Accelerated Postnatal Growth Increases Lipogenic Gene Expression and Adipocyte Size in Low–Birth Weight Mice

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OBJECTIVE—To characterize the hormonal milieu and adipose gene expression in response to catch-up growth (CUG), a growth pattern associated with obesity and diabetes risk, in a mouse model of low birth weight (LBW).

RESEARCH DESIGN AND METHODS—ICR mice were food restricted by 50% from gestational days 12.5–18.5, reducing offspring birth weight by 25%. During the suckling period, dams were either fed ad libitum, permitting CUG in offspring, or food restricted, preventing CUG. Offspring were killed at age 3 weeks, and gonadal fat was removed for RNA extraction, array analysis, RT-PCR, and evaluation of cell size and number. Serum insulin, thyroxine (T4), corticosterone, and adipokines were measured.

RESULTS—At age 3 weeks, LBW mice with CUG (designated U-C) had body weight comparable with controls (designated C-C); weight was reduced by 49% in LBW mice without CUG (designated U-U). Adiposity was altered by postnatal nutrition, with gonadal fat increased by 50% in U-C and decreased by 58% in U-U mice ($P < 0.05$ vs. C-C mice). Adipose expression of the lipogenic genes *Fasn*, *Acc*I, *Lpin1*, and *Srebf1* was significantly increased in U-C compared with both C-C and U-U mice ($P < 0.05$). Mitochondrial DNA copy number was reduced by >50% in U-C versus U-U mice ($P = 0.014$). Although cell numbers did not differ, mean adipocyte diameter was increased in U-C and reduced in U-U mice ($P < 0.01$).

CONCLUSIONS—CUG results in increased adipose tissue lipogenic gene expression and adipocyte diameter but not increased cellularity, suggesting that catch-up fat is primarily associated with lipogenesis rather than adipogenesis in this murine model. *Diabetes* **58:1192–1200, 2009**

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Low–birth weight (LBW) infants are at increased risk for hypertension, type 2 diabetes, and metabolic syndrome (1). Mechanisms remain ill defined but may involve epigenetic regulation of development and gene expression (2,3). Accelerated postnatal, or catch-up growth (CUG), is common in LBW infants and heightens these risks (4,5).

Why CUG has deleterious effects is not well understood, but the rapid adipose tissue expansion accompanying CUG (termed catch-up fat) likely plays a key role. LBW newborns have reductions in both lean and fat mass. While reduced lean mass persists, fat mass accrues preferentially during CUG (6), and LBW adults have increased adiposity (7). Such fat accumulation during childhood is a strong determinant of insulin sensitivity in LBW adults (8). Similar patterns are observed during weight recovery after starvation in adults, where increased food intake and decreased thermogenesis enhance adipose accretion (9). It is unknown whether similar mechanisms contribute to catch-up fat in LBW.

We developed a mouse model of LBW associated with CUG, with obesity and glucose intolerance in adulthood (10). In this model, prevention of early postnatal CUG normalizes glucose intolerance and reduces adiposity (11). To identify mechanisms contributing to catch-up fat, we analyzed hormone secretion, adipose gene expression, and histology in LBW mice with and without CUG. We found that postnatal CUG is associated with lipogenic patterns of gene expression and increased adipocyte size.

RESEARCH DESIGN AND METHODS

Animal protocol. Female ICR mice (Harlan, Indianapolis, IN) aged 6–8 weeks were caged with ICR males; pregnancies were dated by vaginal plug (day 0.5). Pregnant females had ad libitum access to standard chow (Purina 9F; Purina Mills, St. Louis, MO), with 21% of calories from protein, 21% from fat, and 56% from carbohydrate (wheat/corn). On day 12.5, females were randomly assigned to either a control or undernutrition group. Undernutrition group dams were 50% food restricted from days 12.5 to 18.5 (calculated from intake in gestational day–matched controls). At birth, litters were equalized to eight. During suckling, dams were randomly assigned to ad libitum chow or continued 50% food restriction (versus postpartum day–matched dams). This yielded four experimental groups (Fig. 1A): C-C ad libitum chow in utero, ad libitum during suckling; U-C in utero undernutrition, ad libitum during suckling; C-U ad libitum in utero, undernutrition during suckling; and U-U in utero undernutrition, undernutrition during suckling.

Pups were weaned at day 21 to ad libitum Purina 9F chow. Twenty-four–hour food intake was measured in individual mice following a 1-day acclimation in metabolic cages. Comparisons of C-C versus U-C mice are a paradigm for the effects of birth weight, while U-C versus U-U comparisons model the effects of postnatal growth in LBW.

