Am J Physiol Gastrointest Liver Physiol 299: G1023–G1029, 2010.
First published September 2, 2010; doi:10.1152/ajpgi.00052.2010.

Epigenetics of programmed obesity: alteration in IUGR rat hepatic IGF1 mRNA expression and histone structure in rapid vs. delayed postnatal catch-up growth

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Submitted 10 February 2010; accepted in final form 27 August 2010

Tosh DN, Fu Q, Callaway CW, McKnight RA, McMillen IC, Ross MG, Lane RH, Desai M. Epigenetics of programmed obesity: alteration in IUGR rat hepatic IGF1 mRNA expression and histone structure in rapid vs. delayed postnatal catch-up growth. *Am J Physiol Gastrointest Liver Physiol* 299: G1023–G1029, 2010. First published September 2, 2010; doi:10.1152/ajpgi.00052.2010.—Maternal food restriction (FR) during pregnancy results in intrauterine growth-restricted (IUGR) offspring that show rapid catch-up growth and develop metabolic syndrome and adult obesity. However, continued nutrient restriction during nursing delays catch-up growth and prevents development of obesity. Epigenetic regulation of IGF1, which modulates growth and is synthesized and secreted by the liver, may play a role in the development of these morbidities. Control (AdLib) pregnant rats received ad libitum food through gestation and lactation, and FR dams were exposed to 50% food restriction from *days 10* to *21*. FR pups were nursed by either ad libitum-fed control dams (FR/AdLib) or FR dams (FR/FR). All pups were weaned to ad libitum feed. Maternal FR resulted in IUGR newborns with significantly lower liver weight and, with the use of chromatin immunoprecipitation, decreased dimethylation at H3K4 in the IGF1 region was observed. Obese adult FR/AdLib males had decreased dimethylation and increased trimethylation of H3K4 in the IGF1 region. This corresponded to an increase in mRNA expression of IGF1-A (134 ± 5%), IGF1-B (165 ± 6%), IGF1 exon 1 (149 ± 6%), and IGF1 exon 2 (146 ± 7%) in the FR/AdLib compared with the AdLib/AdLib control group. In contrast, nonobese FR/FR had significantly higher IGF1-B mRNA levels (147 ± 19%) than controls with no difference in IGF1-A, exon 1 or exon 2. Modulation of the rate of IUGR newborn catch-up growth may thus protect against IGF1 epigenetic modifications and, consequently, obesity and associated metabolic abnormalities.

intrauterine growth restriction; insulin-like growth factor 1; histone methylation

EPIDEMIOLOGICAL AND EXPERIMENTAL studies have demonstrated an association between suboptimal intrauterine environment, intrauterine growth restriction (IUGR) and the subsequent development of metabolic and cardiovascular disease in later life. Specifically, maternal undernutrition during pregnancy leads to IUGR with asymmetric reduction in organ growth, such that growth of liver is reduced, whereas that of brain and heart is spared. This altered growth of specific organs is associated with metabolic abnormalities in the adult offspring. Additionally, modulation of postnatal nutrition and, hence, subsequent growth of the IUGR newborn determines the ultimate adult phenotype. For example, IUGR newborns nursed by ad libitum-fed dams exhibit rapid catch-up growth and develop insulin resistance, glucose intolerance, central obesity, type 2 diabetes, hypertension, and cardiovascular disease (38, 41). These adverse health consequences are prevented or diminished in severity when the rapid catch-up growth phase of the newborn is delayed by limiting the availability of nutrients through continued nursing by food-restricted dams (16, 17).

IGF1 plays a key role in mediating perinatal growth and development as part of the growth hormone-IGF-insulin axis (32). IGF1-null mutant rodents have a 40% reduction in growth along with decreased postnatal survival (2, 35). The liver is the predominant source of circulating IGF1 (>80%) although most tissues synthesize IGF1 that has both paracrine and autocrine actions (11, 43, 49). Interestingly, tissue-specific knockouts of hepatic IGF1 result in normal body growth, indicating that extrahepatic IGF1 with autocrine/paracrine action may be sufficient to sustain body growth in these animals. Notably, they have hyperinsulinemia, a reduced age-dependant accumulation of adipose tissue and reduction of plasma IGF, and previous studies conclude that liver-derived IGF1 was associated with metabolic function rather than growth (49, 56). Other studies have found that plasma IGF1 correlates directly with fetal growth, and undernutrition studies have shown reduced IGF1 levels in IUGR (27, 34). Therefore, the findings from genetic and nutrition studies suggest that fetal plasma IGF1, potentially from a nonhepatic source, correlates with fetal growth and that aberrant hepatic IGF1 expression may be programmed during the fetal period but may not result in an abnormal phenotype until later adult life. The underlying mechanisms for the programmed changes in hepatic IGF1 expression may include epigenetic modifications, such as histone methylation.

