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Assessment of Body Composition in Wistar Rat Offspring by DXA in Relation to Prenatal and Postnatal Nutritional Manipulation.

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RUNNING TITLE: Perinatal dietary manipulation in rats

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Abstract

Background: This experimental study aims to investigate the impact of combinations of prenatal and postnatal food manipulation on body composition in rat offspring.

Methods: On day 12 of gestation, 100 timed pregnant rats were randomized into two nutritional groups: standard laboratory and 50% starved. Pups born to starved mothers were subdivided, based on birthweight (BiW), into fetal growth restricted (FGR) and non-FGR. Pups were born on day 21, cross-fostered, then left undisturbed lactating until the 26th postnatal day when they underwent dual energy X-ray absorptiometry (DXA) examination.

Results: Prenatally control fed animals had a significantly greater body weight at 26 days postnatally than the prenatally starved groups, irrespective of their postnatal diet ($p<0.001$). Postnatal control diet was associated with significantly increased abdominal and total fat in non-FGR compared to FGR rats ($p<0.001$). non-FGR/CONTROL rats showed higher values of abdominal fat than prenatally starved animals that were starved postnatally irrespective of their birth weight ($p<0.001$). Postnatal control diet significantly increased total bone mineral content (BMC), Head BMC, Head Area, Abdominal BMC in non-FGR compared to FGR rats ($p<0.001$).

Conclusions: Interaction between prenatal and postnatal nutrition affects growth, abdominal adiposity and bone accrual in Wistar rats' offspring at 26 days of life.

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Introduction

A range of genetic and environmental factors regulate growth and metabolism of individuals. Alterations in their interactions can produce adaptations that may permanently program their health and biological vulnerability to disease (1-3). Obesity is associated with an increased risk of developing cardiometabolic disease contributing to an increase in cardiovascular morbidity and mortality worldwide. The risk is profoundly influenced by the pattern of fat distribution, and abdominal obesity is a key predictive factor of the metabolic syndrome. Additionally, visceral adiposity is associated with an unfavorable metabolic, dyslipidemic, and atherogenic obesity phenotype whereas abdominal subcutaneous adipose tissue is related to a more benign phenotype described by moderate association with inflammatory biomarkers and leptin. Moreover, abdominal subcutaneous adiposity has not been associated independently with dyslipidemia, insulin resistance, or atherosclerosis in obese individuals suggesting that abdominal fat distribution defines diverse obesity sub-phenotypes with heterogeneous metabolic and atherogenic risks.(4).

Prenatal exposure to excessive or deficient nutrition alters adipocyte development (adipogenesis), permanently increasing the ability of adipose tissue to form new cells and to store lipids in existing adipocytes (lipogenesis). Adipogenesis is a late prenatal and early postnatal life phenomenon and is highly influenced by the nutritional environment at this time period. The number of adipocytes remains rather stable during adulthood, showing a very low turnover rate of adipose cells, providing evidence that events during

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both fetal and early postnatal life are vital for the overall development of adipose tissue (5, 6).

Body composition and musculoskeletal development begin in embryonic life, when bone and muscle develop from the mesodermal layer. Both bone mass and bone size increase throughout childhood, reaching their peak between 20 and 30 years of age and at the end of puberty, respectively. Optimal bone growth achieved during childhood is not only critical for ensuring optimal development and protection from fractures during childhood, but is also associated with later changes in bone mineral density (BMD) that increase bone fragility and susceptibility to fractures in adulthood (7, 8).

Accumulating data from epidemiologic and experimental studies indicate that ‘‘early-life events’’ (prenatal and early postnatal) can initiate changes in gene expression which determine not only the risk for postnatal disease but also an individual’s response to the postnatal environment (9-12). Nutrition is one of the environmental variables with the widest range of effects on physical growth, metabolism, brain, adipose and musculoskeletal development (13, 14).

