6-30-2015

HIGHER HEPATIC MIR-29 EXPRESSION IN UNDERNOURISHED MALE RATS DURING THE POSTNATAL PERIOD TARGETS THE LONG-TERM REPRESSION OF INSULIN-LIKE GROWTH FACTOR 1

Gurjeev Sohi
Western University, gsohi@uwo.ca

Andrew Revesz

Julie Ramkumar

Daniel B. Hardy
Western University, daniel.hardy@schulich.uwo.ca

HIGHER HEPATIC MIR-29 EXPRESSION IN UNDERNOURISHED MALE RATS DURING THE POSTNATAL PERIOD TARGETS THE LONG-TERM REPRESSION OF INSULIN-LIKE GROWTH FACTOR 1

Gurjeev Sohi[1,2,4,5], Andrew Revesz[1,2,4,5], Julie Ramkumar[4,5], and Daniel B. Hardy[1–5*#]

[1]The Children's Health Research Institute and [2]The Lawson Health Research Institute, [3]Department of Obstetrics & Gynecology and [4]Physiology & Pharmacology, [5]The University of Western Ontario, London, ON, Canada, N6A 5C1

A nutritional mismatch in postnatal life of low birth weight offspring increases the risk of developing the metabolic syndrome. Moreover, this is associated with decreased hepatic insulin-like growth factor 1 (Igf1) expression, leading to impaired growth and metabolism. Previously we have demonstrated that the timing of nutritional restoration in perinatal life can differentially program hepatic gene expression. While microRNAs also play an important role in silencing gene expression, to date, the impact of a nutritional mismatch in neonatal life on their long-term expression has not been evaluated. Given the complementarity of miR-29 to the 3′-UTR of Igf1, we examined how protein restoration in maternal protein restricted rat (MPR) offspring influences hepatic miR-29 and Igf1 expression in adulthood. Pregnant Wistar rats were designated into one of four dietary regimes; 20% protein (Control), 8% protein during lactation only (LP-Lact), 8% protein during gestation only (LP1) or both (LP2). The steady-state expression of hepatic miR-29 mRNA significantly increased in LP2 offspring at postnatal day 21 and 130 and this was inversely related to hepatic Igf1 mRNA and body weight. Interestingly, this reciprocal association was stronger in LP-Lact offspring at postnatal day 21. Functional relevance of this *in vivo* relationship was evaluated by transfection of miR-29 mimics in neonatal clone 9 rat hepatoma cells. Transfection with miR-29 suppressed Igf1 expression by 12 hours. Collectively, these findings implicate that nutritional restoration post-weaning (after liver differentiation) in MPR rat offspring fails to prevent long-term impaired growth, in part, due to miR-29 suppression of hepatic Igf1 expression.

Intrauterine growth restriction (IUGR) has been linked to an increase in the risk for developing the metabolic syndrome (1, 2). Moreover, protein deficiency in the developing fetus is one of the leading causes of IUGR (3). Clinically, IUGR offspring that undergo rapid postnatal catch-up growth due to a mismatch in their postnatal nutritional environment (ie, infant formula diets, over nutrition) exhibit earlier indications of the metabolic syndrome, but to date, the underlying causes for this remain elusive (4–6). Epigenetic mechanisms, such as DNA methylation and posttranslation histone modifications, have been observed to underlie the development of IUGR-linked metabolic disorders (7–9). Specifically, we have previously demonstrated that rat offspring derived from maternal protein restriction (MPR) diet exhibit postnatal catch-up growth and elevated metabolic risk factors, that are mediated, in part, by posttranslational histone modifications (7, 9, 10). Interestingly, this was greatly influenced by the timing of protein restoration in neonatal life. Another established epigenetic mechanism that can influence gene expression is via microRNAs (miRNAs) (11). However, the role of miRNAs on modulating long-term outcomes resulting from nutritional deficits in perinatal life remains elusive.

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in USA

Received January 19, 2015. Accepted June 30, 2015.

Abbreviations:

miRNAs are an endogenous, 20–25 nucleotide long, class of noncoding regulatory RNA (12). Moreover, they can dictate gene expression by repressing protein translation or decreasing messenger RNA (mRNA) stability (13). Currently, limited information is known about how alterations in nutrition during perinatal life (eg, protein restriction/restoration) in developing offspring influence the pattern of miRNA expression along with its downstream targets long-term.

Recently, miR-29 (eg, miR-29a, miR-29b, miR-29c) was identified as a novel inhibitory regulator of insulin-like growth factor 1 (IGF1) via targeting its 3′-UTR sequence in hepatic stellate cells (14, 15). Given that reduced body growth, liver growth and cell proliferation in MPR rat offspring has been previously associated with decreases in insulin-like growth factor 1 (Igf1) (16), an important growth- and metabolism-regulating hormone, we sought to examine if miR-29 expression would inversely correlate with Igf1 expression in vivo and in vitro, respectively. To address this question, three separate long-term dietary regimes were employed in pregnant rats. These included a normal (20%) protein diet administered throughout life (Control), a low (8%) protein diet until birth (low protein; LP1), or until weaning (LP2). An additional cohort examining the influence of protein restriction during lactation only (LP-Lact) was also employed. Moreover, transfection studies using Clone 9 rat hepatocytes were performed to investigate the impact of miR-29 on Igf1 expression in a time-dependent manner.

