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Developmental programming of metabolic syndrome in individuals born with intrauterine growth restriction followed by accelerated postnatal catch-up growth: exploration of outcomes in adulthood

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ABSTRACT

INTRODUCTION

The hypothesis that some noncommunicable diseases may find important parts of their origins early in life is currently known as *Developmental programming* or *Developmental origins of health and disease* (DOHaD). Several epidemiological studies have reported that *intrauterine growth restriction* (IUGR) increases the susceptibility to develop diabetes, obesity and associated disturbances later in life. The risk might even be amplified if a postnatal catch-up growth follows IUGR. The aim of this study is to evaluate in adult rats several metabolic alterations induced by IUGR followed by postnatal catch-up growth in both gender, in order to, thereafter, identify the potentially involved mechanisms.

METHODS

An IUGR rat model was obtained by maternal exposure to a low protein diet (9% casein) throughout the gestation compared to a control diet (23% casein). At birth, litter size was adjusted to 10 or reduced to 4 pups (2 males and 2 females) per mother inducing a transient postnatal *overfeeding* (OF) and catch-up growth due to the surplus of milk availability for each offspring. Body weight measurement, computed tomography adipose tissue quantification, glucose tolerance tests, liver histology and plasma analyses were performed.

RESULTS

Higher visceral fat content, altered tolerance to glucose, hepatic steatosis and fibrosis, increased plasma levels of cholesterol, triglycerides and transaminases were retrieved in male and female rats with either IUGR, postnatal catch-up growth or both IUGR and subsequent postnatal catch-up growth between 6 and 12 months of age compared to controls.

CONCLUSION

IUGR and accelerated postnatal catch-up growth generate lifelong consequences with adverse metabolic outcomes thereafter. Indeed, several metabolic syndrome components were retrieved in adulthood. Therefore, exploring the developmental programming involved mechanisms and finding biomarkers to identify individuals at risk may provide opportunities for lifestyle interventions and disease prevention, especially during the current diabetes and obesity pandemic.

KEYWORDS

Developmental programming; developmental origins of health and disease; metabolic syndrome; intrauterine growth restriction; postnatal catch-up growth

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INTRODUCTION

NONCOMMUNICABLE DISEASES

Noncommunicable diseases (NCDs) are defined as non-infectious health conditions and tend to be long-lasting or recurrent. Accordingly, they are also referred as chronic diseases. NCDs are the leading cause of death globally, responsible for more than two thirds (40 million per year) of the world's deaths and are projected to significantly increase over the next few years. More than one third of them are "premature" deaths under age 70 years and mainly occur in low-income countries. The large majority of NCDs can be subdivided in 4 groups of diseases: cardiovascular, respiratory, cancers and diabetes (fig. 1.1). International strategies are progressively settled trying to control and prevent the global burden of NCDs, essentially by reducing the associated risk factors (2).

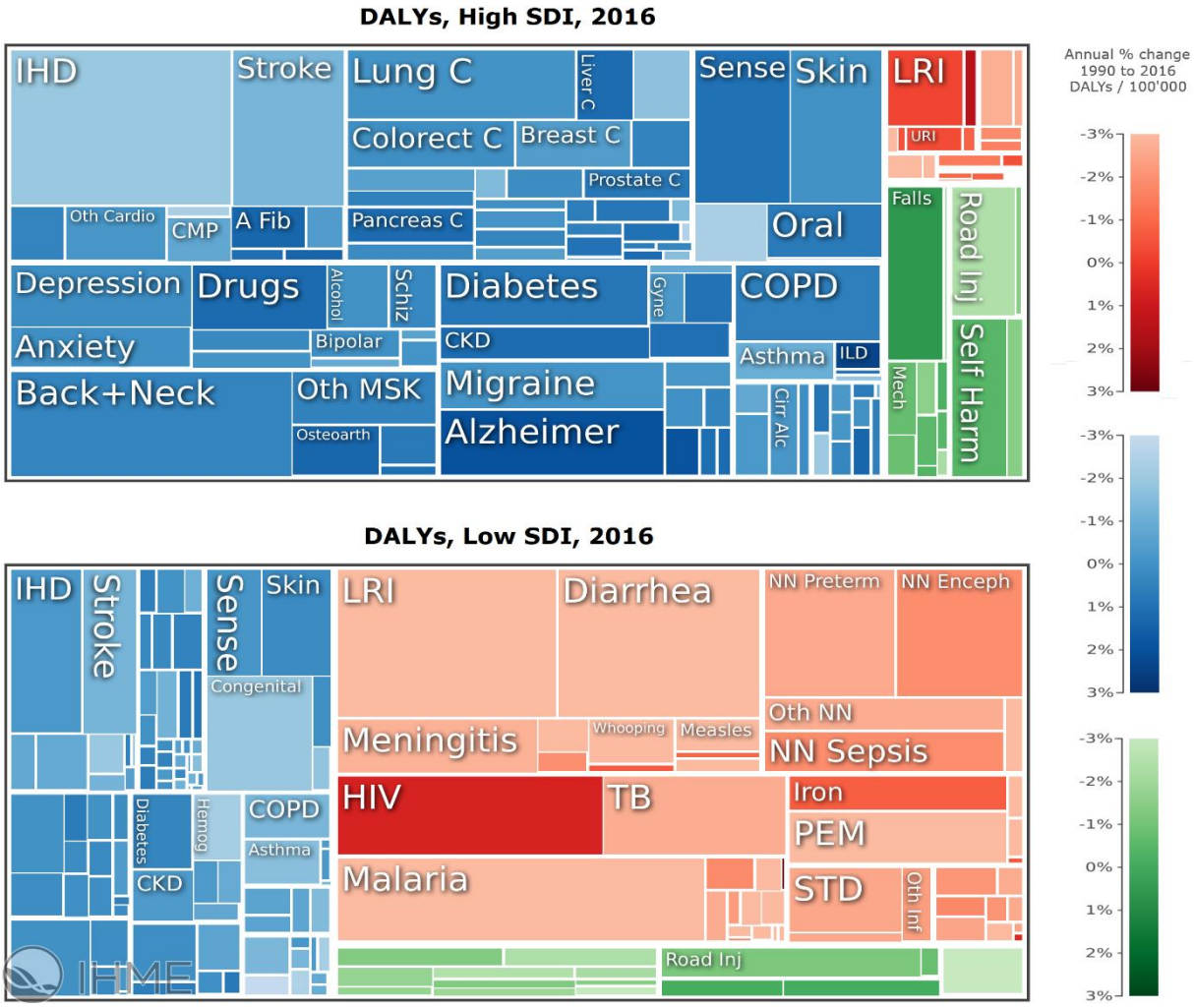


Figure 1.1 Square pie chart: *disability-adjusted life years* (DALYs; years of healthy life lost) in high compared to low *socio-demographic index* (SDI; composite development measure of the incomes per capita, educational attainment and fertility rates) countries in 2016. Proportional size of boxes to the number of DALYs, subdivided in noncommunicable diseases (blue), communicable, maternal, neonatal, nutritional diseases (red) and injuries (green) (1).

METABOLIC SYNDROME

For many decades, the metabolic syndrome remained controversial and many definitions have been proposed. In the late 2000s, the syndrome was harmonized and defined as ≥ 3 of these following criteria: *central obesity* (various waist-circumference thresholds depending on nations, sexes and ethnic groups), increased *fasting glucose* (≥ 5.5 mmol/L), *blood pressure* (≥ 130/85 mmHg), *triglycerides* (≥ 1.7 mmol/L) and reduced *HDL cholesterol* (♀ < 1.3 mmol/L and ♂ < 1.0 mmol/L) (3).

Nowadays, it is estimated that around 20-25% of the world's adult population have metabolic syndrome (4). This syndrome is currently widely validated as a cluster of risk factors to develop diabetes and cardiovascular diseases. However, it is a far stronger predictor of diabetes than cardiovascular diseases (5) and seems not to reveal any better the cardiometabolic risk than the sum of its criteria (no synergistic effect) (6). The mechanism is mainly explained by insulin resistance: initial excessive central adiposity, normoglycemia with hyperinsulinemia as a trade-off for several years, gradually leading to insufficient insulin secretion and metabolic syndrome. Therefore, lifestyle interventions targeting insulin resistance continue to be the primary therapy (physical activity, weight loss and caloric restriction) (7).

