

1 **Effects of postnatal growth restriction and subsequent catch-up growth on**
2 **neurodevelopment and glucose homeostasis in rats**

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21 **ABSTRACT**

22 **Background**

23 There is increasing evidence that poor growth of preterm infants is a risk factor for poor
24 long-term development, while the effects of early postnatal growth restriction are not
25 well known. We utilized a rat model to examine the consequences of different patterns of
26 postnatal growth and hypothesized that early growth failure leads to impaired
27 development and insulin resistance. Rat pups were separated at birth into normal (N,
28 n=10) or restricted intake (R, n=16) litters. At d11, R pups were re-randomized into litters
29 of 6 (R-6), 10 (R-10) or 16 (R-16) pups/dam. N pups remained in litters of 10 pups/dam
30 (N-10). Memory and learning were examined through T-maze test. Insulin sensitivity was
31 measured by i.p. insulin tolerance test and glucose tolerance test.

32 **Results**

33 By d10, N pups weighed 20% more than R pups ($p < 0.001$). By d15, the R-6 group
34 caught up to the N-10 group in weight, the R-10 group showed partial catch-up growth
35 and the R-16 group showed no catch-up growth. All R groups showed poorer scores in
36 developmental testing when compared with the N-10 group during T-Maze test ($p <$
37 0.05). Although R-16 were more insulin sensitive than R-6 and R-10, all R groups were
38 more glucose tolerant than N-10.

39 **Conclusion**

40 In rats, differences in postnatal growth restriction leads to changes in development and in
41 insulin sensitivity. These results may contribute to better elucidating the causes of poor
42 developmental outcomes in human preterm infants.

43

44 Key words: growth restriction; catch-up growth; development; insulin sensitivity

45 **BACKGROUND**

46 In term infants, *in utero* growth restriction or small-for-gestational-age status at birth
47 (SGA) are associated with the development of increased adiposity and impaired insulin
48 sensitivity in later life [1], that may be exacerbated by more rapid catch-up growth in the
49 first 1-2 y of life [1, 2]. In comparison, preterm infants grow much more poorly after
50 birth, a term coined *ex utero* growth restriction, and by term corrected age most are below
51 the 5th weight-for-age centile [3]. This *ex utero*, postnatal, growth failure is common in
52 preterm infants, [3, 4] and is associated with poorer neurocognitive outcomes in later life
53 [5, 6]. Further, it has been shown that neonatal leptin deficiency may contribute to
54 adverse neurodevelopmental outcomes associated with postnatal growth restriction [7].
55 Subsequently, preterm infants have variable amounts of catch-up growth, especially
56 during the first 1-3 y of life [8, 9]. This pattern of small body size at term corrected age,
57 followed by increased rates of growth is similar to that seen in SGA term infants, and
58 there has been concern that this may lead to increased risk of obesity and metabolic
59 disorders arising from impaired glucose tolerance, such as type II diabetes, in preterm
60 infants, similar to the increased risk in term SGA infants [10-12].

61 We have previously described a rodent model of *ex utero* growth restriction and the
62 effects of variable amounts of catch-up growth on early metabolic and neurocognitive
63 outcomes [13]. Changes in litter size lead to *ex utero* growth restriction (EUGR), and in
64 turn, changes in body composition and poorer neurodevelopment. However, no
65 differences in fasting insulin or glucose in early life were seen [13]. In the present study,
66 we used the same model to assess the effects of *ex utero* growth restriction and
67 subsequent catch-up growth on longer-term metabolic outcomes including glucose

68 tolerance and insulin sensitivity.

69 The objectives of our study were to examine the effects of early postnatal growth
70 restriction, followed by varying degrees of postnatal catch-up growth on growth (both
71 body size and body composition), insulin sensitivity, glucose tolerance,
72 neurodevelopment, and brain myelination. We hypothesized that early postnatal growth
73 restriction would result in poorer neurodevelopment and lead to improved glucose
74 tolerance and insulin sensitivity. We further hypothesized that in EUGR rats, early catch-
75 up growth would lead to improved neurodevelopment but reduced insulin sensitivity and
76 glucose tolerance compared to EUGR rats that did not have early catch-up growth.