Materials and Methods

Animals and Dietary Regimes

All procedures were performed in accordance with the guidelines set by the Canadian Council of Animal Care. Female and male Wistar rats at breeding age (250 g) were purchased from Charles River (La Salle, St.Constant, Quebec, Canada). These rats were housed in individual cages and maintained at room temperature. Upon confirmation of impregnation (as previously described (9)), the rats were fed either a control diet containing 20% protein or a LP diet containing 8% protein. The LP diet contained similar fat content and was made isocaloric by a 14% increase in carbohydrates (Bio-Serv, Frenchtown, NJ, USA). See Table 1 for full composition of the diets. At birth, the litter size was reduced to eight animals (four females and four males) to ensure a standard litter size for all mothers. Four different dietary regimes were administered to these offspring. Offspring derived from a maternal LP diet were either administered the LP diet until birth (LP1), the end of weaning (LP2), or during lactation only (LP-Lact). Control offspring received a control diet throughout prenatal and postnatal life. Food and water was provided ad libitum. The food intake of these offspring were monitored by measuring their food consumption every third day. At postnatal day 21 (d21) and day 130 (d130), a subset of the offspring were sacrificed using a lethal dose (LD) (50 mg/kg) of Euthanyl forte pentobarbital sodium (Bmeda-MTC, Cambridge, ON, Canada) and the medial lobe liver tissue were excised and snap frozen for quantitative RT-PCR. We did not examine the female offspring to prevent confounding factors related to their estrous cycle and their hormonal profile.

Table 1. Composition of the diets

Diet	Control Casein	Low Casein
Component	20% Casein	8% Casein
Casein	220	90
Sucrose	213	243
Dextrose	—	—
Cellulose fiber	50	50
Cornstarch	80	80
Vitamin mix	2.5	2.5
Mineral mix	47	48
Soya oil	43	43
Corn oil	—	—
Chlorine chloride	4	40
DL-Methionine	2	0.8

Diets (in g/kg) are from Bioserv (Frenchtown, NJ). Both diets contain the same amount of total calories.

Cell Culture - Transfections

The Clone 9 rat hepatic cell line (derived from 4 week male rat neonates) was obtained from the American Type Culture Collection (ATCC, Manassas, Virginia) and culture procedures were followed as per the supplier's recommendations. Specifically, Clone 9 cells between 9–13 passages were subcultured in F12K were then allowed to grow to 70% confluency and transfected using the HiPerFect transfection reagent and miRNA mimics for miR-29a (Qiagen Canada, Toronto, Ontario). 300 ng of the miRNA mimics were incubated with HiPerFect transfection reagent (Qiagen Canada) in serum free media for 15 minutes to allow for complex formation and added onto the cells according to suppliers instructions. The controls for transfections experiments at 24 hours included nontreated cells, mock transfections lacking miRNA mimics, negative controls lacking complementarities to any mammalian gene, as well as miRNA inhibitors (Qiagen Canada).

RNA Isolation

Total RNA was isolated from frozen liver or from the Clone 9 cells by two methods, first by using a miRNeasy kit (Qiagen Canada) and secondly by the one-step method of Chomczynski and Sacchi (17) All RNA underwent spectrophotometric analysis on a Nanodrop 2000 allowing RNA concentrations, 260/280, and 260/230 ratios to be accurately determined. RNA from these two isolations was then reverse transcribed into cDNA using a Qiagen miScript cDNA synthesis kit and SuperScript II (Invitrogen, Toronto, Canada), respectively.

Real Time PCR Analysis

Isolated RNA was treated with deoxyribonuclease (DNase) to remove any contaminating DNA. 4 μg of the total RNA was reverse transcribed to cDNA using random primers and Superscript II RNase H-reverse transcriptase (Invitrogen, Carlsbad,

CA, USA). Primer sets directed against rat total Igf1 and β-actin were generated via Primer Express software (PE Applied Biosystems, Boston, MA, USA) based on published sequences (Table 2) and Qiagen miRNA primers were purchased and used for miR-29a, miR-29b, miR-29c and the internal control U6B (Qiagen Canada). The relative abundance of each transcript was determined by real-time quantitative PCR as previously published (18). For the quantitative analysis of mRNA expression, the Bio-Rad CFX384 Real Time System was employed using the DNA binding dye IQTM SYBER green supermix (Bio-Rad, Mississauga, Ontario, Canada) or a Qiagen miScript SYBR Green PCR kit. The cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. We calculated the relative fold changes for mRNA and miRNA using the comparative cycle times (Ct) method with β-actin or U6B as the reference guide, respectively. Given that all primer sets had equal priming efficiency, the ΔCt values (primer internal control) for each primer set were calibrated to the experimental samples with the lowest transcript abundance (highest Ct value), and the relative abundance of each primer set compared with calibrator was determined by the formula, $2^{-\Delta\Delta Ct}$, in which ΔΔCt is the calibrated Ct value.

Statistics

All results were expressed as the mean of arbitrary values ± the standard error of the mean (SEM). A Student's unpaired *t* test was performed for total food intake, maternal weight gain, and body weights. One-way analysis of variance (ANOVA) followed by a Tukey HSD multiple comparison post hoc test, was used to evaluate significance of differences for results comparing the effect of all the dietary regimes for postnatal body weights and Q-RT-PCR analysis. GraphPad Prism™ 5 software was used for the statistical analyses and to create the graphs for the manuscript.