Mice were housed in a National Institutes of Health Office of Laboratory Animal Welfare–approved facility, with controlled temperature, humidity, and light-dark cycle (0700 h–1900 h). Protocols approved by the Joslin Diabetes Center Institutional Animal Use and Care Committee (*Principles of Labora-*
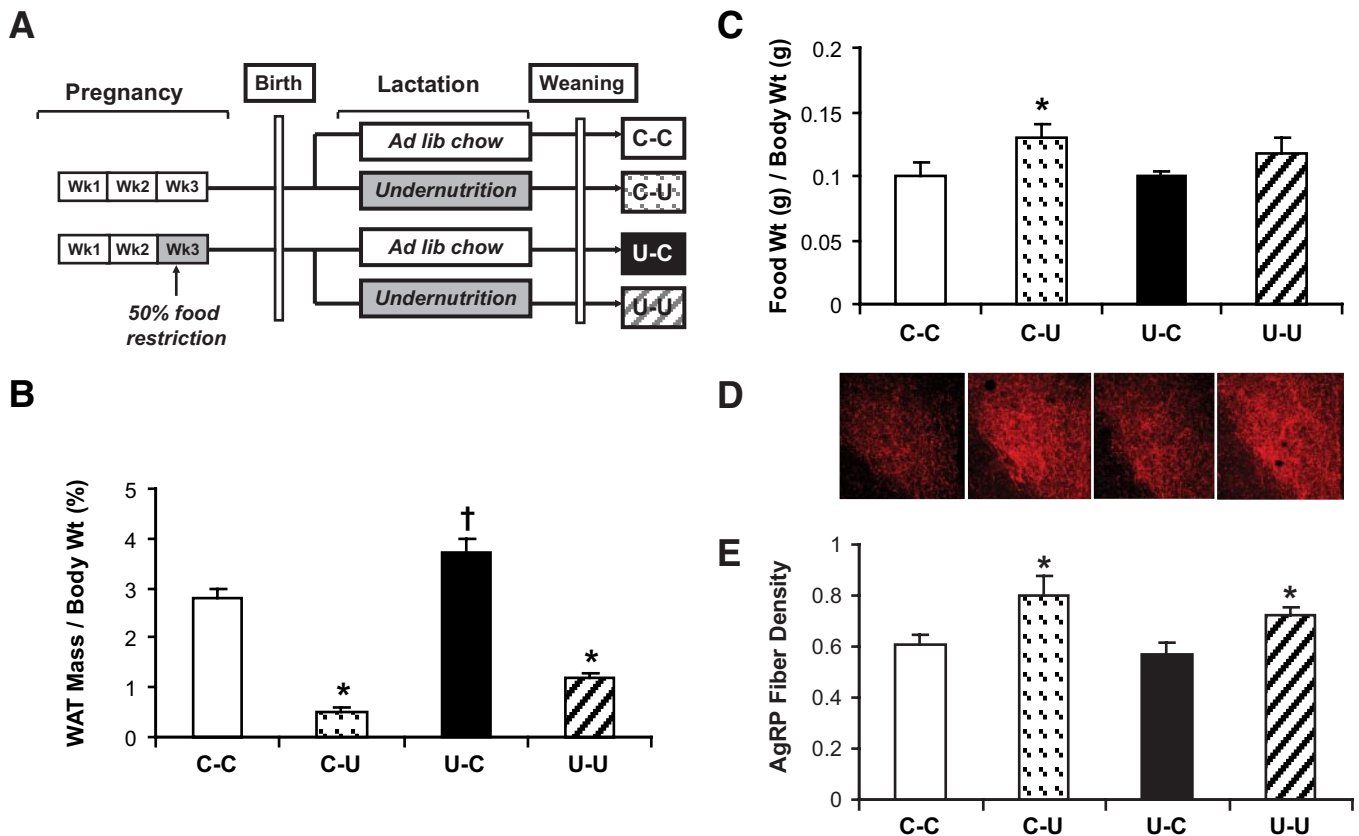
FIG. 1. *A*: Experimental schema. *B*: White adipose tissue mass (WAT mass represents sum of epididymal, perirenal, and flank subcutaneous fad pads), age 3 weeks. * and † denote $P < 0.05$ vs. C-C; groups with different symbols have statistically significant differences ($P < 0.05$). *C*: Daily food intake in 4-week-old animals expressed as grams of food per grams of body weight per 24 h. *Denote $P < 0.05$ vs. C-C. *D*: Agouti-related peptide staining in the periventricular nucleus of the hypothalamus of 6-month-old animals. Note the increase in signal intensity in the C-U and U-U panels. *E*: Agouti-related peptide fiber density, quantitated by image analysis. *Denote $P < 0.05$ vs. C-C. (A high-quality digital representation of this figure is available in the online issue.)

tory Animal Care [available at http://grants1.nih.gov/grants/olaw/references/phspol.htm]) were followed.

Measurement of glucose, hormones, and adipokines. Fed glucose was measured between 9:00 A.M. and 11:00 A.M. in tail vein blood samples (Ascensia Elite; Bayer, Pittsburgh, PA) before pentobarbital anesthesia (150 mg/kg i.p.) and blood sampling via cardiac puncture. Insulin was measured using rat insulin enzyme-linked immunosorbent assay (ELISA) with mouse standards (Crystal Chem, Downers Grove, IL), leptin and adiponectin via ELISA (Crystal Chem and Linco, St. Charles, MO, respectively), and corticosterone by enzyme immunoassay (Immunodiagnostic Systems, Fountain Hills, AZ). Total T4 and T4 charcoal binding were analyzed as described (12), and adipokines (tumor necrosis factor [TNF]-α, interleukin [IL]-6, monocyte chemoattractant protein [MCP]-1, plasminogen activator inhibitor [PAI]-1, and resistin) measured by multiplex ELISA (Millipore, Billerica, MA).

Microarray analysis of gene expression. We chose to study white adipose tissue gene expression at age 3 weeks because white fat is largely absent from neonatal mice, and 3 weeks is the minimum age at which sufficient tissue could be isolated from all four experimental groups. Epididymal fat was dissected from 3-week-old males for RNA extraction and complementary RNA (cRNA) preparation for hybridization to Affymetrix M430A v2 arrays (Santa Clara, CA). Analysis was performed on globally scaled data (MAS 5.0; median 1,500) for samples from five mice in C-C and U-C and four in U-U groups. Multigroup and between-group comparisons of microarray data were performed with significance analysis of microarrays (SAM) (13). MAPPFinder (http://www.genmapp.org) was used for pathway-based analyses. Gene network enrichment analysis (GNEA) was also used to determine whether specific gene sets were overrepresented in cumulatively differentially expressed gene networks in two-way comparisons (14).

Quantitative real-time PCR and mitochondrial DNA copy number. Complementary DNA (cDNA) was generated from 1 μg RNA using oligo-dT primers (Takara Bio, Mountain View, CA) and used for PCR with SYBRGreen (Applied Biosystems, Foster City, CA). Expression was normalized to cyclophilin or TATA box binding protein, as indicated. Primer sequences are available upon request. Mitochondrial DNA (mtDNA) was quantitated in

epididymal fat DNA by RT-PCR (DNAzol extraction, 5 ng DNA input), using ratio of mitochondrial-encoded (NADH dehydrogenase 1, or *ND1*; cytochrome C oxidase, subunit 2; or *Cox2*) to nuclear-encoded genes (β-globin).

Adipocyte quantitation. Epididymal fat was dissected from 3-week-old (C-C and U-C mice, $n = 10–12$/group) and 6-week-old males (all four experimental groups, $n = 8$/group) and collagenase digested (1 mg/ml) for fixation in osmic acid (15) and cell counting (16). This protocol requires a minimum of ~0.1–0.2 g of adipose tissue; 6 weeks is the earliest age at which this could readily be harvested from all four experimental groups.

Measurement of adipocyte size. Epididymal fat from 3-week-old males ($n = 4$/group) was fixed in 10% formalin and paraffin embedded; 4-μm sections were hematoxylin/eosin stained. Eight digital images (600×) from nonoverlapping fields were taken from each slide (total 32 fields per group) and adipocyte diameters calculated (ImageJ [http://rsb.info.nih.gov/ij/]).

Hypothalamic paraventricular nucleus staining for agouti-related peptide. To assess neural regulation of appetite in mature animals, males ($n = 4$/group) were killed at 6 months for quantitation of hypothalamic agouti-related peptide (AgRP) neuronal projections (17). Mice were anesthetized before dissection, postfixation, and cryoprotection (18). Thirty-micrometer coronal sections were blocked, incubated with rabbit anti-AgRP (Phoenix Pharmaceuticals, Burlingame, CA) and then Alexa 594–conjugated donkey anti-rabbit, and mounted onto slides (ProLong Antifade; Invitrogen). Images were obtained with FluoView 500 Confocal Microscope (Olympus, Center Valley, PA) (Image Analysis Core, Diabetes Research and Training Center, University of Michigan). AgRP fiber density was quantified in three representative paraventricular nucleus (PVN) regions using MetaMorph software (Molecular Devices). Background intensities were subtracted and normalized by 1-μm z-stack slice number (28–29/animal) to obtain pixel intensity/μm.