IGF1 is expressed as two isoforms, which arise from alternative splicing of the six IGF1 exons. Exons 1 and 2 both contain promoter regions and are differentially spliced, as are exons 5 and 6, whereas exons 3 and 4 encode the mature IGF1 peptide and are also present in both isoforms. IGF1-A comprises exon 1 or 2, 3, 4, and 6 and IGF1-B comprises exons 1 or 2, 3, 4, 5, and 6, with exon 2 being the predominate leader exon in the liver (26, 41, 48, 50). It has been shown that development, fasting, and diabetes cause differential regulation

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of the different transcription start sites (1), possibly as a result of epigenetic modifications to histone structure that result in changes in the ability of the transcriptional machinery to access the DNA and therefore determine gene expression (13, 21, 44). Histone methylation is a key structural alteration that can impact gene expression, either promoting or repressing gene expression, depending on the location (10, 33, 54). Methylation of either DNA or histone proteins requires methyl donors from dietary folate and requires the presence of vitamins B6 and B12, choline, methionine, and a range of methyltransferases (25, 44). Aberrant hypomethylation has been linked with cancer, diabetes, developmental and neurological abnormalities, and cardiovascular and cerebrovascular diseases (9, 30, 31), diseases that are also associated with IUGR offspring (6, 47, 53).

Histone methyltransferase mediates the addition of a methyl group to the lysine in the NH_2 terminus tail of histones, and lysine tails can be mono-, di-, or trimethylated. Trimethylation of H3 Lys4 (H3K4) has been well established as a marker on promoter regions of transcriptionally active genes, whereas dimethylation of H3K4 is associated with other areas in the vicinity of active genes and is thought to play an indirect role in promoting expression by recruiting transcriptional machinery (8, 13, 45). Therefore, increased H3K4 di- and trimethylation may be a long-term, heritable modification that is directly linked with active gene expression.

We have established a rat model of maternal food restriction during pregnancy that results in IUGR pups. IUGR pups exposed to standard nursing and postweaning diet (food restriction and ad libitum, FR/AdLib) demonstrate rapid catch-up growth and develop adult obesity with increased body fat, insulin resistance, and elevated plasma triglyceride levels. In contrast, IUGR pups that are nursed by FR dams (FR/FR) exhibit delayed catch-up growth and notably do not develop adult obesity and metabolic syndrome (17, 18). We therefore hypothesized that maternal undernutrition-induced IUGR would result in epigenetic modifications of histone structure, specifically H3K4, in the regions of the IGF1 gene of the liver. We further hypothesized that these epigenetic changes, including increased hepatic IGF1 mRNA and plasma IGF1 levels, would be evident in IUGR offspring that develop adult obesity.

MATERIALS AND METHODS

Animals and diet. A model of maternal FR (50%) during pregnancy was used as previously described (18). Studies were approved by the Animal Research Committee of Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center and were in accordance with the American Association for Accreditation of Laboratory Care and National Institutes of Health guidelines. First-time-pregnant Sprague Dawley rats (Charles River Laboratories, Hollister, CA) were housed in a facility with constant temperature and humidity and a controlled 12-h:12-h light/dark cycle. At 10 days of gestation, pregnant rats were provided either an ad libitum diet of standard laboratory chow (LabDiet 5001; LabDiet, Brentwood, MO; 23% protein, 4.5% fat, 3,030 kcal/kg of metabolizable energy) or a 50% FR diet determined by quantification of normal intake in the ad libitum-fed rats. The respective diets were given from *day 10* of pregnancy to term.

Offspring. Rat dams gave birth normally, and, at *day 1* after birth, pups were limited to eight (4 males and 4 females) per litter to normalize rearing although only male offspring were used in this report. Control pups were cross fostered onto ad libitum-fed mothers to continue the control group (AdLib/AdLib). Maternally FR pups (FR) were cross fostered onto either ad libitum-fed mothers (FR/AdLib) or 50% FR mothers (FR/FR). At 3 wk of age, offspring in the three groups were housed individually and weaned to ad libitum feed. Thus two groups of offspring were studied at *day 1*, control ($n = 6$) and FR ($n = 6$), and three groups were studied at 9 mo of age (adult), AdLib/AdLib ($n = 6$), FR/AdLib ($n = 6$), and FR/FR ($n = 6$), to investigate the impact of rapid and delayed catch-up growth, respectively ($n = 6$ represents 6 offspring from 6 mothers).

Body and liver weights. Body and liver weights of *day 1* newborns and 9-mo adult offspring were measured.

RNA isolation. Total RNA was extracted from *day 1* and adult male liver using the RNA Stat-60 (Tel-Test, Friendswood, TX) according to manufacturer's instructions, treated with DNase-I (Qiagen, Valencia, CA), and then quantified by absorption at A_{260nm} measured by UV spectrophotometry. Gel electrophoresis confirmed the integrity of the samples.

Quantitative real-time PCR. Liver mRNA levels of IGF1-A, IGF1-B, IGF1 exon 1 (IGF1-E1), IGF1 exon 2 (IGF1-E2), and GAPDH were measured using real-time RT-PCR. cDNA was synthesized from 5 μg of DNase-treated total RNA using a Superscript III First-Strand kit (Invitrogen, Carlsbad, CA), with the inclusion of no superscript and no RNA samples to confirm both RNA and reagent purity. Target primers and probes were designed using Primer Express