Results

Low protein diet during pregnancy and lactation leads to reduced growth by postnatal day 130

In this animal cohort, placing the dams on an 8% low protein diet had no significant impact on overall food intake (Control 557.4 ± 27 g vs LP 591.9 ± 30 g, Figure 1A) or maternal weight gain (Figure 1B) from gestation day 1 to 21, but did lead to a significant decrease (~20%) in birth weight (Figure 1C). Although postnatal food intake appeared not be not to be different between dietary groups (Figure 1D), by day 21 the male LP1 offspring (with restored maternal dietary proteins) had completely recovered in weight whereas LP2 and LP-Lact offspring were still significantly smaller (Figure 1E). Interestingly, by postnatal d130, male LP2 offspring remained significantly smaller in weight while LP1 offspring were slightly larger, although not significant (Figure 1F).

Table 2. Real Time PCR Primers

Gene	Primer (5′-3′)	Reference No.
Igf1	FWD TCA ACA AGC CCA CAG GCT AT	X06043
	REV GTC TTG GGC ATG TCA GTG TG	X06043
β-actin	FWD CAG CCT TCC TTC CTG GGT AT	NM 031 144
	REV AGG AGC CAG GGC AGT AAT CT	NM 031 144

Higher steady-state miR-29 expression corresponded with lower Insulin-like growth factor 1 (Igf1) mRNA in vivo at postnatal day 130

At 130 days, the steady-state hepatic mRNA of miR-29a, miR-29b, and miR-29c were significantly augmented in LP2 offspring (Figure 2A-C, $P < .05$), where normal protein diet was restored postweaning but recovery in body weight did not completely occur (Figure 1F). Moreover, the increases in miR-29 expression was found to correlate in LP2 offspring with decreases in Igf1 mRNA ($P < .05$) in vivo (Figure 2D). Conversely, LP1 offspring did not exhibit any significant change in steady-state miR-29 or Igf1 mRNA.

In vivo increases in steady-state miR-29 and decreases in Igf1 expression were observed exclusively in LP-Lact and LP2 offspring at postnatal day 21

To determine whether the significant increases in hepatic miR-29 and decreases in Igf1 mRNA observed in day 130 LP2 offspring occurred before the normal protein diet was restored at weaning, Q-RT-PCR analysis was performed at day 21. In the LP2 offspring at day 21, there was a significant increase in the steady-state mRNA of miR-29b ($P < .05$) and miR-29c ($P < .05$), while miR-29a had a trend towards an increase in the LP2 offspring (Figure 3A-C). This increase corresponded with a decrease in steady-state Igf1 mRNA (Figure 3D). Intriguingly, protein restriction during lactation only (LP-Lact) had more profound effects on the expression of all three miR-29ab/c ($P < .01$) and Igf-1 ($P < .01$) transcripts (Figure 3A-D). When maternal protein was restored (20%) postpartum (LP1) the expression of hepatic miR-29 and Igf1 expression was not significantly altered (Figure 3A-D).

miR-29a downregulated Igf1 in vitro

To validate that the decrease in steady state Igf1 mRNA observed in vivo was indeed due to the increases in steady-state miR-29a expression in the liver, miR-29a mimics were transected in 4 week rat neonatal clone 9 hepatoma cells. Although miR-29a, miR-29b, and miR-29c were all demonstrated to be increased in vivo in MPR offspring, we utilized miR-29a as the candidate microRNA given it is the most abundant isoform in the rodent liver (14). Time course experiments revealed that 24 hours post transfection was most effective for observing the greatest Igf1

knock-down by miR-29a in these cells compared to mock control or scrambled miRNAs (Figure 4A). Upon selecting 24 hours as the incubation time for subsequent transfection experiments, anti-miR-29a compared to miR-29a transfections was performed. Intriguingly, transfection of anti-miR-29a alone did not increase Igf1 expression relative to the nontransfected clone 9 cells (Figure 4B).