77

78 **RESULTS**

79 **Growth**

80 Growth differed significantly between the normal (N) and restricted (R) intake groups by
81 d5 (14.2 ± 0.19 g vs. 11.4 ± 0.10 g, $p < 0.001$) onwards. By d10 the R groups were
82 approximately 20% smaller than the N groups ($p < 0.001$, Fig 2).

83 On d10, R animals were re-randomized to litters of 6 (R-6), 10 (R-10) or 16 (R-16), while
84 N pups remained in litters of 10 (N-10). The R-16 group remained significantly smaller
85 than the N-10 group throughout the study. The weight of the R-6 pups "caught-up" with
86 the N-10 pups by d15 and were statistically indistinguishable from them for the rest of
87 the study.

88 The R-10 group grew intermediate to the N-10 and R-16 animals until d21 (Fig 3), and
89 was similar to the N-10 and R-6 groups thereafter.

90 By d40, the R-16 group remained significantly smaller than the three other groups, which
91 were all statistically similar. On d60, the R-16 rats remained significantly smaller than
92 the other three groups. This was seen for both males and females (Fig 4).

93 **Body Composition**

94 Body composition was assessed in a subset of animals (N-10 = 10, R-10 = 10, R-6 = 6,
95 R-16 = 16) at d60. There were no significant differences in percentage water, protein, fat,
96 or ash between the four groups (Fig 5).

97 **Serum Hormones**

98 On d10, serum leptin was significantly higher in the N group (3.93 ± 0.33 ng/ml) than the
99 R group (1.09 ± 0.31 ; $p < 0.0001$). Serum triglycerides on d10 were similar in the N
100 (1370 ± 330 mg/L) and R (860 ± 360 mg/L; $p = 0.77$) groups.

101 Serum leptin on d60 differed significantly between groups, with the R-16 group having
102 the lowest levels (Table 2). Serum triglycerides on d60 were similar among groups, but
103 hepatic triglycerides on d60 differed with the lowest level in the R-10 and R-16 groups
104 and the highest in the R-6 group. Serum insulin values did not differ between groups.

105 **Insulin Sensitivity**

106 Fasting blood glucose on d50 was similar in all four groups ($p = 0.07$). When expressed
107 as the area under the curve (AUC), the two catch-up groups, R-6 (7635 ± 189 , $n = 23$) and
108 R-10 (7531 ± 147 , $n = 38$), had significantly higher AUC than the R-16 group ($6870 \pm$
109 119 , $n = 58$), while the N-10 group was intermediate between the others (7229 ± 132 , $n =$
110 47) (Fig 6). Similar patterns were seen for the AUC between 0-30 min and between 30-
111 120 min. When individual time-points were considered, the R-6 and R-10 groups had

112 higher blood glucose concentrations than the other groups (N-10 and R-16) at 30, 45 and
113 60 min.

114 When the data were examined as change in glucose concentration from baseline, the area
115 under baseline (AUB) between 0-30 min was significantly greater for the N-10 group
116 (593 ± 43 , $n = 47$) than for the R-6 group (387 ± 61 , $n = 58$), while the R-10 (428 ± 48 , n
117 $= 38$) and R-16 groups (489 ± 37 , $n = 58$) were intermediate between the two. There were
118 no differences in AUB among the groups for the time period 30 min to 120 min.

119 **Glucose Tolerance**

120 Fasting blood glucose on d55 was significantly affected by sex ($M > F$; $p = 0.0061$) and
121 by group ($p = 0.0022$). Fasting glucose was lower in the N-10 (99.0 ± 1.4 mg/L) and the
122 R-16 (99.1 ± 1.3 mg/L) groups than in the R-10 group (105.3 ± 1.5 mg/L), with the R-6
123 group being intermediate between them (102.8 ± 2.0 mg/L).

124 The AUC was significantly different between groups ($p = 0.0079$) and was greater in
125 males than females ($p = 0.06$). The AUC for the N-10 group (10778 ± 413 , $n = 47$) was
126 significantly greater than both the R-16 (9210 ± 368 , $n = 59$) and the R-10 (8819 ± 453 , n
127 $= 39$) groups, with the R-6 group being intermediate (9620 ± 577 , $n = 24$) (Fig 7). There
128 were no significant group effects between 0 and 30 min, but were seen subsequently
129 during the remainder of the GTT for the time period 30 to 180 min. A similar pattern was
130 seen in AUB among the groups.