Statistical analyses. Data are presented as means ± SE. Multigroup comparisons of body weight, hormonal parameters, adipocyte measurements, and expression data were assessed by ANOVA (Statview, Cary, NC), with post hoc pairwise comparisons assessed by Fisher's protected least-significant difference. $P < 0.05$ was considered significant.

TABLE 1
Growth and hormonal data, age 3 weeks

	C-C	C-U	U-C	U-U
Birth weight (g)	1.59 ± 0.01	1.56 ± 0.03	1.23 ± 0.05*	1.09 ± 0.02*†
Body weight (g)	16.4 ± 1.1	7.7 ± 0.8*‡	17.8 ± 0.7	8.4 ± 0.9*‡
Body length (cm)	7.8 ± 0.1	6.0 ± 0.2*‡	7.7 ± 0.1	6.2 ± 0.2*‡
Gonadal fat weight (g)	0.18 ± 0.02	0.018 ± 0.004*‡	0.25 ± 0.03§	0.041 ± 0.005*‡
Gonadal fat/body weight (%)	1.1 ± 0.1	0.22 ± 0.04*‡	1.4 ± 0.1§	0.40 ± 0.04*‡
Sum of fat pad weights/body weight (%)#	2.8 ± 0.2	0.5 ± 0.1*‡	3.7 ± 0.3§	1.2 ± 0.1§
Blood glucose (mmol/l)	8.9 ± 0.2	5.5 ± 0.5§	8.2 ± 0.3	6.1 ± 1.1§
Corticosterone (ng/ml)	151 ± 18	234 ± 14	167 ± 21	341 ± 63‖¶
Insulin (pmol/l)	445 ± 70	115 ± 35§	545 ± 100	275 ± 40§
T4 (μg/dl)	8.7 ± 0.7	7.1 ± 0.9	8.8 ± 0.8	6.1 ± 0.2*†
T4 resin binding (%)	40.8 ± 1.2	40.0 ± 1.6	41.5 ± 1.5	36.2 ± 1.6*†
Leptin (ng/dl)	6.1 ± 0.4	0.8 ± 0.2*‡	5.9 ± 0.4	2.0 ± 0.5*‡
Adiponectin (ng/ml)	7.8 ± 1.2	13.3 ± 4.1*¶	5.2 ± 0.9	8.9 ± 1.1
Resistin (ng/ml)	2.6 ± 0.3	1.3 ± 0.1*	2.0 ± 0.2§	1.9 ± 0.3§
IL-6 (pg/ml)	4.0 ± 0.3	5.2 ± 0.9	4.8 ± 1.1	4.7 ± 0.5
MCP-1 (pg/ml)	47 ± 4	60 ± 8	36 ± 6	49 ± 8
PAI-1 (ng/ml)	4.5 ± 0.4	5.9 ± 0.2§	3.6 ± 0.3	4.4 ± 0.6
TNF-α (pg/ml)	9.5 ± 0.5	8.4 ± 0.4	9.1 ± 0.4	10.9 ± 1.2

Data are means ± SE. Growth data, glucose homeostasis, and hormonal data in male mice, age 3 weeks. $n = 5$–9 per group, depending on the assay. Multigroup comparisons were done by ANOVA, and pairwise comparisons were done by Fisher's protected least-significant difference. *$P < 0.001$ vs. C-C; †$P < 0.05$ vs. U-C; ‡$P < 0.001$ vs. U-C; §$P < 0.05$ vs. C-C; ‖$P < 0.01$ vs. C-C; ¶$P < 0.01$ vs. U-C; #sum of weight of gonadal, subcutaneous flank, and perirenal fat pads.

RESULTS

Growth and body composition. Food restriction of pregnant dams reduced birth weight by 25% (control: 1.58 ± 0.06 g, LBW: 1.18 ± 0.04; $P < 0.0001$) (Table 1). By 3 weeks of age, body weight and length were equivalent in C-C and U-C offspring, indicating CUG in U-C mice. Weight and length were significantly reduced in mice with postnatal food restriction ($P < 0.0001$, C-U and U-U mice vs. C-C and U-C mice) (Table 1). By 3 weeks, U-C mice had increased gonadal and total white adipose weight ($P < 0.05$ vs. all other groups) (Table 1) (Fig. 1*B*).

Glucose, adipogenic hormones, and adipokines. Because insulin is a potent adipogenic hormone and alterations in insulin sensitivity have been linked to CUG (19), we assessed glucose homeostasis in LBW versus controls, with and without CUG, at age 3 weeks. Fed glucose was comparable in U-C and C-C mice but significantly decreased in C-U and U-U mice ($P < 0.01$ vs. C-C) (Table 1). Serum insulin levels were reduced by 74% in C-U mice ($P = 0.04$ vs. C-C) but tended to be higher in U-C mice.

Both corticosteroids and thyroid hormone have adipogenic effects, and altered regulation has been associated with LBW (20,21). Serum corticosterone was similar in C-C, C-U, and U-C mice but increased twofold in U-U mice ($P < 0.05$ vs. C-C) (Table 1). Total T4 did not differ in LBW mice with CUG, but both total and free T4 were significantly reduced in LBW mice with postnatal food restriction (Table 1).

To evaluate adipocyte secretory function, we measured serum adipocytokines. Leptin paralleled nutritional status and was decreased in postnatally food-restricted C-U and U-U groups (87 and 67% decrease, respectively, $P < 0.0001$ vs. C-C) (Table 1). Conversely, both adiponectin and PAI-1 were increased in C-U mice (71 and 31% increase, respectively, $P < 0.05$ vs. C-C). Resistin was reduced in all groups ($P < 0.05$ vs. C-C). TNF-α, IL-6, and MCP-1 were not altered by prenatal or postnatal nutrition.

Early postnatal nutrition alters orexigenic hypothalamic AgRP fiber density in adult life. We next asked whether altered food intake contributes to increased adiposity in mice with CUG. At age 4 weeks, the earliest time point at which mice could tolerate individual metabolic cages, we detected no differences in weight-adjusted 24-h food intake between C-C and U-C groups, consistent with prior data at older ages (10,11). Interestingly, C-U mice had higher food intake than either C-C or U-C mice ($P < 0.05$) (Fig. 1*C*). Because subtle differences in appetite can be difficult to detect through measurements of food intake, we performed immunostaining for AgRP neuronal projections in the PVN in fully mature animals. AgRP fiber density was not altered in LBW mice with CUG compared with controls. However, mice exposed to postnatal undernutrition had increased AgRP fiber density ($P < 0.05$, C-U and U-U vs. C-C) (Fig. 1*D* and *E*).