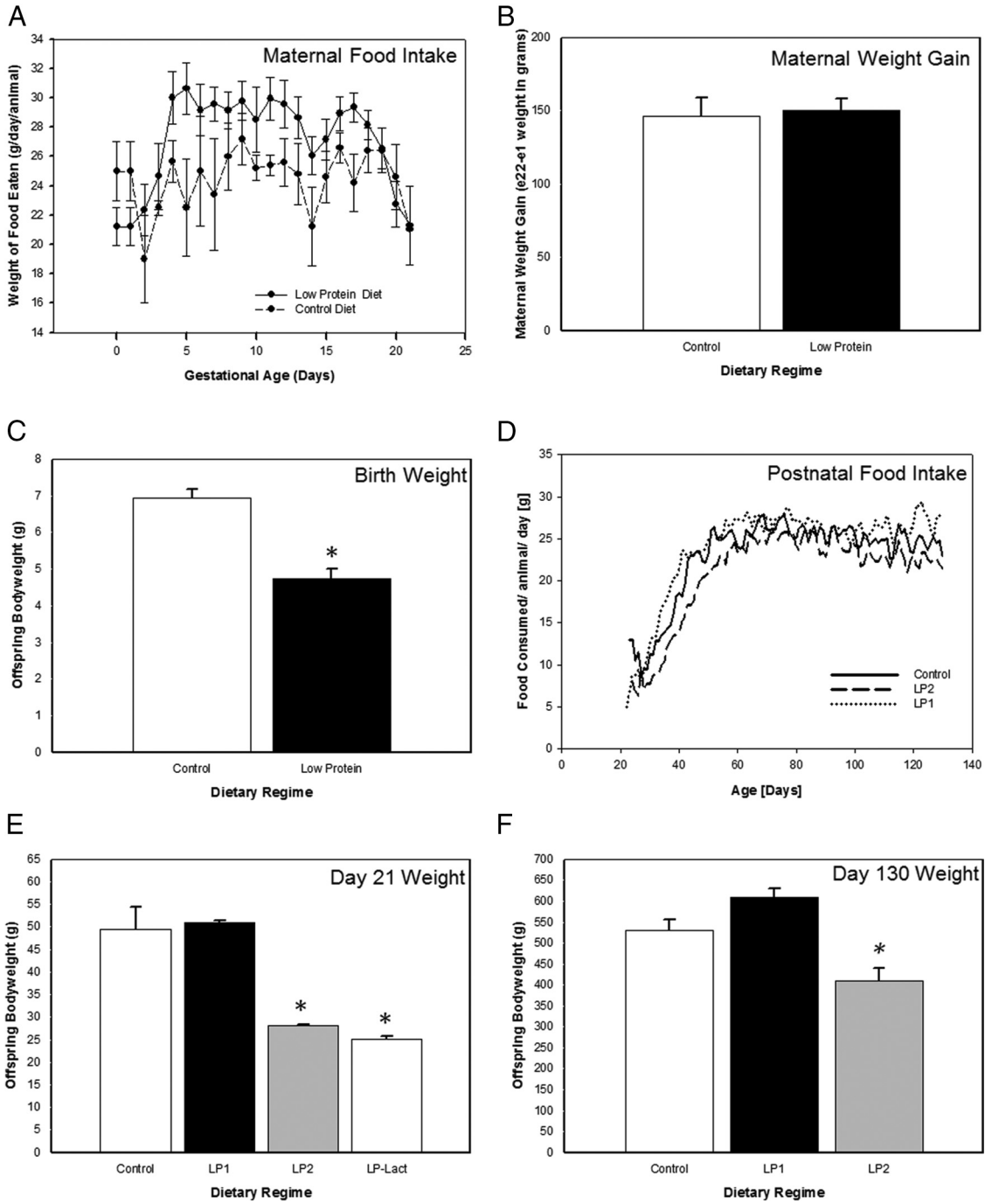

Figure 1. Effect of maternal low protein diet on **A.** Maternal Food Intake, **B.** Maternal Weight, **C.** Birth weight, **D.** Food Intake of Offspring, **E.** Weight of Offspring at Day 21, and **F.** Weight of Offspring at Day 130. Pregnant rats were given either a control diet (20% protein) or a low protein diet (8% protein) during gestation only (LP1) and lactation (LP2). Weight of food eaten in g/d/animal and maternal weight gain from gestation day 1 to gestation day 22 in grams were measured, respectively. Total maternal food intake, maternal weight gain and birth weight results are expressed as the mean ± SEM and significance was assessed using Student's unpaired *t* test. For postnatal day 21 and 130 wt analysis, the dietary groups were compared by ANOVA and significant difference was determined by a Tukey HSD post hoc test for individual pairwise comparisons (*$P < .05$, indicates significance between both the control and LP1 group). n = 5–8/group, where each n represents an offspring derived from a different mother.

Discussion

In this study we examined how a nutritional mismatch in postnatal life alters miR-29 regulation of Igf1 expression in the livers of MPR derived offspring in adulthood. Indeed, several clinical studies have linked reduced circulating Igf1 concentrations to stunted growth, impaired glucose metabolism, risk of type II diabetes and cardiovascular disease (19–21). The MPR rat model used in this study is a relevant model to study the role of Igf1 in the fetal origins of the metabolic syndrome given placental insufficiency (eg, IUGR) in humans leads to protein deficiency in the fetus (22). While both MPR groups had normal protein diets restored at some point in postnatal life, it was exclusively the LP2 offspring of dams administered a low protein diet through pregnancy and weaning which exhibited a long-term (eg, 3 weeks and 4 months) upregulation of miR-29 expression. This corresponded with the long-term repression of Igf1 in these offspring. Interestingly, a low protein diet exclusively during lactation had a greater effect to both induce hepatic miR-29 expression and repress Igf-1 compared to LP1 or LP2 offspring at 3 weeks, suggesting that the neonatal developmental window likely plays a greater role in the long-term regulation of these microRNAs. This neonatal window appears to be critical given protein restriction in adult mice (eg, 5–8 months) does not alter Igf-1 levels (23). Collectively, this study supports for the role of microRNAs during lactation and adulthood on the expression of this critical gene for growth (24, 25). Interestingly, miR-29-induced decreases in Igf1 may also mediate our previously reported decreases in hepatic phosphorylation of Akt1 (Serine 473), a marker of insulin sensitivity, exclusively in these LP2 offspring (9, 10). Indeed, Sun et al recently demonstrated that transfection of miR-29 in C4–2 cells negatively regulated Igf1 expression and consequently decreased phosphorylation of Akt1 (26).