131 **T-Maze Test**

132 Memory and learning was assessed using spontaneous alternation in a T-maze. The N-10
133 group scored significantly better (6.86 ± 0.13 successes ($n = 69$); $p < 0.05$) than any of

134 the other groups (R-6 5.6 ± 0.18 (n = 36), R-10 5.6 ± 0.26 (n = 50), R-16, 5.14 ± 0.15
135 successes (n = 96)). The effects of group were similar in both sexes (Fig 8).

136 **Brain Histology**

137 The area of MBP-positive fibers in the R-16 group appeared smaller than that in the N-10
138 group on d60, but no significant differences could be detected. These results suggest that
139 myelination within the hypothalamus and corpus callosum may have been completed by
140 d60 (Fig 9).

141

142 **DISCUSSION**

143 Since poor growth in preterm infants occurs postnatally, we aimed to produce a postnatal
144 model of growth restriction in neonatal rats. Many animal models have been used to
145 examine effects of *in utero* growth restriction with or without catch-up growth on
146 metabolic outcomes, and though these models have provided great insight into infants
147 born small for gestational age or who experience intrauterine growth restriction [14, 15],
148 they do not represent the type of growth that is experienced by most preterm infants.
149 Further, the effects of growth restriction and subsequent catch-up growth on cognition
150 and metabolism have not been examined concurrently. We therefore developed a model
151 of post-natal growth restriction in rat pups based on manipulations in litter size, that we
152 have shown leads to reproducible levels of *ex utero* growth restriction and catch-up
153 growth [13]. This model leads to changes in both milk intake and in growth. However, it
154 is possible that other factors may also be changed by modifications in litter size, for
155 example dam-pup interactions and pup-pup interactions, as seen in other rodents [16, 17].

156

157 The initial intervention in our study was carried out from birth until d10 of age, as this
158 period in rats is believed to be equivalent to the third trimester of pregnancy in humans
159 [18], or the period when reduced intake and poor growth are common in premature
160 infants. The increased milk volume intake that occurs as litter size is decreased in the
161 second intervention represents the increased volume intake that preterm infants who
162 experience catch-up growth encounter after hospital discharge. Further, dams of large
163 litters have been shown to produce milk with unaltered protein composition, and thus
164 litter size manipulation results in modified volume intake without altered milk
165 composition [19].

166

167 The current study confirms our previous findings that R-6 pups catch-up with N-10 pups
168 by d21, R-10 pups show partial catch-up by d21, and R-16 pups remain smaller than the
169 other three groups [13]. The current study confirms this, but also demonstrates that the R-
170 10 group does ultimately show complete catch-up in body weight by d60. The R-16
171 group, however, remained significantly smaller than the N-10 group until at least d60.

172

173 We have previously shown that catch-up growth in R-6 pups comes at the cost of changes
174 in body composition with R-6 pups having significantly greater percentage body fat, and
175 significantly lower percent lean mass on d21 [13]. The current study demonstrates that by
176 d60, body composition in the R-6 pups has normalized, and is similar to the N-10 pups.
177 Furthermore, although the R-10 pups catch-up to the N-10 pups by d60, the two groups
178 have similar body composition on d60, just as they have at d21. The early changes in

179 body composition related to catch-up group are therefore not maintained over time.

180

181 We have previously shown that the R-16 group has lower percentage body fat in d21. By
182 d60, however, the differences in body composition are lost, and all groups have similar
183 percent body fat despite the fact that the R-16 rats remain smaller. Once again, early
184 differences in body composition are not sustained over time. These findings are
185 consistent with the human data, which suggests that although preterm infants with catch-
186 up growth have increased adiposity at term corrected age, those changes are not
187 maintained during the rest of the first year of life [20].