Early nutrition alters adipose gene expression. To determine whether adipose gene expression is dysregulated during CUG, we performed microarray analysis in adipose tissue from C-C, U-C, and U-U mice ($n = 4$–5/group). While growth data, glucose levels, and hormonal analyses were similar to the entire cohort, gonadal fat weight was not significantly different between U-C and C-C mice in this subgroup (online appendix Table 2 [available at http://diabetes.diabetesjournals.org/cgi/content/full/db08–1266/DC1]). Primary microarray data are available at http://www.ncbi.nlm.nih.gov/geo. Official names of genes are provided in online appendix Table 1.

To identify individual genes differentially expressed between groups, we performed multigroup comparisons using SAM (13). A total of 1,778 genes were differentially regulated with false discovery rate (FDR) ≤0.25 (393 with FDR <0.15) (online appendix Table 3). To determine pathways overrepresented in these comparisons, we performed GNEA. Top-ranking gene sets included insulin signaling, T-cell receptor, and IL-3 through IL-9, but these

TABLE 2
Pathway analysis (MAPPFinder)

| Regulation by antenatal nutrition | | | Regulation by postnatal nutrition | | |
| Upregulated in U-C versus C-C | | | Upregulated in U-C versus U-U | | |
Pathway	Z score	$P_{permuted}$	Pathway	Z score	$P_{permuted}$
Cholesterol biosynthesis	3.9	0.003	Krebs-TCA cycle	6.4	<0.001
			Pentose phosphate pathway	4.4	0.001
			Electron transport chain	3.6	0.004
			Glycolysis and gluconeogenesis	3.6	0.003
Proteasome degradation	2.5	0.026	Fatty acid β-oxidation BiGCaT	2.9	0.012
			Prostaglandin synthesis regulation	2.9	0.009
			Fatty acid synthesis BiGCaT	2.9	0.02
			Triacylglycerol synthesis BiGCaT	2.7	0.017
Downregulated in U-C versus C-C			Downregulated in U-C versus U-U		
Pathway	Z score	$P_{permuted}$	Pathway	Z score	$P_{permuted}$
Ribosomal proteins	10.8	<0.001	mRNAprocessing	3.3	0.001
Translation factors	5.2	<0.001			
mRNA processing	4.4	<0.001			

Results of MAPPFinder pathway analysis comparing patterns of gene expression in C-C versus U-C and U-C versus U-U. Genes are considered up- or downregulated if expression is changed by ≥25% and permuted P value ($P_{permuted}$) is <0.05. The frequency of up- or downregulated genes in a pathway is compared with the frequency in the array as a whole; a Z score and a P value is generated for each pathway. All pathways with Z score >2.5 are presented in the table.

reached only marginal significance (FDR = 0.259) (online appendix Table 4).

Effect of LBW on adipose gene expression (U-C versus C-C mice). No individual genes were significantly upregulated in adipose tissue as a function of birth weight (U-C versus C-C mice) (SAM, FDR ≤0.25). However, pathway analyses (MAPPFinder) demonstrated that cholesterol biosynthesis (Z score = 3.9, $P_{permuted}$ = 0.003) and proteasome degradation (Z score = 2.5, $P_{permuted}$ = 0.026) pathways were upregulated in LBW (Table 2). Fifteen genes were downregulated in LBW (U-C vs. C-C mice, FDR ≤0.25) (online appendix Table 5A). Significantly downregulated pathways in LBW (U-C versus C-C mice) included ribosomal proteins (Z score = 10.8, P < 0.001), translation factors (Z score = 5.2, P < 0.001), and messenger RNA (mRNA) processing (Z score = 4.4, P < 0.001) (Table 2; online appendix Table 6A).

Effect of CUG on adipose gene expression (U-C versus U-U mice). To identify genes for which adipose expression is modulated by CUG, we compared array data in LBW mice with CUG (U-C) to those without CUG (U-U). A total of 157 genes were upregulated in U-C versus U-U (FDR <0.25) (online appendix Table 5B). Multiple gene sets related to oxidative and lipid metabolism, including Krebs cycle, electron transport, glycolysis and gluconeogenesis, β-oxidation, and triglyceride/fatty acid synthesis, were upregulated in mice with CUG (Z score >2.5, $P_{permuted}$ <0.05) (Table 2; online appendix Table 6C). A total of 516 genes were significantly downregulated in LBW mice with CUG (U-C versus U-U mice, FDR ≤0.25) (online appendix Table 5B); pathways downregulated in U-C mice included mRNA processing (Z score = 3.3, P = 0.01) (Table 2; online appendix Tables 6D and 8C).

LBW and CUG alter expression of genes regulating adipocyte differentiation and function. Because gene and pathway analyses indicated that genes involved in adipocyte metabolism were upregulated as a function of CUG in LBW mice (Table 2), we assessed expression of genes related to differentiation and function by RT-PCR in adipose from all four experimental groups (Fig. 2). While

expression of the adipogenic transcription factors *Cebpa*, *Cebpb*, and *Pparg* tended to be higher in U-C mice, expression of the insulin-responsive glucose transporter *Glut4* was increased by 60% in U-C mice (P = 0.03 vs. C-C) and reduced by 20% in U-U mice (P = 0.02). Expression of the differentiated adipocyte marker *aP2* did not differ significantly. Ribosomal proteins were also identified in pathway analysis as downregulated in U-C mice. However, differences in expression were low in magnitude for individual genes, and differences were not confirmed by PCR.

We observed marked differences in expression of two imprinted genes linked to early developmental patterning in adipose tissue. Consistent with array data, expression of *Mest*, a paternally imprinted adipocyte gene associated with increased cell size (22), was increased ninefold in LBW mice with CUG compared with LBW mice without CUG (P = 0.005 U-C vs. U-U) (Fig. 2A). Moreover, expression of *Pref1*, an inhibitor of adipogenesis expressed by preadipocytes, was significantly increased in mice with postnatal undernutrition (P = 0.002 C-U vs. C-C; P < 0.001 U-U vs. C-C) (Fig. 2A).

We next assessed expression of nuclear receptors known to regulate adipocyte differentiation and/or lipid metabolism. We detected no significant differences in expression of *Fxr*, *Lxr*α, *Lxr*β, Smrt/Ncor2, or *Nrip1/Rip140* as a function of either LBW or CUG (not shown). However, expression of several nuclear receptors involved in retinol signaling was increased in LBW mice and normalized by postnatal food restriction, including *Rxra* (increased twofold in U-C mice; P = 0.008 vs. C-C) and *Rarb* (increased threefold in U-C vs. U-U, P < 0.05 trend for increase vs. C-C, P = 0.07) (Fig. 2B). In addition, retinol binding protein 4 (*Rbp4*), a marker and potential inducer of insulin resistance (23), was increased nearly twofold in U-C mice (P < 0.05 vs. both C-C and U-U) (Fig. 2B).

