BASIC SCIENCE ARTICLE

Catch-up growth in juvenile rats, fat expansion, and dysregulation of visceral adipose tissue

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BACKGROUND: Accelerated catch-up growth following intrauterine restriction increases the risk of developing visceral adiposity and metabolic abnormalities. However, the underlying molecular mechanisms of such metabolic programming are still poorly understood.

METHODS: A *Wistar* rat model of catch-up growth following intrauterine restriction was used. A gene expression array was performed in the retroperitoneal adipose tissue sampled at postnatal day (PD) 42.

RESULTS: Five hundred and forty-six differentially expressed genes (DEGs) were identified (adjusted p value < 0.05). Gene ontology enrichment analysis identified pathways related to immune and lipid metabolic processes, brown fat cell differentiation, and regulation of PI3K. *Ccl21*, *Npr3*, *Serpina3n*, *Pnpla3*, *Slc2a4*, and *Serpina12* were validated to be upregulated in catch-up pups (all $p < 0.01$) and related to several fat expansion and metabolic parameters, including body weight at PD42, postnatal body weight gain, white and brown adipose tissue mass, plasma triglycerides, and insulin resistance index (all $p < 0.05$).

CONCLUSIONS: Genes related to immune and metabolic processes were upregulated in retroperitoneal adipose tissue following catch-up growth in juvenile rats and were found to be associated with fat expansion and metabolic parameters. Our results provide evidence for several dysregulated genes in white adipose tissue that could help develop novel strategies to prevent the metabolic abnormalities associated with catch-up growth.

IMPACT:

- Catch-up growth presents several dysregulated genes in white adipose tissue related to metabolic abnormalities.
- *Ccl21*, *Npr3*, *Serpina3n*, *Pnpla3*, *Slc2a4*, and *Serpina12* were validated to be upregulated in catch-up pups and related to visceral fat expansion and metabolic parameters.
- Profiling and validation of these dysregulated genes in visceral adipose tissue could help develop novel strategies to prevent the metabolic abnormalities associated with catch-up growth.

INTRODUCTION

Obesity is a relevant medical condition that has the dimension of a global epidemic and carries the risk of developing other noncommunicable diseases, such as type 2 diabetes (T2D) and coronary artery disease.[1] Accumulating evidence reveals that nutrition has a critical role during crucial periods of development, including the intrauterine life.[2,3] The concept of the "thrifty phenotype hypothesis" states that nutritional insults affecting intrauterine growth make the fetus adopt survival strategies, through metabolic adaptations, to optimize the use of nutrients and prioritize the growth of certain organs, such as the brain, at the expense of others, mostly peripheral organs.[4] Although these adjustments can benefit the individual in the short term, they will be potentially detrimental under subsequent conditions of abundant nutrient supply since the organism remains programmed to scarcity conditions.[5–9] Negative consequences of such metabolic programming include the development of obesity and the associated metabolic diseases later in life.[5,8]

Studies in low birth weight children have pointed out that early postnatal catch-up growth, rather than having reduced prenatal growth, increases the risk of developing cardiometabolic diseases later in life.[10,11] Animal models showed that even before any catch-up growth is evident, prenatally restricted rats exhibit physiologic, morphologic, and transcriptomic defects that can be considered as the initial signs of metabolic programming, which can be worsened or readapted with catch-up growth.[12] Several

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studies have reported that low birth weight children with catch-up growth manifest abnormalities already at school age, such as precocious puberty, overweight, augmented adiposity, insulin resistance, and low-grade inflammation.[13–17] Despite the robust associations of these studies, the molecular mechanisms mediating the interaction between catch-up growth and metabolic-related conditions are still largely unknown.

Adipose tissue has been identified as one of the principal targets of such metabolic programming, as emerging evidence has linked catch-up growth to the excess of adipose tissue and impaired metabolism in animal models. Pigs that experienced early catch-up growth developed a remarkable increase in adiposity and an abnormal lipid profile after both chow diet feeding[18] and high fat diet feeding.[19] Studies in rats have come to similar results: restricted rats following high caloric or chow diets increased their fat storage and showed a deleterious metabolic phenotype.[20]

Previous results in our laboratory showed that catch-up growth following intrauterine restriction in rats increased the adipocyte size in the retroperitoneal fat pad, and presented a higher amount of circulating triglycerides and higher homeostatic model assessment of insulin resistance (HOMA-IR), indicating the role of catch-up in fat accumulation and metabolism alterations.[21] Several studies of adult animals with intrauterine restriction and subsequent catch-up have been developed to describe the genes in the adipose tissue playing important roles in the development of catch-up-associated metabolic disorders and their comorbidities.[22,23] However, little is known about the gene profiling at earlier stages, such as at school age. We hypothesize that the gene profile of retroperitoneal adipose tissue already at a juvenile timepoint will be dysregulated in catch-up rats.

Herein, we aimed to study the gene expression profile in the retroperitoneal adipose tissue of juvenile rats with intrauterine restriction and postnatal catch-up growth compared to those without catch-up growth in order to better understand the molecular mechanisms involved in catch-up growth.