188

189 In our previous study there were no differences between groups in fasting insulin or
190 glucose of d21. In the current study we carried out more detailed investigations of
191 glucose homeostasis in older animals. Fasting blood glucose prior to the glucose
192 tolerance test (after a 12 h fast) was significantly greater in the two catch-up groups (R-6
193 and R-10) than in the groups without catch-up growth (N-10 and R-16). The difference in
194 fasting blood glucose prior to the insulin tolerance test (after a 4 h fast) failed to reach
195 statistical significance. Insulin sensitivity was higher in the groups without catch-up
196 growth (N-10 and R-16) than in the groups that changed their dietary intakes on d10 (R-6
197 and R-10) and experienced catch-up growth, as shown by their higher AUC values. This
198 occurred even though all groups had similar body composition at the end of the study. It
199 is possible that early changes in body composition may be responsible for the poorer
200 insulin sensitivity seen in the R-6 and R-10 groups in later life, or that changes in early
201 dietary intake or growth lead to long-term changes in insulin sensitivity, possibly via

202 epigenetic mechanisms. Growth restriction may result in improved insulin sensitivity in
203 adulthood since it has been suggested that early undernourishment may enhance insulin
204 sensitivity, as well as fatty acid oxidation [21]. It has been shown that children born
205 prematurely have decreased insulin sensitivity immediately after birth, and those who
206 experience greater weight gain remain having lower insulin sensitivity compared to
207 infants born at term [22].

208

209 Conversely, glucose tolerance by GTT was significantly worse in the N-10 group than the
210 R-16 group, as shown by AUC. The two catch-up groups were intermediate between the
211 N-10 and R-16 groups. The differences in fasting blood glucose among the groups is
212 consistent with the findings that mice who are small at birth and have postnatal catch-up
213 growth are at high risk of glucose intolerance [23]; however, there was no significant
214 group effect in AUC for the first 30 min of the GTT, and differences in glucose tolerance
215 were only apparent after 30 min.

216

217 Growth before weaning, specifically before d11, could be a critical window for later
218 programming. The developmental origins of disease hypothesis suggests that prenatal
219 development is critical to metabolic adaptation later in life [24]. However, the postnatal
220 environment may be “mismatched” to the early *in utero* environment, creating a
221 disadvantageous phenotype [25]. Cognitive outcomes were worse in the three groups
222 with early growth restriction (R-6, R-10, R-16), and highest in the group with greater
223 early growth (N-10). We thus show that growth restriction, despite catch-up growth, may
224 predispose poor cognition. Though there were no differences in MBP expression at d60,

225 this may be due to the fact that the maximum rate of myelin accumulation in the rat
226 occurs around d20 [26, 27]. Myelin accumulation does continue into adulthood in the rat,
227 though it occurs at a decreasing rate [28]. Several animal studies have shown that dietary
228 restriction during the suckling period results in decreased myelination in early life [29-
229 31]. In our study, early postnatal growth restriction and possible undernutrition due to
230 large litter size may be a cause for the developmental impairments seen in the R groups.

231

232 We also examined the effects of growth restriction and catch-up growth on serum
233 hormones, specifically insulin and leptin. Neonatal overfeeding of pups by litter size
234 manipulation has been shown to result in a significant elevation of serum insulin
235 concentration and alterations in hepatic enzymes involved in carbohydrate and lipid
236 metabolism [32]. However, we did not find a significant difference in serum insulin
237 concentrations. Interestingly, serum leptin at d22 and d60 differed significantly between
238 groups, with the R-16 group having the lowest levels. This is consistent with our previous
239 data on d21 [13]. The association of low leptin concentrations in the R litters and poor T-
240 maze score suggests that reduced leptin levels may be a mechanism behind the
241 differences seen in cognition. Leptin has recently been proposed to play a role in brain
242 development during the prenatal and neonatal periods [33]. Administration of leptin to
243 *ob/ob* mice, which are leptin deficient, has been shown to increase brain weight, total
244 brain DNA, and increase MBP-mRNA expression in rodents [34, 35], further suggesting
245 a role for leptin in brain development.

246

247 Finally, we demonstrated that hepatic triglyceride content was highest in the group with

248 early catch-up growth (R-6). Hepatic lipid accumulation may be one of the earliest
249 findings in the metabolic syndrome in humans. This, combined with the differences in
250 fasting glucose and in insulin sensitivity, suggests that catch-up growth in this model may
251 be associated with increased risk of metabolic syndrome.