Postnatal nutrition modulates mtDNA copy number. Pathway analyses demonstrated increased expression of nuclear-encoded genes involved in mitochondrial oxidative function in LBW mice with CUG, the group at highest
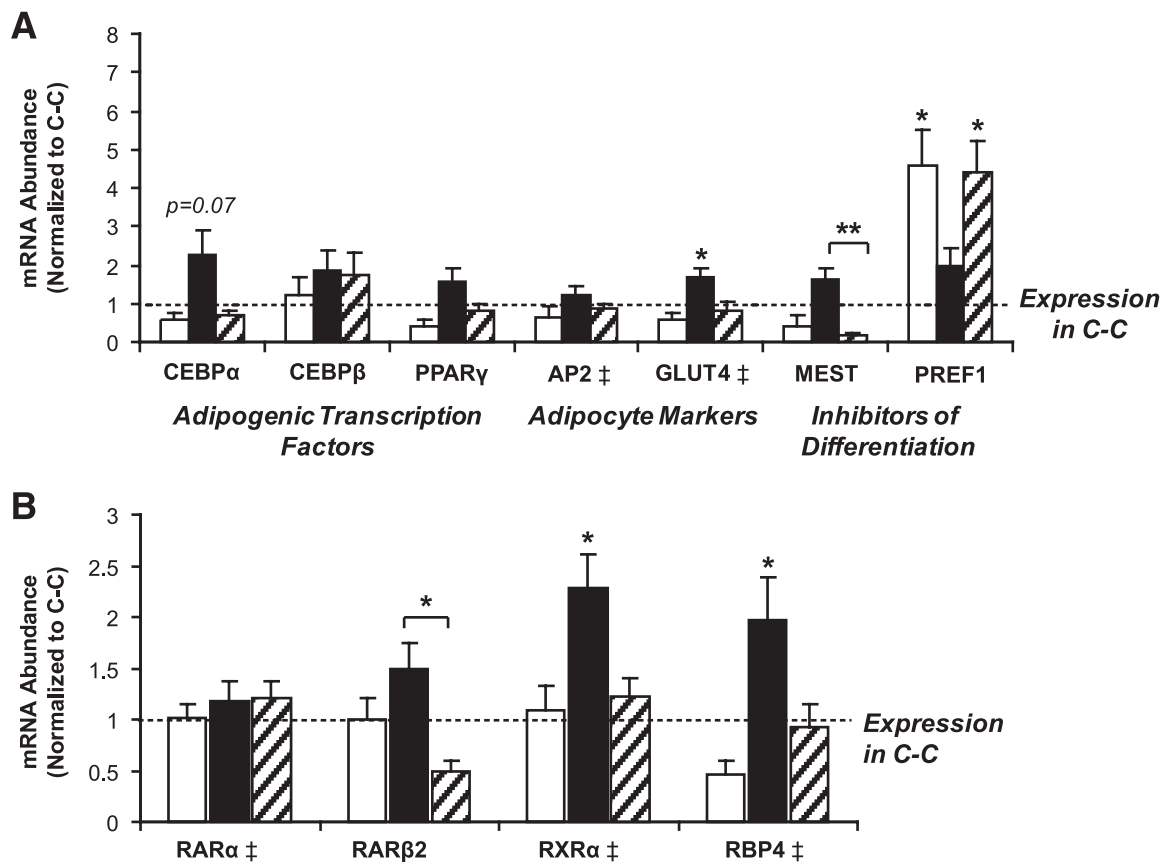
FIG. 2. *A*: Expression of genes related to adipocyte differentiation by RT-PCR. *B*: Expression of genes related to retinol signaling by RT-PCR. *$P <$ 0.05 vs. C-C; comparisons of U-C vs. U-U also reached $P < 0.05$ for Rxr-α and Rar-β2. ‡Data are normalized to expression of cyclophilin or TBP. Gene expression in the C-C group is set as 1; □, C-U group; ■, U-C group; ▨, U-U group.

risk for obesity and diabetes. However, we did not observe any significant differences in expression of regulators of mitochondrial biogenesis or function, including *Pgc-1α*, *Esrra*, *Pprc1*, *Dio2*, or *Thrb* (Fig. 3*A*), or in *Ucp1* or genes encoding components of the electron transport chain (*Atp5c*, *Cox8b*, not shown). By contrast, mtDNA copy number, assessed by the relative expression of Cox2 to β-globin, was reduced by >50% in LBW mice with CUG (U-C) versus LBW mice without CUG (U-U) ($P = 0.014$). Similar patterns were observed for *ND1*, which tended to be reduced in U-C versus U-U mice ($P = 0.055$) (Figs. 3*B* and *C*).

Lipogenic gene expression is increased in LBW mice with CUG. A consistent pattern of gene expression in LBW mice with CUG was upregulation of lipogenic and cholesterol synthesis genes. PCR confirmed that expression of *Fasn*, *Hmgcs1*, *Acc*I, and *Acss2* was increased two- to threefold in U-C mice ($P < 0.05$ vs. C-C). *Scd1* was not differentially expressed in U-C but was increased in C-U mice ($P < 0.05$ vs. C-C) (Fig. 4*A*).

To determine which transcriptional regulators might contribute to increased lipogenic gene expression in LBW, we analyzed expression of *Adpn*, *Chrebp*, *Insig1*, *Ir*, *Igf1r*, *Lipin-1α*, *Lipin-1β*, and *Srebf1* by RT-PCR (Fig. 4*B*). Expression of both *Srebf1* and *Adpn* was increased two- to threefold in U-C mice ($P = 0.013$ vs. CC); similar trends were observed for *Chrebp* ($P = 0.08$ C-C vs. U-C). Both isoforms of lipin, a triacylglycerol synthesis enzyme and regulator of lipogenesis (24), were upregulated in U-C mice (lipin 1α: two-fold increase, lipin 1β: 2.5-fold increase, both $P = 0.02$ vs. C-C). Expression of *Insig1*, a negative regulator of *Srebf*-mediated lipogenesis (25), was

also increased twofold in U-C mice ($P = 0.026$ vs. C-C). While expression of insulin receptor mRNA tended to be increased in U-C versus U-U mice ($P = 0.05$), expression of *Igf1r* was not altered.

Adipocyte size, but not number, is increased in LBW with CUG. We next asked whether LBW followed by CUG alters adipocyte number (implicating altered differentiation) or increases adipocyte size (suggesting increased lipogenesis). Adipocyte number was similar in LBW mice with CUG (U-C) and in controls (C-C) at both 3 weeks (not shown) and 6 weeks of age (Fig. 5*A*). By contrast, adipocyte number was reduced in C-U mice ($P = 0.004$ vs. C-C) and tended to be reduced in U-U mice ($P = 0.1$ C-C vs. U-U) at 6 weeks of age (Fig. 5*A*).

While adipocyte number did not differ as a function of antenatal nutrition, adipocyte size was significantly increased in LBW mice with CUG (U-C) (Fig. 5*B* and *C*). Adipocyte diameter was reduced in C-U and U-U mice (C-C: 24.5 ± 0.7 μm; C-U: 9.9 ± 0.2; U-C: 41.5 ± 1.4; and U-U: 16.9 ± 0.3; $P < 0.0001$ between all groups). In parallel, we noted a marked increase in adipocytes larger than 30 μm in U-C mice (rightward distribution shift) (Fig. 5*B*), whereas C-C, C-U, and U-U mice had relatively few or no large adipocytes. Adipocytes in C-U mice tended to be small and multilocular, whereas adipocytes in U-C mice were large and unilocular.