MATERIALS AND METHODS
Animal model
All studies were approved by the Animal Research Ethics Committee (C.E.E.A., #DAAM 6911) of the University of Barcelona according to the principles and procedures outlined in the Catalan Government guideline 214/97. Wistar rats (Janvier, Le Genest Saint Isle, France) were bred and maintained under control conditions (22 °C, 12:12 light–dark cycle, humidity 50%, free access to water) and fed a standard laboratory chow (Teklad Global Rodent Maintenance Diet, Harlan Laboratories Inc.; 14% protein, 4% fat, 2900 kcal/kg). A model of intrauterine restriction induced by caloric restriction throughout gestation was developed as described previously[21] (Supplementary Fig. S1). Briefly, virgin females were housed with males overnight and mating was confirmed by observation of spermatozoa in vaginal smears. From day 1 of gestation, the dams were then housed individually. Dams were fed on a 50% calorie-restricted diet during gestation to obtain pups with low birth weight (R). During suckling, dams were fed with standard rat chow to obtain catch-up growth pups (R/C) or were kept on a calorie-restricted diet to obtain restricted pups (R/R). An additional control group (C/C) was also present to confirm both catch-up growth and restriction events.[21] At postnatal day 22 (PD22), weaning pups were weighed, separated from their mothers, and provided with a chow diet ad libitum till PD42. Body weights from PD1 until PD42 were recorded. At the end of the experiment, pups that were fasted overnight were anesthetized with 3% isoflurane and euthanized by cardiac exsanguination. After dissection, tissues (retroperitoneal and interscapular brown adipose tissues) were weighed for assessment of body composition and stored at −80 °C until analysis.[21] Plasma

samples were obtained from blood extracted during the cardiac exsanguination, collected in heparinized syringes, and centrifuged at 3500 r.p.m. for 15 min at 4 °C. Glucose and triglycerides were determined by dry chemistry technique (Spotchem II, Spotchem EZ SP-4410, Menarini Diagnostics-Akray, Japan) and insulin and total adiponectin levels were measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Ultrasensitive Rat Insulin ELISA kit and Rat Adiponectin ELISA kit, Crystal Chem Inc.) as previously described.[21] HOMA-IR was also calculated as published before.[24] As sex differences in anthropometric or biochemical parameters were not found, both females and males were used in the study.

Gene expression array
Gene expression profiling was performed using retroperitoneal adipose tissue of eight R/C samples and eight R/R samples (four females and four males, randomly selected from different litters per each group) using Affymetrix Rat Expression Array (Clariom S Array, Affymetrix, California). Total RNA was extracted from homogenized retroperitoneal adipose tissue with Qiazol lysis reagent (Qiagen, Venlo, The Netherlands), subsequently purified by RNAeasy mini kit (Qiagen) and coupled with on-column DNase digestion by RNase-free DNase Set (Qiagen) according to the manufacturer's instructions. RNA integrity was tested with the Agilent 2100 Bionanalyzer (Agilent Technologies, California).

Arrays were performed at the High Technology Unit in Vall d'Hebron Research Center (Barcelona, Spain) following the Affymetrix standard protocols. Raw data were extracted and analyzed using Affymetrix Transcriptome Analysis Console 4.0 software (TAC, Affymetrix, California). Briefly, the probe signal values were log transformed, background corrected, and quantile normalized. Comparison between catch-up and non-catch-up groups was set and differentially expressed genes (DEGs) were identified using the following criteria $\log_2$ fold change (FC) >3 and <−3, and false discovery rate (FDR)-adjusted p value < 0.05 to avoid false-positive issues. Gene Ontology (GO) enrichment analysis of DEGs was performed with *Gene Ontology enRIchment anaLysis and visuaLizAtion tool* (GOrilla, Multi Knowledge Project)[25] considering an FDR-adjusted p value < 0.05. Afterwards, *Reduce and Visualize Gene Ontology* (REVIGO) software[26] was used to remove redundancy and cut down the number of significant pathways. Afterwards, the pathways containing any of the 20 top-ranked upregulated or downregulated DEGs were selected. Finally, all DEGs present in the selected pathways were chosen to be validated.

Real-time quantitative PCR (RT-qPCR) analysis
Genes selected for validation were analyzed in 16 rat samples per group (R/C, R/R, and C/C samples; 1/2 females and 1/2 males). Total RNA was isolated from adipose tissue as described above and was retro-transcribed to complementary DNA (cDNA) by High-capacity cDNA Reverse Transcription kit (Applied Biosystems, Thermo Fisher Scientific). Gene expression was assessed by RT-qPCR using the LightCycler 480 Real-Time PCR System (Roche Diagnostics, Indianapolis), according to the manufacturer's protocol. Taqman Gene Expression Assays were purchased from Applied Biosystems for *Adrb3* (Rn00565393_m1), *Agt* (Rn00593114_m1), *Apod* (Rn00562832 _m1), *Ccl19* (Rn01439563_m1), *Ccl21* (Rn01764651_g1), *Cd28* (Rn00565469_m1), *Cd79b* (Rn00591795_m1), *Cr2* (Rn04244351 _m1), *Igf1* (Rn00710306_m1), *Itk* (Rn01496863_m1), *Npr3* (Rn00563495_m1), *Pck1* (Rn01529014_m1), *Plac8* (Rn01450126 _m1), *Pnpla3* (Rn01502361_m1), *Pparg* (Rn00440945_m1), *Slc2a4* (Rn00562597_m1), *Sema4d* (Rn01435039_m1), *Serpina3n* (Rn00755832_mH), *Serpina12* (Rn01518409_m1), and *Ucp1* (Rn00562126_m1) genes. Data obtained were normalized to the expression of housekeeping gene *Gapdh* (Rn01775763_g1). Relative gene expression was retrieved using the comparative threshold (Ct) method and is presented as relative expression ($2^{-\Delta Ct}$).