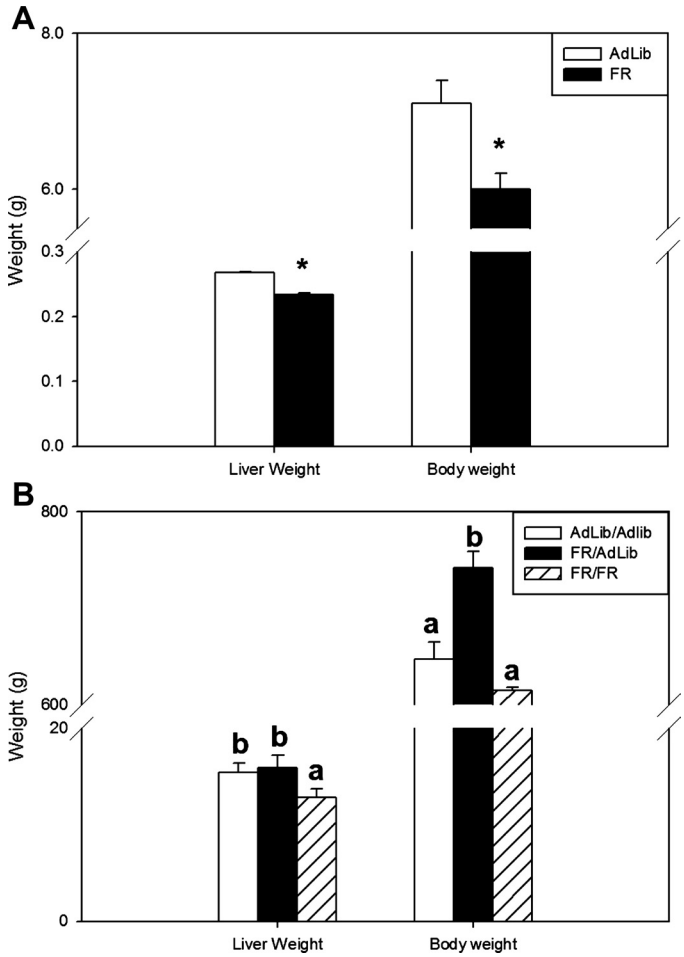

Fig. 1. *A*: newborn liver weight and body weight (g). Open bars represent ad libitum-fed animals (AdLib), and solid bars represent food-restricted animals (FR); asterisk denotes differences between AdLib and FR newborns, $P < 0.01$. *B*: adult liver weight and body weight (g). Open bars represent AdLib/AdLib (control), solid bars represent FR/AdLib (FR during pregnancy), and hatched bars represent FR/FR (FR during pregnancy and lactation); *a* and *b* denote a significant difference between the adult groups AdLib/AdLib, FR/AdLib, and FR/FR, $P < 0.05$.

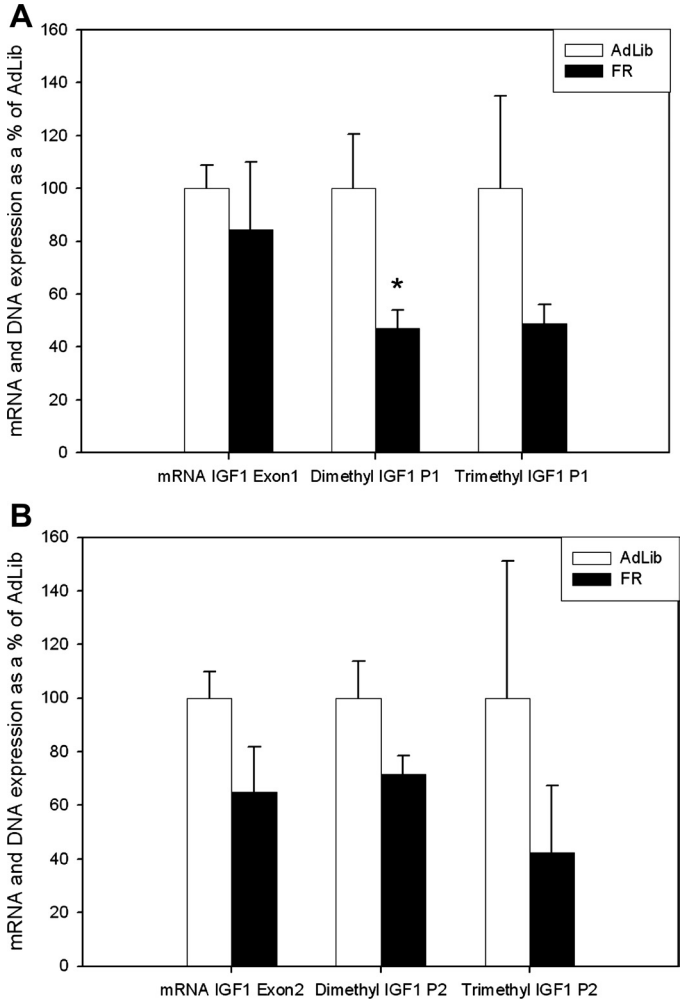

Fig. 2. *A*: newborn IGF1 exon 1 mRNA expression and H3K4 dimethylation and trimethylation of promoter 1 (P1) on exon 1. Open bars represent AdLib, and solid bars represent FR; asterisk denotes differences between AdLib and FR newborns, $P < 0.05$. *B*: newborn IGF1 exon 2 mRNA expression and H3K4 dimethylation and trimethylation of H3K4 promoter 2 (P2) on exon 2. Open bars represent AdLib, and solid bars represent FR.