CENTRAL OBESITY

Overweight and obesity, defined as a *body mass index* (BMI) ≥ 25 kg/m^2 and ≥ 30 kg/m^2 respectively, are related to an increased overall mortality and likelihood of several diseases including diabetes, cardiovascular diseases, stroke, some cancers, sleep apnoea and osteoarthritis (2). In addition to BMI, waist circumference is another easily measured anthropometric indicator of obesity, especially for abdominal, also known as central obesity. However, adipose tissue is not uniformly distributed in the body and accumulates into *subcutaneous* (SAT) or *visceral adipose tissue* (VAT) compartments (fig. 1.3). SAT and VAT are highly correlated with each other, as well as with both BMI and waist circumference. Though, VAT appears to be the most accurate predictor of cardiometabolic risk providing further information to anthropometric measurements (8). Indeed, VAT may be seen as a unique pathogenic endocrine fat depot releasing excess adipokines, inflammatory cytokines and free fatty acids directly into the portal vein and liver, worsening insulin resistance and thereby, metabolic syndrome (9, 10).

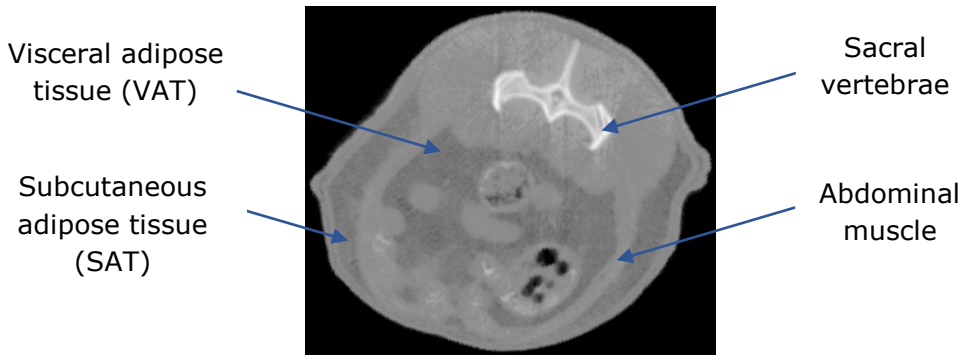


Figure 1.3 Computed tomography: slice passing through 1st sacral vertebrae of a 6-month-old rat with *subcutaneous* (SAT) and *visceral adipose tissue* (VAT) discrimination.

In small animal experimental models, invasive and non-invasive methods are available to assess adipose tissue. Invasive methods require sacrificing the animal to measure fat deposits ex vivo and provide details concerning fat content and distribution. Non-invasive methods include imaging techniques such as computed tomography or magnetic resonance enabling valuable longitudinal assessment (11).

DEVELOPMENTAL PROGRAMMING

BACKGROUND

Retrospective human epidemiological studies for the last three decades have progressively set up the hypothesis that some noncommunicable diseases could find an important part of their origins early in life. This concept, currently known as *Developmental Programming* or *Developmental Origins of Health and Disease* (DOHaD), emerged in England around 1990 as a result of David Barker and co-worker's observations. They were the first to notice an inverse correlation between birthweight and death rates due to ischemic heart disease in adulthood (12).

Indeed, birthweight is an easily measured proxy reflecting quality of intrauterine development and has only a minor genetic component (13). Environmental perturbations (such as undernutrition or overnutrition, exposition to toxicants or endocrine disruptors, stress, etc.) during critical time window of vulnerability extending approximately from conception to the child's second anniversary (also referred as *the 1000 days period*) might steer individuals through different risk pattern to develop some noncommunicable diseases thereafter (14, 15). Consequently, these diseases are being increasingly recognized as not only triggered by genetic predispositions (innate) and adult lifestyle (acquired), but also the encountered environment during early development.

Since the Barker theory was put forward, worldwide human and animal studies have validated the initial epidemiological evidence. All biological systems appear to be influenced by developmental programming, including mostly cardiovascular, metabolic and kidney diseases but also pulmonary diseases, immunity, fertility, hormone-dependant cancers, lifespan or even behavioural functions (15-18).

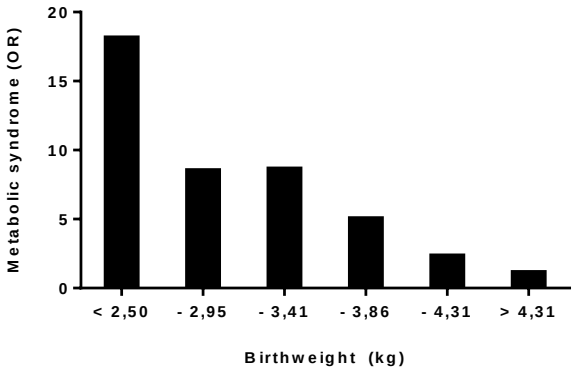


Figure 1.2 Odds ratio for metabolic syndrome in adulthood according to birthweight among 407 men born in Hertfordshire (England) between 1920 and 1930 (adjusted for adult body mass index) (19).

Although the magnitude of this concept is awfully arduous to assess, it may be considerable. Among a cohort of infants born between 1911 and 1930 in England, those who weighted 8 kilograms or less at one year had almost three times greater deaths rate from ischaemic heart disease sixty years later compared to those who attained 12 kilograms or more (12). Concerning prevalence of metabolic syndrome in adulthood, the risk was 10 times higher among those whose birthweights were 2.95 kilograms or less than those who weighted more than 4.31 kilograms (fig. 1.2). Thus, Barker et al. suggested to rename metabolic syndrome "the small-baby syndrome" (19).

UNDERNUTRITION

The *thrifty phenotype hypothesis* suggests that in a deficient fetal environment, such as maternal undernutrition or placental insufficiency, the fetus will adapt his growth with selective development of vital organs (brain and heart) to the detriment of non-vital organs (pancreatic β-cell, nephron number or skeletal muscle) resulting as a trade-off in *intrauterine growth restriction* (IUGR) or being *small for gestational age* (SGA; weight < 10^{th} percentile), revealed or not with a low birthweight (< 2.5 kg) (20-22). This *developmental plasticity* may be considered as an attempt to set the most suited phenotype to assist survival in the future predicted environment, before adaptations become irreversible (13). Nevertheless, inappropriate predictions or adaptations to a threatening environment during critical periods of development provoke permanent structural and functional body changes which may increase the risk of metabolic, cardiovascular or renal diseases thereafter (23).

CATCH-UP GROWTH

Postnatal rapid weight gain looks like a serious stage of malprogramming, increasing susceptibility for later obesity and associated metabolic disturbances (24). Each 100 grams gain in absolute weight during the first week of life increased the risk of 28% becoming an overweight adult in a cohort of 653 formula-fed infants born between 1965 and 1978 in Iowa (USA) (25). Some evidences demonstrate an even worse susceptibility if accelerated postnatal growth (*2^{nd} hit*) follows a transient period of growth inhibition such as IUGR or a preterm birth (*1^{st} hit*). Indeed, in accordance with the concept stated above, the amplified mismatch between early environmental cues predicting sparse conditions and the actual plentiful ones, as we might encounter in our modern societies, become awfully maladaptive and significantly amplify the risk of disease from unexpected excess (13). Hence, rapid postnatal catch-up growth, especially in IUGR or preterm babies, should be avoided and exclusive breastfeeding promoted for the first 6 months of life (26).

OVERNUTRITION

Maternal diabetes and obesity exposing the fetus to excess nutrients results in macrosomia (birthweight > 4 kg) or being *large for gestational age* (LGA; weight > 90^{th} percentile). According to Pedersen's hypothesis, maternal blood sugar acts directly on fetus growth and indirectly through fetal insulin secretion acting as a growth hormone (27). Although the mechanisms seem different than in IUGR infants, overnourished fetuses possibly undergo similar increased risk to develop metabolic, cardiovascular and renal diseases later in life (28, 29). Subsequently, female offspring of diabetic or obese mothers are more likely to develop diabetes and obesity by the time they reach childbearing years, perpetuating an ever-expanding vicious cycle, explaining part of the current obesity and diabetes pandemic (30).

MECHANISMS

Incriminated mechanisms of developmental programming are still not plainly identified. Consistent with the thrifty phenotype hypothesis, tissue differentiation including structural and functional alterations in pancreatic β-cell, liver, muscle, kidney, vascular homeostasis, appetite regulatory network, hypothalamic-pituitary-adrenal axis and sympathetic system may be involved (28).

Oxidative stress, characterised by an imbalance between cellular production of reactive oxygen species (ROS) and antioxidant defences might play a major role in developmental programming pathogenesis. Increased superoxide anion production, oxidative DNA and protein damage, lipid peroxidation and reduced levels of endogenous antioxidant enzymes as superoxide dismutase, catalase, glutathione peroxidase and reductase have been described in IUGR and overnourished individuals (14, 31, 32). Excessive ROS have also been reported to possibly contribute in premature cellular senescence, inducing notably liver dysfunctions thereafter (33). Consequently, both growth restriction and overnutrition, acting as pro-oxidative conditions, could possibly lead to later elevated risks for cardiometabolic diseases through this unified mechanism.

