

***Dlk1* expression relates to visceral fat expansion and insulin resistance in male and female rats with postnatal catch-up growth**

Cite this article as: Gemma Carreras-Badosa, Xavier Remesar, Anna Prats-Puig, Sílvia Xargay-Torrent, Esther Lizarraga-Mollinedo, Francis de Zegher, Lourdes Ibáñez, Judit Bassols and Abel López-Bermejo, *Dlk1* expression relates to visceral fat expansion and insulin resistance in male and female rats with postnatal catch-up growth, *Pediatric Research* doi:10.1038/s41390-019-0428-2

This Author Accepted Manuscript is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication but has not been copyedited or corrected. The official version of record that is published in the journal is kept up to date and so may therefore differ from this version.

Terms of use and reuse: academic research for non-commercial purposes, see here for full terms. <https://www.nature.com/authors/policies/license.html#AAMtermsV1>

Author accepted manuscript

***Dlk1* expression relates to visceral fat expansion and insulin resistance in male and female rats with postnatal catch-up growth**

Gemma Carreras-Badosa^{1*}, Xavier Remesar^{2,3*}, Anna Prats-Puig^{1,4}, Sílvia Xargay-Torrent¹, Esther Lizarraga-Mollinedo¹, Francis de Zegher⁵, Lourdes Ibáñez^{6,7}, Judit Bassols^{8§}, Abel López-Bermejo^{1,9§}

¹ Pediatric Endocrinology Group, Girona Institute for Biomedical Research, Girona, Spain;

² Department of Biochemistry and Molecular Biomedicine, Universitat de Barcelona, Barcelona, Spain;

³ CIBEROBN, Instituto de Salud Carlos III, Madrid, Spain;

⁴ Department of Physiotherapy, EUSES University School, Girona, Spain;

⁵ Department of Development & Regeneration, University of Leuven, Leuven, Belgium;

⁶ Sant Joan de Déu Children's Hospital Pediatric Institute, University of Barcelona, Barcelona, Spain;

⁷ CIBERDEM, Instituto de Salud Carlos III, Madrid, Spain;

⁸ Maternal-Fetal Metabolic Group, Girona Institute for Biomedical Research, Girona, Spain and

⁹ Pediatrics, Dr. Josep Trueta Hospital, Girona, Spain.

* These authors contributed equally to this work and are therefore first co-authors

§ These authors are both co-senior and corresponding authors.

Running title: *Dlk1* expression relates to visceral fat and insulin resistance.

Contact info:

Judit Bassols, PhD & Abel López-Bermejo, MD
Girona Institute for Biomedical Research
Av. França s/n
Girona-17007. Spain
Tel: +34-972-940200. Ext. 2810
Fax: +34-972-940270
Email: jbassols@idibgi.org & alopezbermejo@idibgi.org

None of the authors has any potential conflict of interest regarding to this work.

The study was supported by grant no. PI13/01257 (to A.L.-B.) from the National Institute of Health Carlos III (Fund for Health Research FIS, Spain), project co-financed by FEDER.

Type of manuscript: Basic science article

Abstract

Background: Although prenatal and postnatal programming of metabolic diseases in adulthood is well established, the mechanisms underpinning metabolic programming are not. *Dlk1*, a key regulator of fetal development, inhibits adipocyte differentiation and restricts fetal growth.

Methods: Assess *Dlk1* expression in a *Wistar* rat model of catch-up growth following intrauterine restriction. Dams fed *ad libitum* delivered control pups (C) and dams on a 50% calorie-restricted diet delivered pups with low birth weight (R). Restricted offspring fed a standard rat chow showed catch-up growth (R/C) but those kept on a calorie-restricted diet did not (R/R).

Results: Decreased *Dlk1* expression was observed in adipose tissue and skeletal muscle of R/C pups along with excessive visceral fat accumulation, decreased circulating adiponectin, increased triglycerides and HOMA-IR (from $p < 0.05$ to $p < 0.0001$). Moreover, in R/C pups the reduced *Dlk1* expression in adipose tissue and skeletal muscle correlated with visceral fat ($r = -0.820$, $p < 0.0001$) and HOMA-IR ($r = -0.745$, $p = 0.002$).

Conclusions: Decreased *Dlk1* expression relates to visceral fat expansion and insulin resistance in a rat model of catch-up growth following prenatal growth restriction. Modulation of *Dlk1* expression could be among the targets for the early prevention of fetal programming of adult metabolic disorders.

Introduction

Fetal programming is a process by which the fetus' adaptations to the adverse conditions during gestation and early postnatal life have long-term effects on the newborn (1). Clinical studies, and subsequent work in animal models, have demonstrated that adverse conditions in these critical developmental windows can program physiological processes and cause metabolic alterations leading to future adult diseases, such as obesity, type 2 diabetes, metabolic syndrome and cardiovascular disease (1),(2),(3).