252

253 **CONCLUSION**

254 In summary, we have demonstrated that early growth restriction leads to profound and
255 long-lasting adverse effects on neurodevelopment. Catch-up growth occurs after early
256 postnatal growth restriction, and complete catch-up in weight can occur if it begins before
257 d21 in the rat (equivalent to the first 2-3 y in humans). Postnatal growth restriction
258 without catch-up growth (R-16) leads to short-term reductions in body adiposity, while
259 postnatal growth restriction with catch-up growth (R-6) leads to short-term increases in
260 body adiposity. Neither of these changes in body composition is maintained long-term.
261 Postnatal growth restriction without catch-up growth leads to improved glucose
262 tolerance. However, insulin sensitivity is reduced if catch-up growth occurs after
263 postnatal growth restriction. These finding reinforce the concerns that *ex utero* growth
264 restriction in preterm infants reduces long-term neurocognitive outcomes, and that
265 subsequent catch-up growth may impair insulin sensitivity without improving
266 development.

267

268 **METHODS**

269 **Animals**

270 Timed pregnant CD dams were obtained from Charles River (Wilmington, MA) at 14 d
271 of gestation. Rats were housed in solid plastic hanging cages under constant conditions
272 (temperature, 22°C; humidity, 62%) with a 12-h dark-light cycle and were allowed to
273 consume food and water *ad libitum*. On d2, rat pups were randomized to litters of 10 pups
274 per dam (Normal growth, N) or 16 pups per dam (Restricted growth, R). On d11, R pups
275 were re-randomized into litters creating catch-up (R-6, 6 pups/dam), normal (R-10, 10
276 pups/dam) or reduced growth (R-16, 16 pups/dam) groups. N pups remained in litters of
277 10 pups/dam (N-10). Equal numbers of males and females were included in all litters (Fig
278 1). Pups were weaned at d21 to a standard, non-purified rodent diet (LabDiet 5001,
279 Purina, Hayward, CA) fed *ad libitum*. Weights were monitored until d60. The University
280 of California Institutional Animal Care and Use Committee approved all animal
281 procedures.

282 The period from d2-10 in rats is typically taken to represent the period between the early
283 third trimester and term in humans, and therefore represents early *ex utero* life in preterm
284 infants. The period from d11-21 in the rat is broadly representative of the first 2 y of life
285 in humans, and therefore reflects the period where catch-up growth is common in human
286 preterm infants [18].

287 **Body Composition**

288 A subset of animals had body composition assessed at d60 by carcass analysis. Frozen
289 carcasses were cut and freeze-dried for 24 h to determine water content, calculated from

290 change in weight before and after freeze-drying. Fat content was measured from the
291 change in weight after diethyl ether (Fisher Scientific, Pittsburgh, PA) extraction for 7 d
292 using a Soxhlet apparatus, followed by acetone (Fisher Scientific, Pittsburg, PA)
293 extraction for an additional 7 d. Total ash content was determined following muffle
294 furnace incineration for 72 h at 540°C and desiccation for 24 h. Protein was calculated as
295 the difference between post-fat extraction weight and ash content. Water, protein, fat and
296 ash content of each animal were expressed as a percentage of total body weight.

297 **Biochemical Analysis**

298 Blood samples were collected at time of sacrifice on d22 and d60. Specimens were
299 centrifuged at 1000 x g for 15 min at 4°C, and serum samples stored at -80°C until
300 analysis. Serum insulin and serum leptin were measured using ELISA kits (Millipore,
301 Billerica, MA). Serum and hepatic triglycerides were measured with Triglyceride
302 Reagent (Fisher Scientific, Pittsburg, PA) and read at 540 nm at 37°C.

303 **Insulin and Glucose Tolerance Tests**

304 An intraperitoneal insulin tolerance test (ITT) was performed on d50 after 4 h of food
305 deprivation. Insulin (0.5 U/kg body weight [36]) was injected intraperitoneally and blood
306 glucose levels were measured in tail vein blood using a glucometer (Easy Plus, Home
307 Aid Diagnostics, Deerfield Beach, FL) at 0, 15, 30, 45, 60, 90, and 120 min after insulin
308 injection. The area under the blood glucose curve (AUC) was calculated using a
309 rhomboid rule. The primary comparison between groups was the total AUC for the entire
310 study (120 min); secondary comparisons were for the AUC between 0 min and 30 min,
311 and between 30 min and 120 min. Larger values for AUC denote poorer insulin

312 sensitivity. In addition, the change in blood glucose from baseline (0 min) was examined.
313 The area under baseline (AUB) was calculated for the entire period, and for the first 30
314 min and last 90 min separately.