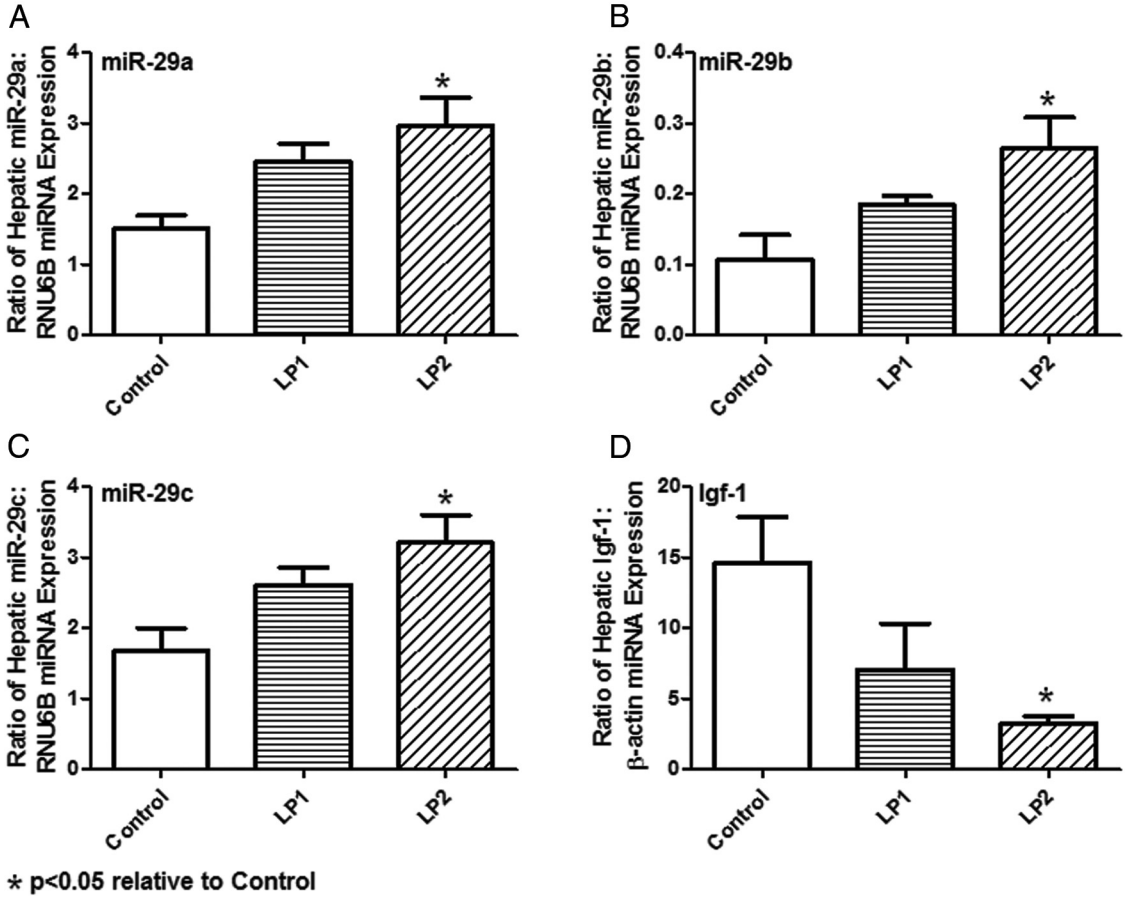

Figure 2. Quantitative RT-PCR microRNA analysis of **A.** miR-29a, **B.** miR-29b, **C.** miR-29c along with and **D.** Igf1 mRNA in the livers of rat offspring (Control, LP1, and LP2) derived at postnatal d130. The relative amounts of miR-29a, -29b and -29c mRNA were normalized to that of the expression of RNU6B. The relative expression of each Igf1 mRNA transcript was normalized to that of the each β-actin mRNA transcript. Results were expressed as the mean ± SEM. The groups were compared by ANOVA and significant difference was determined by a Tukey HSD post hoc test for individual pairwise comparisons (*$P < .05$, indicates significance between control and LP2 cohort). For Figure 2D, given the variances were not equal, the Tukey HSD post hoc test was performed on log-transformed data. n = 5–8/group, where each n represents an offspring derived from a different mother.

Consistent with previous studies, our in vitro transfection experiments in Clone 9 cells further support the hypothesis that miR-29a is involved with the down-regulation of steady-state Igf1 mRNA (14, 15). While the findings of this study provide insight into the regulation of Igf1 by a postnatal diet and miR-29, the mechanisms underlying the long-term up-regulation of miR-29 in IUGR offspring remain elusive. Given that LP2 or LP-Lact offspring at postnatal day 21 had increased hepatic miR-29 expression compared to control, this would imply that the low (8%) protein environment induces miR-29 expression. However, the timing of this low protein insult during lactation in LP2 or LP-Lact rat offspring seems critical given the LP1 offspring (eg, with restored protein at birth) at day 21 did not exhibit increases in miR-29, nor did fetal livers at e19 (data not shown). This is likely attributed to postnatal liver development in rodents. Unlike humans, most liver differentiation occurs postpartum (27). Moreover, the rat liver has a high degree of plasticity in the first three weeks of neonatal life. As a result the rat liver can incur further insult or recovery during this postpartum period. Therefore, while maintaining a low protein diet during weaning (LP2) significantly alters miR-29 and Igf1 expression long-term, restoring a normal protein diet at birth (LP1) prevents these long-term changes. We have previously observed a similar recovery in hepatic gene expression and cholesterol in LP1 offspring in studies examining the repression of Cyp7a1 expression in MPR offspring (9).