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Table 1. Body composition and metabolic parameters of the studied groups.

	R/C $n = 16$	R/R $n = 16$	C/C $n = 16$	P value
Body composition				
Body weight PD1 (g)	5.17 ± 0.29	5.11 ± 0.25	6.51 ± 0.27	NS
Body weight PD22 (g)	51.64 ± 4.78	18.28 ± 1.16	41.81 ± 3.91	**<0.001**
Body weight PD42 (g)	178.90 ± 21.58	86.33 ± 11.50	165.56 ± 20.78	**<0.001**
Postnatal body weight gain (g)	173.72 ± 21.56	81.22 ± 11,58	159.05 ± 20.62	**<0.001**
Postnatal body weight gain (%)	33.67 ± 4.40	15.97 ± 2.67	24.39 ± 2.72	**<0.001**
Retroperitoneal AT (g)	1.02 ± 0.33	0.26 ± 0.08	0.74 ± 0.24	**<0.001**
Retroperitoneal AT/BW PD42 (%)	0.57 ± 0.14	0.31 ± 0.09	0.44 ± 0.10	**<0.001**
Brown AT (g)	0.36 ± 0.06	0.17 ± 0.03	0.30 ± 0.09	**<0.001**
Brown AT/BW PD42 (%)	0.21 ± 0.03	0.19 ± 0.04	0.18 ± 0.05	NS
Serum parameters				
Glucose (mmol/L)	9.73 ± 0.49	8.91 ± 0.89	9.98 ± 0.74	**0.016**
Insulin (pmol/L)	329.1 ± 21.1	195.1 ± 53.5	247.1 ± 23.1	**0.010**
HOMA-IR index	3.55 ± 1.12	1.25 ± 0.37	3.05 ± 0.87	**<0.001**
Triglycerides (mmol/L)	1.41 ± 0.49	0.75 ± 0.27	1.25 ± 0.40	**0.001**
Total adiponectin (mg/g)	10.5 ± 5.9	21.4 ± 12.6	25.7 ± 12.6	**0.015**

Values are expressed as mean ± standard deviation.
Significant *p* value < 0.05 comparing R/C vs R/R groups are shown in bold.
R/R non-catch-up group, *R/C* catch-up group, *PD* postnatal day, *AT* adipose tissue, *BW* body weight, *HOMA-IR* index homeostatic model assessment of insulin resistance, *NS* nonsignificant.

Statistical analysis

SPSS 22.0 (SPSS software, IBM) was used for statistical analyses. For array analysis, *p* values were calculated by analysis of variance (ANOVA) using the TAC 4.0 software. For validation analysis, unpaired *t* test was used to establish significant differences between R/C and R/R groups. Differences with *p* value < 0.05 were considered significant. Spearman's correlation was performed to analyze associations between gene expression and body composition and metabolic parameters. Bonferroni correction for multiple comparisons was made by applying a *p* value ≤ 0.01 (0.05 divided by 5 validated genes). Multiple linear regression analyses adjusting by sex were done to confirm significant correlations seen between gene expression and body composition or metabolic parameters. When necessary, logarithmic transformation was used to achieve the normal distribution of continuous variables.

RESULTS

Animal model

In this catch-up animal model, previously used and characterized by our laboratory,[21] R/C and R/R had similar birth weight at PD1 but body weight at PD22 and PD24, as well as body weight gain was higher in R/C compared to R/R (Supplementary Fig. S1), indicating that R/C was caught up in weight. Moreover, in a previous publication, we also showed that R/C pups presented accelerated catch-up growth in comparison to C/C control group, and R/R pups presented restriction in comparison to C/C control group.[21] R/C pups also showed higher accumulation of retroperitoneal adipose tissue, were more insulin resistant (higher glucose, insulin, and HOMA-IR index), and had higher levels of plasma triglycerides and lower total adiponectin compared to R/R (Table 1).

Gene expression profile of retroperitoneal adipose tissue: array analysis

The Rat Clariom S Array (Affymetrix) performed in retroperitoneal adipose tissue analyzed 21,848 coding gene transcripts. From all

of them, 546 (2.5%) were found to be DEGs between the R/C and R/R groups ($|\log_2 FC| > 3$ and FDR-adjusted *p* value < 0.05) (Fig. 1a). Of the 546 DEGs, 327 were upregulated and 219 were downregulated (Fig. 1b and Supplementary Table S1).