Epigenetic regulation, likely the molecular basis of developmental origins of adult disease, can be define as gene expression changes in response to environmental cues without affecting DNA sequence (15). Therefore, a range of many phenotypes can develop from a single genotype. Epigenetic marks, particularly vulnerable during early development, either silencing (downregulating) or enhancing (upregulating) gene expression, are reversible acquired characteristics that can be inherited over several generations via germ cells imprinting. DNA methylation usually leading to repression of transcription, histone modifications modulating access to DNA transcription factors and non-coding RNAs regulating post-transcriptional expression are the three main pathways of epigenetic regulations (14, 18). Increasing evidence emphasizes the potential epigenetic involvement in developmental programming of adult disease.

OBJECTIVES

The aim of the present study is to evaluate in an adult rat model several metabolic alterations induced by IUGR and accelerated postnatal catch-up growth in both gender, in order to, thereafter, identify the potential involved mechanisms triggering these malfunctions.

METHODS

ETHICAL STATEMENT AND PROCEDURES

The use of animals in this study was approved by the swiss cantonal authority (VD 3050) in conformity with the federal law concerning the protection of animals 2005 (LPA, art. 18), the ordinance concerning the protection of animals 2008 (OPAn, art. 141) and the ordinance concerning experimentation in animals 2010 (OExAn, art. 30).

Throughout the experimentation, care was taken to avoid suffering and ensure animal welfare. Sprague Dawley rats were housed in a light-cycle room from 7 a.m. to 7 p.m. at 22°C temperature and caged during one night at the age of 10 weeks for mating with one male per female. Animals had always free access to tap water. Pellet diets SAFE U8958 version 1-control and U8959 version 40-hypoproteinic (Augy, France) were used.

MODEL OF IUGR AND POSTNATAL CATCH-UP GROWTH

An experimental IUGR rat model was attained feeding dams throughout the gestation with an isocaloric *low protein diet* (LPD; containing 9% casein) compared to a *control* diet (CTRL; containing 23% casein). At birth, accelerated postnatal catch-up growth was randomly performed in half the litters by reducing litter size to 4 pups (2 males and 2 females) per mother inducing a transient *overfeeding* (OF) due to the surplus of milk availability for each offspring during lactation period. On the other half, a control litter size of 10 pups per mother was set to mimic normal postnatal growth. At *postnatal day* (PND) 21, corresponding to weaning, rats were separated from mothers and had free access to the control diet ad libitum. Therefore, 4 different groups are obtained (fig. 2.1). Several metabolic explorations were performed from the period of birth up to 12 months of age as explained below.

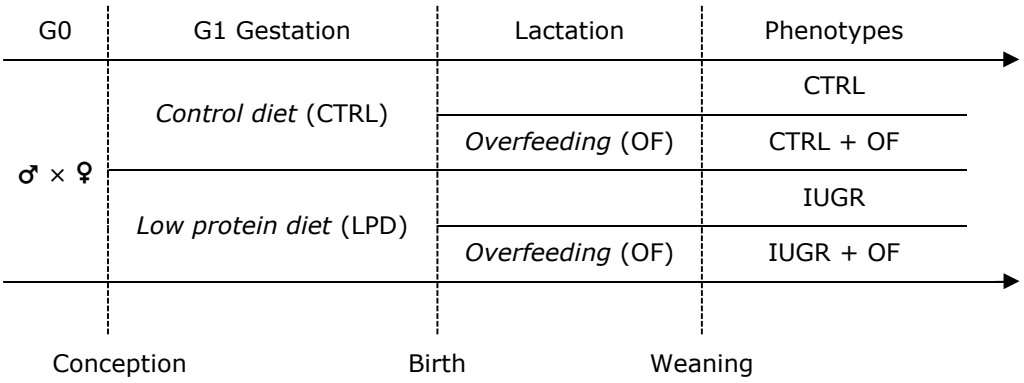


Figure 2.1 Experimental animal model through time of IUGR attained with LPD during gestation in half the mothers. Accelerated postnatal catch-up growth performed with OF during lactation in half the litters. Therefore, 4 different groups are obtained: CTRL, CTRL+OF, IUGR, IUGR+OF.

BODY WEIGHTS

Weight of every rat was usually measured weekly. Depending on date of birth, animals from different litters might have a ± 3 days time lag. Consequently, body weights were proportionally adapted for comparison at a same time point.

ADIPOSE TISSUE QUANTIFICATION

Computed tomography (CT) was used in this study to assess adipose tissue quantity. Rats were anaesthetized by isoflurane inhalation (3-4%) and kept under (1-2%) during image acquisition. At 2, 6 and 12 months of age, adipose tissue was quantified using a micro-CT from Albira Bruker. Images were acquired at 200 µA, 45 kV, 125 µM voxel size, with 1000 projections, and reconstructed by FBP algorithm. Image processing was achieved with the PMOD 3.7 (Zürich, Switzerland) software.

Initially performed in the abdominal volume of interest between 1st and 6th lumbar vertebrae, methodology was adjusted to improve accuracy in a slice passing through the inferior articular facet of 6th lumbar vertebrae. Due to their difference in X-ray attenuation, fat and lean mass can be segmented from each other. A segmentation range for *total adipose tissue* (TAT) was set between -500 and -100 *Hounsfield Units* (HU). To segregate *subcutaneous* (SAT) from *visceral adipose tissue* (VAT), semi-automated tools were used to demarcate SAT. VAT surface was obtained by subtracting SAT to TAT. Indeed, SAT is much more easily delineable under the skin than VAT scattered between the abdominal organs. Then VAT can be expressed as a percentage of total slice surface (fig. 2.2).

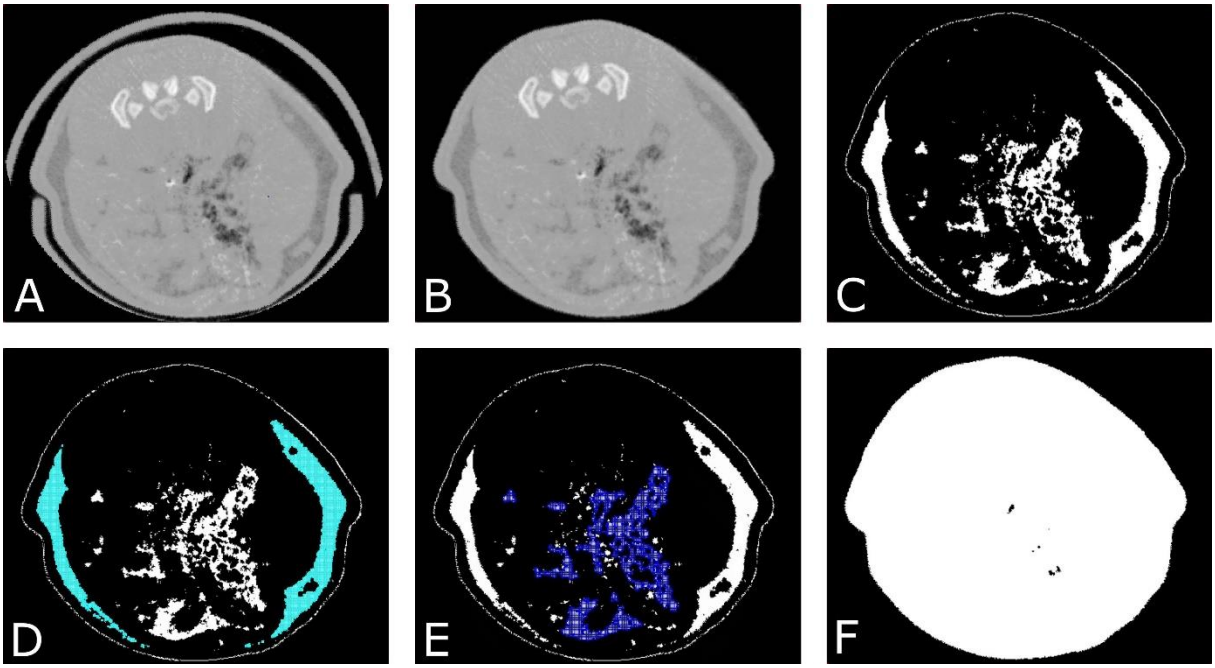
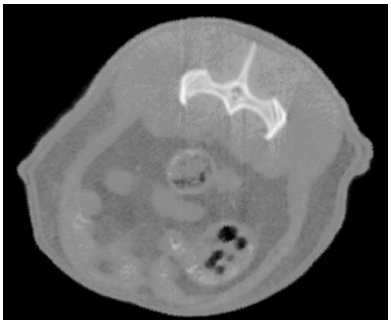
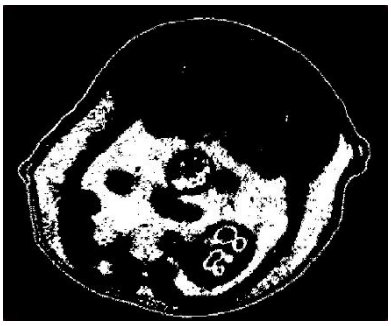