Another mechanism for the increase in hepatic miR-29 expression in these LP2 offspring may be attributed to endoplasmic reticulum (ER) stress. A recent study has implicated that ER stress induces miR-29 expression in vivo in mice (28). This is of great interest considering we have demonstrated that our LP2 offspring at 4 months exhibit increased hepatic ER stress due to the nutritional mismatch in postnatal life (10).

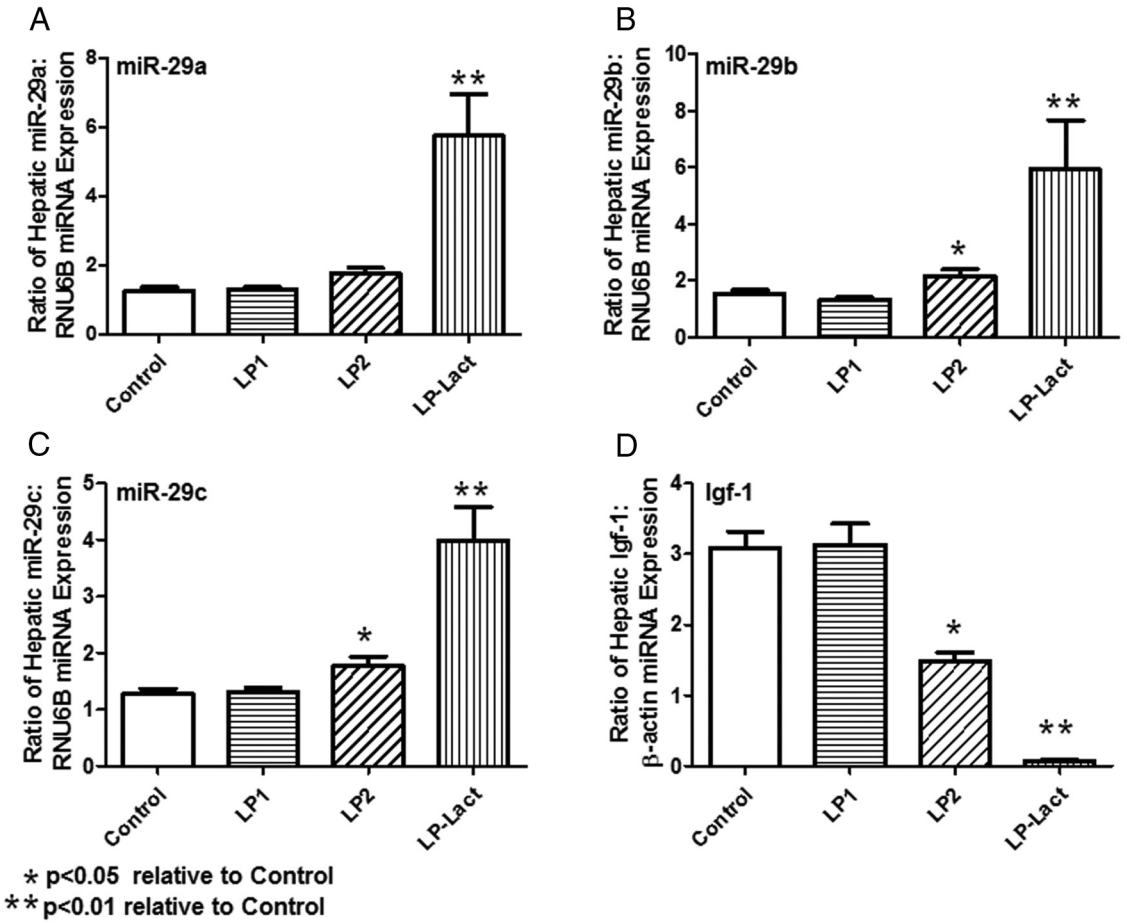

Figure 3. Quantitative RT-PCR microRNA analysis of **A.** miR-29a, **B.** miR-29b, **C.** miR-29c and **D.** Igf1 mRNA expression in the livers of rat offspring (Control, LP1, LP2, and LP-Lact) derived at postnatal d21. The relative amounts of miR-29a, -29b and -29c mRNA were normalized to that of the expression of RNU6B. The relative expression of each Igf1 mRNA transcript was normalized to that of each β-actin mRNA transcript. Results were expressed as the mean ± SEM. The groups were compared by ANOVA and significant difference was determined by a Tukey HSD post hoc test for individual pairwise comparisons (*$P < .05$, **$P < .01$, indicates significance from the control group). n = 5–8/group, where each n represents an offspring derived from a different mother.