Fetal development is sensitive to the nutritional environment. Adaptations to adverse conditions serve to optimize and improve the chances of fetal survival. However, when there is a mismatch between fetal and postnatal nutrition, this adapted thrifty phenotype is inappropriate, becomes detrimental and contributes to metabolic disorders in adulthood (3),(4). Consequently, the major risk of developing these disorders later in life has been associated with postnatal catch-up growth following low birth weight, a condition occurring in approximately 8% of pregnancies worldwide (1),(2),(5).

The impact of early life insults on childhood and adult health is certainly apparent; however, the mechanisms underlying the alterations in metabolic programming remain largely unknown. The study of genes that regulate fat storage, whose alterations lead to insulin resistance, are of central importance for understanding the pathophysiology of catch-up growth.

Delta-like protein 1 (*Dlk1*) gene is a key regulator of fetal development and it is involved in energy and nutrient metabolism. In adults, its expression is limited to

endocrine tissues: *Dlk1* inhibits adipocyte differentiation in adipose tissue and is involved in regeneration processes in skeletal muscle (6),(7),(8). Our group has previously linked prenatal and postnatal growth with the placental imprinted *DLK1-DIO3* domain in human studies (9); others have described that impairments in fetally derived *DLK1* cause fetal growth restriction (10). Studies, however, linking *Dlk1* gene expression and catch-up growth following prenatal restriction are lacking.

The aim of the study was to assess whether changes in *Dlk1* gene expression relate to metabolic risk markers in an animal model of postnatal catch-up growth following fetal weight restriction.

Methods

Rat model of catch-up growth following prenatal growth restriction

A *Wistar* rat model of intrauterine growth restriction induced by calorie restriction throughout gestation was used. Studies were approved by the Animal Research Ethic Committee (C.E.E.A., #DAAM 6911) of the University of Barcelona and were in accordance with the Catalan Government guideline 214/97. First-time-pregnant *Wistar* rats (Janvier, Le Genest Saint Isle, France) were housed in a facility with constant temperature ($21 \pm 1^\circ\text{C}$) and humidity (50-60%) and a controlled 12:12-h light-dark cycle. The first day of gestation was determined by observation of spermatozoa in vaginal smears. From day 1 of pregnancy to term, control dams (C) were provided an ad libitum diet consisting of a standard laboratory chow (Teklad Global Rodent Maintenance Diet, Harlan Laboratories Inc.; 14% protein, 4% fat, 2900 kcal/kg) and restricted dams (R) were fed a 50% less of diet used in control dams (Supplementary Figure S1). Rat dams gave birth normally and pups were limited to ten per litter at day

1 after birth to normalize pups feeding. Throughout the 21-day lactation period, different control or restricted diets were provided in order to create 3 experimental groups: control dams were fed an *ad libitum* diet (C/C) and restricted dams were fed either an *ad libitum* diet (R/C) or a restricted diet (R/R) to create catch-up or non-catch-up growth groups, respectively (Supplementary Figure S1). At 22-days of age, after the lactation period, offspring in all groups were weaned to *ad libitum* feed. All pups were weighed twice a week from postnatal day 1 (PD1) until PD42. At PD42 all pups (N=14 per group; 7 females and 7 males) fasted overnight were anesthetized with 3% isoflurane and euthanized by cardiac exsanguination. Blood was collected via cardiac puncture in heparinized syringes and centrifuged at 3500 rpm for 15 minutes at 4°C to obtain plasma samples which were stored at -80°C. Adipose tissue from different locations in the abdomen and skeletal gastrocnemius muscle samples were immediately frozen in liquid nitrogen. Additionally, an approximation to body composition was obtained by calculating visceral fat tissue mass (sum of weights of perigonadal, retroperitoneal and mesenteric adipose tissues). For gene and protein expression studies, rat retroperitoneal adipose tissue was selected as being equivalent to human omental adipose tissue.

Plasma metabolic profile

Plasma adiponectin and insulin concentrations were determined by ELISA using a commercial kit (*Rat Adiponectin ELISA Kit*, and *Ultrasensitive Rat Insulin ELISA kit*; Crystal Chem Inc.). For adiponectin, the sensitivity of the kit was 0.25 mg/l and intra-assay CVs were less than 10% and for insulin, the sensitivity was 0.1 ng/ml and intra-assay CVs were less than 10%. Plasma glucose, total cholesterol, HDL-cholesterol and

triglycerides were determined by dry chemistry technique (*Spotchem II, Spotchem EZ SP-4410*, Menarini Diagnostics-Akroy, Japan). Homeostatic model assessment of insulin resistance (HOMA-IR) was assessed using the following formula:
$$\text{HOMA-IR index} = \frac{\text{glucose} \times \text{insulin}}{2430} \text{ (11).}$$

Gene and protein expression

For gene expression, RNA (*RNAeasy mini kit*, Qiagen) was extracted and 2 µg of total RNA was retro-transcribed to cDNA (High-capacity cDNA Reverse Transcription Kit, Applied Biosystems, Thermo Fisher Scientific Inc.). cDNA samples were used to quantify the relative expression by real-time PCR (*Taqman Assays 20X [Dlk1: Rn00587011_m1 and endogenous control GAPDH: Rn01775763_g1]*, Applied Biosystems, Thermo Fisher Scientific Inc.). For protein quantification, total protein was extracted from tissue (*DC protein assay*, Bio-Rad Laboratories Inc.). Total protein concentration was quantified by the Lowry method and Dlk1 protein concentrations by an ELISA commercial kit (*Rat Delta Like 1 Homolog Dlk1 ELISA kit*, MyBioSource, Inc.). The sensitivity of the kit was 0.27 ng/ml and intra-assay CVs were less than 9%.