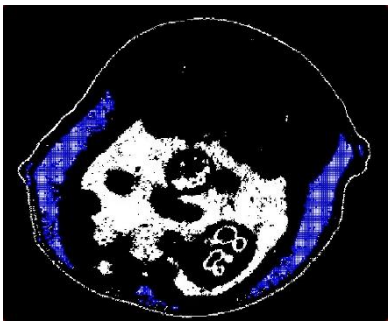
Figure 2.2 Computed tomography of a slice passing through 6th lumbar vertebrae, before (**A**) and after (**B**) rat holding structure removal. TAT segmentation expressed in white (**C**). SAT demarcation in light blue (**D**). Impossible complete VAT demarcation in dark blue because scattered between the organs (**E**). Thus, VAT was obtained by subtracting SAT (D) to TAT (C) and can be expressed as a percentage of total slice surface (**F**).



Native computed tomography



Adipose tissue segmentation



SAT (blue) and VAT (white)

GLUCOSE TOLERANCE TESTS

Intraperitoneal glucose tolerance tests (IPGTT) were performed at 6 months of age. After a 12-hour fasting period, an intraperitoneal glucose solution was injected (1 g of glucose / kg of rat). Blood droplets were collected from the saphenous vein just prior to glucose injection (time 0), at 15, 30, 60, 90 and 120 minutes following the injection. Blood glucose concentrations were measured using a glucometer with test strips (Accu-Chek, Roche, Basel, Switzerland). Blood glucose concentrations are presented in mmol/L (mM).

LIVER HISTOLOGY

At 6 months of age, rats were sacrificed by abdominal aortic exsanguination after pentobarbital (Esconarkon-Streuli Pharma, Switzerland) intraperitoneal injection. Livers were harvested, immediately fixed in formalin 4% for 24-48h and then paraffin-embedded. 5 µm sections were stained with *haematoxylin and eosin* (HE) for hepatic structure evaluation. *Masson's trichrome* (MT) staining was performed to assess hepatic fibrosis. In addition, ImageJ open source software (https://imagej.nih.gov/ij) was used to quantify fibrosis with a "stack" image from MT staining and report results as fibrotic percentage of total area.

PLASMA ANALYSES

Blood samples from saphenous vein were collected at 6 months of age after a 12-hour fasting period. After centrifugation at 5000 rpm during 10 min, plasma levels of cholesterol, triglycerides, *alanine aminotransferase* (ALAT), *aspartate aminotransferase* (ASAT) and lipase were quantified.

STATISTICAL ANALYSES

All values are reported as mean ± SD. GraphPad Prism 6 (La Jolla, USA) software was used to perform statistical analyses and create graphics. Wilcoxon-Mann-Whitney test and one-way *analysis of variance* (ANOVA) with multiple comparisons Tukey post-hoc test were used. Significance level was set at a p-value < 0.05.

BODY WEIGHTS

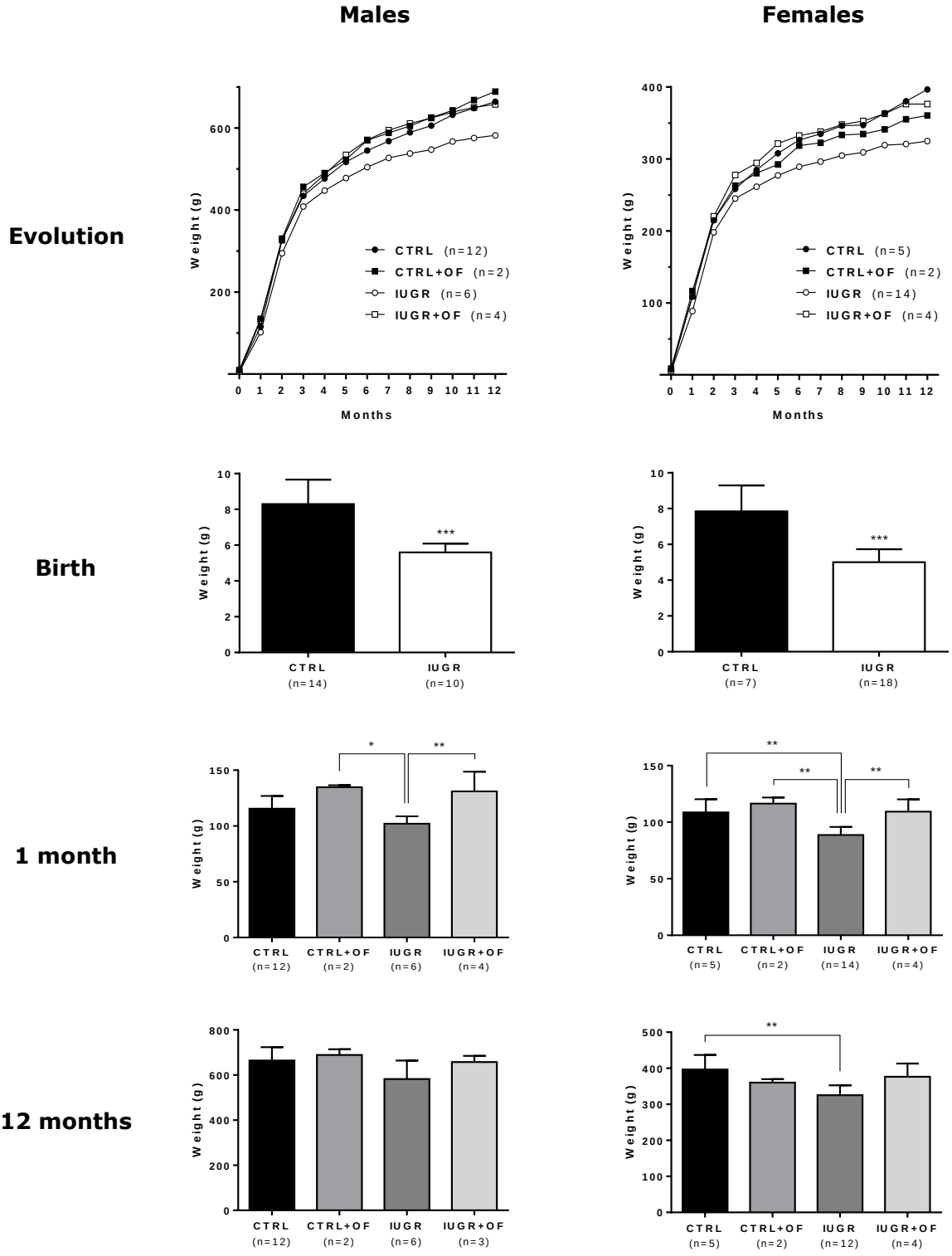


Figure 3.1 Body weights evolution through time, at birth, 1 month and 12 months of age in males (left panel) and females (right panel). Values are reported as mean ± SD. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Numerical values are presented in table 1.

Experimental IUGR model was successively performed with a significant smaller body weight at birth compared to CTRL group: – 48% in males ($p < 0.001$) and – 57% in females ($p < 0.001$). The effect persisted during growth and maturation, but to a lesser extent: – 14% in males ($p > 0.05$) and – 22% in females ($p < 0.01$) at 12 months of age (fig. 3.1).

Transient postnatal *overfeeding* (OF) induced a rapid weight gain during suckling period. Body weights at one month of age in CTRL+OF pups compared to *controls* (CTRL) were: + 17% in males ($p > 0.05$) and + 7% in females ($p > 0.05$). IUGR pups exposed to OF (IUGR+OF) caught up the weight of controls by the end of first month of age. Body weights at one month of age in IUGR+OF compared to IUGR were: + 28% in males ($p < 0.01$) and + 23% in females ($p < 0.01$). Hence, body weight gain in catch-up growth groups (CTRL+OF and IUGR+OF) was proportionally higher in IUGR+OF than CTRL+OF rats, revealing the increased susceptibility to accelerated postnatal growth in IUGR pups. The OF effect persisted through time in all groups, except the female CTRL+OF one in which body weight was smaller than controls at 12 months of age (fig. 3.1).

