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RESEARCH ARTICLE | *Model Systems for the Study of Integrative Physiology: The Rebirth of Translational Biology*

Maternal nutrient restriction in guinea pigs as an animal model for studying growth-restricted offspring with postnatal catch-up growth

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Nevin CL, Formosa E, Maki Y, Matushewski B, Regnault TR, Richardson BS. Maternal nutrient restriction in guinea pigs as an animal model for studying growth-restricted offspring with postnatal catch-up growth. *Am J Physiol Regul Integr Comp Physiol* 314: R647–R654, 2018. First published January 10, 2018; doi:10.1152/ajpregu.00317.2017.—We determined the impact of moderate maternal nutrient restriction (MNR) in guinea pigs with fetal growth restriction (FGR) on offspring body and organ weights, hypothesizing that FGR-MNR animals will show catch-up growth but with organ-specific differences. Guinea pig sows were fed ad libitum (Control) or 70% of the control diet from 4 weeks preconception, switching to 90% at midpregnancy (MNR). Control newborns >95 g [appropriate for gestational age (AGA); $n = 37$] and MNR newborns <85 g (FGR; $n = 37$) were monitored until neonatal (~25 days) or adult (~110 days) necropsy. Birth weights and body/organ weights at necropsy were used to calculate absolute and fractional growth rates (FRs). FGR-MNR birth weights were decreased ~32% compared with the AGA-Controls. FGR-MNR neonatal whole body FRs were increased ~36% compared with Controls indicating catch-up growth, with values negatively correlated to birth weights indicating the degree of FGR leads to greater catch-up growth. However, the increase in organ FRs in the FGR-MNR neonates compared with Controls was variable, being similar for the brain and kidneys indicating comparable catch-up growth to that of the whole body and twofold increased for the liver but negligible for the heart indicating markedly increased and absent catch-up growth, respectively. While FGR-MNR body and organ weights were unchanged from the AGA-Controls by adulthood, whole body growth rates were increased. These findings confirm early catch-up growth in FGR-MNR guinea pigs but with organ-specific differences and enhanced growth rates by adulthood, which are likely to have implications for structural alterations and disease risk in later life.

fetal growth restriction; maternal undernourishment; postnatal catch-up growth

INTRODUCTION

Fetal growth restriction (FGR) is a major contributor to perinatal morbidity and mortality and for later adverse health outcomes, including cardiovascular disease, diabetes, and neu- rodevelopmental disability (2, 20, 33, 37, 44). This has led to the notion that restriction of fetal growth as an adaptation to impaired nutrient delivery can adversely affect the structure and functional development of major tissues or organs thereby increasing risk for later health adversities (1, 2, 18, 20). Moreover, dietary conditions and feeding activity in the early postnatal period are likely to contribute to this aberrant devel- opment since FGR offspring who display acceleration of growth, termed catch-up growth, compared with those who do not, are at even greater risk for later health impairment as well as obesity (1, 2, 5, 16, 40).

Guinea pigs deliver precocial young after a relatively long pregnancy with peak brain growth during the latter half of pregnancy similar to that in humans with peak brain growth at birth, whereas in other rodents this process occurs postnatal (4, 34). Guinea pig fat content of ~10% at birth is similar to that in humans and much more than that in rats and sheep. Addi- tionally, placental development being hemomonochorial is more similar to human placentation than that in other nonpri- mate species (4, 15). Accordingly, moderate maternal nutrient restriction (MNR) in guinea pigs at 70% of an ad libitum diet from 4 weeks preconception until midpregnancy increasing to 90% thereafter has been utilized for inducing FGR and study- ing maternal, placental, and fetal growth and developmental outcomes. We (13, 14) and others (32, 39) have shown that this experimental paradigm in guinea pigs leads to moderate to severe FGR with fetal weights decreased by 28–40% near term and with aberrant placental development, asymmetrical growth, polycythemia, hypoglycemia, and evidence of chronic hypoxia in visceral tissues. These findings support the utility of this model for inducing FGR with many similarities to that in humans with moderate to severe growth restriction whether resulting from maternal undernourishment or placental insuf- ficiency (11, 19, 33, 38). Moderate MNR as outlined also targets the insult preconception, periconception, and through- out pregnancy analogous to the human situation since most undernourished women do not improve their dietary and life- style patterns in pregnancy (6). This feeding regime has added relevance for comparative study in humans since periconcep- tion and early pregnancy nutrient availability are known to be important determinants of embryonic inner cell mass and fetal growth trajectory (20).

While fetal body and organ weights in males and females have been well described with moderate MNR in guinea pigs

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(13, 14, 32, 39), there has been no comparable study in FGR offspring and the extent to which catch-up or overgrowth occurs during the early neonatal period to adulthood. We have therefore studied moderate MNR in guinea pigs and report on our pregnancy outcomes in animals delivering spontaneously near or at term and the distribution of newborn weights and means for denoting FGR to further characterize the utility of this model. We have also determined the impact on absolute (AGR) and fractional growth rates (FR) through the neonatal period and on select organ weights at neonatal and later adult necropsy in both males and females, postulating that FGR-MNR animals will show catch-up growth as is often seen in human infants with growth restriction but with organ-specific differences that might have implications for later disease risk.