Altered maternal nutrition may induce long-term metabolic consequences in offspring. However, the effects of maternal undernutrition during different developmental windows on body composition in offspring are not well defined. We investigated the effect of maternal undernutrition during pregnancy and/or lactation on postnatal growth, abdominal adiposity and bone accrual in offspring. Animal studies commonly involve nutritional interventions of fetal or neonatal environment to investigate diseases with

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developmental origins. These interventions are mainly dietary and include global caloric restriction, dietary fat supplementation or alterations of dietary protein content (15-17). The majority of studies have not distinguished between the effects of maternal diet during pregnancy and those during the lactating period since the same diet continues postnatally until weaning. The contribution of maternal diet during suckling is also important as organ development and maturation continues after birth. Moreover, mismatch between fetal and postnatal environments, through manipulation of postnatal diet could be the basis of disease manifestation according to the Predictive Adaptive Response (PAR) hypothesis. The (PAR) hypothesis refers to a form of developmental plasticity in which cues received in early life influence the development of a phenotype that is normally adapted to the environmental conditions of later life (18).

The aim of this study was to assess the effect of prenatal and postnatal food manipulation on weight status and body composition of the offspring during the lactating period. In particular, to examine the effects of prenatal starvation combined with postnatal food restriction or standard diet on growth and body composition of 26 days old Wistar rats, compared to siblings of a standard nutritional perinatal environment. We hypothesized that mismatch of prenatal and postnatal nutritional status might have adverse effects on body composition in early postnatal life.

Results

In total, 100 animals were studied. Table 1 shows the number of animals in each study group. The mean values for all DXA outcomes and comparisons between the different study groups are presented in Table 2. The DXA output provides information about the

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following masses (in grams): fat, lean tissue, and bone mineral content (BMC) of the total body and body regions.

CONTROL/CONTROL group had significantly greater values on Weight, tot. Lean, tot. BMC, Hd BMD, Hd BMC, Hd Area, Abd. T.Mass, Abd. Lean than FGR /CONTROL ($p<0.001$), indicating that normal postnatal diet does not allow complete catch-up growth of FGR pups by day 26.

Furthermore, CONTROL/CONTROL group in comparison with non-FGR / CONTROL had significantly greater values on Weight (g), tot. Lean, Abd. BMD, Abd. BMC, Abd. Area and Abd. Lean and significantly lower values on Abd. Fat and tot. Fat. Prenatally starved cases remained smaller than controls even if they reached normal body weight and received a normal postnatal diet, indicating that the effect of prenatal starvation persists to day 26.

In comparison with FGR/FR and non-FGR/FR group, CONTROL/CONTROL group had significantly greater values on Weight, tot. T.Mass, tot. Lean, tot. BMC, Hd BMD, Hd BMC, Hd Area, Abd. BMD, Abd. T.Mass and Abd. Lean.

FGR/ CONTROL group had significantly lower values on Weight, tot. Fat, tot. Lean, tot. BMC, Hd BMC, Hd Area, Abd. BMC, Abd. Area, Abd. T.Mass, Abd. Fat and Abd. Lean than the non-FGR/CONTROL group.

Furthermore, FGR/CONTROL group had significantly greater values on Weight, tot. Lean, tot. BMC, Hd BMD, Hd BMC, Hd Area, Abd. BMD, Abd. T.Mass and Abd. Lean than FGR/FR and non-FGR/FR group.

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non-FGR/CONTROL group had significantly greater values on Weight, tot. T.Mass, tot. Lean, tot. BMC, Hd BMD, Hd BMC, Hd Area, Abd. BMD, Abd. BMC, Abd. Area, Abd. T.Mass, Abd. Fat and Abd. Lean than the FGR/FR and non-FGR/FR groups.

Weight was the only parameter that was greater in the non-FGR/FR group than the FGR/FR group.

% T. Fat was lower in FGR/CONTROL than the non-FGR/CONTROL, non-FGR/FR and FGR/FR groups and lower in CONTROL/CONTROL than the FGR/FR and non-FGR/FR groups.

Abd. Fat was greater in non-FGR/CONTROL group than the FGR/CONTROL, FGR/FR and non-FGR/FR groups, while tot. Fat was greater in non-FGR/CONTROL group than the FGR/CONTROL group.

Multiple regression analyses (Table 3) for body weight showed lower values in the FR than the CONTROL group, in the FGR than the non-FGR group and in females than in males.