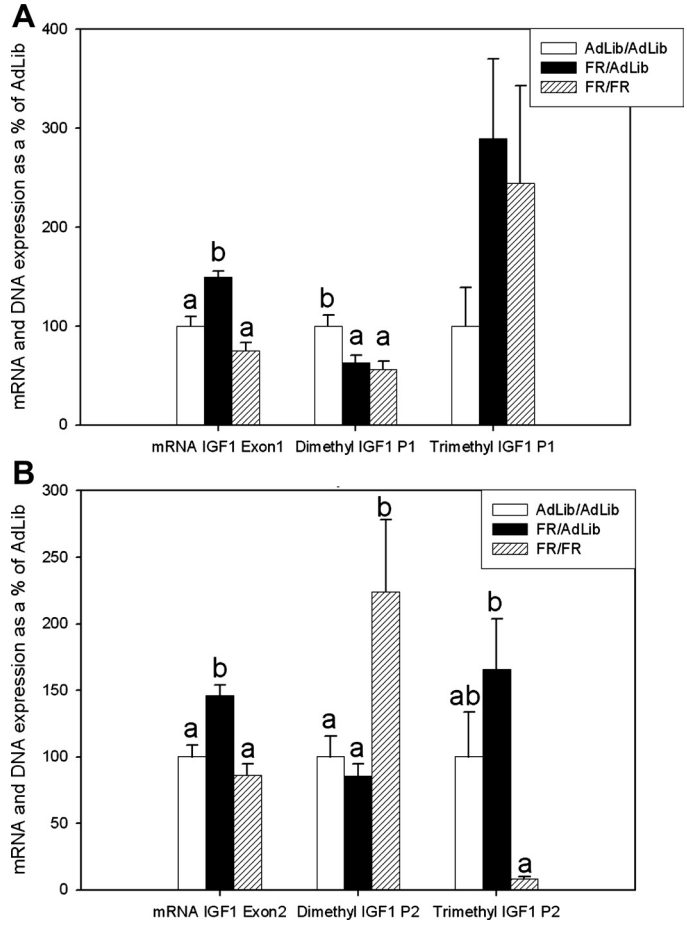

Fig. 3. *A*: adult IGF1 exon 1 mRNA expression and H3K4 dimethylation and trimethylation of P1 on exon 1. Open bars represent AdLib/AdLib (control), solid bars represent FR/AdLib (FR during pregnancy), and hatched bars represent FR/FR (FR during pregnancy and lactation); *a* and *b* denote a significant difference between the adult groups AdLib/AdLib, FR/AdLib, and FR/FR, $P < 0.05$. *B*: adult IGF1 exon 2 mRNA expression and H3K4 dimethylation and trimethylation of P2 on exon 2. Open bars represent AdLib/AdLib (control), solid bars represent FR/AdLib (FR during pregnancy), and hatched bars represent FR/FR (FR during pregnancy and lactation); *a* and *b* denote a significant difference between the adult groups AdLib/AdLib, FR/AdLib, and FR/FR, $P < 0.05$.

software (Applied Biosystems, Foster City, CA) as previously described (26); target probes were labeled with carboxyfluorescein fluorescent reporter dye. cDNA, probe, and primers were added to TaqMan Universal PCR Master Mix (Applied Biosystems), and samples were run on an ABI Prism 7000. GAPDH C_T values were assessed for stable expression across all groups to ensure suitability for use as a reference gene, and relative quantification of PCR products was calculated on the basis of value differences between the target and GAPDH control using the comparative C_T method. This method is predicated on an amplification efficiency of 100 (46). Cycle parameters were 50°C for 2 min, 95°C for 10 min, and then 40 cycles at 95°C for 15 s and 60°C for 60 s.

Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) with antidimethyl-H3K4 and antitrimethyl-H3K4 (Millipore,

Table 1. *Newborn and adult mRNA expression of regions of IGF1*

Treatment	Age	IGF1 Exon 1	IGF1A	IGF1 Exon 2	IGF1B
AdLib	Newborn	100 ± 8.8	100 ± 22.8	100 ± 9.9	100 ± 9.0
FR	Newborn	84.3 ± 25.8	211.9 ± 98.5	64.8 ± 17.1	104 ± 30.7
AdLib/AdLib	Adult	100 ± 9.6*	100 ± 6.9*	100 ± 8.8*	100 ± 5.0*
FR/AdLib	Adult	149.3 ± 6.6†	133.7 ± 5.3†	146 ± 7.7†	164.9 ± 6.3†
FR/FR	Adult	74.6 ± 8.3*	99.3 ± 6.8*	86.3 ± 8.3*	146.8 ± 21.0†

Values are means ± SE. mRNA expression of regions of IGF1 relative to GADPH is expressed as a percentage of expression levels of controls for the age group. The results are from newborns at 1 day of age of ad libitum-fed animals (AdLib, controls) vs. food-restricted pregnancy animals (FR), adults at 9 mo of age of AdLib/AdLib (control) vs. FR/AdLib (food-restricted pregnancy and AdLib lactation), and FR/FR (food-restricted pregnancy and lactation). * and †Differences between adult treatment groups, $P < 0.05$.