MATERIALS AND METHODS

Animal feeding and breeding. An established model of moderate MNR in guinea pigs (13, 14, 32, 39) was used with all experimental procedures approved by The University of Western Ontario Animal Care Committee (Animal Use Protocol 2014–027) and followed the guidelines of the Canadian Council on Animal Care. Animal care, feeding, and breeding have previously been described (13). Briefly, 39 young adult guinea pig sows (Dunkin-Hartley ~4–6 mo of age, from Charles River Laboratories, Sherbrooke, QC, Canada) were randomly assigned to either a Control group fed ad libitum (Guinea Pig Diet 5025; LabDiet, St. Louis, MO) or an MNR group fed 70% of the average food intake per kilogram of body weight of the ad libitum fed animals from 4 weeks preconception until midpregnancy at 35 days of gestation, increasing to 90% thereafter. Throughout the experiment, daily food intake and body weight of the animals were monitored three to four times per week, and the dietary intake of the MNR animals was adjusted as needed to maintain their food intake at 70 or 90% of the average food intake per kilogram of body weight of the ad libitum fed animals.

Pupping, postnatal feeding, and growth measurements. Sows delivered spontaneously near or at term (~68 days of gestation). The number of live born and demised newborns was noted, and body weights (g) were measured on all pups within 24 h of birth. Crown rump length from the tip of the nose to the rump (cm) and abdominal circumference at the level of the umbilicus (cm) were additionally measured on all live born pups using calibrated string. Newborns were considered to be appropriate for gestational age (AGA) if >95 g and FGR if <85 g, which was extrapolated from our fetal study using moderate MNR (13).

Control and MNR sows remained on their respective feeding regimes until 14 days postnatal to mimic the human situation where undernourished mothers are likely to remain undernourished through the lactation period (3). However, at this time, MNR sows were placed on 100% of the average food intake per kilogram body weight of the ad libitum-fed animals since pups from both animal groups were beginning to wean and were noted to be eating the mother's food allocation.

Neonatal weights were measured in the morning before the daily allocation of maternal feed at the end of *weeks 1, 2,* and *3* postnatal. Neonatal AGRs (g/day) were determined from the *week 3* postnatal weight minus respective birth weights divided by 21 days as a measure of early growth up until weaning (7, 31). Neonatal FRs (%/day) were calculated as the neonatal AGR divided by the birth weight × 100, which is a better measure of the anabolic partitioning of nutrients toward growth and thereby of early catch-up growth (7, 31).

Neonatal and adult necropsies, tissue collection, and growth measurements. Neonatal necropsies were undertaken between 23 and 27 days corrected postnatal age so that postconceptual age was comparable for all neonates at the time of necropsy. This was done to

limit the effect of preterm delivery per se on growth measurements obtained at neonatal necropsy. Only AGA newborns from Control group litters and FGR newborns from MNR group litters were subjected to neonatal necropsy with no more than two males and one female or one male and two females per litter utilized. These neonatal offspring were also subjected to brain magnetic resonance imaging (MRI) no less than 72 h before necropsy. Remaining AGA-Control and FGR-MNR offspring that were not subjected to neonatal necropsy or culled were separated from their mothers and allowed ad libitum access to the guinea pig diet. Adult necropsies were undertaken on these animals between 99 and 124 days of postnatal age (AGA-Controls 109 ± 2, FGR-MNR 111 ± 1), again with no more than two males and one female or one male and two females per litter utilized. These adult offspring were also subjected to neurobehavioral study and brain MRI no less than 72 h before necropsy. Before necropsy, all animals were weighed and then euthanized with 0.3 ml intraperitoneal injection of Euthanol (pentobarbital sodium; MTC Pharmaceuticals, Cambridge, ON, Canada). Cardiac puncture was then carried out to obtain ~2 ml of blood in a heparinized syringe, which was cold centrifuged, and the plasma was collected and stored at −80°C for later analysis. This was followed by dissection and weighing of the brain (including cerebral hemispheres, cerebellum and brainstem), heart, liver, and both kidneys and gonads.

Neonatal body and organ FRs (%/day) were calculated for the AGA-Control and FGR-MNR animals undergoing neonatal necropsy. These were determined from the necropsy body weights and respective birth weights, from the necropsy organ weights and respective estimated organ weights at birth, and from postnatal age in days at necropsy as previously described. Estimated organ weights at birth were determined using each animal's birth weight and organ/body weight percentages from fetal animal groups subjected to the same dietary regimes as in the present study and necropsied near term (13, 32), assuming these remain unchanged until birth. Accordingly, these organ growth rates are also estimates and are presented by animal group for males and females combined with the primary intent of assessing both body and organ catch-up growth in the FGR-MNR neonates as reflected by their FRs. Postweaning AGRs and FRs were also calculated for the AGA-Control and FGR-MNR animals undergoing adult necropsy. These were determined from the body weights at adult necropsy along with their respective weights at 3 wk postnatal and time in days between these weightings. Accordingly, these are actual growth rates and are shown by animal group for males and females separately to allow for comparison of neonatal and postweaning values.