ADIPOSE TISSUE QUANTIFICATION

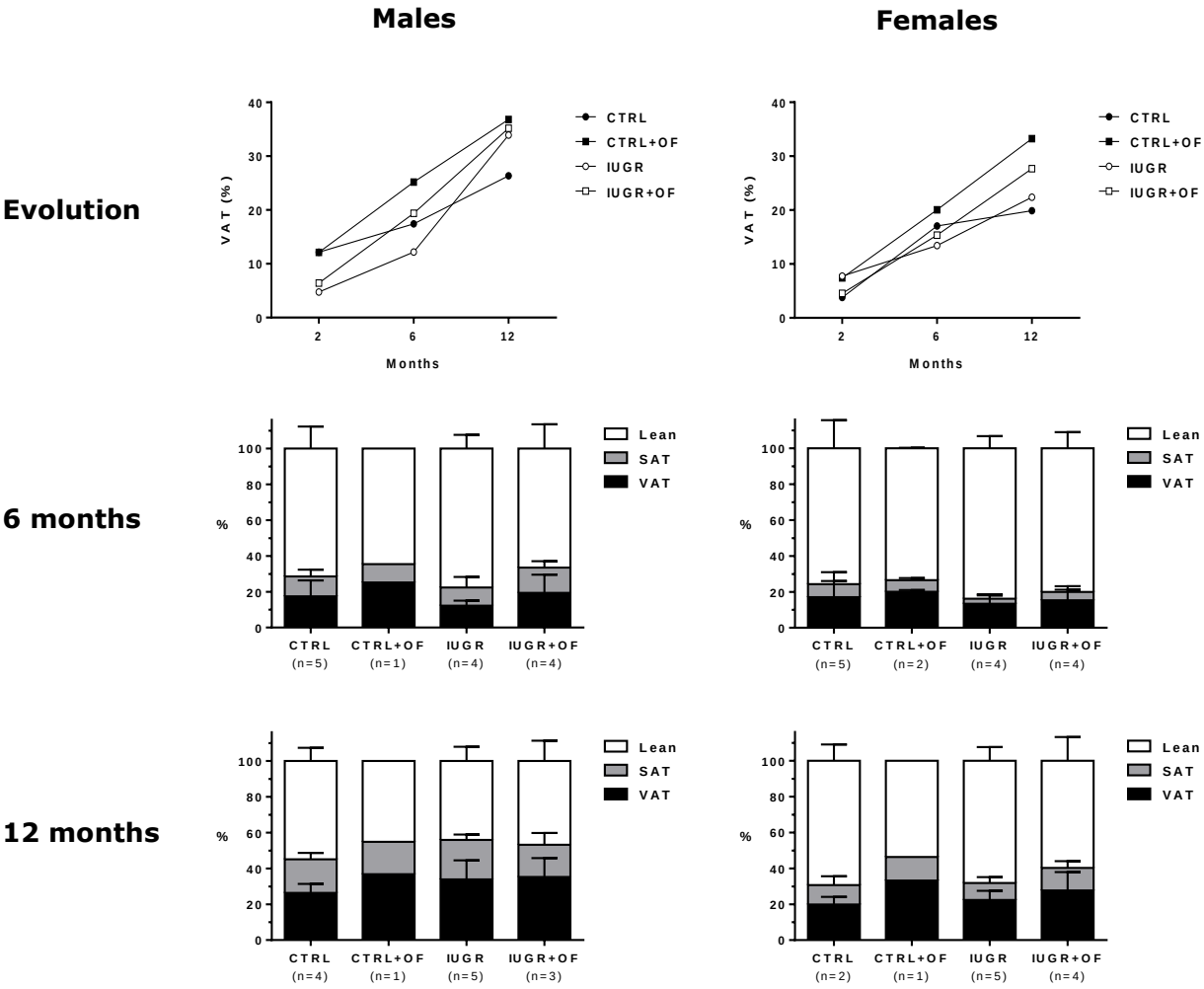


Figure 3.2 VAT evolution through time, body composition at 6 and 12 months of age in males (left panel) and females (right panel). SAT = *subcutaneous adipose tissue* and VAT = *visceral adipose tissue*. Values are reported as mean ± SD. No statistical significant results. Numerical values are presented in table 1.

At 6 months of age, both male and female IUGR groups had the lowest adipose, especially *visceral adipose tissue* (VAT) percentage compared to other groups: + 107% and + 50% in CTRL+OF, + 59% and + 15% in IUGR+OF, + 43 % and + 28% in CTRL male and female groups respectively ($p > 0.05$) (fig 3.2).

At 12 months of age, the IUGR group VAT percentage had exceeded the CTRL one. Actually, the 3 experimental male and female groups had more adipose, especially VAT than controls: + 40% and + 67% in CTRL+OF, + 33% and + 39% in IUGR+OF, + 29% and + 13% in IUGR male and female groups respectively ($p > 0.05$) (fig. 3.2).

GLUCOSE TOLERANCE TESTS

Intraperitoneal glucose tolerance tests (IPGTT) were performed at 6 months of age. In both male and female groups, *area under the curve* (AUC) of blood glucose concentrations in CTRL+OF and IUGR groups were higher than controls: + 22% and + 5% in CTRL+OF ($p > 0.05$), + 50% ($p < 0.01$) and + 13% ($p > 0.05$) in IUGR male and female groups respectively. Although, IUGR+OF groups had lower AUC than IUGR alone: – 27% in males ($p < 0.05$) and – 31% in females ($p > 0.05$) (fig. 3.3).

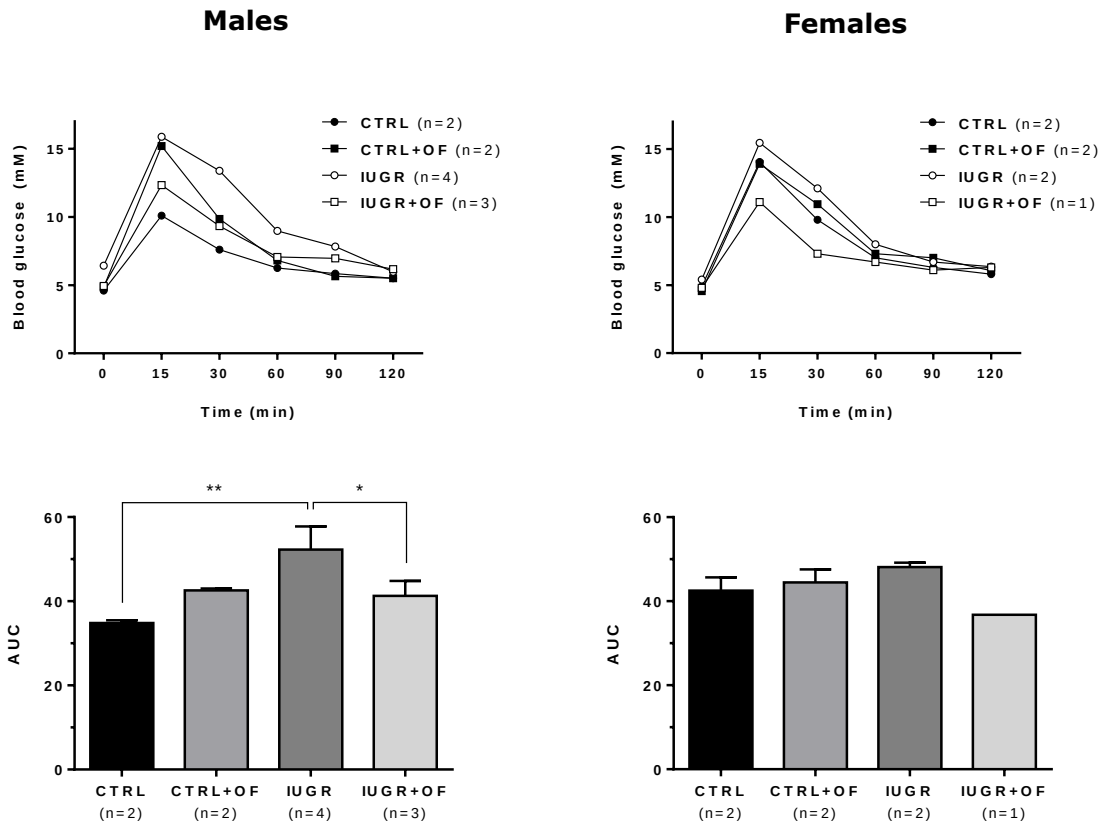


Figure 3.3 *Intraperitoneal glucose tolerance tests* (IPGTT) at 6 months of age in males (left panel) and females (right panel). Blood glucose evolution through time (top) and *area under the curve* (AUC) (bottom) after a 1g/kg glucose intraperitoneal injection. Values are reported as mean ± SD. * = $p < 0.05$, ** = $p < 0.01$. Numerical values are presented in table 1.