Billerica, MA) was performed as described previously (25). A quantity of 100 mg of liver tissue from *day 1* and adult male rats was ground in liquid nitrogen and cross linked with formaldehyde, with a final concentration of 1%, for 10 min. The chromatin was sonicated (Sonic Dismembrator, model 100; Fisher Scientific, Pittsburgh, PA) 12 times for 10 s on ice at the highest level to generate chromatin fragments of 500–2,000 bp. The sonicated chromatin was quantified on the basis of DNA content at A_{260}, and on the basis of this estimate the chromatin equivalent of 100 μg of DNA was used in each immunoprecipitation (IP). Fifty microliters of antidimethyl-H3K4 and antitrimethyl-H3K4 (Millipore) were used for each IP. DNA samples without the addition of an antibody and a sample without the addition of DNA were included to verify the purity of the DNA and the reagents, respectively. Following IP, cross linking was reversed, and a DNeasy Tissue Kit (Qiagen) was used to purify the DNA from the IP samples. Real-time PCR was used to quantitate the amount of IGF1-promoter 1 (IGF1-P1), promoter 2 (IGF1-P2), exon 5 (IGF1-E5), exon 6 (IGF1-E6), 3′UTR-3 (IGF1-U3), and 3′UTR-4 (IGF1-U4) sequence (26) that was immunoprecipitated.

Western blot. Protein was extracted from *day 1* and adult liver using a radioimmuno precipitation assay buffer that contained a protease inhibitor cocktail (Roche, Manheim, Germany). Supernatant protein concentration was determined by BCA assay (Pierce, Rockford, IL). An IGF1 antibody (Upstate Biotechnology, Lake Placid, NY) diluted 1:1,000 and a GAPDH (Chemicon, Temecula, CA) diluted 1:5,000 were used. One hundred micrograms of protein were mixed with a loading buffer and reducing agent, boiled for 10 min, and separated on a 12% Bis-Tris gel (BioRad, Richmond, CA). The protein was transferred to a PVDF membrane for 1 h in an ice-water bath. Nonspecific antibody binding was blocked by incubation for 1 h at room temperature with 5% nonfat dry milk in 0.1% Tris-buffered saline with Tween (TBST). The membrane was then incubated with the appropriate antibody in 5% milk in TBST for 1 h at room temperature and then washed three times for 5 min in TBST. The membrane was washed with the secondary antibody goat anti-rabbit horseradish peroxidase conjugate (Upstate Biotechnology) diluted 1:5,000 for 1 h at room temperature and then washed three times for 5 min in TBST. Supersignal West Pico Chemiluminescent Substrate (Pierce) was used to detect the targeted protein. The band intensity was analyzed using a BioRad GS-800 densitometer, and IGF1 intensity was normalized to GAPDH.

Plasma. Excess pups at 1 day of age during culling (3–5 pups per litter from 6 litters per group) were decapitated, and blood was collected in heparinized capillary tubes. At 9 mo of age, one male from each adult litter ($n = 6$ per group) was fasted overnight, and blood was collected via cardiac puncture in heparinized and EDTA-aprotinin (780 KIU/ml of blood) tubes for determination of plasma IGF1 levels. IGF1 concentrations were determined by a radioimmunoassay rat-specific IGF1 kit (Diagnostic Systems Laboratories, Webster, TX).

Statistics. Data from newborn control and FR groups were compared using a Student's unpaired *t*-test. Data from the AdLib/AdLib, FR/AdLib, and FR/FR groups in adult life were compared using one-way ANOVA. All data are the means ± SE with weights in grams and plasma in nanograms/milliliter and are presented as a percentage of the age-matched control group for mRNA, ChIP DNA, and protein.

RESULTS

Body and liver weights. At *day 1*, FR newborns had significantly decreased body weights compared with control newborns (6.0 ± 0.2 vs. 7.1 ± 0.3 g, $P < 0.01$). The liver weight in FR newborns was also decreased compared with controls (233 ± 3 vs. 277 ± 2 mg, $P < 0.01$) (Fig. 1*A*).

At 9 mo, FR/AdLib males were heavier than AdLib/AdLib controls (742 ± 17 vs. 647 ± 18 g, $P < 0.01$), whereas liver weight in the animals was not different (15.9 ± 1.3 vs. 15.4 ± 1.0 g). In contrast, the FR/FR males had body weights (615 ± 150 g) similar to that of AdLib/Adlib, whereas the liver weight in this group remained significantly lower than controls (12.8 ± 0.9 g, $P < 0.01$) (Fig. 1*B*).

Hepatic GAPDH and IGF1 mRNA expression. No significant difference was found between GAPDH C_T values in newborn AdLib [25.5 ± 0.1 Cycle number (CN)] or FR (25.6 ± 0.2 CN) groups. Similarly there was no significant difference between GAPDH C_T values in the AdLib/AdLib (23.8 ± 0.3 CN), FR/AdLib (24.4 ± 0.2 CN), or FR/FR (23.6 ± 0.4 CN) groups. At 1 day of age there was no significant difference in hepatic IGF1 mRNA expression between FR and AdLib groups (Fig. 2 and Table 1).

At 9 mo of age there was significantly increased mRNA expression of IGF1-A (134 ± 5%), IGF1-B (165 ± 6%), IGF1-E1 (149 ± 6%, Fig. 3*A*), and IGF1-E2 (146 ± 7%, Fig. 3*B*) in FR/AdLib compared with AdLib/AdLib. In contrast, in the FR/FR group there was only a significant difference in IGF1-B (147 ± 19%) (Table 1).