Data acquisition and statistical analysis. Litter size and weight were based on live born and demised newborns noted at birth. Overall Control and MNR growth characteristics included data from all Control sows and their live born newborns, and all MNR sows and their live born newborns, excluding data from animals that failed to conceive. AGA-Control and FGR-MNR growth characteristics included data from all AGA-Control and FGR-MNR newborns that were live born and met the birth weight and litter selection criteria noted. Maternal and newborn/neonatal/adult characteristic findings are presented as group means ± SE. Overall Control and MNR population characteristics and AGA-Control and FGR-MNR growth and necropsy characteristics were compared using two-way ANOVA to determine the effects of MNR and sex. Where interactions between MNR and sex were present, post hoc tests (Tukey) were carried out to determine the effect of MNR in males and females separately. Associations between neonatal FRs and birth weight were assessed by Pearson's correlation analysis (Graphpad Software, San Diego, CA). For all analysis, statistical significance was assumed for $P < 0.05$. Neonatal and adult neurobehavioral and brain MRI findings are reported separately.

Table 1. *Maternal and newborn population characteristics*

	Control (16 sows/44 live born)	MNR (17 sows/52 live born)	AGA-Control (37 live born)	FGR-MNR (41 live born)
Maternal wt, g				
Conception	812 ± 16	822 ± 11		
Delivery	$1{,}247 \pm 41$	$1{,}094 \pm 18^{**}$		
Gestational age at delivery, days	68 ± 0	$67 \pm 0^{*}$		
Litter size	3.3 ± 0.3	3.4 ± 0.2		
Total litter wt, g	342 ± 28	$271 \pm 9^{*}$		
Birth wt, g	105 ± 2	$79 \pm 2^{***}$	109 ± 1	$74 \pm 1^{***}$
Crown rump length, cm	16.8 ± 0.2	$15.2 \pm 0.2^{***}$	17.0 ± 0.2	$14.9 \pm 0.2^{***}$
Body wt/length, g/cm	6.3 ± 0.1	$5.2 \pm 0.1^{***}$	6.4 ± 0.1	$5.0 \pm 0.1^{***}$
Abdominal circumference, cm	10.8 ± 0.2	$9.9 \pm 0.2^{***}$	10.9 ± 0.1	$9.6 \pm 0.1^{***}$

Data presented as means $\pm$ SE. MNR, maternal nutrient restriction; AGA, appropriate for gestational age; FGR, fetal growth restriction. AGA-Controls included all Control live born >95 g; FGR-MNRs included all MNR live born <85 g. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. corresponding Control group value.

RESULTS

Breeding and pregnancy outcomes. Two of 18 guinea pig sows bred under ad libitum-feeding conditions and 4 of 21 guinea pig sows bred under MNR feeding conditions failed to become pregnant despite up to four breeding attempts. The 16 pregnant Control sows delivered at 68 ± 0 days of gestation (range of $67-71$ days) with 44 live born and 9 newborn demises, which formed the overall Control population. The 17 pregnant MNR sows delivered a day earlier at 67 ± 0 days of gestation (range of $64-71$ days) ($P < 0.05$) with 52 live born and 6 newborn demises, which formed the overall MNR population.

Maternal and newborn population characteristics. The maternal and newborn population characteristics from all ad libitum and MNR pregnancies are shown in Table 1 with the findings for the male and female live born combined since there were no sex differences evident. While maternal weights were not different at conception, at delivery MNR sows were 12% lighter than Control sows ($P < 0.01$). Litter size did not differ between the two study groups, although the combined newborn weight per litter was 21% less for the MNR animals than that of the Control animals ($P < 0.05$). Newborn birth weights were 25% less in the MNR pregnancies at 79 ± 2 g than in the Control pregnancies at 105 ± 2 g ($P < 0.001$). Crown rump lengths were decreased in the MNR newborns by 10% ($P < 0.001$), with body weight/length as a measure of leaness thereby decreased by 17% versus that of the Control newborns ($P < 0.001$). Abdominal circumference as an additional measure of leaness was also decreased in the MNR newborns by 8% vs. the Control newborns ($P < 0.001$).

Control newborns ranged in weight from 80 to 132 g, while MNR newborns ranged in weight from 58 to 126 g, indicating overlap in the population weight distributions as seen in Fig. 1. This is expected since litter size, number of fetuses per uterine horn, and fetal position within the horn are also known to impact fetal growth (9, 45). We therefore chose to establish a cohort of AGA offspring from the Control pregnancies and a cohort of FGR offspring from the MNR pregnancies for a more in-depth comparative study of growth-related parameters. As noted, we used >95 and <85 g as our thresholds for categorizing these respective cohorts at birth, which were close to the 20th and 10th percentiles for the population weight distribution of the Control newborns at ~97 and 87 g (Fig. 1). Accordingly, 84% of all Control newborns were deemed to be AGA (15 of

19 males and 22 of 25 females), while 79% of all MNR newborns were deemed to be FGR (16 of 20 males and 21 of 28 females, with 4 unsexed due to postnatal demise). The newborn population characteristics from these AGA-Control and FGR-MNR offspring are also shown in Table 1 with the male and female findings combined since there were no sex differences. FGR-MNR birth weights were now decreased by 32% at 74 ± 1 g compared with those of the AGA-Control newborns at 109 ± 1 g ($P < 0.001$), while FGR-MNR body weight/length and abdominal circumference were decreased by 22 and 12%, respectively, compared with those of the AGA-Control newborns (both $P < 0.001$).