In summary, this is the first study to demonstrate that the timing of the nutritional insult during perinatal development can alter long-term expression of Igf1, an important gene in growth and metabolism, via changes to miRNA expression in IUGR offspring. The findings support the notion that aside from posttranslational histone modifications, alterations in miRNA regulation may underlie the predisposition of undernourished offspring to an increased risk of developing the metabolic syndrome. Moreover, timely restoration of diet during a period of organ plasticity may help prevent some of the long-term adverse effects exhibited in these MPR offspring. However, this is likely to be species and organ-specific. Future studies are warranted to investigate the role of miR-29 in other organ systems in predisposing undernourished offspring to an increase risk of developing the metabolic syndrome.

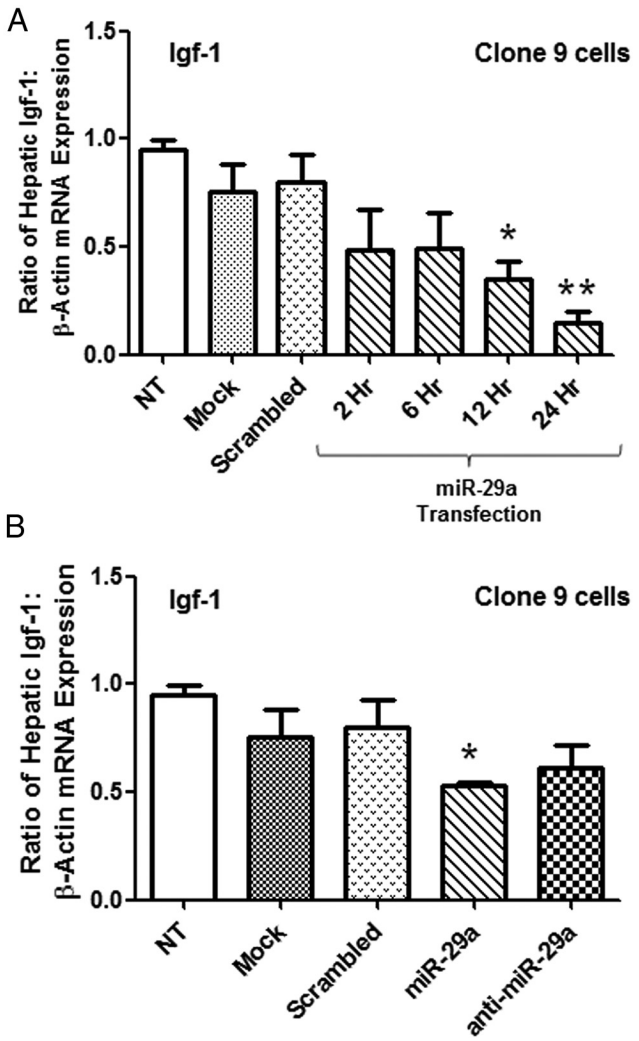

Figure 4. Quantitative RT-PCR assessed the steady state mRNA of Igf1 in Clone 9 cells **A.** after a time course experiment following transfection with miR-29a (n = 5) and **B.** after 24 hours following individual transfections with miR-29a and anti-miR-29a (n = 5). The relative expression of each Igf1 mRNA transcript were normalized to that of β-actin mRNA. The mock group lacked mimics, while the scrambled group lacked complementarity to any known mammalian gene. The groups were compared by ANOVA and significant difference was determined by a Tukey HSD post hoc test for individual pairwise comparisons ($*P < .05$, $**P < .01$ indicates significance from the nontreated control group).

Acknowledgments

We thank Dr. Lin Zhao (Department of Obstetrics and Gynecology, Western University) for technical assistance with molecular analysis.

Address all correspondence and requests for reprints to: * Corresponding author, The Department of Physiology & Pharmacology, The University of Western Ontario, London, ON, Canada, N6A 5C1, telephone: 519–661-2111 ext.84238, fax: 519–661-3827; email: Daniel.Hardy@schulich.uwo.ca,.

Reprint requests should be sent to the Corresponding author

Declaration of interest: There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Authors Contributions: Participated in Research Design Study: Sohi, Ramkumar, Revesz, Hardy, Conducted Experiments: Revesz, Ramkumar, Contributed new reagents or analytic tools: Hardy, Performed data analysis: Sohi, Revesz, Hardy, Wrote or contributed to the writing of the manuscript: Sohi, Hardy

This work was supported by **Financial Support:** This work was supported by a CIHR Operating Grant (MOP 111 001) and an NSERC Discovery Grant (RGPIN-04 090).

References

1. **Lamarche B, Lemieux S, Dagenais GR, Despres JP.** Visceral obesity and the risk of ischaemic heart disease: insights from the Quebec Cardiovascular Study. *Growth Horm IGF Res*. 1998;8 Suppl B:1–8.
2. **Ross MG, Beall MH.** Adult sequelae of intrauterine growth restriction. *Semin Perinatol*. 2008;32:213–218.
3. **Petry CJ, Ozanne SE, Hales CN.** Programming of intermediary metabolism. *Mol Cell Endocrinol*. 2001;185:81–91.