GO analysis retrieved enrichment in 172 different biological pathways (Supplementary Table S2). After summarizing and removing the redundant GO terms with REViGO, 56 pathways were grouped according to semantic similarity (Fig. 1c and Supplementary Table S2). In order to validate genes with functional relevance, we identified the pathways that contained genes from the 20 top-ranked upregulated or downregulated DEGs. Using this combined approach, six pathways were selected: immune system process, together with regulation of leukocyte proliferation and response to cytokines, lipid metabolic process, brown fat cell differentiation, and regulation of PI3K (Fig. 1d).

Then, all genes from our DEGs from these six selected pathways were chosen to be validated in all study samples: *Cd79b*, *Itk*, *Cr2*, *Npr3*, *Ccl19*, *Ccl21*, and *Serpina3n* related to the immune system; *Apod*, *Pck1*, and *Pnpla3* related to lipid metabolism; *Adrb3*, *Plac8*, *Pparg*, *Slc2a4*, and *Ucp1* for brown fat-related pathways; and *Agt*, *Cd28*, *Igf1*, *Sema4d*, and *Serpina12* for positive regulation of PI3K (Fig. 1e, f).

DEGs between R/C and R/R: validation analysis

The validation by RT-qPCR demonstrated that *Ccl21* (response to cytokines) was significantly downregulated and *Npr3* (regulation of leukocyte proliferation); *Serpina3n* (response to cytokines) *Pnpla3* (lipid metabolic process), *Slc2a4* (related to brown fat cell differentiation), and *Serpina12* (positive regulation of PI3K) were significantly upregulated in R/C vs R/R group (Fig. 2). Differential gene expression was not confirmed for the remaining selected genes. Available C/C control group from the experimental setting was also assessed for confirmation. In all cases, the C/C control group gene expression was in between gene expression of R/R and R/C groups (Fig. 2).

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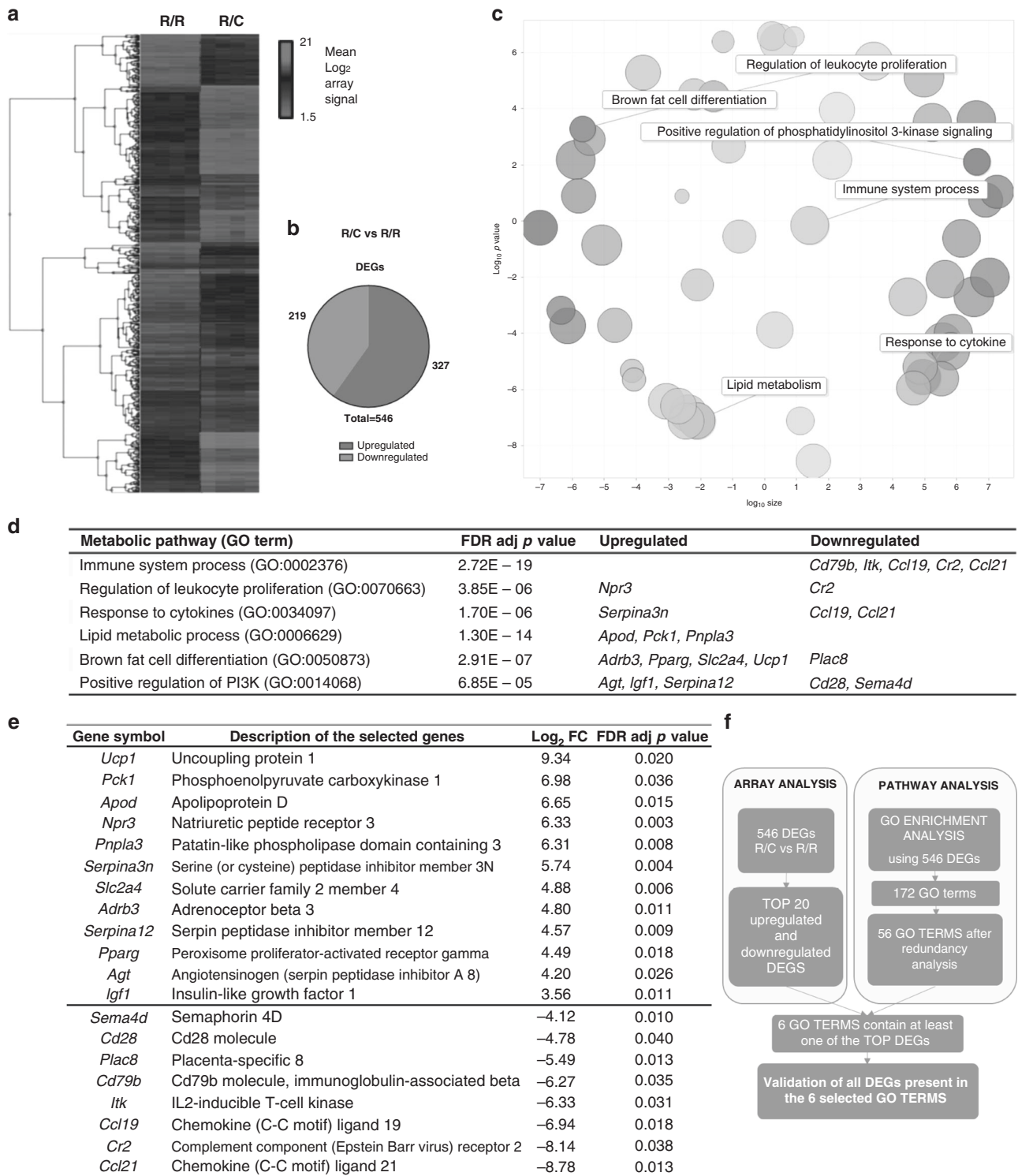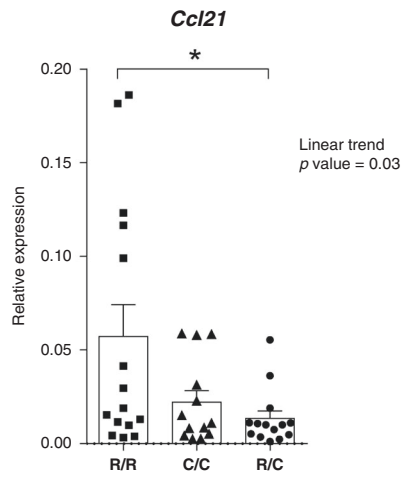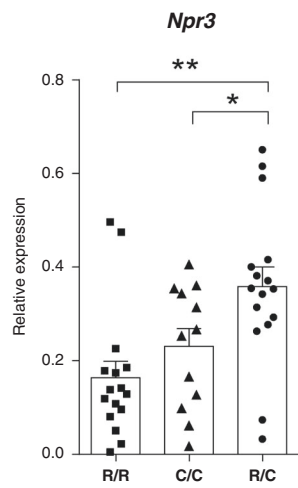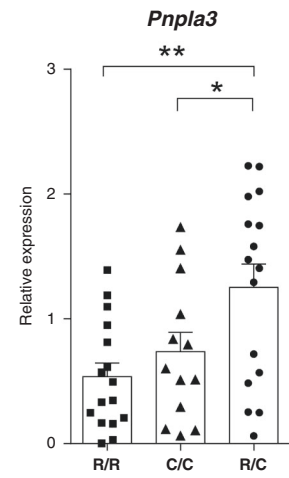E Lizarraga-Mollinedo et al.