Neonatal body weights and growth measurements. While all AGA-Control newborns survived out to 3 wk, 4 of the FGR-MNR newborns succumbed during the first week postnatal precluding further study in these animals (birth weights of 58, 62, 62, and 72 g). Birth weights and weekly neonatal weights for remaining offspring over the first 3 wk postnatal along with associated growth rates are shown by animal group and sex in Table 2. FGR-MNR birth weights were decreased 31% compared with those of the AGA-Controls ($P < 0.001$) and while both animal groups showed increased weight through *week 1*
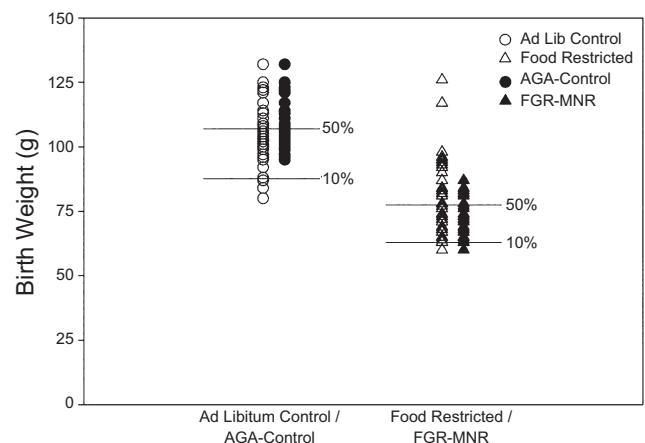

Fig. 1. Scatter plot showing the birth weights for all 44 live born Control newborns (○) and all 52 live born maternal nutrient restricted (MNR) newborns (△) along with the 50th and 10th percentiles for each of these cohort populations. Additionally shown are the distribution of newborn weights for the 37 appropriate for gestational age (AGA)-Control newborns (●) and 41 fetal growth restriction (FGR)-MNR newborns (▲).

Table 2. *Neonatal body weights and growth rates*

	Male		Female		P (ANOVA)		
	AGA-Control ($n = 15$)	FGR-MNR ($n = 16$)	AGA-Control ($n = 22$)	FGR-MNR ($n = 21$)	MNR	S	MNR × S
Birth wt, g	109 ± 2	76 ± 2	108 ± 2	74 ± 2	***	NS	NS
Week 1 wt, g	153 ± 7	100 ± 4	147 ± 4	99 ± 3	***	NS	NS
Week 2 wt, g	227 ± 11	164 ± 6	214 ± 6	160 ± 5	***	NS	NS
Week 3 wt, g	301 ± 11	232 ± 6	280 ± 5	223 ± 6	***	*	NS
AGR, g/day	8.9 ± 0.4	7.3 ± 0.2	8.2 ± 0.2	7.1 ± 0.2	***	*	NS
FR, %/day	8.2 ± 0.3	9.6 ± 0.2	7.6 ± 0.2	9.6 ± 0.4	***	NS	NS

Data presented as means ± SE. AGA, appropriate for gestational age; FGR, fetal growth restricted; MNR, maternal nutrient restricted; S, sex; NS, not significant; AGR, absolute growth rate; FR, fractional growth rate. *$P < 0.05$; ***$P < 0.001$.

postnatal, FGR-MNR offspring continued to be 33% smaller with no sex differences. However, by the end of *week 2* postnatal FGR-MNR offspring were 26% smaller and by *week 3* postnatal they were 21% smaller indicating a degree of catch-up growth. Moreover, sex differences were now evident with *week 3* weights ~6% greater in males than females for both animal groups ($P < 0.05$). Neonatal AGRs over the first 3 wk postnatal were decreased 15% in the FGR-MNR offspring compared with those of the AGA-Controls ($P < 0.001$) with males again showing higher values in both animal groups ($P < 0.05$). Conversely, neonatal FRs in the FGR-MNR offspring were increased by 22% compared with those of the AGA-Controls ($P < 0.001$) and showed a modest negative correlation to birth weights, $r = -0.38$ ($P < 0.05$), which was not seen in the AGA-Control offspring, $r = -0.16$ (NS). Of note, the 11 AGA offspring from MNR pregnancies with mean birth weights of 99 ± 3 g had neonatal AGR and FR values of 8.5 ± 0.4 g/day and 8.6 ± 0.3%/day, respectively, while the 7 FGR offspring from Control pregnancies with mean birth weights of 86 ± 1 g had neonatal AGR and FR values of 7.2 ± 0.4 g/day and 8.3 ± 0.4%/day, respectively.