DISCUSSION

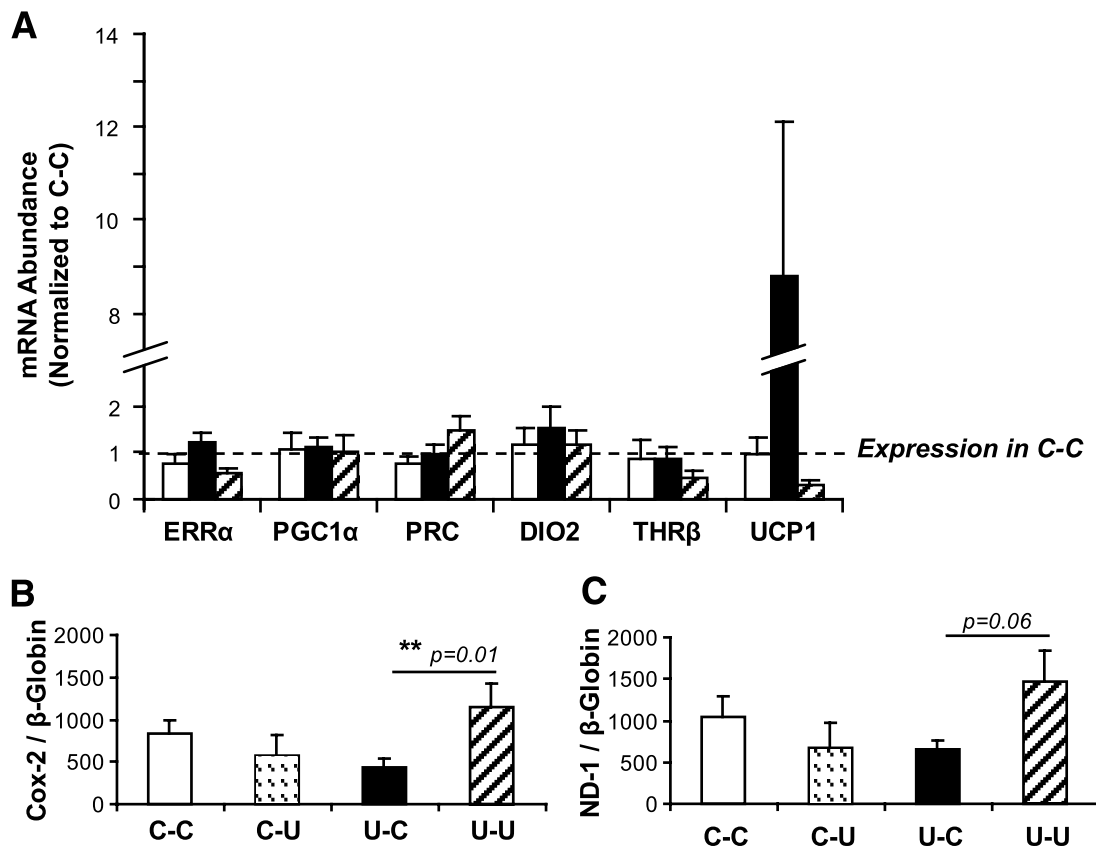LBW and postnatal CUG are associated with increased adiposity in humans (7) and animal models (26). To

FIG. 3. *A*: Gonadal fat expression of genes related to mitochondrial biogenesis and thermogenesis by RT-PCR. *$P < 0.05$ vs. CC. mRNA abundance is normalized to expression of the housekeeping gene cyclophilin. Gene expression in the C-C group is set as 1. *B*: Abundance of the mitochondrial-encoded gene Cox-2 relative to the nuclear-encoded gene β-globin in gonadal fat pads. The relative proportion of Cox-2 to β-globin is proportional to the amount of mitochondrial DNA per cell. *$P < 0.05$. *C*: Abundance of the mitochondrial-encoded gene ND-1 relative to the nuclear-encoded gene β-globin in gonadal fat pads. The relative proportion of ND-1 to β-globin is proportional to the amount of mitochondrial DNA per cell. ▨, C-U; ■, U-C; ▨, U-U.

examine hormonal and molecular processes contributing to increased adiposity, we utilized our mouse model of LBW induced by maternal caloric restriction. LBW pups with CUG have greater adipose mass than controls, similar to human catch-up fat phenotypes. In this model, early postnatal CUG is more closely linked to adult phenotypes than birth weight, and prevention of CUG by early post-natal caloric restriction prevents subsequent obesity and type 2 diabetes (11). We now demonstrate that CUG results in several dominant patterns in fat: *1*) increased adipocyte size, *2*) minimal alterations in adipogenic gene expression, *3*) increased expression of lipogenic genes, and *4*) reduced mtDNA content. Importantly, each of these phenotypes was reversed by attenuation of early postnatal weight gain. Together, these data suggest that increased lipogenesis and/or impaired oxidative function are key features of catch-up fat.

LBW mice with CUG have significantly increased adipocyte diameter but similar numbers of adipocytes per fat depot. The early postnatal environment plays a key role in LBW-associated adipocyte enlargement, as attenuation of postnatal growth rates normalizes cell size. Adipocyte hypertrophy may be an important contributor to LBW phenotypes, as it has been described in other models of developmental programming (27), and may be associated with inflammation (28), insulin resistance (29), and type 2 diabetes, independent of fat mass (30). Altered secretion of adipokines, including leptin, TNF-α, IL-6, and MCP-1 (31), has been postulated to link adipocyte size and insulin

resistance. In our model, leptin levels were highest in C-C and U-C mice, which had larger adipocytes, than in C-U or U-U mice. While leptin levels at age 3 weeks did not differ as a function of birth weight, we cannot exclude dynamic differences in leptin secretion or in the neonatal leptin surge (32). Although array analysis using GNEA suggested a modest proinflammatory signature associated with CUG, we found no significant differences in circulating TNF-α, IL-6, or MCP-1. Whether inflammatory phenotypes become more prominent with advancing age and increasing obesity will be an important question for future study. Interestingly, expression of *Rbp4* was significantly increased in U-C mice, possibly reflecting larger adipocyte size (33).

In parallel, lipogenic genes and pathways were markedly upregulated in adipose tissue of LBW mice with CUG (U-C). For example, we observed significant upregulation of the prolipogenic genes *Fasn, Acc*I, *Acss2, Hmgcs1, Adpn, Insig1, Lpin1,* and *Srebf1.* Furthermore, expression of these genes was largely normalized in LBW mice without CUG. By contrast, we noted minimal changes in expression of adipogenic genes by RT-PCR. These data, together with the similar numbers of adipocytes in U-C versus C-C, suggest that lipogenesis may be a more dominant contributor to catch-up fat than adipogenesis. Our data contrasts with observations in antenatally protein-restricted rats, where increased adiposity was associated with increased preadipocyte proliferation capacity (34).