ChIP. At 1 day of age H3K4 dimethylation was lower in the FR compared with the AdLib newborn at IGF1-P1 (47.2 ± 6.7%), IGF1-E5 (67.2 ± 7.4%), and IGF1-U3 (61.1 ± 6.7%). There were, however, no significant differences in the level of trimethylation at any IGF1 site in the FR and AdLib groups (Fig. 2, Table 2).

At 9 mo of age there was significantly decreased H3K4 dimethylation at IGF1-P1 (62.6 ± 7.7%) and IGF1-E5 (49.6 ±

Table 2. *Newborn and adult levels of IGF1 regions of DNA associated with H3K4 dimethylation and trimethylation*

Group	Methyl	Promoter 1	Promoter 2	Exon 5	Exon 6	UTR 3	UTR 4
AdLib	Di	100 ± 20.4	100 ± 13.7	100 ± 13.0	100 ± 21.3	100 ± 13.2	100 ± 27.8
FR	Di	47.2 ± 6.7[a]	71.4 ± 7.1	67.2 ± 7.4[a]	70.9 ± 7.6	61.1 ± 6.7[a]	74.7 ± 15.6
AdLib	Tri	100 ± 34.9	100 ± 51.0	100 ± 32.2	100 ± 32.6	100 ± 41.6	100 ± 37.4
FR	Tri	48.7 ± 7.3	42.3 ± 25.0	65.7 ± 11.0	60.7 ± 6.6	25.7 ± 4.3	31.25 ± 6.6
AdLib/ AdLib	Di	100 ± 11.0[c]	100 ± 15.6[b]	100 ± 14.9[c]	100 ± 37.1	100 ± 28.6[b]	100 ± 20.0
FR/AdLib	Di	62.6 ± 7.7[b]	85.3 ± 9.5[b]	49.6 ± 3.8[b]	50.0 ± 11.3	127.8 ± 28.6[b]	70.3 ± 9.5
FR/FR	Di	55.7 ± 8.3	223.7 ± 54.5[c]	95.7 ± 11.6[c]	36.9 ± 4.7	233.3 ± 28.6[c]	64.9 ± 12.0
AdLib/AdLib	Tri	100 ± 39.3	100 ± 33.6[d,e]	100 ± 40.7	100 ± 48.8	100 ± 33.3	100 ± 20.1[d]
FR/AdLib	Tri	289.5 ± 81.0	165.7 ± 37.9[e]	181.4 ± 42.5	98.8 ± 58.7	204.8 ± 90.5	419.1 ± 134[e]
FR/FR	Tri	244.5 ± 98.2	8.1 ± 2.0[d]	82.3 ± 20.9	130.6 ± 75.2	54.8 ± 30.0	152.3 ± 36.1[d]

Values are means ± SE. DNA is normalized to input level and expressed as a percent of control. Results are shown from 1 day of age of AdLib (controls) vs. FR (food-restricted pregnancy), from 9 mo of age of AdLib/AdLib (control) vs. FR/AdLib (food-restricted pregnancy and AdLib lactation), and FR/FR (food-restricted pregnancy and lactation). [a]Differences between AdLib and FR newborns. [b] and [c]Differences between the adult groups AdLib/AdLib, FR/AdLib, and FR/FR in dimethylation. [d] and [e]Differences between the adult groups AdLib/AdLib, FR/AdLib, and FR/FR in trimethylation, $P < 0.05$.

3.8%) and increased H3K4 trimethylation at IGF1-U4 (419.1 ± 134.0%) in the FR/AdLib group compared with AdLib/AdLib. Adult FR/FR offspring also had decreased H3K4 dimethylation at IGF1-P1 (55.7 ± 8.3%) and additionally had increased dimethylation at IGF1-P2 (223.7 ± 54.5%) and IGF1-U3 (233.3 ± 28.6%), and, whereas there were no differences in trimethylation compared with AdLib/AdLib, IGF-P2 (8.1 ± 2.0%) was significantly decreased compared with FR/AdLib (165.7 ± 37.9%) (Fig. 3, Table 2).

IGF1 protein. At 1 day of age, FR newborns had increased hepatic IGF1 protein levels compared with AdLib offspring (126.4 ± 18.9 vs. 100 ± 18.7%, $P < 0.05$) (Fig. 4*A*). At 9 mo of age, hepatic IGF1 protein was significantly increased in FR/AdLib compared with FR/FR and AdLib/AdLib (990 ± 190 vs. 98 ± 20 and 100 ± 30%, $P < 0.001$) (Fig. 4*A*).

