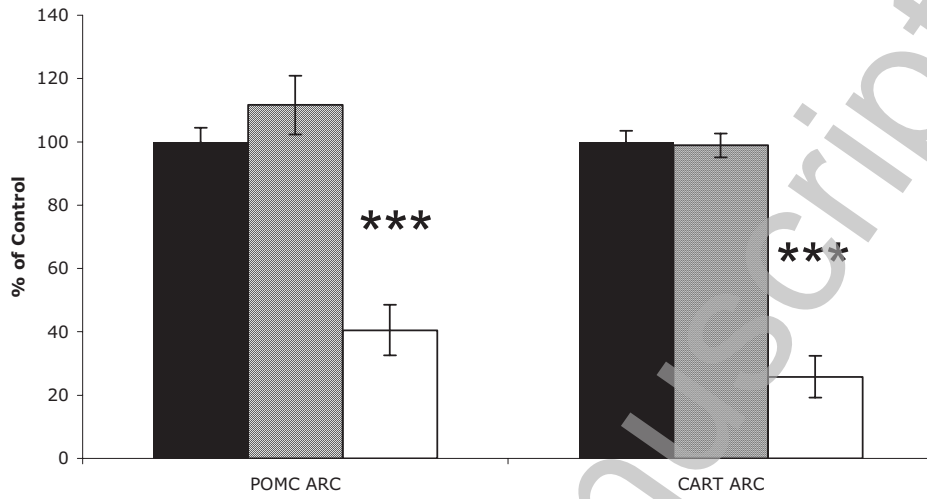


Figure 4

A



B