Tissue morphology

For morphometric studies, retroperitoneal adipose tissue from the abdominal cavity and skeletal gastrocnemius muscle from a set of 12 animals (N=4 animals per each treatment, 2 males and 2 females) were used. Tissues were paraffin-embedded, tissue sections were deparaffinized by the xylene-ethanol sequence and processed using the hematoxylin-eosin dye method. Images were taken at 200X (Olympus BX51, XC50 CellVe). One histological section of each animal was randomly selected from the histological slides. Twelve randomly chosen fields per section were considered for the

measurement of both adipocyte area and density (more than 250 adipocytes were analyzed per group) and skeletal muscle cross-sectional myofiber density and cross-sectional myofiber area. The measurements were analyzed in a blinded manner and both manual and automatic tools of the Image J software (NIH, public domain) were used.

Statistical analysis

All statistics were performed with SPSS 22.0 (SPSS software, IBM, Inc.) and graphs were done with Prism 6.0 (GraphPad Software, Inc.). Differences between experimental groups were compared using ANOVA test and least significance difference (LSD) *post hoc* tests. Spearman correlation was used to analyze associations between variables. Combined data for males and females are shown, since no sex differences were evident. Data are expressed as mean \pm standard deviation (SD) and p value was set at 0.05.

Results

Catch-up growth Wistar rats following prenatal growth restriction exhibited increased fat mass and higher HOMA-IR

At birth, R/R and R/C pups showed less body weight ($p < 0.0001$, Table 1) relative to C/C pups: both R/R and R/C pups showed a 22% lower value in body weight at birth in relation to C/C pups. At weaning (PD22), R/C pups showed higher body weight ($p < 0.0001$; Table 1) and at PD42 these pups experienced further postnatal weight gain ($p < 0.0001$; Table 1), together with higher retroperitoneal adipose tissue weight and higher visceral fat (from $p < 0.05$ to $p < 0.0001$; Table 1), as compared to other pup

groups. At PD42, R/C pups showed also lower total adiponectin, higher plasma triglycerides (both $p < 0.01$; Table 1) and higher insulin concentration and HOMA-IR index (both $p < 0.0001$; Table 1), as compared to R/R pups.

Decreased Dlk1 expression in adipose tissue and skeletal muscle relates to visceral fat and HOMA-IR in catch-up growth Wistar rats following prenatal growth restriction.

Relative *Dlk1* expression in R/C pups was decreased in both retroperitoneal adipose tissue and skeletal muscle compared to R/R pups ($p < 0.0001$; Table 1). Likewise, *Dlk1* protein concentrations in retroperitoneal adipose tissue were lower in R/C pups ($p < 0.001$; Table 1) compared to other pup groups. In adipose tissue, negative correlations between *Dlk1* relative expression and body weight, postnatal body weight gain and visceral fat at PD42 were observed (all between $r = -0.820$ and $r = -0.895$, $p < 0.0001$; Figure 1 and Supplementary Table S1). In muscle, negative correlations between *Dlk1* relative expression and plasma insulin and HOMA-IR index were observed ($r = -0.701$, $p = 0.005$ and $r = -0.745$, $p = 0.002$; respectively; Figure 1 and Supplementary Table S1).

Catch-up growth Wistar rats following prenatal growth restriction showed hypertrophied adipocytes and enlarged skeletal muscle myofibers

The histological studies showed higher adipocyte area ($4933 \mu\text{m}^2 \pm 1336$; $3277 \mu\text{m}^2 \pm 900$; $2144 \mu\text{m}^2 \pm 533$; $p < 0.0001$) and lower adipocyte density ($242 \text{ n}^\circ/\text{mm}^2 \pm 85$; $340 \text{ n}^\circ/\text{mm}^2 \pm 83$; $531 \text{ n}^\circ/\text{mm}^2 \pm 153$; $p < 0.0001$) in retroperitoneal adipose tissue and lower density or number of myofibers per area ($491 \text{ n}^\circ/\text{nm}^2 \pm 90$; $474 \text{ n}^\circ/\text{nm}^2 \pm 45$; $726 \text{ n}^\circ/\text{nm}^2 \pm 95$; $p = 0.003$) in skeletal muscle of R/C pups, as compared to C/C and R/R groups, respectively (Figure 2).