LIVER HISTOLOGY

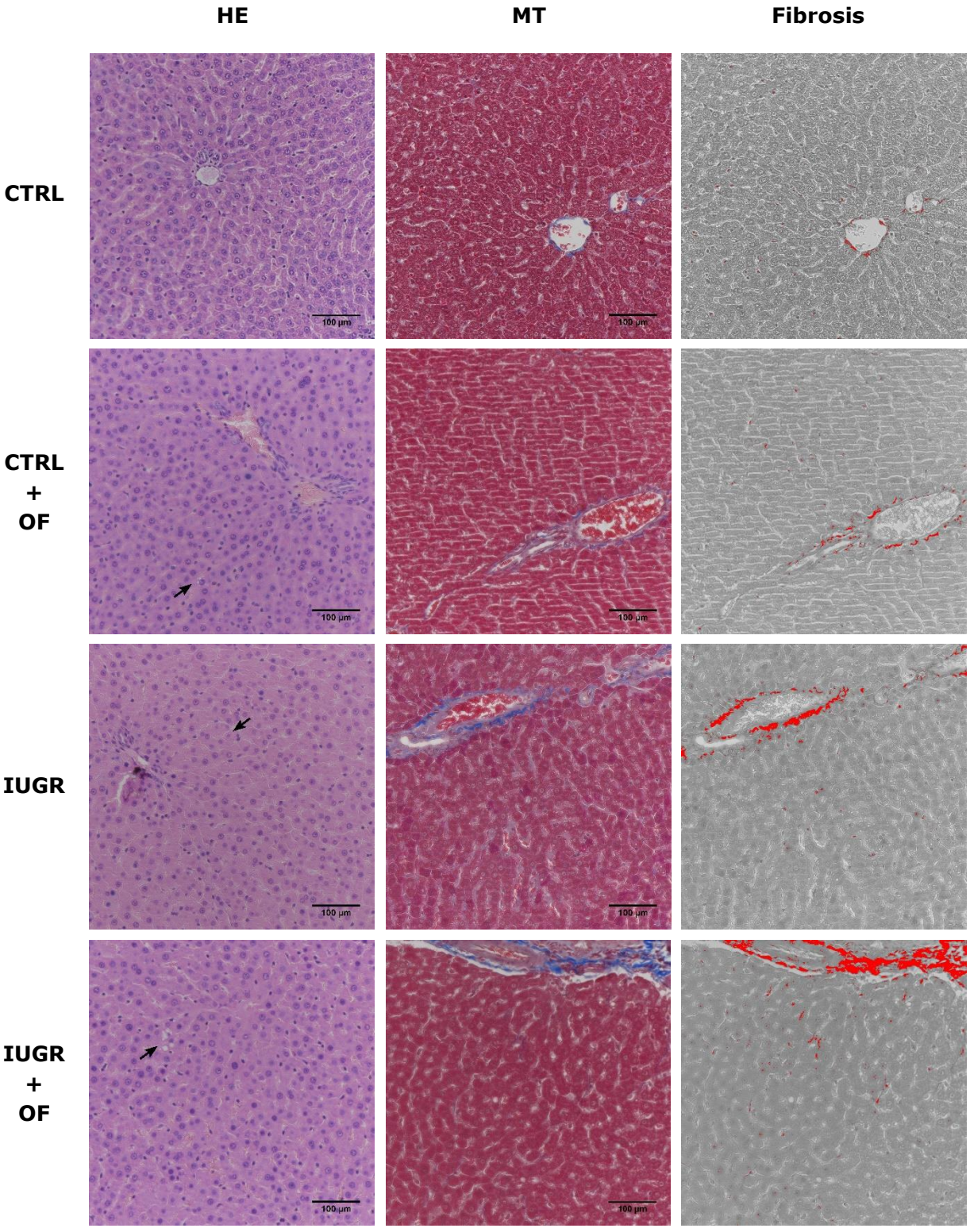


Figure 3.4 Liver photomicrographs at 6 months of age and same magnification (×20) with *haematoxylin and eosin* (HE) and *Masson's trichrome* (MT) staining. In addition, fibrosis assessment with a "stack" image from MT staining was generated with ImageJ software. These micrographs are representative of male and female CTRL (♀), CTRL+OF (♀), IUGR (♂) and IUGR+OF (♂) rats. Microvesicular steatosis is showed with arrows (↗).

Liver structure was assessed using histological stains at 6 months of age. Similar outcomes were observed in male and female groups. *Haematoxylin and eosin* (HE) staining revealed a few hepatocyte cytoplasmic lipid droplet accumulations, characterizing a mild microvesicular steatosis in the three experimental CTRL+OF, IUGR and IUGR+OF groups compared to controls. OF groups (CTRL+OF and IUGR+OF) seem to present higher lipid accumulation than IUGR groups (fig. 3.4).

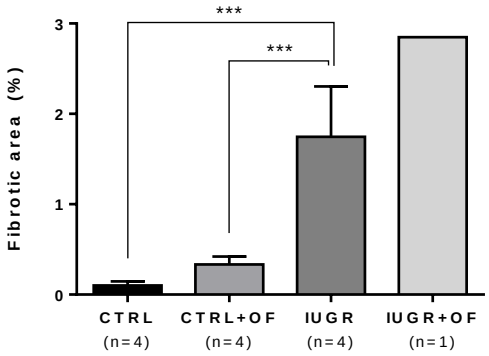


Figure 3.5 Fibrosis quantification in liver at 6 months of age using *Masson's trichrome* (MT) staining photomicrographs (fig. 3.4) and ImageJ software. Values are reported as fibrotic percentage of total area, mean ± SD, of both male and female rats. *** = $p < 0.001$. Numerical values are presented in table 1.

Hepatic fibrosis was identified with *Masson's trichrome* (MT) staining and quantified using ImageJ software. Increased fibrotic areas were observed in the three experimental groups compared to controls (mean (%) ± SD: 0.10 ± 0.04 in CTRL (n=4), 0.33 ± 0.09 in CTRL+OF (n=4), 1.75 ± 0.56 in IUGR (n=4) and 2.85 ± 0 (n=1) in IUGR+OF groups) (fig. 3.5).

PLASMA ANALYSES

At 6 months of age, several plasma parameters were measured. Cholesterol levels were higher in both male and female OF (CTRL+OF and IUGR+OF) compared to corresponding non-OF groups (CTRL + IUGR): + 16% and + 12% in CTRL+OF ($p > 0.05$), + 10% ($p > 0.05$) and + 40% ($p < 0.05$) in IUGR+OF male and female groups respectively (fig. 3.6).

Triglycerides levels in OF groups were also higher than non-OF corresponding groups, excepted the male CTRL+OF one in which they are identical than controls: + 0% ($p > 0.05$) and + 100% ($p < 0.05$) in CTRL+OF, + 50% ($p > 0.05$) and + 80% ($p < 0.05$) in IUGR+OF male and female groups respectively (fig. 3.6).

Alanine (ALAT) and *aspartate aminotransferases* (ASAT) both showed in a similar trend higher levels in CTRL+OF and IUGR groups compared to controls: + 40% and + 74% in CTRL+OF, + 72% and + 131% in IUGR male and female groups respectively for ALAT ($p > 0.05$) and + 72% and + 113% in CTRL+OF, + 144% and + 460% in IUGR male and female groups respectively for ASAT ($p > 0.05$). Although, IUGR+OF groups had lower ALAT and ASAT levels than IUGR: – 44% in males and – 169% in females for ALAT and – 59% in males and – 592% in females for ASAT ($p > 0.05$). All groups had an ASAT/ALAT ratio > 2, apart from the male CTRL group (fig. 3.6).

Concerning lipase, levels were nearly 6 times higher in male IUGR and IUGR+OF groups compared to controls: + 469% ($p < 0.05$) in IUGR and + 500% ($p > 0.05$) in IUGR+OF male groups. Values were not statistically significant in female groups (fig. 3.6).