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Fig. 1 Functional enrichment analysis of differentially expressed genes (DEGs) using Gene Ontology (GO) analysis. a Heatmap of the 546 DEGs detected by the gene expression profile of retroperitoneal adipose tissue. **b** Summary of upregulated and downregulated DEGs in R/C as compared to R/R. **c** Semantic similarity-based scatterplot of the relevant pathways using all DEGs performed with REViGO. Bubble color indicates the user-provided *p* value, and size indicates the frequency of the GO term in the underlying GO analysis database. **d** Table of the selected pathways and DEGs presented within each pathway. **e** Array analysis Log$_2$ fold change (FC) and false rate discovery (FDR) adjusted *p* value of the selected genes for validation. **f** Diagram of the selection of candidate genes for validation.
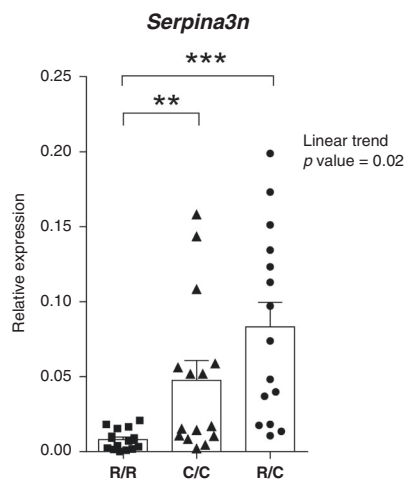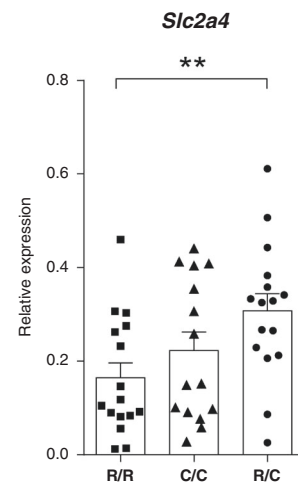
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Metabolic pathway (GO term)	FDR adj *p* value	Upregulated	Downregulated
Immune system process (GO:0002376)	2.72E − 19		Cd79b, Itk, Ccl19, Cr2, Ccl21
Regulation of leukocyte proliferation (GO:0070663)	3.85E − 06	Npr3	Cr2
Response to cytokines (GO:0034097)	1.70E − 06	Serpina3n	Ccl19, Ccl21
Lipid metabolic process (GO:0006629)	1.30E − 14	Apod, Pck1, Pnpla3	
Brown fat cell differentiation (GO:0050873)	2.91E − 07	Adrb3, Pparg, Slc2a4, Ucp1	Plac8
Positive regulation of PI3K (GO:0014068)	6.85E − 05	Agt, Igf1, Serpina12	Cd28, Sema4d