Discussion

In the present model of catch-up growth following fetal growth restriction, we observed decreased *Dlk1* in both visceral fat and skeletal muscle in pups experiencing catch-up growth. Moreover, in these pups, reduced *Dlk1* gene expression in retroperitoneal adipose tissue was related to visceral fat mass accumulation, and reduced *Dlk1* gene expression in skeletal muscle was associated with insulin resistance.

As expected, in our experimental model of catch-up growth following fetal weight restriction, R/C pups successfully presented catch-up growth, as judged by higher body weight gain and visceral adiposity at P42. Previous studies have shown excessive visceral fat accumulation during catch-up growth (12). It has also been suggested that permanent changes in the expression of regulatory proteins in liver, muscle and adipose during catch-up growth relate to insulin resistance and metabolic disorders later in life (4). In our study, R/C pups showed not only increased visceral fat but also decreased circulating adiponectin and increased plasma triglycerides. It is well known that circulating adiponectin concentrations decrease along with visceral fat accumulation (13). Consequently, the observed visceral fat mass accumulation, low adiponectin levels and high triglycerides during catch-up growth may reflect dysregulation of adipose tissue function, which may plausibly contribute to the development of insulin resistance in these animals. This is substantiated by previous studies relating catch-up growth and insulin resistance in *Wistar* rats (14) and by our results showing higher HOMA-IR values in R/C pups. Catch-up growth in these pups caused both visceral fat accumulation and insulin resistance as early as at postnatal

period PD42. On the contrary, R/R pups kept postnatally under restricted conditions, in order to prevent the mismatch between fetal and postnatal nutrition, exhibited rather insulin sensitivity. Metabolic programming has been described to present both altered fat expansion and disrupted insulin signaling pathway leading to insulin resistance, although the molecular mechanisms implicated in these processes are not fully understood.

Dlk1 is a regulator of adipocyte differentiation and adipogenesis (6),(15). The decrease in *Dlk1* relative gene expression and *Dlk1* protein concentrations in retroperitoneal adipose tissue at P42 together with the negative correlations between *Dlk1* expression and body weight, body weight gain and visceral fat mass in R/C pups point to a relevant role of *Dlk1* in the control of adipocyte growth and differentiation during catch-up growth. It was shown that mice with *Dlk1* deficiency presented higher amounts of visceral fat, circulating lipids and adipocyte cell size at weaning and developed obesity and fatty liver disease (6),(16),(8). Histologically, hypertrophied adipocytes have been consistently found in adipose tissue of individuals with obesity (17) as well as in restricted pups at weaning (12),(18),(19). This observation has been related to a lower number of progenitor adipocytes capable of differentiating into healthy adipocytes (20). Accordingly, our results showed hypertrophy of the retroperitoneal adipose tissue in R/C pups at P42, which was characterized by higher adipocyte area and lower adipocyte density. The low *Dlk1* expression observed in R/C pups may thus predispose these pups to postnatal adipose tissue expansion, especially of visceral fat, with a low number of preadipocytes being capable of differentiating into mature adipocytes, in turn contributing to adipocyte dysfunction. Indeed, low *Dlk1* gene expression in adipose tissue is likely to cause excessive adipocyte lipid

storage, inflammation and altered adipokine secretion, all known to be involved in the development of insulin resistance and obesity (18). As it has been described that cell size and/or lipid droplet locularity influence cortical actin structures that mediate GLUT4 translocation (21), we hypothesize that low *Dlk1* and related adipocyte hypertrophy could directly influence the development of insulin resistance.

Dlk1 is known to be a crucial regulator of the myogenic program in skeletal muscle, and it has been shown that *Dlk1* has a dual biological function in this tissue, both as an enhancer of muscle development and as an inhibitor of adult muscle regeneration (22). The observed low *Dlk1* expression in skeletal muscle could be involved in restraining of muscle development as we observed histologically lower number of myofibers per area (myofiber density) in skeletal muscle of R/C pups at P42. In this sense, catch-up growth towards fat mass accumulation in detriment of muscular mass has been described in humans (23). Low birth weight following free access to food during the growing-finishing period in pigs also showed enlarged cross-sectional myofiber areas due to enhanced fat deposition (24). Moreover, fat mass accumulation in detriment of muscular mass has been related to insulin resistance and overexpression of insulin receptor in skeletal muscle in experimental animals (25). In our model we observed decreased *Dlk1* expression in skeletal muscle in R/C pups that was related to increased HOMA-IR values. We therefore suggest that *Dlk1* expression in skeletal muscle is contributing to the development of insulin resistance during catch-up growth. It is tempting to speculate that lower *Dlk1* gene expression could enhance C/EBP α , a known downstream *Dlk1* target molecule, which in turn would regulate GLUT4 and affect glucose homeostasis (26). Then, *Dlk1* dysregulation would

be among the mechanisms explaining why skeletal muscle presents lower insulin-stimulated glucose utilization and develops insulin resistance during catch-up growth.