Neonatal necropsy body and organ weights and growth measurements. Eighteen AGA-Control offspring (8 males and 10 females) and 18 FGR-MNR offspring (9 males and 9 females) were selected for neonatal necropsy measurements that were collected at 25 ± 1 and 26 ± 1 days postnatal, respectively. Birth weights and neonatal necropsy weights for these offspring are shown by animal group and sex in Table 3. While birth weights in these FGR-MNR animals were decreased 35% compared with the AGA-Controls ($P < 0.001$), at

the time of neonatal necropsy FGR-MNR body weights were only decreased by 16% ($P < 0.001$). Mean organ weights were all decreased in the FGR-MNR neonates compared with those of the AGA-controls, with brain weights decreased 6% ($P < 0.01$), heart weights decreased 24% ($P < 0.001$), liver weights decreased 10% (NS), kidney weights decreased 16% ($P < 0.001$), testes weights decreased 24% ($P < 0.05$), and ovary weights decreased 26% ($P < 0.05$). Organ weights as a percentage of body weight were also assessed with FGR-MNR brain/body weights increased by 14% compared with the AGA-Controls ($P < 0.01$), whereas liver/body weights and kidney/body weights were not significantly changed, while heart/body weights were decreased by 10% ($P = 0.06$).

Neonatal body and organ FRs were calculated for the AGA-Control and FGR-MNR animals undergoing neonatal necropsy and are shown in Table 4. Body FRs were increased 36% in these FGR-MNR neonates at 9.8 ± 0.4%/day compared with the AGA-Controls at 7.2 ± 0.2%/day ($P < 0.001$). Brain FRs were much lower than whole body values, and although they increased by ~31% in the FGR-MNRs vs. those of the AGA-Controls, this was not significant. Heart FRs were comparable to whole body values in the AGA-Controls but were decreased rather than increased in the FGR-MNRs. Liver FRs were slightly lower than whole body values in the AGA-Controls and were increased ~69% in the FGR-MNRs ($P < 0.001$), while kidney FRs were slightly higher than whole body values in the AGA-Controls and were increased ~32% in the FGR-MNRs ($P < 0.001$).

Table 3. *Neonatal necropsy body and organ weights (23–27 days postnatal)*

	Male		Female		P (ANOVA)		
	AGA-Control ($n = 8$)	FGR-MNR ($n = 9$)	AGA-Control ($n = 10$)	FGR-MNR ($n = 9$)	MNR	S	MNR × S
Birth wt, g	112 ± 4	73 ± 2	109 ± 3	70 ± 3	***	NS	NS
Necropsy wt, g	324 ± 13	258 ± 12	297 ± 5	261 ± 17	***	NS	NS
Brain wt, g	3.35 ± 0.06	3.15 ± 0.08	3.30 ± 0.07	3.11 ± 0.06	**	NS	NS
Heart wt, g	1.85 ± 0.06	1.46 ± 0.09	1.95 ± 0.10	1.45 ± 0.12	***	NS	NS
Liver wt, g	13.9 ± 0.9	11.8 ± 0.7	12.2 ± 0.6	11.3 ± 1.0	NS	NS	_
Kidney wt, g	3.11 ± 0.16	2.66 ± 0.10	2.96 ± 0.10	2.44 ± 0.14	***	NS	NS
Testes wt, g	0.75 ± 0.06	0.57 ± 0.04			*		
Ovary wt, g			0.15 ± 0.01	0.11 ± 0.01	*		
Brain/body wt, %	1.05 ± 0.05	1.24 ± 0.05	1.11 ± 0.03	1.23 ± 0.08	**	NS	NS
Heart/body wt, %	0.58 ± 0.02	0.56 ± 0.02	0.66 ± 0.04	0.56 ± 0.03	0.06	NS	NS
Liver/body wt, %	4.3 ± 0.1	4.6 ± 0.1	4.1 ± 0.2	4.3 ± 0.2	NS	NS	
Kidney/body wt, %	0.96 ± 0.03	1.04 ± 0.03	0.99 ± 0.03	0.94 ± 0.03	NS	NS	

Data presented as means ± SE. AGA, appropriate for gestational age; FGR, fetal growth restricted; MNR, maternal nutrient restricted; S, sex; NS, not significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 4. *Neonatal body and organ weights and growth rates*

	AGA-Control ($n = 18$)	FGR-MNR ($n = 18$)
Body wt, g		
Birth/necropsy	110 ± 2/309 ± 7	71 ± 2***/259 ± 10***
FR, %/day	7.2 ± 0.2	9.8 ± 0.4***
Brain wt,† g		
Birth/necropsy	2.97 ± 0.06/3.32 ± 0.05	2.70 ± 0.07**/3.13 ± 0.05**
FR, %/day	0.49 ± 0.10	0.64 ± 0.10
Heart wt,† g		
Birth/necropsy	0.65 ± 0.01/1.90 ± 0.06	0.51 ± 0.01***/1.46 ± 0.08***
FR, %/day	7.7 ± 0.4	7.1 ± 0.3
Liver wt,† g		
Birth/necropsy	5.1 ± 0.1/13.0 ± 0.5	3.1 ± 0.1***/11.6 ± 0.6
FR, %/day	6.1 ± 0.4	10.3 ± 0.5***
Kidney wt,‡ g		
Birth/necropsy	0.95 ± 0.02/3.03 ± 0.09	0.63 ± 0.02***/2.55 ± 0.09***
FR, %/day	8.7 ± 0.4	11.5 ± 0.4***