e

Gene symbol	Description of the selected genes	Log$_2$ FC	FDR adj *p* value
Ucp1	Uncoupling protein 1	9.34	0.020
Pck1	Phosphoenolpyruvate carboxykinase 1	6.98	0.036
Apod	Apolipoprotein D	6.65	0.015
Npr3	Natriuretic peptide receptor 3	6.33	0.003
Pnpla3	Patatin-like phospholipase domain containing 3	6.31	0.008
Serpina3n	Serine (or cysteine) peptidase inhibitor member 3N	5.74	0.004
Slc2a4	Solute carrier family 2 member 4	4.88	0.006
Adrb3	Adrenoceptor beta 3	4.80	0.011
Serpina12	Serpin peptidase inhibitor member 12	4.57	0.009
Pparg	Peroxisome proliferator-activated receptor gamma	4.49	0.018
Agt	Angiotensinogen (serpin peptidase inhibitor A 8)	4.20	0.026
Igf1	Insulin-like growth factor 1	3.56	0.011
Sema4d	Semaphorin 4D	−4.12	0.010
Cd28	Cd28 molecule	−4.78	0.040
Plac8	Placenta-specific 8	−5.49	0.013
Cd79b	Cd79b molecule, immunoglobulin-associated beta	−6.27	0.035
Itk	IL2-inducible T-cell kinase	−6.33	0.031
Ccl19	Chemokine (C-C motif) ligand 19	−6.94	0.018
Cr2	Complement component (Epstein Barr virus) receptor 2	−8.14	0.038
Ccl21	Chemokine (C-C motif) ligand 21	−8.78	0.013

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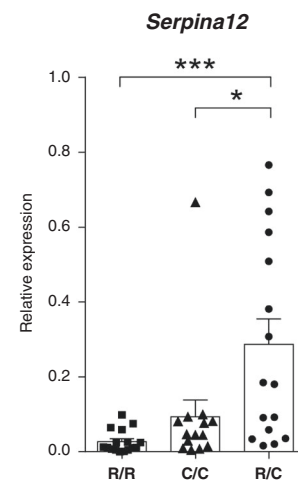E Lizarraga-Mollinedo et al.

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Fig. 2 Expression levels of validated genes in retroperitoneal adipose tissue of catch-up (R/C), non-catch-up (R/R), and control (C/C) samples. Data are expressed as mean ± SEM, $n = 16$ rats per group. Linear ANOVA trend p value and unpaired T test, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

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Table 2. Correlations between *Ccl21*, *Npr3*, *Serpina3n*, *Pnpla3*, *Slc2a4*, and *Serpina12* gene expression and body composition and metabolic parameters in all R/C and R/R studied samples (N = 32).

	Ccl21		*Npr3*		*Serpina3n*	
	r	p	r	p	r	p
Body weight PD42 (g)	−0.338	0.05	0.514	**0.003**	0.660	**<0.0001**
Postnatal body weight gain (g)	−0.338	0.05	0.514	**0.003**	0.660	**<0.0001**
Retroperitoneal AT (g)	−0.325	NS	0.353	0.04	0.598	**<0.0001**
Brown AT (g)	−0.265	NS	0.477	**0.007**	0.695	**<0.0001**
Triglycerides (mmol/L)	−0.484	**0.01**	0.580	**0.005**	0.660	**0.001**
HOMA-IR	−0.415	0.05	0.529	**0.01**	0.647	**0.001**
	Pnpla3		*Slc2a4*		*Serpina12*	
	r	p	r	p	r	p
Body weight PD42 (g)	0.524	**0.002**	0.366	0.03	0.616	**<0.0001**
Postnatal body weight gain (g)	0.524	**0.002**	0.366	0.03	0.616	**<0.0001**
Retroperitoneal AT (g)	0.466	**0.007**	0.177	NS	0.595	**<0.0001**
Brown AT (g)	0.616	**<0.0001**	0.383	0.03	0.668	**<0.0001**
Triglycerides (mmol/L)	0.393	NS	0.573	**0.005**	0.654	**0.001**
HOMA-IR	0.530	**0.01**	0.179	NS	0.639	**0.001**

Values are expressed as ρ of Spearman's bivariate correlation.
Significant associations after correcting for multiple comparisons (p ≤ 0.01) are shown in bold.
PD postnatal day, *AT* adipose tissue, *HOMA-IR index* homeostatic model assessment of insulin resistance, *NS* nonsignificant, *p* p value.

Association of DEGs with body composition and metabolic parameters

Correlation analysis showed significant associations between the validated genes and several metabolic parameters, after correcting for multiple comparisons (Table 2). *Ccl21* showed negative correlations with body weight at PD42, postnatal body weight gain, plasma triglycerides, and HOMA-IR (all *p* < 0.05). *Npr3* showed positive correlations with body weight at PD42, postnatal body weight gain, amount of brown adipose tissue, plasma triglycerides, and HOMA-IR (all *p* < 0.01). *Serpina3n* and *Serpina12* showed positive correlations with body weight at PD42, postnatal body weight gain, amount of white and brown adipose tissue, plasma triglycerides, and HOMA-IR (all between *p* < 0.0001 and ≤0.001); and *Serpina3n* was negatively correlated with total plasma adiponectin (Supplementary Fig. S2). *Pnpla3* showed positive correlations with body weight at PD42, postnatal body weight gain, and the amount of white and brown adipose tissue and HOMA-IR (all *p* ≤ 0.01). *Slc2a4* showed positive correlations with plasma triglycerides (all *p* < 0.05). Most of these associations were maintained in multivariate regression analyses, adjusting for the sex of the animals, with the exception of *Slc2a4* that maintained association only with triglycerides (data not shown).