We acknowledge some limitations in our study. Major differences were observed between R/C and R/R pups, with fewer differences being evident between R/C and C/C pups. It should be highlighted, however, that there were strong linear differences between the 3 groups of pups in most of the examined parameters, which give relevance to our results. In future studies, our goal will be to examine more severe fat mass accumulation or obesity phenotypes during catch-up growth as well as to extend the growth period since P42 may have been rather a short juvenile time-point to examine the association between *Dlk1* and metabolic risk markers. Interestingly though, our findings indicate that these metabolic abnormalities take place at an early age following catch-up growth. Future studies assessing these parameters for a longer period should take into account the age-related sex dimorphism in insulin sensitivity that is evident starting at PD90 (3 months), and which has not been described in early stages, such as at PD26 or PD40, when the juvenile rats are still not sexually mature (27) (28) (29). Finally, we could not analyze the direction of causality of the molecular changes observed at PD42 in pups experiencing catch-up growth since the *Wistar* rat model of intrauterine growth restriction induced by calorie restriction throughout gestation used in the present study did not include tissues at birth: a few pups were removed to equal litter sizes within groups but they were insufficient in number to provide tissue samples at birth. Despite these limitations, we have observed novel molecular changes in *Dlk1* gene expression during catch-up growth.

In conclusion, decreased gene expression of *Dlk1* in adipose tissue and skeletal muscle may contribute to visceral fat expansion and development of insulin resistance during catch-up growth following fetal growth restriction. It is tempting to speculate that future intervention strategies based on *Dlk1* activation may improve the outcome of catch-up growth following prenatal growth restriction.

Author accepted manuscript

Acknowledgements

The authors would like to thank Dr. Glòria Oliveras Serrat and Ms. Yaiza Martin Gonzalez for the preparation of the tissue histological sections.

All authors read and approved the final manuscript. G.C.-B., X.R., J.B. and A.L.-B. designed research; G.C.-B. and A.L.-B. wrote the first draft of the manuscript; G.C.-B., X.R. and A.P.-P. conducted research; G.C.-B., S.X.-T. and E.L.-M. analyzed data; and F.dZ. and L.I. contributed to review the manuscript.

Author accepted manuscript

References

1. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ*. 1989 Mar 4;298(6673):564–7.
2. Ravelli AC, van der Meulen JH, Michels RP, et al. Glucose tolerance in adults after prenatal exposure to famine. *Lancet*. 1998 Jan 17;351(9097):173–7.
3. Bol V V, Delattre A-I, Reusens B, Raes M, Remacle C. Forced catch-up growth after fetal protein restriction alters the adipose tissue gene expression program leading to obesity in adult mice. *Am J Physiol Regul Integr Comp Physiol*. 2009 Aug;297(2):R291-9.
4. Hales CN, Ozanne SE. The dangerous road of catch-up growth. *J Physiol*. 2003 Feb 15;547(Pt 1):5–10.
5. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia*. 1993 Jan;36(1):62–7.
6. Sul HS. Minireview: Pref-1: role in adipogenesis and mesenchymal cell fate. *Mol Endocrinol*. 2009 Nov;23(11):1717–25.
7. Mortensen SB, Jensen CH, Schneider M, et al. Membrane-tethered delta-like 1 homolog (DLK1) restricts adipose tissue size by inhibiting preadipocyte proliferation. *Diabetes*. 2012 Nov;61(11):2814–22.
8. Falix FA, Aronson DC, Lamers WH, Gaemers IC. Possible roles of DLK1 in the Notch pathway during development and disease. *Biochim Biophys Acta*. 2012 Jun;1822(6):988–95.
9. Prats-Puig A, Carreras-Badosa G, Bassols J, et al. The placental imprinted DLK1-DIO3 domain: a new link to prenatal and postnatal growth in humans. *Am J Obstet Gynecol*. 2017 Sep;217(3):350.e1-350.e13.
10. Geach T. DLK1 levels predict fetal growth restriction. *Nat Rev Endocrinol*. 2017 Jan 4;13(1):4–4.
11. Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *Am J Physiol Endocrinol Metab*. 2008 Nov;295(5):E1269-76.
12. De Toro-Martín J, Fernández-Millán E, Lizárraga-Mollinedo E, et al. Predominant role of GIP in the development of a metabolic syndrome-like phenotype in female Wistar rats submitted to forced catch-up growth. *Endocrinology*. 2014 Oct;155(10):3769–80.
13. Turer AT, Khera A, Ayers CR, et al. Adipose tissue mass and location affect circulating adiponectin levels. *Diabetologia*. 2011 Oct;54(10):2515–24.
14. Berends LM, Fernandez-Twinn DS, Martin-Gronert MS, Cripps RL, Ozanne SE. Catch-up growth following intra-uterine growth-restriction programmes an insulin-resistant phenotype in adipose tissue. *Int J Obes*. 2012 Dec 11;37(8):1051–7.