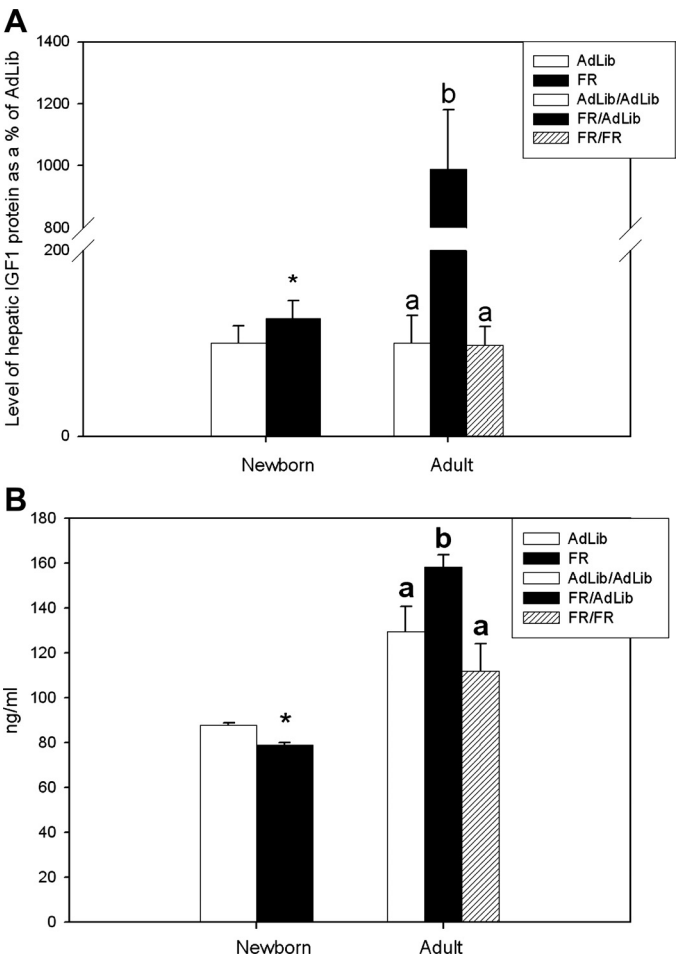

Fig. 4. *A*: quantification of newborn and adult liver IGF1 peptide relative to GAPDH and expressed as a percentage of AdLib (newborn) and AdLib/Adlib (adult). Newborn open bar represents AdLib, and solid bars represent FR; adult open bars represent AdLib/AdLib (control), solid bars represent FR/AdLib (FR during pregnancy), and hatched bar represents FR/FR (FR during pregnancy and lactation); asterisk denotes differences between AdLib and FR newborns; *a* and *b* denote a significant difference between the adult groups AdLib/AdLib, FR/AdLib, and FR/FR, $P < 0.05$. *B*: newborn and adult plasma IGF1 concentrations (ng/ml). Newborn open bar represents AdLib, and solid bars represent FR; adult open bar represents AdLib/AdLib (control), solid bar represents FR/AdLib (FR during pregnancy), and hatched bar represents FR/FR (FR during pregnancy and lactation); asterisk denotes differences between AdLib and FR newborns, $P < 0.01$; *a* and *b* denote a significant difference between the adult groups AdLib/AdLib, FR/AdLib, and FR/FR, $P < 0.05$.

Plasma IGF1. At 1 day of age, FR newborns had decreased plasma IGF1 compared with control offspring (79 ± 1 vs. 88 ± 1 ng/ml, $P < 0.01$) (Fig. 4*B*). Conversely at 9 mo of age, FR/AdLib males had significantly increased plasma IGF1 levels compared with FR/FR and control (158.5 ± 5.3 vs. 111.9 ± 12.2 and 129.4 ± 11.4 ng/ml, $P < 0.01$) (Fig. 4*B*).

DISCUSSION

We have previously shown that maternal FR during pregnancy results in IUGR offspring that when normally nursed show rapid catch-up growth and develop adult obesity and insulin resistance. However, continued nutrient restriction during nursing delays catch-up growth and prevents development of adult obesity. This study presents novel findings of epigenetic modifications to the histone structure associated with the IGF1 gene in IUGR offspring with differing adult phenotypes. Notably, IUGR newborns that undergo rapid catch-up growth and develop adult obesity (FR/AdLib) have increased postnatal hepatic IGF1 mRNA levels, likely a result of IGF1 histone and chromatin structure modifications to H3K4 trimethylation. Conversely, IUGR animals with delayed catch-up growth and absence of adult obesity (FR/FR) have levels similar to that of controls. Thus modulation of the rate of IUGR newborn catch-up growth may partially protect against IGF1 epigenetic modifications.

Consistent with previous studies (17), the IUGR newborns have reduced body and liver weights, including reduced plasma IGF1 levels. Notably, IUGR newborns clearly demonstrate disrupted hepatic histone methylation status in the IGF1 region. Specifically, H3K4 dimethylation was significantly decreased at the IGF1-P1, IGF1-E5, and IGF1-U3, whereas dimethylation of IGF1-P2, IGF1-E6, and IGF1-U4 as well as trimethylation of all IGF1 regions showed a decreased trend. Of particular interest from the methylation data is the large variation seen within the AdLib animals compared with the relatively small variation among the FR animals. This may demonstrate a critical window in which AdLib control animals are beginning to have increased dimethylation and trimethylation of IGF1, yet the developmentally delayed FR animals have yet to reach this period of growth. Despite the decreased H3K4 methylation in IUGR newborns, hepatic IGF1 mRNA expression was unaltered although protein expression was increased, suggesting a disruption in translation efficiency and exocytosis of the IGF1 protein. This concept is supported by the decreased plasma IGF1 level seen in IUGR, which is predominantly of liver origin.