Increases in lipogenic gene expression and adipocyte hypertrophy may result from increased energy intake.
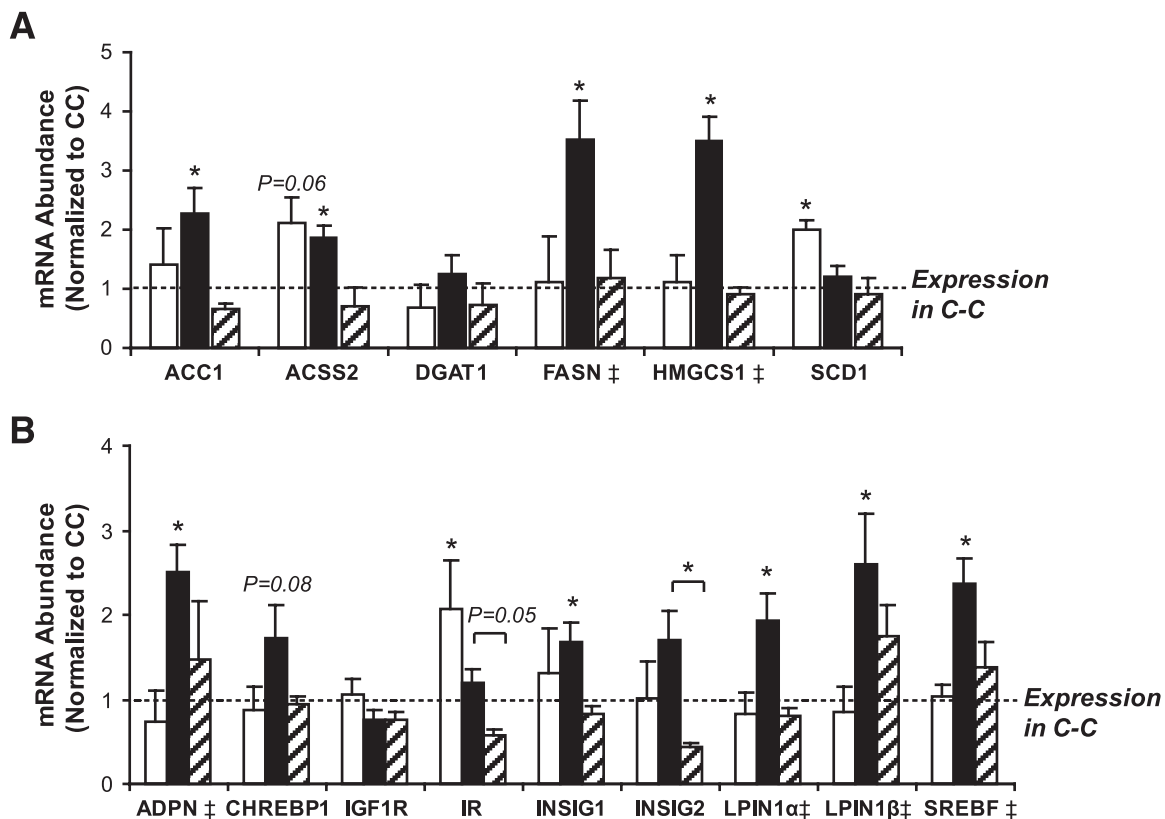
FIG. 4. *A*: Gonadal fat expression of genes related to lipogenesis by RT-PCR. *B*: Gonadal fat expression of genes regulating lipogenesis by RT-PCR. *$P < 0.05$ vs. C-C; comparisons of U-C vs. U-U also reached $P < 0.05$ for *Acc*I, Acss2, Fasn, Hmgcs1, Insig1, Insig2, and Lpin1α. ‡mRNA abundance is normalized to expression of cyclophilin or TBP. Gene expression in the C-C group is set as 1. □, C-U; ■, U-C; ▨, U-U.

However, we observed no alterations in food intake or orexigenic AgRP fiber density in LBW mice with CUG. The more dominant determinant of energy intake in our model appears to be the postnatal nutritional environment, as food intake is increased in C-U mice at 4 weeks, and orexigenic AgRP projections are increased in adult C-U and U-U animals. Together, these results suggest that LBW-related adiposity is more likely to stem from a lipogenic hormonal environment or alterations in adipocyte transcriptional regulation.

One potent hormonal regulator of lipogenesis is insulin. We have previously described significant increases in fed glucose and insulin in LBW mice with CUG at 2 months of age (10), together with increased basal insulin secretion in isolated islets. In the current study, we noted a trend to higher insulin in U-C mice and reduction with postnatal nutritional modifications in the U-U group at 3 weeks. Alterations in insulin sensitivity during early life could contribute to enhanced lipogenesis. Indeed, studies in LBW human infants suggest that insulin sensitivity is increased during CUG (35). Although we were unable to formally assess insulin sensitivity in neonatal mice, expression of both *Ir* and *Glut4* was increased in LBW mice with CUG. Moreover, GNEA analysis suggested that genes differentially expressed between LBW mice with and without CUG were enriched for genes related to insulin signaling. Downregulation of ribosomal synthesis in U-C adipose tissue may also represent an insulin-dependent process, as increased ribosomal synthesis has been noted in mice with muscle-specific insulin receptor ablation (36). It is possible that whole-body and tissue-specific insulin sensitivity varies throughout the lifespan, with early increases in

lipogenesis and adipose tissue accretion and subsequent reductions in insulin sensitivity with aging (37). Further assessement of insulin sensitivity and glucose tolerance will help define the temporal and tissue-specific alterations in glucose homeostasis in our model.

Thyroxine (T4) and corticosterone could also contribute to early adipose tissue phenotypes (20,21,38). We observed no differences in T4 or corticosterone in LBW mice with CUG. Moreover, mice with reduced postnatal growth and lipogenic expression patterns (C-U and U-U mice) had increased levels of corticosterone; since corticosteroids enhance adiposity, it is unlikely this plays a major role in our model. Conversely, since total T4 is reduced in U-U mice, decreased T4-driven lipogenesis may contribute to the obesity-protective effects of postnatal food restriction.

Transcriptional regulators of lipogenesis may also contribute to adipocyte hypertrophy and expression patterns. Sterol regulatory element–binding protein (SREBP)-1c, a dominant insulin-regulated mediator of lipogenic gene expression (39), was significantly upregulated in LBW mice with CUG (U-C). SREBP-1c coactivators, including CAAT/enhancer-binding protein-β or carbohydrate-responsive element–binding protein (ChREBP), may also play an important role in mediating lipogenic expression in adipocytes (40). Interestingly, adipose tissue *Chrebp* expression also tended to be increased in mice with CUG.