4. **Yajnik C.** Interactions of perturbations in intrauterine growth and growth during childhood on the risk of adult-onset disease. *Proc Nutr Soc*. 2000;59:257–265.
5. **Eriksson JG.** Early growth and coronary heart disease and type 2 diabetes: findings from the Helsinki Birth Cohort Study (HBCS). *Am J Clin Nutr*. 2011;94:1799S–1802S.
6. **Martin RM, McCarthy A, Smith GD, Davies DP, Ben-Shlomo Y.** Infant nutrition and blood pressure in early adulthood: the Barry Caerphilly Growth study. *Am J Clin Nutr*. 2003;77:1489–1497.
7. **Vo TX, Revesz A, Sohi G, Ma N, Hardy D.** LXR Mediates Enhanced Hepatic Gluconeogenic Gene Expression in Adult Male Rat MPR Offspring. *J Endocrinol*. 2013.
8. **van Straten EM, Bloks VW, Huijkman NC, Baller JF, van Meer H, Lutjohann D, Kuipers F, Plosch T.** The liver X-receptor gene promoter is hypermethylated in a mouse model of prenatal protein restriction. *Am J Physiol Regul Integr Comp Physiol*. 2010;298: R275–82.
9. **Sohi G, Marchand K, Revesz A, Arany E, Hardy DB.** Maternal protein restriction elevates cholesterol in adult rat offspring due to repressive changes in histone modifications at the cholesterol 7alpha-hydroxylase promoter. *Mol Endocrinol*. 2011;25:785–798.
10. **Sohi G, Revesz A, Hardy DB.** Nutritional mismatch in postnatal life of low birth weight rat offspring leads to increased phosphorylation of hepatic eukaryotic initiation factor 2 alpha in adulthood. *Metabolism*. 2013;62:1367–1374.
11. **Fabian MR, Sonenberg N, Filipowicz W.** Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem*. 2010; 79:351–379.
12. **Lee RC, Feinbaum RL, Ambros V.** The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*. 1993;75:843–854.
13. **Fabian MR, Sonenberg N, Filipowicz W.** Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem*. 2010; 79:351–379.
14. **Hand NJ, Horner AM, Master ZR, Boateng LA, LeGuen C, Uvaydova M, Friedman JR.** MicroRNA profiling identifies miR-29 as a regulator of disease-associated pathways in experimental biliary atresia. *J Pediatr Gastroenterol Nutr*. 2012;54:186–192.
15. **Kwiecinski M, Elfimova N, Noetel A, Tox U, Steffen HM, Hacker U, Nischt R, Dienes HP, Odenthal M.** Expression of platelet-derived growth factor-C and insulin-like growth factor I in hepatic stellate cells is inhibited by miR-29. *Lab Invest*. 2012;92:978–987.
16. **El-Khattabi I, Gregoire F, Remacle C, Reusens B.** Isocaloric maternal low-protein diet alters IGF-I, *IGFBPs, and hepatocyte proliferation in the fetal rat. Am J Physiol Endocrinol Metab*. 2003;285: E991–E1000.
17. **Chomczynski P, Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem*. 1987;162:156–159.
18. **Hardy DB, Janowski BA, Corey DR, Mendelson CR.** Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of nuclear factor-kappaB activation of cyclooxygenase 2 expression. *Mol Endocrinol*. 2006;20:2724–2733.
19. **Paolisso G, Tagliamonte MR, Rizzo MR, Carella C, Gambardella A, Barbieri M, Varricchio M.** Low plasma insulin-like growth factor-1 concentrations predict worsening of insulin-mediated glucose uptake in older people. *J Am Geriatr Soc*. 1999;47:1312–1318.
20. **Ong KK, Petry CJ, Emmett PM, Sandhu MS, Kiess W, Hales CN, Ness AR, Dunger DB.** ALSPAC study team 2004 Insulin sensitivity and secretion in normal children related to size at birth, postnatal growth, and plasma insulin-like growth factor-I levels. *Diabetologia*. 47:1064–1070.
21. **Juul A, Scheike T, Davidsen M, Gyllenborg J, Jorgensen T.** Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease: a population-based case-control study. *Circulation*. 2002;106:939–944.
22. **Crosby WM.** Studies in fetal malnutrition. *Am J Dis Child*. 1991; 145:871–876.
23. **Mitchell SE, Delville C, Konstantopedos P, Hurst J, Derous D, Green C, Chen L, Han JJ, Wang Y, Promislow DE, Lusseau D, Douglas A, Speakman JR.** The effects of graded levels of calorie restriction: II. Impact of short term calorie and protein restriction on circulating hormone levels, glucose homeostasis and oxidative stress in male C57BL/6 mice. *Oncotarget*. 2015.
24. **Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A.** Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol*. 2001;229:141–162.
25. **Clemmons DR.** Metabolic actions of insulin-like growth factor-I in normal physiology and diabetes. *Endocrinol Metab Clin North Am*. 2012;41:425–43, vii-viii.
26. **Sun F, Chen HG, Li W, Yang X, Wang X, Jiang R, Guo Z, Chen H, Huang J, Borowsky AD, Qiu Y.** Androgen receptor splice variant AR3 promotes prostate cancer via modulating expression of autocrine/paracrine factors. *J Biol Chem*. 2013.
27. **Kung JW, Currie IS, Forbes SJ, Ross JA.** Liver development, regeneration, and carcinogenesis. *J Biomed Biotechnol*. 2010;2010: 984248.
28. **Nolan K, Mitchem MR, Jimenez-Mateos EM, Henshall DC, Concannon CG, Prehn JH.** Increased expression of microRNA-29a in ALS mice: functional analysis of its inhibition. *J Mol Neurosci*. 2014;53:231–241.