15. Lee K, Villena JA, Moon YS, et al. Inhibition of adipogenesis and development of glucose intolerance by soluble preadipocyte factor-1 (Pref-1). *J Clin Invest*. 2003 Feb;111(4):453–61.
16. Villena JA, Kim KH, Sul HS. Pref-1 and ADSF/resistin: two secreted factors inhibiting adipose tissue development. *Horm Metab Res*. 2002 Jan;34(11–12):664–70.
17. Bieswal F, Ahn M-T, Reusens B, et al. The importance of catch-up growth after early malnutrition for the programming of obesity in male rat. *Obesity*. 2006 Aug;14(8):1330–43.
18. Duque-Guimarães DE, Ozanne SE. Nutritional programming of insulin resistance: causes and consequences. *Trends Endocrinol Metab*. 2013 Oct;24(10):525–35.
19. Somm E, Vauthay DM, Guérardel A, et al. Early metabolic defects in dexamethasone-exposed and undernourished intrauterine growth restricted rats. *PLoS One*. 2012 Jan;7(11):e50131.
20. Lafontan M. Adipose tissue and adipocyte dysregulation. *Diabetes Metab*. 2014 Feb;40(1):16–28.
21. Kim JI, Huh JY, Sohn JH, et al. Lipid-overloaded enlarged adipocytes provoke insulin resistance independent of inflammation. *Mol Cell Biol*. 2015 May 15;35(10):1686–99.
22. Andersen DC, Laborda J, Baladron V, Kassem M, Sheikh SP, Jensen CH. Dual role of delta-like 1 homolog (DLK1) in skeletal muscle development and adult muscle regeneration. *Development*. 2013 Sep;140(18):3743–53.
23. Mullis P-E, Tonella P. Regulation of fetal growth: consequences and impact of being born small. *Best Pract Res Clin Endocrinol Metab*. 2008 Feb;22(1):173–90.
24. Gondret F, Lefaucheur L, Juin H, Louveau I, Lebret B. Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the longissimus muscle in pigs. *J Anim Sci*. 2006 Jan;84(1):93–103.
25. Ozanne SE. Metabolic programming in animals. *Br Med Bull*. 2001 Jan;60:143–52.
26. Cha HC, Oak NR, Kang S, et al. Phosphorylation of CCAAT/Enhancer-binding Protein α Regulates GLUT4 Expression and Glucose Transport in Adipocytes. *J Biol Chem*. 2008 Jun 27;283(26):18002–11.
27. Amengual-Cladera E, Lladó I, Proenza AM, Gianotti M. Sex dimorphism in the onset of the white adipose tissue insulin sensitivity impairment associated with age. *Biochimie*. 2014 Nov;106:75–80.
28. Guarda DS, de Moura EG, Carvalho JC, et al. Maternal flaxseed oil intake during lactation changes body fat, inflammatory markers and glucose homeostasis in the adult progeny: role of gender dimorphism. *J Nutr Biochem*. 2016 Sep;35:74–80.
29. Garcia-Carrizo F, Priego T, Szostaczk N, Palou A, Picó C. Sexual Dimorphism in the Age-Induced Insulin Resistance, Liver Steatosis, and Adipose Tissue Function in Rats. *Front Physiol*. 2017 Jul 11;8:445.

Figure 1. Correlation graphs between *Dlk1* expression in adipose and skeletal muscle tissues and studied parameters in a *Wistar* rat catch-up growth model. Correlation graphs of different R/R, C/C and R/C groups between *Dlk1* relative expression in retroperitoneal adipose tissue (AT) and **(a)** body weight at PD42, **(b)** postnatal body weight gain and **(c)** visceral fat; as well as *Dlk1* relative expression in skeletal muscle and **(d)** insulin and **(e)** HOMA-IR values. Spearman r and p values are shown, P value was set at $p < 0.05$ and significant correlations are marked in bold style. Gene expression variables have been logarithmically transformed (\log_{10}). R/R: restricted, C/C: control, R/C: catch-up growth.

Author accepted manuscript

Figure 2. Adipose tissue and skeletal muscle morphometric parameters in a *Wistar* rat catch-up growth model. Representative images of **(a)** retroperitoneal adipose tissue and **(c)** skeletal muscle at PD42 are shown (scale bar: 50um). Bar graphs showing **(b)** adipocyte area and adipocyte density (more than 250 adipocytes per group were measured), **(d)** myofibers area and myofiber density (more than 20 fields per group were measured) are shown (* $p < 0.05$ compared to C/C and # $p < 0.05$ compared to R/R).

Author accepted manuscript

Table 1. Somatometric, metabolic and gene expression characteristics of the studied groups according to pre and postnatal growth: restricted pups (R/R), control pups (C/C) and catch-up growth pups (R/C).