Data are presented as means ± SE. AGA, appropriate for gestational age; FGR, fetal growth restriction; MNR, maternal nutrient restricted; FR, fractional growth rate. **$P < 0.01$; ***$P < 0.001$ vs. corresponding Control group value. †Organ weights at birth were obtained using the present birth weights and the organ/fetal weight percentages of Elias et al. (13) for similar fetal animal groups necropsied near term and with organ weights at neonatal necropsy obtained using the present animals. ‡Organ weights at birth were obtained using the present birth weights and the organ/fetal weight percentages of Kind et al. (32) for similar fetal animal groups necropsied near term and with organ weights at neonatal necropsy obtained using the present animals.

Adult necropsy body and organ weights and growth measurements. Eighteen AGA-Control offspring (6 males and 12 females) and 16 FGR-MNR offspring (6 males and 10 females) were available for adult necropsy measurements after culling of AGA-Control (1 male) and FGR-MNR (1 male and 2 females) offspring at weaning from litters with three or more offspring of the same sex. Nine of these AGA-Control adults and nine of these FGR-MNR adults were litter mates of animals selected for neonatal necropsy with initial priority given to establishment of the neonatal cohorts. Birth weights, postnatal *week 3* weights, and adult necropsy weights for these offspring are shown by animal group and sex in Table 5. While *week 3* weights in these FGR-MNR animals were decreased 22% compared with the AGA-Controls ($P < 0.001$), at the time of necropsy as young adults, FGR-MNR weights were not different from that of the AGA-Controls. However, body weights were now ~17% greater in males than females in the AGA-Control and FGR-MNR animal groups ($P < 0.001$). Mean organ weights were also not different between the two groups and were not different for the organ weights as a percentage of body weight except for that the heart where heart/body weights were decreased 12% in the FGR-MNR animals ($P < 0.05$). Sex differences were again evident with all organ weights greater in males than females for both animal groups (brain, heart, and liver, all $P < 0.01$; kidney, $P < 0.05$), while the brain/body weight was greater in females than males for both animal groups ($P < 0.05$).

Postweaning body AGRs and FRs were calculated for the AGA-Control and FGR-MNR animals undergoing adult necropsy and are shown by animal group and sex in Table 5. Postweaning AGRs were all lower than respective rates seen in neonates, with males again showing higher AGRs in both animal groups ($P < 0.001$). However, FGR-MNR AGRs were now increased ~18% compared with those of the AGA-Controls ($P < 0.05$) rather than decreased as seen in neonates. Postweaning FRs were likewise all lower than respective values seen in neonates but with males showing higher FRs in both animal groups ($P < 0.05$). Additionally, the increase in FGR-MNR FRs vs that of the AGA-Controls at ~56% ($P < 0.001$) was much greater than that seen in neonates.

DISCUSSION

MNR sows delivered a day earlier on average with preterm delivery also seen in our fetal MNR study (13) and in that of Kind et al. (32). This is similar to the human situation where preterm birth and FGR are increased in underweight mothers with low body mass index and/or weight gain during pregnancy (12, 29, 41, 42). However, the mechanisms underlying the initiation of preterm labor in underweight mothers and the added role played by FGR in this process remain poorly understood (41). Demise rates for the Control and MNR newborns were higher than the demise rates we noted with fetal necropsy at 60/61 days at ~3% (13). While the causes for these newborn demises were not determined, they are likely to be multiple and include difficulties during delivery and initiating

Table 5. *Adult necropsy body and organ weights (99–124 days postnatal) and growth rates*

	Male		Female		*P* (ANOVA)		
	AGA-Control ($n = 6$)	FGR-MNR ($n = 6$)	AGA-Control ($n = 12$)	FGR-MNR ($n = 10$)	MNR	S	MNR × S
Birth wt, g	106 ± 2	81 ± 1	108 ± 2	77 ± 2	***	NS	NS
Week 3 wt, g	305 ± 20	242 ± 7	287 ± 9	220 ± 9	***	NS	NS
Necropsy wt, g	756 ± 52	784 ± 30	631 ± 15	642 ± 25	NS	***	NS
AGR, g/day	5.2 ± 0.5	6.1 ± 0.2	4.0 ± 0.2	4.7 ± 0.3	*	***	NS
FR, %/day	1.7 ± 0.1	2.6 ± 0.1	1.4 ± 0.1	2.2 ± 0.2	***	*	NS
Brain wt, g	4.17 ± 0.08	4.43 ± 0.09	4.13 ± 0.07	3.91 ± 0.07	NS	**	**
Heart wt, g	3.96 ± 0.14	3.51 ± 0.15	3.21 ± 0.16	2.99 ± 0.22	NS	**	NS
Liver wt, g	28.6 ± 2.6	27.8 ± 1.6	21.4 ± 1.0	24.8 ± 1.5	NS	**	NS
Kidney wt, g	5.41 ± 0.31	5.20 ± 0.27	4.50 ± 0.14	4.40 ± 0.15	NS	*	NS
Testes wt, g	4.33 ± 0.29	4.41 ± 0.29			NS		
Ovary wt, g			0.13 ± 0.02	0.13 ± 0.03	NS		
Brain/body wt, %	0.56 ± 0.04	0.57 ± 0.02	0.66 ± 0.02	0.62 ± 0.03	NS	*	NS
Heart/body wt, %	0.53 ± 0.03	0.45 ± 0.02	0.51 ± 0.03	0.46 ± 0.02	*	NS	NS
Liver/body wt, %	3.8 ± 0.2	3.5 ± 0.1	3.4 ± 0.1	3.8 ± 0.1	NS	NS	
Kidney/body wt, %	0.73 ± 0.06	0.67 ± 0.03	0.71 ± 0.02	0.69 ± 0.02	NS	NS	