A significant interaction effect of groups based on postnatal diet (CONTROL or FR) with FGR indicated that differences between FGR and non-FGR group are more evident in CONTROL group. When multiple regression analyses were conducted with dependent variables the DXA outcomes (Table 3), there was a significant group effect on almost all outcomes. Specifically, for all DXA variables except for Abd. %T.Fat, in the FR group had significantly lower values than the CONTROL group. Additionally, FGR animals had significantly lower values on all DXA outcomes except for % T. Fat, tot. T. Mass, Hd BMD, Abd. BMD and Abd. %T.Fat. The effect of sex was significant on body weight,

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tot. Lean and Abd. Lean, and females had significantly lower values. No significant interaction effect of sex with groups based on prenatal or postnatal diet was found. Furthermore, the interaction effect of groups based on postnatal diet (FR and CONTROL) with FGR was significant for tot. Fat, tot. Lean, tot. BMC, Hd BMC, Hd Area, Abd. BMC, Abd. Area, Abd. Fat and Abd. Lean indicating that differences between FGR and non-FGR groups were more evident with the postnatal CONTROL diet.

Discussion

DXA is an established method for body composition assessment that effectively characterizes lean and fat volume and bone mineral density both in rodents and humans (19-21). Our study demonstrated that a combination of specific prenatal and postnatal nutritional statuses produces distinct body composition profiles in the offspring that may have potential health implications in childhood or even later in adult life.

Regarding growth, maternal control fed animals during pregnancy had a significantly greater body weight at 26 days postnatally than the prenatally starved groups irrespective of their postnatal food manipulation. The non-FGR/CONTROL animals had significantly greater body weight than the FGR/CONTROL animals, the non-FGR/FR had significantly higher body weight than the FGR/FR subjects showing that starved Rats that were born >-2 SD maintained their higher body weight compared to starved rats that were born <-2 SD, at 26 days postnatally, irrespectively of their postnatal food manipulation. Our data show that the effects of prenatal nutrition (either normal diet or starvation) on postnatal growth persist during the lactation period irrespectively of the

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postnatal environment, indicating the importance of *in utero* adverse events on postnatal growth.

Mismatch between fetal and postnatal environments could lead to adult disease (22); thus, the manipulation of postnatal diet - by exposing the developing organism postnatally to the same amount of nutrition it was exposed prenatally - could theoretically prevent adverse metabolic consequences. As current medical interventions for FGR are mainly focused on the prevention of adverse perinatal complications (23), whereas postnatal therapeutics for FGR are lacking, it might be essential for FGR infants to implement both early nutritional interventions such as the promotion of breastfeeding and the avoidance of overeating in order to catch-up and lifelong lifestyle interventions aiming at avoiding exposure to conditions of "plenty" (low fat diet consumption, regular body exercise).

Clinical studies have demonstrated a strong relation between abdominal fat and metabolic diseases, such as type 2 diabetes mellitus, dyslipidemia, and cardiovascular disease (24-26). Indeed, abdominal obesity is strongly associated with metabolic disorders in humans, and body composition analysis and fat distribution are important to study and understand the mechanisms involved in metabolic regulation. A strong relation between abdominal fat accumulation and metabolic alterations has been documented even in subjects with normal BMI (24-26).

In a recent study where DXA was used to assess abdominal obesity in an adolescent population, the strongest association was observed between abdominal obesity and insulin resistance, suggesting the key impact of abdominal obesity on the

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development of metabolic syndrome (27).

It has been postulated that the detrimental effects of visceral adipocytes on metabolism are due to their macrophage infiltration and proinflammatory cytokine production, which then influence liver metabolism and increase metabolic risk factors (28, 29). In a prospective cohort study regarding fetal and infant growth patterns and their association with total and abdominal fat distribution in childhood, growth during both fetal life and infancy affected childhood body mass index, whereas only infant growth directly affected measured total body and abdominal fat. Interestingly, fetal growth deceleration followed by infant growth acceleration may lead to an adverse body fat distribution in childhood (30). Experimental studies have also established abdominal obesity as a potential biomarker for metabolic abnormalities (liver fat accumulation, insulin resistance/diabetes), similar to that described in clinical studies (31).