	R/R	C/C	R/C		
<i>Somatometric parameters</i>	N=14	N=14	N=14	<i>p</i>	<i>p for trend</i>
Body weight PD1 g	5.10 ± 0.23 *	6.47 ± 0.29	5.08 ± 0.28 *	<0.0001	0.912
Body weight PD22 g	17.81 ± 1.13	43.08 ± 3.56 #	51.32 ± 3.94 #*	<0.0001	<0.0001
Body weight PD42 g	79.43 ± 11.64	170.62 ± 20.37 #	179.42 ± 20.43 #	<0.0001	<0.0001
Postnatal body weight gain g	74.33 ± 11.69	164.15 ± 20.28 #	173.62 ± 20.66 #	<0.0001	<0.0001
Postnatal body weight gain %	14.62 ± 2.54	25.36 ± 3.04 #	33.87 ± 4.17 #*	<0.0001	<0.0001
Retroperitoneal AT g	0.22 ± 0.06	0.75 ± 0.26 #	1.02 ± 0.33 #*	<0.0001	<0.0001
Mesenteric AT g	0.17 ± 0.04	0.35 ± 0.07 #	0.41 ± 0.09 #	<0.0001	<0.0001
Visceral fat g	0.67 ± 0.16	2.12 ± 0.60 #	2.71 ± 0.87 #*	<0.0001	<0.0001
<i>Metabolic parameters</i>	N=14	N=14	N=14	<i>p</i>	<i>p for trend</i>
Total adiponectin mg/g	19.81 ± 9.33	12.41 ± 4.71 #	10.42 ± 5.65 #	0.002	0.001
Glucose mmol/L	8.28 ± 0.74	10.15 ± 0.90 #	9.04 ± 0.56 #*	<0.0001	0.011
Insulin pmol/L	159.1 ± 73.6	267.2 ± 24.6 #	360.1 ± 26.8 #*	<0.0001	<0.0001
HOMA-IR	1.62 ± 0.77	3.53 ± 0.67 #	3.97 ± 1.34 #	<0.0001	<0.0001
Total cholesterol mmol/L	2.47 ± 0.36	2.31 ± 0.16	2.34 ± 0.42	0.448	0.318
HDL cholesterol mmol/L	0.91 ± 0.19	0.80 ± 0.09	0.89 ± 0.17	0.211	0.787
Triglycerides mmol/L	0.82 ± 0.27	1.41 ± 0.40 #	1.38 ± 0.58 #	0.001	0.002
<i>Gene expression</i>	N=14	N=14	N=14	<i>p</i>	<i>p for trend</i>
<i>Retroperitoneal adipose tissue</i>					
Dlk1 relative expression 2-ACT	0.93 ± 0.93	0.02 ± 0.02 #	0.01 ± 0.01 #	<0.0001	<0.0001
Dlk1 protein ng/g	392.3 ± 169.5	209.3 ± 148.2 #	82.3 ± 46.1 * #	<0.0001	<0.0001
<i>Skeletal muscle</i>					
Dlk1 relative expression 2-ACT	1.87 ± 1.55	0.39 ± 0.24 #	0.30 ± 0.28 #	<0.0001	<0.0001

Values are expressed in mean ± deviation score. R/R: restricted, C/C: control, R/C: catch-up growth, PD: postnatal day; AT: adipose tissue, HOMA-IR: homeostatic model assessment of insulin resistance, HDL: high-density lipoprotein. Postnatal body weight gain percentage in relation to birth body gain, specific adipose tissue weights percentage in relation to total body weight and total adiponectin was corrected for body weight. Anova p and p for trend values are shown (LSD post-hoc tests are shown with *p<0.05 compared to C/C and # p<0.05 compared to R/R).