Data presented as means ± SE. AGA, appropriate for gestational age; FGR, fetal growth restricted; MNR, maternal nutrient restricted; S, sex; NS, not significant; AGR, absolute growth rate; FR, fractional growth rate. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

early feeding, given the range in birth weights with normally grown as well as FGR pups in both animal groups. Difficulties with feeding are also likely to have played a role in the four FGR-MNR neonatal demises, with three of these pups at or below the 10th percentile for birth weights in the MNR newborns. Newborn weights for all live born animals in the MNR pregnancies were decreased by 25% with asymmetrical growth restriction as indicated by the associated decrease in body weight/length and abdominal circumference compared with the Control newborns, as we (13) and others (32, 39) have previously reported with this animal model for inducing FGR.

Birth weight thresholds of >95 g for AGA born offspring from Control pregnancies and <85 g for FGR born offspring from MNR pregnancies were close to the 20th and 10th percentiles for the population weight distribution of the live born Control newborns. This establishment of AGA-Control and FGR-MNR offspring groups has the advantage of avoiding any confounding effects of study in AGA fetuses from MNR pregnancies and FGR fetuses from Control pregnancies and better reflects the human situation where AGA and FGR birth weight distributions are separate and often delineated by the 10th percentile adjusted for gestational age (38). Of note, the decrease in FGR-MNR newborn weights at ~32% was similar to that in our FGR-MNR fetal weights necropsied at 60/61 days in comparison to respective AGA-Controls (13) indicating that growth trajectories for these cohort groups remain relatively unchanged with respect to one another over the last week of guinea pig pregnancy.

While FGR-MNR newborn weights were decreased ~32%, by the end of *week 3* postnatal, FGR-MNR weights were only decreased by 21% compared with AGA-Control weights indicating a degree of catch-up growth. Increased neonatal FRs in the FGR-MNR offspring likewise indicate higher growth rates relative to initial size thereby reflecting a greater partitioning of nutrients toward growth in these animals. This finding of early catch-up growth in guinea pigs born FGR after moderate MNR is similar to that seen in other species with induced FGR (7, 21, 24, 31, 36, 46, 50) and in humans born growth restricted (1, 2, 5, 16, 17, 28, 30, 39), albeit with the degree of catch-up growth also dependent on postnatal dietary conditions. Neonatal FRs were negatively correlated to birth weights in the FGR-MNR animals, which was also reported by Kind et al. (31) in FGR guinea pigs, indicating that the degree of growth restriction at birth also impacts the degree of catch-up growth. This relationship likely involves in utero programming of increased postnatal feeding activity or hyperphagia in association with increased insulin and leptin signaling activity, which have been shown to predict catch-up growth in relation to size at birth (5, 7, 8, 21, 31, 36, 46). The AGA-MNR and FGR-Control offspring with birth weights and FR values that were both intermediary between those of the AGA-Control and FGR-MNR offspring further support a relationship between size at birth and postnatal catch-up growth. However, AGA-MNRs with birth weights greater than the FGR-Controls also had FR values that were higher rather than lower, suggestive of enhanced programming of postnatal catch-up growth with maternal under nutrition vs. normal nutrition with lower size at birth.

Mean organ weights obtained at neonatal necropsy were all decreased in the FGR-MNR animals compared with respective AGA-Control values, but this was variable, being greatest for the heart and gonads and least for the brain and liver. The change in FGR-MNR organ weights as a percentage of body weight was also variable being increased for the brain, unchanged for the liver and kidneys, and decreased for the heart. While these findings suggest differential rates for organ catch-up growth in the FGR-MNR offspring, these rates will also be dependent on organ and body weights at birth and asymmetrical growth occurring in utero (13, 31, 32). Additionally, increasing adiposity in FGR-MNR fetuses (32) and offspring (31) will impact body weights and distort the ability to discern organ catch-up growth using organ/body weight measurements. To further assess this aspect of catch-up growth, we estimated organ weights at birth for each AGA-Control and FGR-MNR animal undergoing neonatal necropsy using their actual birth weights and the organ/body weight percentages obtained near term for fetal animal groups similarly studied with moderate MNR (13, 32). An assumption here is that these fetal organ/body weight percentages remain unchanged until birth. This seems reasonable with the decrease in FGR-MNR fetal weights at 60/61 days (13), being similar to that in the FGR-MNR newborns presently studied, and allows for the qualitative assessment of organ catch-up growth in the FGR-MNR neonates as reflected by their FRs.