Lipin (*Lpin1*) is an important transcriptional regulator of lipogenic gene expression and triglyceride synthesis (41,42). Both lipin-α and -β splicing isoforms (42) were upregulated in LBW mice with CUG (U-C), suggesting another potential mechanism for adipocyte hypertrophy. *Lpin1* overexpression in adipose tissue increases diet-
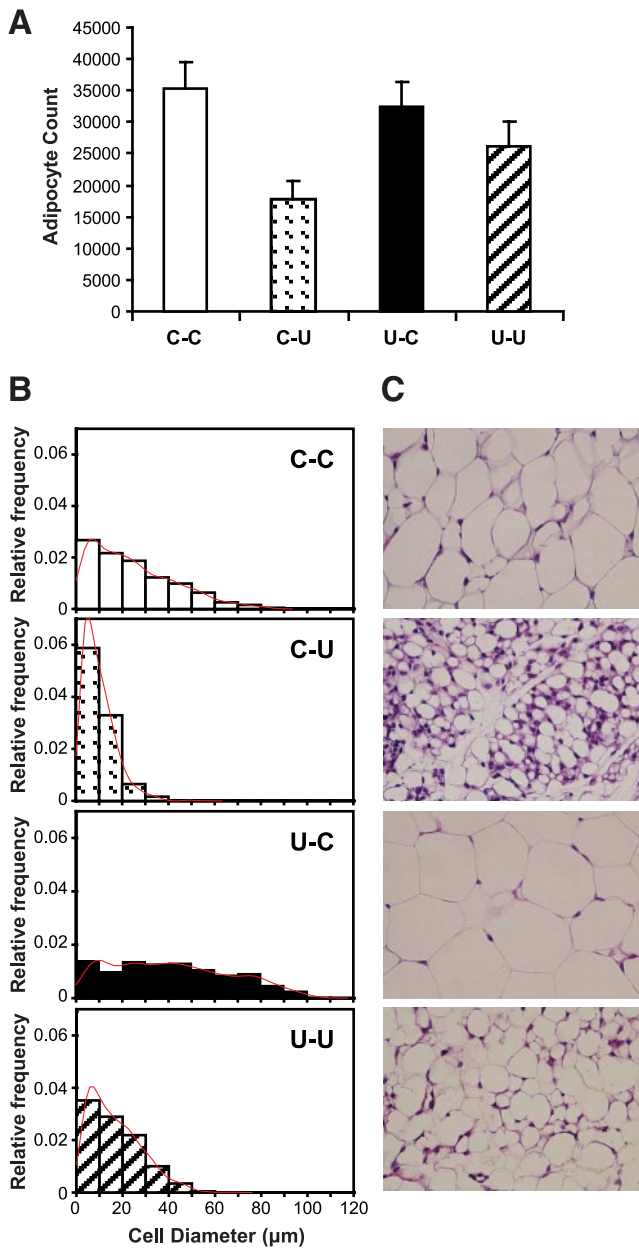
A

B   **C**

FIG. 5. *A*: **Quantitation of osmium-fixed adipocytes per gonadal fat depot. *P < 0.05 vs. C-C. *B*: Distribution of adipocyte diameter (microns) in gonadal fat depots; data obtained by image analysis of histologic sections. *C*: Histologic sections of formalin-fixed gonadal adipose tissue, hematoxilin and eosin stained, shown at ×600 magnification. Note the small, multiloculated adipocytes in C-U and the increased adipocyte diameter in U-C. (A high-quality digital representation of this figure is available in the online issue.)**

induced obesity (24). Moreover, *Lpin1* expression correlates with insulin-stimulated glucose uptake and *Glut4* expression in adipocytes (43). Further experiments will be essential to determine whether increased SREBP-1c and/or lipin transcriptional activity is responsible for the upregulation of lipogenic gene expression in LBW mice with CUG.

A transcriptional signature of LBW with CUG (U-C) was upregulation of mitochondrial oxidative genes, including tricarboxylic acid cycle, oxidative phosphorylation, and fatty acid oxidation, compared with LBW mice with reduced postnatal growth (U-U). Although upstream regulators of many of these genes (e.g., peroxisome proliferator–

activated receptor γ coactivator 1 family, *Esrra*, and *Dio2*) have been linked to both adipocyte metabolic function and type 2 diabetes risk (44), we observed no differences in adipose expression of these genes in LBW mice with or without CUG.

mtDNA content, a potential regulator of adipose oxidative capacity, was decreased in LBW mice as a function of CUG. Reduced mtDNA content may also contribute to increased nuclear-encoded oxidative gene expression patterns (Table 2), also a feature of human mtDNA deletion syndromes (45). Similar reductions in mtDNA content have been observed in muscle, liver, and pancreas, following antenatal protein restriction, (46,47) and in umbilical cord cells from LBW babies (48). While adipocyte mitochondrial number may also regulate lipogenic capacity (49), our data suggest that regulation is likely to be more complex in the LBW setting. Interestingly, prevention of postnatal CUG increases adipose mtDNA content in LBW animals, again indicating a key role for the postnatal environment to modulate phenotypes associated with LBW. Interestingly, this increase in adipose mitochondrial content is also observed in rodent models with prolonged lifespan (50).

Our analysis of adipose histology and gene expression was performed in samples obtained from mice at ≥3 weeks of age. Therefore, a limitation of our analysis is that we are identifying patterns that may be a result of catch-up fat rather than the primary mechanisms. However, our approach was guided by practical limitations, as neonatal mice have minimal white adipose tissue available for analysis. Furthermore, whole-body growth rates in LBW mice continue to exceed that of controls up to 4 weeks of age (11), suggesting that processes mediating catch-up fat are likely to be operative at 3 weeks of age.

In summary, we have characterized the hormonal milieu, adipose histology, and patterns of gene expression in a mouse model of LBW and modulated postnatal growth achieved through nutrient restriction during gestation and lactation. In this model, LBW mice with accelerated postnatal growth have increased adiposity in early life (catch-up fat) and increased risk of type 2 diabetes and obesity in adulthood. By contrast, LBW mice with reduced postnatal growth are protected from these complications. We observed increased adipocyte diameter and upregulation of lipogenic pathways and transcriptional regulators (*Srebf*, *Lpin1*) in LBW mice with CUG, suggesting that lipogenesis, to a greater degree than adipogenesis, contributes to increased adiposity. Such patterns may contribute to lifelong risk of type 2 diabetes in LBW mice. Importantly, attenuation of postnatal weight gain normalized lipogenic gene expression and adipocyte morphology and increased adipose mitochondrial content, illustrating the importance of the postnatal nutritional environment in shaping lifelong disease risk. A clearer understanding of the mechanisms that drive adipose tissue growth during catch-up fat will be important for the eventual development of therapies to prevent the long-term risks of LBW and could also shed new insight into the pathophysiology of early adiposity rebound and childhood obesity.

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