DISCUSSION

Using the same rat model of catch-up growth that we previously validated,[21] we show for the first time a gene expression profile in adipose tissue of juvenile rats with catch-up growth following intrauterine restriction and report associations of several dysregulated genes in adipose tissue with fat expansion and metabolic parameters. We have compared restricted pups with and without catch-up growth in order to study the exclusive effect of postnatal growth on metabolic alterations. Given that low birth weight children with catch-up growth can manifest early abnormalities at school age[15] and that adipose tissue has been identified as one of the principal targets affected by catch-up growth,[19,21] the data

obtained in this rat model provide interesting information regarding the molecular mechanisms involved in the visceral fat expansion and metabolic abnormalities following catch-up growth.

The present results showed changes in the gene expression of retroperitoneal adipose tissue in R/C group as compared to R/R. Pathway enrichment analysis showed that DEGs were mainly involved in six metabolic pathways, including immune and inflammatory processes (regulation of leukocyte proliferation and response to cytokines), lipid metabolic process, brown fat cell differentiation, and upregulation of PI3K signalling. Dysregulated gene expression of the studied genes occurred in parallel to (a) fat expansion, as catch-up pups showed increased weight gain from birth, increased weight at PD42, increased retroperitoneal adipose tissue mass, and also hypertrophy of adipocytes in the retroperitoneal adipose tissue;[21] and (b) lipid and glucose metabolism as catch-up pups showed increased levels of triglycerides and HOMA-IR.[21]

Ccl21 expression has been described in adipocytes as well as in dendritic cell/macrophage populations.[27] Many articles report that high fat diet-induced obesity enhances the expression of tissue chemokines, including CCL21 and CCL19, during the course of low-grade inflammation.[28] Moreover, *Ccl21* has been closely related to atherosclerosis as its expression is elevated in ruptured lesions of coronary arteries and in carotid plaques.[29] Then, dysregulated *Ccl21* expression in retroperitoneal fat during catch-up growth may contribute to atherosclerotic events[29] or impair immune system tissue responses.[30]

High *Npr3* levels in adipose tissue of obese mice increased the clearance of lipolytic atrial natriuretic peptide (ANP), abrogating its locally lipolytic effect and thus contributing to obesity.[31] The regulation of Npr3 over lipolytic ANP may only be manifested in states of low insulin action or in combination with other factors (e.g., obesity) that contribute to pre-existing insulin resistance.[31] As already shown by our research group, R/C rats exhibited an insulin-resistant state;[21] thus high *Npr3* expressions in this context

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may facilitate the observed altered metabolism and fat expansion. Our results showed positive correlations of *Npr3* with HOMA-IR and also with body weight gain and the amount of brown adipose tissue. The association with brown adipose tissue is in line with observations from other authors describing that *Npr3* can confer thermogenic characteristics to rodent adipose tissues.[32] *Npr3* contribution to both obesity and enhancing thermogenesis may seem contradictory at first sight, but a plausible explanation may lie in the potential effect of *Npr3* on beige adipocytes. Beige adipocytes (basically white adipocytes that present brown adipocyte characteristics) are observed during the browning of white adipose tissues through the activation of natriuretic peptides.[32] Despite observing a similar expansion of both white and brown adipose tissues in the catch-up growth group, the browning of white adipose tissue may be different under catch-up growth conditions. Thus, higher *Npr3* during catch-up could at the same time modulate white adipose tissue expansion and enhance its thermogenic potential through a browning effect.

We also showed that *Serpina3n* was upregulated in retro-peritoneal adipose tissue of R/C rats and was positively correlated with postnatal body weight, weight gain, the amount of visceral adipose tissue, and plasma triglycerides. *Serpina3n*, a serine protease inhibitor that forms part of the SERPINA clade, is the murine ortholog of the human anti-chymotrypsin and has been identified as an inhibitor of both human and rodent granzyme B in vitro.[33] Recently, *Serpina3n* has been identified as a serum biomarker in the development of the early stages of T2D and it is believed that the increased expression of *Serpina3n* might contribute to impaired insulin secretion in the pancreas in the diabetic state.[34,35] The same authors pointed out the possibility that the increase in *Serpina3n* might reflect the obesity-related phenotype, as the weight gain was more prominent in diabetic than control rats. This is in line with the associations we observed between *Serpina3n* and body composition during catch-up growth in our juvenile rats. It is known that rats with intrauterine restriction and postnatal catch-up growth show increased susceptibility to diabetes mellitus in adulthood;[36] therefore, increased *Serpina3n* expression could facilitate the appearance of T2D in these rats. Increase in *Serpina3n* expression was also observed in response to inflammation in the hypothalamus of rats subjected to high fat diet.[37] Adiponectin, an anti-inflammatory cytokine of the adipose tissue, is related to obesity-induced chronic inflammation. In our study, total plasma adiponectin was negatively correlated with *Serpina3n*, supporting that over-expression of *Serpina3n* in R/C rats may be involved in inflammatory responses under low-grade inflammation during catch-up growth.