IUGR newborns suckled by a normally fed control dam (FR/AdLib) exhibit rapid catch-up growth and as adults develop obesity and insulin resistance (17, 18). Consistent with increased growth, FR/AdLib offspring have markedly elevated plasma IGF1 levels, upregulation of hepatic IGF1 protein, and increased mRNA expression of multiple IGF1 regions. Furthermore, there was a persistent decrease in dimethylation of H3K4 at the IGF1-P1 and IGF1-E5. In contrast, trimethylation of H3K4 at IGF1-U4 is increased. Across all regions of IGF1, FR/AdLib offspring showed a trend toward decreased dimethylation, as these previously dimethylated lysine tails become further methylated as evidenced by the corresponding increased trimethylation. This overall increase in methylation can result in a structural change to the histone complex that has

been shown to correspond with an increase in expression as we have seen also.

IUGR newborns continued to be suckled by a FR dam (FR/FR) exhibited delayed catch-up growth and absence of adult obesity (17, 18). FR/FR adult offspring have body weights comparable to the control although liver weight is lower. Nonetheless, plasma IGF1 levels and hepatic IGF1 protein expression are similar to the control offspring. Expression of hepatic mRNA for IGF1-E1, IGF1-E2, and IGF1A is the same as controls; however, mRNA from IGF1B shows an increase similar to the rapid catch-up group. Consistent with FR/AdLib, the FR/FR offspring too showed decreased dimethylation at IGF1-P1. However, in contrast to FR/AdLib, FR/FR had increased dimethylation at IGF1-P2 and IGF1-U3 and decreased trimethylation at IGF1-P2. Whereas the FR/AdLib offspring demonstrated decreasing dimethylation and increasing trimethylation across all regions of IGF1, the FR/FR offspring show trends of increasing dimethylation and decreasing trimethylation at IGF1-P2 and U3 and decreasing dimethylation and increased trimethylation at IGF1-P1, E6, and U4, possible evidence of a chromatin structure that is not as transcriptionally active as the more open FR/AdLib group, yet with increased activity compared with controls.

The link between IUGR and the development of adult morbidities such as metabolic syndrome has been extensively demonstrated in humans and various animal models (5, 20, 38, 55) although the etiology of these diseases remains unknown. Epigenetic changes may be one of the underlying mechanisms (8, 10, 21, 24, 26, 36, 53), as numerous studies have linked epigenetic modifications of DNA and histones with the development of cancer and aging (7, 19, 22, 23, 39, 44). Epigenetic instability, such as loss of imprinting and relaxation of the inactive X chromosome (3, 7, 37) as well as loss of methylation in mice at a rate of 0.01% per month (4, 22), has been associated with aging and specifically linked to environmental differences, such as nutrition and toxic substances during postnatal life (21, 23). In addition, mutations in the IGF1 pathway leading to reduced IGF1 levels have been shown to double the lifespan of *Caenorhabditis elegans* (29, 34) and extend the lifespan of *drosophilia* by 48% (14, 52). In humans, high levels of IGF1 are related to the development of cancer although some studies have shown that increased IGF1 has a beneficial impact on numerous diseases associated with aging. These studies have focused on the association of IGF1 with age-related disease, rather than actual lifespan (12, 51). It is apparent in humans that, although IGF1 is involved in aging and the development of age-associated morbidities, it is a dynamic system that requires a balance for the maximum benefit. Accordingly it could be postulated that overly increased IGF1 levels could thereby decrease lifespan, and aberrant epigenetic modifications that persist through life may further confound the problem.

Although it is clear that epigenetics plays a role in the alteration of transcription of the IGF1 gene into mRNA, other additional mechanisms may also be altered that would lead to the observed discordance between mRNA and peptide levels within the liver of IUGR newborns. Altered mRNA stability arising from different splice variants of IGF1 (40) and disruption in transcription or peptide exocytosis may account for the build up of the IGF1 peptide in IUGR livers, where no explanatory increase in mRNA is present. Goya et al. (28) have shown that the treatment of underfed diabetic rats with insulin leads to the partial recovery of IGF1 mRNA expression, and insulin combined with refeeding returned mRNA levels to normal. They also demonstrated, using fetal rat hepatocytes, that insulin treatment leads to increased stability of IGF1 mRNA as opposed to an increase in gene transcription (28). We have previously shown that plasma insulin levels in FR/AdLib offspring are decreased at 3 wk and increased at 9 mo of age (18). Although no data for plasma insulin have been collected in our animals, on the basis of data from 3 wk and 9 mo, it is possible that a reduced insulin level in the IUGR newborn may correspond with a reduction in IGF1 mRNA stability at *day 1*; similarly, an increase in IGF1 stability in the adult may occur in accordance with the increased insulin. IGF1 is not normally considered to be stored in vesicles within the cell but is rather secreted as it is produced (15, 42). A decreased rate of exocytosis from the hepatocyte may account for a build up of peptide even in the absence of increased mRNA and plasma IGF1.

In conclusion, this study demonstrates that IUGR leads to epigenetic modifications in the liver and alterations in hepatic IGF1 mRNA and peptide and plasma IGF1 levels. Most importantly, rapid catch-up growth during the postnatal period in IUGR offspring leads to increased H3K4 trimethylation with increased hepatic mRNA and plasma IGF1, which may partly contribute to an increase in liver and body weights. Thus modulation of the rate of IUGR newborn catch-up growth may protect against IGF1 epigenetic modifications.

ACKNOWLEDGMENTS

The authors thank Linda Day and Stacy Behare for assistance with animals.

GRANTS

This work was supported by National Institute of Child Health and Human Development Grant R03HD060241 and American Heart Association Grant 0865125.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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