315 After a 3-d recovery period, an intraperitoneal glucose tolerance test (GTT) was
316 performed after 12 h of food deprivation. Rats were injected intraperitoneally with 2 g/kg
317 of glucose solution (Sigma, St. Louis, MO) and blood glucose was measured at 0, 15, 30,
318 45, 60, 90, 120, 150, and 180 min after glucose injection [37]. As before, the blood
319 glucose concentrations were used to calculate the area under the blood glucose versus
320 time curve (AUC) for the entire study (0 min to 180 min), as well as for the first 30 min
321 and the last 150 min. Changes in blood glucose from the 0 min baseline were also
322 calculated and the area over the baseline (0 min) value calculated using a rhomboid rule
323 for the time periods 0-180 min, 0-30 min, and 30-180 min.

324 **T-Maze**

325 Memory and learning were examined by spontaneous alternation in a T-maze on d35. In
326 the T-maze test, rats were tested on their capability to alternate between two directions of
327 an enclosed apparatus in the form of a T placed horizontally, as previously described
328 [38]. Upon successful alternation of direction, animals were given a score of 1. This was
329 repeated ten times, with the maximum score being 9.

330 **Brain Histology and Immunohistochemistry**

331 For brain histology studies, rats (d60) were deeply anaesthetized with pentobarbital (100
332 mg/kg) and fixed by transcardial perfusion with 4% paraformaldehyde. Total brains were
333 removed and placed in 4% paraformaldehyde solution overnight at 4°C. Samples were

334 next placed in serial dilutions until fixed in 100% ethanol and embedded in paraffin.
335 Coronal sections were cut into 8-10 μm sections and immunohistochemically stained
336 with goat polyclonal anti-MBP antibody (sc-13914, Santa Cruz Biotechnology, Santa
337 Cruz, CA) at 1:100 dilution in blocking buffer and donkey anti-goat secondary antibody
338 (sc-2020, Santa Cruz, Biotechnology, Santa Cruz, CA) at 1:500 in 1% BSA. The staining
339 was developed with DAB substrate (Vector Laboratories, Burlingame, CA) and sections
340 were counterstained with toluidine (0.1%) blue. Images were acquired under microscope
341 at 40X magnification (DP Olympus BX51). Areas of MBP fibers were assessed as MPB-
342 positive per high power field and quantified using ImageJ software (NIH, Bethesda,
343 MD).

344 **Data Analysis**

345 **Glucose Homeostasis**

346 Blood glucose data for the glucose tolerance test are expressed as area under the curve
347 (AUC), calculated using a rhomboid rule. AUC was calculated for the entire study period
348 (AUC_{0-180}), for the first 30 min (0-30 min, AUC_{0-30}) of the study and for the last 150 min
349 (30-180 min, AUC_{30-180}) of the study. Changes in blood glucose from the time-0 baseline
350 are expressed as the area over the time-0 baseline (AOB) for the same time intervals.

351 Blood glucose data for the insulin tolerance test were converted to AUC, and are
352 expressed for the entire study period (AUC_{0-120}), for the first 30 min (AUC_{0-30}), and for
353 30-120 min (AUC_{30-120}). Changes in blood glucose data for the insulin tolerance test are
354 expressed as the area under the baseline (AUB) for the same time intervals.

355 The primary outcome measure for the glucose tolerance test and for the insulin tolerance

356 tests was the area under the curve (AUC) for the entire study period (AUC₀₋₁₂₀).
357 Secondary outcomes for the glucose tolerance test and for the insulin tolerance test was
358 the area under the curve (AUC) for the first 30 min, and for the rest of the study, and the
359 changes in glucose from baseline.

360 **Statistical analysis**

361 Weight data were analyzed by repeated-measures ANOVA with age, sex, and group as
362 independent variables.

363 The effect of group on other continuously distributed outcomes was assessed by ANOVA
364 with sex as a covariant. If main effects ANOVA showed a significant effect of "group",
365 post-hoc testing to assess differences between the groups was carried out when needed
366 using Tukey's HSD. All statistical analyses were performed using JMP Pro 11.0 (SAS
367 Institute, Cary, NC) and statistical significance was accepted at $P < 0.05$.