Author accepted manuscript

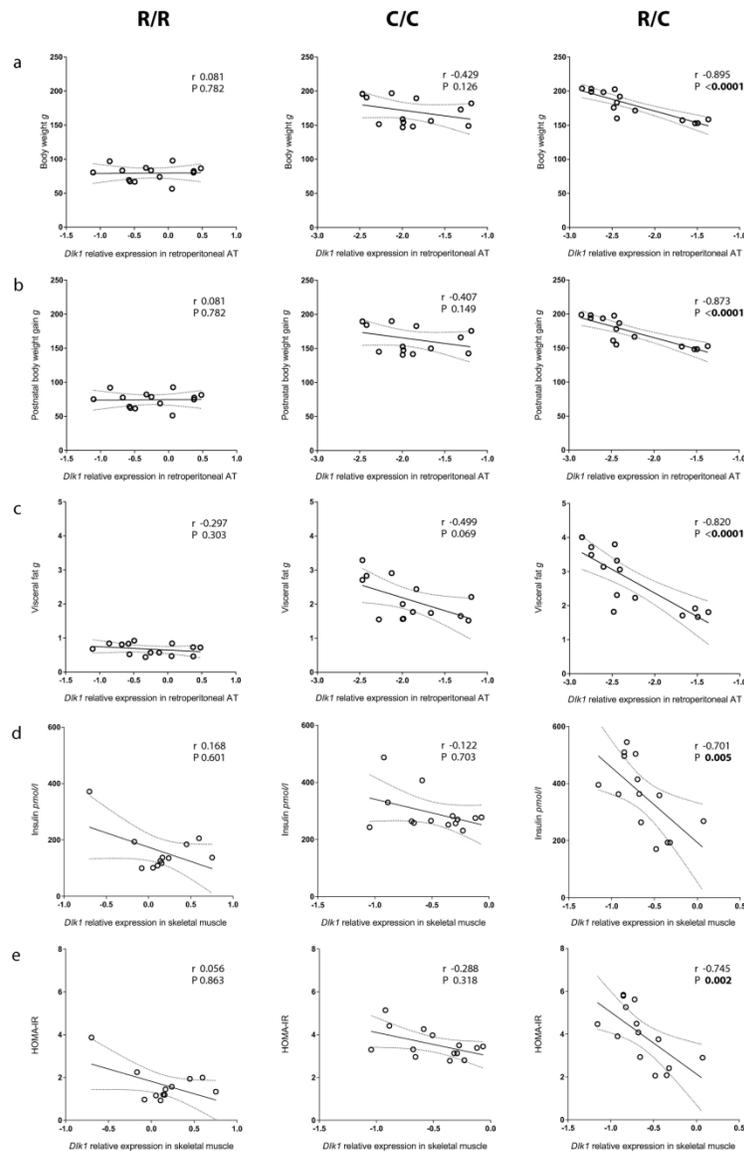


Figure 1. Correlation graphs between *Dlk1* expression in adipose and skeletal muscle tissues and studied parameters in a Wistar rat catch-up growth model. Correlation graphs of different R/R, C/C and R/C groups between *Dlk1* relative expression in retroperitoneal adipose tissue (AT) and (a) body weight at PD42, (b) postnatal body weight gain and (c) visceral fat; as well as *Dlk1* relative expression in skeletal muscle and (d) insulin and (e) HOMA-IR values. Spearman r and p values are shown, p value was set at $p < 0.05$ and significant correlations are marked in bold style. Gene expression variables have been logarithmically transformed (\log_{10}). R/R: restricted, C/C: control, R/C: catch-up growth.

181x271mm (300 x 300 DPI)

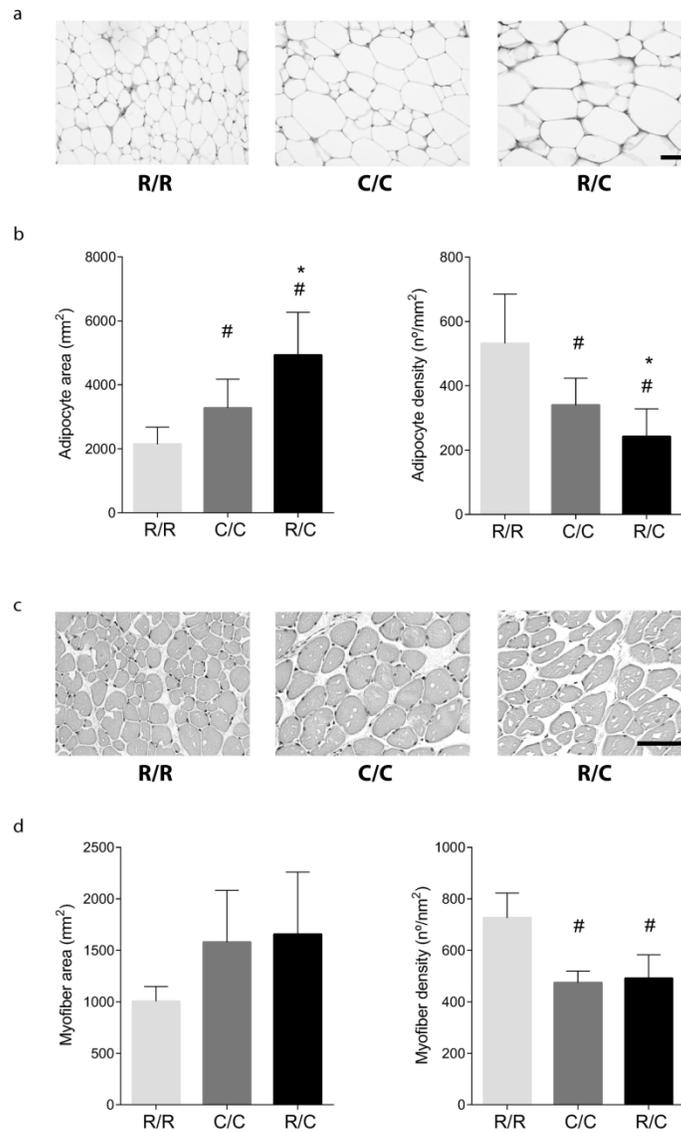


Figure 2. Adipose tissue and skeletal muscle morphometric parameters in a Wistar rat catch-up growth model. Representative images of (a) retroperitoneal adipose tissue and (c) skeletal muscle at PD42 are shown (scale bar: 50um). Bar graphs showing (b) adipocyte area and adipocyte density (more than 250 adipocytes per group were measured), (d) myofibers area and myofiber density (more than 20 fields per group were measured) are shown (*p<0.05 compared to C/C and # p<0.05 compared to R/R).

159x257mm (300 x 300 DPI)