Brain FRs were much lower than whole body values consistent with the slowing of brain growth relative to body growth in guinea pigs over the neonatal period and their designation as "prenatal brain developers" (10, 34). While not significant, these growth rates were increased in the FGR-MNRs by ~31%, thereby supporting a degree of early postnatal catch-up growth for the brain. This finding is reported in sheep where body weight and skull length FRs are both increased ~33% in FGR offspring after placental restriction (7), as well as in humans where growth rates in terms of body weight and head size both increase postnatal in FGR offspring (26, 30). Heart FRs were comparable to whole body values in the AGA-Controls indicating similar growth trajectories through the neonatal period with the need for increasing cardiac output with increasing body size presumably driving a parallel increase in heart size. However, these growth rates were decreased in the FGR-MNR neonates compared with the AGA-Controls indicating an absence of catch-up growth. Since induced FGR in sheep has been shown to delay binucleation and increase hypertrophy of fetal cardiomyocytes (35, 47), this in utero adaptation might help to maintain heart size in the fetus but hamper the required need for increased heart size with rapid catch-up growth postnatal, thus predisposing to left ventricular hypertrophy as seen in FGR offspring (35, 47). Liver FRs were increased ~69% in the FGR-MNR neonates indicating a greater degree of catch-up growth than in any of the other organs studied. Enhanced growth in the liver has also been reported in low birth weight lambs (22) and is again likely to involve increased insulin signaling activity and thereby glycogen and/or lipid accumulation (50), with increased risk for nonalcoholic fatty liver disease as seen in FGR offspring (17, 50). Kidney FRs were increased in a similar manner to that of the whole body in the FGR-MNR neonates indicating similar catch-up growth. This is likely to involve increased insulin-signaling activity as for other visceral tissues and whole body catch-up growth (5, 8, 21, 31, 36, 46, 47, 50). Testes and ovary weights were decreased ~25% in the FGR-MNR neonates and more so than the other organs studied except for the

heart. While FRs could not be computed here, permanent growth alterations are likely contributing to changes in reproductive function as seen in other animal studies with induced growth restriction (23, 48, 49).

FGR-MNR body and organ weights at adult necropsy were unchanged from the AGA-Controls but were increased in males compared with females for both animal groups. As such, the catch-up growth in these FGR offspring after moderate MNR was sufficient to normalize body and organ weights by young adulthood, which is similar to that reported by Kind et al. (31) in guinea pigs after moderate MNR and by De Blasio et al. (7) in sheep after placental restriction. However, increased visceral adiposity was also seen in these FGR offspring, which related to the degree of postnatal catch-up growth (7, 31), and is similarly seen in humans born FGR (5, 17, 28, 40). It is thus of note that postweaning AGRs and FRs were both increased in the FGR-MNR offspring compared with the AGA-Controls and considerably more than those seen in neonates. These growth rate findings thus account for the catch-up growth seen in the FGR-MNR offspring at young adulthood but also predict enhanced growth in these animals relative to that of the AGA-Controls and in keeping with increasing adiposity with advancing age. While FGR-MNR heart weights at adult necropsy were not significantly decreased, heart/body weight values were, in keeping with the lower heart FRs seen in these neonates as well increasing adiposity in these adults.

Perspectives and Significance

Studies using data from famine and longitudinal cohorts have shown that maternal undernutrition before and during pregnancy alters fetal growth and development, predisposing FGR offspring to cardiovascular, metabolic, and cognitive disease in later life (1, 2, 16, 19, 20, 25, 27, 28, 43). These studies additionally support the concept that predisposition to later life disease is greater in those FGR infants whose postnatal nutrition is increased from that during fetal life and leading to early catch-up growth (1, 2, 16, 28). In the present study, we have further characterized pregnancy outcomes and offspring growth in guinea pigs subjected to moderate MNR before and through pregnancy and continuing during the early postnatal period as a useful model for inducing FGR with similarities to that seen in humans. Findings confirm that early catch-up growth does occur in FGR guinea pig offspring after moderate MNR but with organ-specific differences and enhanced growth rates by young adulthood, which are likely to have implications for structural alterations and disease risk as discussed.

AUTHOR CONTRIBUTIONS

T.R.R. and B.S.R. conceived and designed research; C.L.N., E.F., Y.M., and B.M. performed experiments; C.L.N., Y.M., and B.M. analyzed data; C.L.N., E.F., T.R.R., and B.S.R. interpreted results of experiments; B.M. prepared figures; C.L.N. and B.S.R. drafted manuscript; C.L.N., E.F., Y.M., B.M., T.R.R., and B.S.R. edited and revised manuscript; C.L.N., E.F., Y.M., B.M., T.R.R., and B.S.R. approved final version of manuscript.

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