368 Data are expressed as means \pm SEM.

369

370 **COMPETING INTERESTS**

371 The authors declare that they have no competing interests.

372

373 **AUTHORS' CONTRIBUTIONS**

374 EEA carried out the animal experiments and assays, interpreted data, and drafted the
375 manuscript. BL participated in the study design, data interpretation, and revised the
376 manuscript. IJG conceived the study and performed the statistical analysis, participated in

377 data interpretation, and also revised the manuscript. All authors read and approved the
378 final manuscript.

379

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- 491

492 **FIGURE LEGENDS**

493 **Figure 1.** Design of the animal study. On d2, rat pups were randomized to litters of
494 10/dam (Normal growth (N), 5 males and 5 females) or 16/dam (Restricted growth (R), 8
495 males and 8 females). On d11, R pups were re-randomized into litters creating catch-up
496 (R-6, 6 pups/dam), normal (R-10, 10 pups/dam) or reduced growth (R-16, 16 pups/dam)
497 groups. N pups remained in litters of 10 pups/dam (N-10).

498

499 **Figure 2.** Postnatal weight (g) from d1-10. By d5, R pups were ~ 20% smaller than N
500 pups. Values are means \pm SEM. *Different from N litters, $p < 0.05$.

501

502 **Figure 3.** Postnatal weight (g) from d11-21. All R groups diverged by d12. By d15, the
503 N-10 and R-6 groups were similar, the R-16 group showed no catch-up growth, and the
504 R-10 group caught-up half-way between the N-10 and R-16 groups. Error bars represent
505 ± 1 SEM, if not visible they are smaller than the plot symbol.

506

507 **Figure 4.** Postnatal weight (g) at d60. The R-16 rats were significantly smaller than the
508 other three groups, and this was seen in males and females. * $P < 0.05$. Error bars
509 represent ± 1 SEM, if not visible they are smaller than the plot symbol.

510

511 **Figure 5.** Percentage of water, protein, fat, and ash for each study group at d60. Error
512 bars represent mean \pm SEM.

513

514 **Figure 6.** Blood glucose during an intraperitoneal insulin tolerance test (ITT) at d50.

515 Fasting blood glucose was similar in all four groups. Error bars represent ± 1 SEM, if not

516 visible they are smaller than the plot symbol.

517

518 **Figure 7.** Blood glucose during an intraperitoneal glucose tolerance test (GTT) at d55.

519 Fasting blood glucose was significantly affected by sex ($M > F$; $p = 0.0061$) and by group

520 ($p = 0.0022$). Error bars represent ± 1 SEM, if not visible they are smaller than the plot

521 symbol

522

523 **Figure 8.** T-maze test success. The N-10 animals scored significantly better (6.86 ± 0.13

524 successes, $p < 0.05$) than any of the other groups (R-6 5.6 ± 0.18 , R-10 5.6 ± 0.26 , R-16,

525 5.14 ± 0.15 successes). Different letters denote significance.

526

527 **Figure 9.** Myelin basic protein (MBP) staining at d60 of (A) N-10, (B) R-10, (C) R-6,

528 and (D) R-16 groups. No significant differences in MBP-positive fibers could be

529 detected. Scale bar = 1000 μm .

530

531

532

533 **TABLES**

534 **Table 1.** Fasting glucose, insulin, leptin, and triglycerides in the four groups on d60. Data
535 are expressed as mean \pm SEM. *P*-values represent the overall ANOVA *p*-values. *Denote
536 significant difference from the N-10 group, $p < 0.05$.

Group	N-10	R-10	R-6	R-16	<i>P</i> value
Fasting glucose (mg/L)	1084.7 \pm 34.4	1057.3 \pm 19.3	1080 \pm 31.8	1075.2 \pm 22.0	NS
Serum insulin (ng/mL)	2.24 \pm 0.43	2.41 \pm 0.26	2.36 \pm 0.61	2.46 \pm 0.71	NS
Leptin (ng/mL)	3.74 \pm 0.37	4.13 \pm 1.12	4.27 \pm 0.77	2.79 \pm 0.58*	<i>P</i> = 0.0037
Serum TG (mg/L)	1603 \pm 307	1220 \pm 238	1794 \pm 487	1450 \pm 373	NS
Hepatic TG (mg/L)	1550 \pm 263	1100 \pm 139*	2130 \pm 270	1138 \pm 194*	<i>P</i> = 0.0203
<i>n</i>	20	20	12	32	

537

538