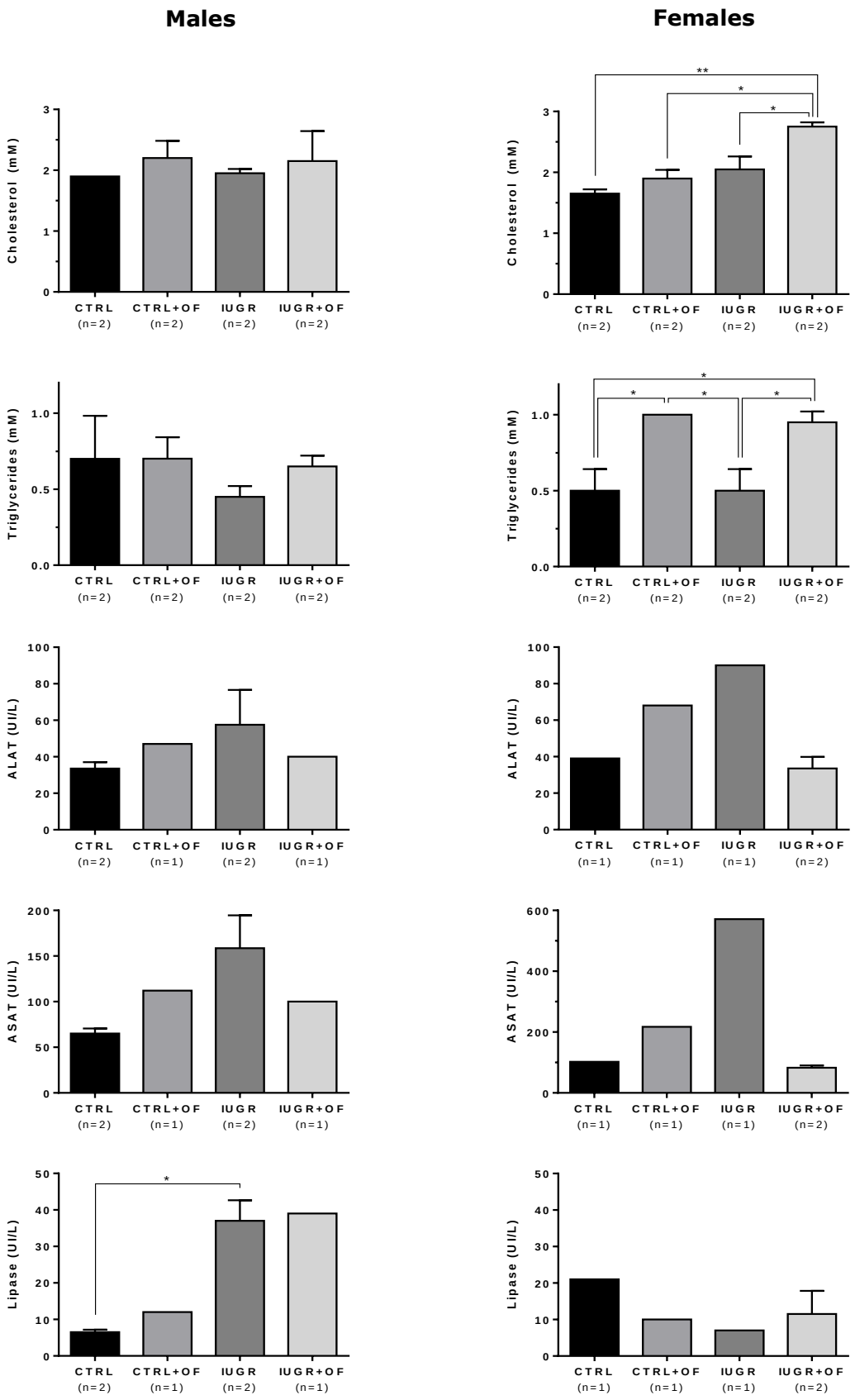


Figure 3.6 Plasma levels of cholesterol, triglycerides, *alanine aminotransferase* (ALAT), *aspartate aminotransferase* (ASAT) and lipase at 6 months of age in males (left panel) and females (right panel). Values are reported as mean ± SD. * = $p < 0.05$, ** = $p < 0.01$. Numerical values are presented in table 1.

Males		CTRL	CTRL+OF	IUGR	IUGR+OF
Body weight (g)	**Birth**	8.3 ± 1.4 (n=14)		5.6 ± 0.5 (n=10)	
	1 month	115.5 ± 11.4 (n=12)	134.8 ± 1.9 (n=2)	102.1 ± 6.6 (n=6)	131.1 ± 17.6 (n=4)
	12 months	664.1 ± 59.3 (n=12)	688.8 ± 25.7 (n=2)	582.1 ± 82.3 (n=6)	657.5 ± 27.7 (n=3)
VAT (%)	**6 months**	17.4 ± 8.9 (n=5)	25.2 ± 0 (n=1)	12.2 ± 2.9 (n=4)	19.4 ± 10.1 (n=4)
	12 months	26.4 ± 5.0 (n=4)	36.8 ± 0 (n=1)	33.9 ± 10.6 (n=5)	35.2 ± 10.6 (n=3)
SAT (%)	**6 months**	11.1 ± 3.8 (n=5)	10.3 ± 0 (n=1)	10.1 ± 5.9 (n=4)	14.1 ± 3.5 (n=4)
	12 months	18.7 ± 3.6 (n=4)	18.0 ± 0 (n=1)	22.0 ± 3.0 (n=5)	18.1 ± 6.5 (n=3)
Lean (%)	**6 months**	71.5 ± 12.2 (n=5)	64.6 ± 0 (n=1)	77.7 ± 7.6 (n=4)	66.5 ± 13.5 (n=4)
	12 months	54.9 ± 7.3 (n=4)	45.2 ± 0 (n=1)	44.0 ± 7.9 (n=5)	46.8 ± 11.3 (n=3)
Glucose	**AUC**	34.8 ± 0.6 (n=2)	42.6 ± 0.4 (n=2)	52.3 ± 5.5 (n=4)	41.3 ± 3.6 (n=3)
Liver	**Fibrosis** (%)	0.10 ± 0.06 (n=2)	0.28 ± 0.03 (n=2)	1.5 ± 0.7 (n=2)	2.9 ± 0 (n=1)
Plasma	**Cholesterol**	1.9 ± 0 (n=2)	2.2 ± 0.3 (n=2)	2.0 ± 0.1 (n=2)	2.2 ± 0.5 (n=2)
	Triglyceride	0.7 ± 0.3 (n=2)	0.7 ± 0.1 (n=2)	0.4 ± 0.1 (n=2)	0.6 ± 0.1 (n=2)
	ALAT (UI/L)	33.5 ± 3.5 (n=2)	47.0 ± 0 (n=1)	57.5 ± 19.1 (n=2)	40.0 ± 0 (n=1)
	ASAT (UI/L)	65.0 ± 5.7 (n=2)	112.0 ± 0 (n=1)	158.5 ± 36.1 (n=2)	100.0 ± 0 (n=1)
	Lipase (UI/L)	6.5 ± 0.7 (n=2)	12.0 ± 0 (n=1)	37.0 ± 5.7 (n=2)	39.0 ± 0 (n=1)

Females		CTRL	CTRL+OF	IUGR	IUGR+OF
Body Weight (g)	**Birth**	7.8 ± 1.5 (n=7)		5.0 ± 0.7 (n=18)	
	1 month	108.7 ± 11.6 (n=5)	116.5 ± 5.4 (n=2)	88.7 ± 7.1 (n=14)	109.3 ± 10.8 (n=4)
	12 months	396.6 ± 40.4 (n=5)	360.3 ± 9.4 (n=2)	325.0 ± 27.8 (n=12)	376.3 ± 36.8 (n=4)
VAT (%)	**6 months**	17.1 ± 9.0 (n=5)	20.1 ± 0.9 (n=2)	13.4 ± 4.6 (n=4)	15.4 ± 5.9 (n=4)
	12 months	19.9 ± 4.2 (n=2)	33.2 ± 0 (n=1)	22.4 ± 5.2 (n=5)	27.7 ± 10.3 (n=4)
SAT (%)	**6 months**	7.2 ± 6.7 (n=5)	6.4 ± 1.2 (n=2)	2.8 ± 2.6 (n=4)	4.6 ± 3.1 (n=4)
	12 months	10.8 ± 4.9 (n=2)	13.1 ± 0 (n=1)	9.5 ± 3.3 (n=5)	12.6 ± 3.7 (n=4)
Lean (%)	**6 months**	75.7 ± 15.7 (n=5)	73.5 ± 0.2 (n=2)	83.8 ± 6.8 (n=4)	80.0 ± 8.9 (n=4)
	12 months	69.3 ± 9.1 (n=2)	53.7 ± 0 (n=1)	68.1 ± 7.6 (n=5)	59.7 ± 13.3 (n=4)
Glucose	**AUC**	42.5 ± 3.2 (n=2)	44.5 ± 3.1 (n=2)	48.1 ± 1.0 (n=2)	36.8 ± 0 (n=1)
Liver	**Fibrosis** (%)	0.11 ± 0.05 (n=2)	0.39 ± 0.1 (n=2)	1.0 ± 0.5 (n=2)	
Plasma	**Cholesterol**	1.7 ± 0.1 (n=2)	1.9 ± 0.1 (n=2)	2.0 ± 0.2 (n=2)	2.8 ± 0.1 (n=2)
	Triglyceride	0.5 ± 0.1 (n=2)	2.0 ± 0.0 (n=2)	0.5 ± 0.1 (n=2)	0.9 ± 0.1 (n=2)
	ALAT (UI/L)	39.0 ± 0 (n=1)	68.0 ± 0 (n=1)	90.0 ± 0 (n=1)	33.5 ± 6.4 (n=2)
	ASAT (UI/L)	102.0 ± 0 (n=1)	217.0 ± 0 (n=1)	571.0 ± 0 (n=1)	82.5 ± 7.8 (n=2)
	Lipase (UI/L)	21.0 ± 0 (n=1)	10.0 ± 0 (n=1)	7.0 ± 0 (n=1)	11.5 ± 6.4 (n=2)