Pnpla3 encodes for a protein called adiponutrin or triacylgly-cerol lipase involved in several lipid metabolic processes, including lipid hydrolysis, and possibly also in energy balance and storage in adipocytes.[38] *Pnpla3* has been described to drive fatty acids excess into tissue lipid droplet storage,[39] which strengthens our data on its relation to increased visceral fat depots and/or hypertrophy. *Pnpla3* has also been related to liver fat deposition.[40] In fact, a well-known PNPLA3 polymorphism has been identified as a childhood predictor of adulthood fatty liver.[41,42] The same variant of *Pnpla3* has been associated with metabolic and anthropometric T2D traits in adult patients with metabolic syndrome.[43] Moreover, increased *Pnpla3* mRNA expression has been previously described in adult obese rats (both in brown and white adipose tissue) and has been attributed to vesicular targeting and protein transport between both types of adipose tissue.[44,45] These findings are consistent with our results showing increased expression of *Pnpla3* in catch-up rats and its positive correlation with the amount of retroperitoneal (white) and brown adipose tissue.

The expression of *Slc2a4* was found to be significantly increased in R/C as compared to R/R rats. *Slc2a4* gene is a member of the solute carrier family and encodes the GLUT4 protein. *Slc2a4/Glut4*, highly expressed in adipose tissue and skeletal muscle, functions as an insulin-regulated facilitative glucose transporter and is a key regulator of whole-body glucose homeostasis.[46] Several studies in animal models showed decreased *Slc2a4/Glut4* expression in offspring adipose tissue after maternal undernutrition,[23,47] although its expression has also been described as being downregulated in adipose tissue of obese subjects.[48,49]

We hypothesize that the *Slc2a4* upregulation in adipose tissue of R/C vs R/R rats may have a compensatory role to improve the dysregulated insulin-resistant state observed in these catch-up rats. An excess of glucose can be up-taken by adipocytes and converted to glycerol as previously described in vitro in primary cell cultures of adipocytes.[50] The upregulation of *Slc2a4* could thus be a compensatory mechanism used by white adipose tissue to convert the excess of glucose input into glycerol, not only to dispose of the glucose excess but also to produce a more readily usable metabolite and redistribute this secondary product into other peripheral tissues.

In addition to being crucial for glucose uptake,[51] augmented PI3K signalling may cause exacerbated lipogenesis by increasing the assortment of the required substrates from the citric acid cycle. *Serpina12* also known as vaspin (visceral adipose tissue-derived serine protease inhibitor) has been associated with insulin resistance, negative regulation of gluconeogenesis, and increased carotid artery intima–media thickness in subjects with metabolic syndrome.[52] Moreover, this vaspin adipokine negatively regulates lipid biosynthesis as it contributes to the metabolic regulation of triglycerides in vitro[51] and in vivo.[53] Human studies have also shown that mRNA expression of vaspin in the adipose tissue could be induced by an increased fat mass.[54] Our results head in the same direction as previous investigations as *Serpina12* positively correlates with body weight and plasma triglycerides. In our model, we observed increased *Serpina12* expression in the retroperitoneal adipose tissue in R/C rats, which was positively correlated with both brown and white adipose tissue weight. We, therefore, suggest that higher *Serpina12* expression could contribute to the visceral fat and brown adipose tissue expansion in R/C rats.

To summarize, we have identified several dysregulated genes (*Ccl21*, *Npr3*, *Serpina3n*, *Pnpla3*, *Slc2a4*, and *Serpina12*) in adipose tissue of juvenile rats that could be involved in catch-up induced visceral fat expansion and glucose and lipid metabolism. However, we acknowledge some limitations in our study. The study is mainly descriptive; nevertheless, the information obtained from our array and validation increases knowledge and may inspire other studies on adipose tissue during catch-up growth. Moreover, other significant DEG genes could be chosen for further studies such as angiopoietin-like proteins. The selected Agt (angiotensinogen) did not get validated from our array results; nonetheless, their implication in vascularization and/or blood pressure regulation could be of study using models assessed at later time-points until adulthood or aging. On the other hand, retroperitoneal adipose tissue availability was scarce in these animals, precluding the assessment of protein expression as well as the separation of different cell population fractions (e.g., adipocytes) from whole adipose tissue samples, in order to complement the transcript gene expression profile described in this study.

In conclusion, genes related to immune/inflammatory and metabolic processes as well as brown fat cell differentiation were upregulated in retroperitoneal adipose tissue following catch-up growth in juvenile rats and were found to be associated with fat expansion and metabolic parameters. Our results provide evidence for several dysregulated genes in white adipose tissue that could help develop novel strategies to prevent the metabolic abnormalities associated with catch-up growth.

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AUTHOR CONTRIBUTIONS
E.L.-M. contributed to the study design, carried out experiments, analyzed data, and wrote the first draft of the manuscript. G.C.-B. contributed to the study design, carried out experiments, analyzed data, and participated in the writing of the manuscript. S.X.-T., X.R., B.M.-P., and A.P.-P. carried out experiments and reviewed the manuscript. F.d.Z. and L.I. reviewed the manuscript. A.L.-B. and J.B. designed and supervised the study and participated in the writing of the manuscript.

ADDITIONAL INFORMATION

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