Table 1 Numerical result values reported as mean ± SD in male (top) and female (bottom) tables.

DISCUSSION

Developmental programming suggests that conditions affecting specific sensitive periods from conception throughout the pregnancy to early infancy, might permanently *program* organs structure and function (14). Consistent with the thrifty phenotype hypothesis, these adaptations may be suited for short-term survival in the future predicted environment, but possibly not to further life course (20). Several epidemiological and experimental studies reported that either IUGR or postnatal rapid weight gain both increase susceptibility to develop diabetes, obesity and associated disturbances later in life (12, 17, 19, 23-25, 31-33). Hypothetically, IUGR and subsequent postnatal catch-up growth might even amplify this risk (13, 16). In the present study, an IUGR and accelerated postnatal catch-up growth rat model obtained by low protein diet maternal exposure and transient postnatal *overfeeding* (OF) respectively, revealed several metabolic syndrome components in adulthood.

Reflecting experimental model attainment, body weight differences were significant in both IUGR and postnatal catch-up growth groups compared to controls. Actually, due to litter size reduction, catch-up growth groups have a smaller rat number. Consequently, an important limitation in the current study is the poor rat number, especially in these groups.

Adipose tissue accumulation is subdivided into *subcutaneous* (SAT) and *visceral adipose tissue* (VAT) compartments. Both were measured using computed tomography. However, VAT may play a key role and is strongly associated with metabolic syndrome pathogenesis (8, 9). Due to their deficient fetal environment, both IUGR male and female rats had the lowest adipose tissue (SAT and VAT) content at 6 months of age. At 12 months of age, though having the smallest body weight, IUGR rats had more VAT than controls. Thus, VAT in IUGR rats caught up and exceeded controls VAT between 6 and 12 months of age. It has been shown that postnatal catch-up growth causes an increase in adiposity rather than muscle and skeletal growth (24, 28). Indeed, catch-up growth groups (CTRL+OF and IUGR+OF) had the highest VAT content in both gender at 12 months of age. Consequently, having higher VAT content than controls, IUGR and catch-up growth groups are particularly at risk of cardiometabolic events. Regarding VAT curves evolution through time, we may hypothesize that from 12 months of age, IUGR+OF VAT percentage will be the highest, in agreement with the suspected amplified risk of postnatal catch-up growth following IUGR.

IUGR and accelerated postnatal catch-up growth revealed impaired glucose tolerance at 6 months of age in male and female rats. Similar data have been reported in both human and animal, IUGR (23) and transient postnatal overfed (33) individuals. IUGR rats had a worse glucose tolerance than catch-up growth rats, indicating a plausible IUGR superiority on catch-up growth in glucose homeostasis malprogramming. Unexpectedly, IUGR and subsequent catch-up growth male and female rats revealed better glucose tolerance than IUGR alone. Postnatal β-cells maturation was shown to be tightly influenced by early nutritional shift occurring at weaning, notably from a high fat maternal milk to a carbohydrate-rich chow diet (18, 34). A hypothetical explanation might be that transient postnatal overfeeding during lactation period partially reversed the altered β-cell programming induced by IUGR, in a compensatory mechanism. Further investigations regarding the potential incriminated mechanisms are needed to explain this observation.

Non-alcoholic fatty liver disease (NAFLD), a continuous spectrum of excessive fat accumulation, steatohepatitis, fibrosis and in worst cases cirrhosis and hepatocellular carcinoma, may be seen as the hepatic manifestation of metabolic syndrome (35). Liver histology at 6 months of age displayed a mild microvesicular steatosis in both gender, IUGR and catch-up growth groups (CTRL+OF and IUGR+OF). In accordance with their higher visceral fat content, catch-up growth rats seem to present more cytoplasmic lipid droplet accumulations than in IUGR hepatocytes. Although operator-dependent and challenging to select representative photomicrographs, increased amount of hepatic fibrosis was noticed in IUGR and catch-up growth groups compared to controls. In addition to the poor rat number in these groups, such findings should be interpreted with caution. However, they may indicate early stage of NAFLD in adulthood induced by IUGR and catch-up growth. Similar results have been reported in a transient postnatal overfeeding mice model, associated with notably hepatic oxidative stress and premature senescence (33).

Consistent with their increased adiposity, plasma analyses at 6 months of age retrieved elevated cholesterol and triglycerides plasma levels in catch-up growth rats (CTRL+OF and IUGR+OF). Human studies frequently describe altered plasma lipid profiles in obese patients (8, 10, 19), but less commonly in IUGR or catch-up growth individuals (23). The elevation was higher and statistically significant in females compared to males. Indeed, although poorly understood, sex differences mechanisms may include a probable higher amount of VAT lipolysis and hepatic free fatty acids delivery in women than in men (10). This would explain the reported female stronger correlation between VAT volume and cardiometabolic risk (8).

Several epidemiological studies reported an association between elevated circulating liver enzymes and metabolic risk, even in the absence of liver injury. Increased plasma *alanine* (ALAT) and *aspartate aminotransferases* (ASAT), as markers of abnormal liver metabolism, might play a role, or at least reflect pathogenesis of insulin resistance and metabolic syndrome (36). In fact, both ALAT and ASAT showed in a similar trend elevated levels in IUGR and catch-up growth (CTRL+OF and IUGR+OF) male and female rats. In the same way as glucose tolerance, IUGR and subsequent catch-up growth rats revealed lower transaminases levels than in IUGR alone. Moreover, higher ASAT / ALAT ratios, associated with increased mortality in cirrhosis and especially non-alcoholic cirrhosis (37), were observed in IUGR and catch-up growth rats.

Elevated circulating lipase levels, a known diagnosis criteria for pancreatitis, have also been correlated with many other intra-abdominal pathologies including hepatobiliary disorders, kidney injury due to reduced clearance, infections and diabetes (38). Of interest, lipase concentrations have been reported as approximately three-fold higher in type 2 diabetes patients in the absence of pancreatitis, although the etiology and significance of this elevation is still unknown (39). Despite great intra- and inter-individual concentration variations, significant lipase elevations were observed in male IUGR and catch-up growth groups (CTRL+OF and IUGR+OF) compared to controls. However, no increase in lipase levels were detected among females.

Taken together, these observations may possibly indicate a greater impact of accelerated catch-up growth than IUGR in increased visceral adiposity, liver steatosis, cholesterol and triglycerides levels thereafter. On the other hand, IUGR might proportionally more severely program glucose intolerance later in life than catch-up growth itself. Although surprisingly, in IUGR individuals, a rapid postnatal weight gain may potentially improve later glucose tolerance. However, such observations should be interpreted with caution until further investigations are done.

In conclusion, the present study demonstrates that IUGR and accelerated postnatal catch-up growth generate lifelong consequences with adverse metabolic outcomes thereafter. Indeed, several metabolic disturbances were retrieved in adulthood including higher visceral fat content, altered tolerance to glucose, hepatic steatosis and fibrosis, increased plasma levels of cholesterol, triglycerides, transaminases and lipase. These results highlight the latency period between early stimuli and later consequences, characterizing altered developmental programming. Therefore, more research needs to be done, particularly regarding long-term animal studies, the potentially involved mechanisms and interactions between prenatal and subsequent postnatal growth. Moreover, finding biomarkers to identify individuals at risk may provide important opportunities for lifestyle interventions and disease prevention, especially assuming the developmental programming implication in the current diabetes and obesity pandemic.

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