In our experimental model, prenatal food restriction had a significant impact on abdominal adiposity. Starved Rats that were born >-2 SD and received standard diet postnatally showed greater values of Abd. Fat and tot. Fat. than starved animals that were born <-2 SD and that received standard diet postnatally, indicating that under standard postnatal nutrition, birth weight in prenatally starved rats may contribute significantly in adipose tissue accumulation. Starved Rats that were born >-2 SD and received standard diet postnatally showed greater values of Abd. Fat than prenatally starved animals which were starved postnatally ,irrespectively of their birthweight. Additionally the same group of prenatally starved animals showed greater values of Abd. Fat and tot. Fat than controls, whereas postnatally starved groups showed no difference in Abd. Fat and tot. Fat

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compared to controls, supporting the concept of fetal and neonatal environmental mismatch as a cause of increased adiposity and metabolic disease later in life. Our results suggest that postnatal prevention and care should be given not only to those with low birth weight, but also to offspring of mothers having experienced adverse events during pregnancy, regardless of the size of birth (16).

Fetal growth restriction (FGR) modifications to postnatal bone metabolism and skeletal growth have been associated with low bone mass in infancy, reduced bone mass and density in adulthood and increased risk for osteoporosis development in adult life, suggesting that the lack of nutrients early in life may compromise the adult skeleton (32-34). It has also been demonstrated that the fetal growth pattern affects BMC not only in small for gestational age infants, but also when birth weight is maintained within the normal range, suggesting the importance of prenatal environment in postnatal bone development (35).

Experimental studies have also demonstrated a negative long-term effect of FGR on bone size, mineral content, and strength in weaning and adult rats, speculating that FGR decreases endochondral ossification responsiveness, and in turn, postnatal linear skeletal growth, bone mineralization and strength (36). In our study, prenatal food restriction had a significant impact on bone mineral accrual, and rats that were born to food restricted mothers showed lower BMC and BMD values irrespectively of their birth weight.

Starved Rats that were born <-2 SD showed lower tot BMC, Hd BMD, Hd BMC, Hd Area whereas those born >-2 SD showed lower Abd. BMD and Abd. BMC at 26 days of life, although they received standard diet postnatally, than animals that were on a

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standard diet both pre- and postnatally. Since the nutritional postnatal environment was the same it was prenatal food restriction that adversely affected both pathophysiological mechanisms (physical growth and bone mineral accrual) either independently or in an interaction manner.

Starved Rats that were born <-2 SD and received standard diet postnatally showed lower tot. BMC, Hd BMC, Hd Area, Abd. BMC than those those born >-2 SD, indicating that under standard postnatal nutrition, birth weight contributes significantly to bone development. Interestingly, postnatal starvation eliminated the effect of birth weight on bone development between the two prenatally starved groups. However, postnatal starvation caused significantly lower values of tot. BMC, Hd BMD, Hd BMC, Hd Area, Abd. BMD than postnatal standard diet in offspring at 26 days of life.

To our knowledge, this is the first experimental study in which body composition was studied in prenatally starved nutritional groups divided by birthweight in FGR and non-FGR, with subsequent postnatal food manipulation during the lactation period. The findings of the study conclude that prenatal diet critically contributes to the determination of body composition during the early postnatal stages of life, regardless of birthweight. Pediatricians might consider not only birthweight but also prenatal adverse events *per se* for the estimation of both the metabolic risk and possible inadequate bone accrual of infants and children, promoting adequate prevention and intervention strategies. Early detection of possible pregnancy complications (diabetes mellitus, preeclampsia, fetal macrosomia, FGR etc.) (37-40) and appropriate follow-up, in addition to nutritional interventions in early infancy (breastfeeding, avoidance of overeating in order to catch-

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up), may program adult metabolic and bone health and ameliorate diseases with developmental origins, such as the metabolic syndrome and osteoporosis.

Material and Methods

This is a part of a larger study involving the effects of prenatal and postnatal food manipulation on metabolism, body composition, organ weight and tissue morphology of the offspring. Earlier publications have emanated from this study (16). The study was initiated at the Fetal Medicine Foundation and the Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK and was collaboratively conducted at the Experimental Laboratory of Aretaieion University Hospital in Athens, Greece.

Rat Model of Prenatal and Postnatal Food Manipulation

The study was approved by the Animal Research Committee of the Aretaieion University Hospital, Athens, Greece, and by the Veterinary Directorate of the Prefecture, Athens, Greece. Every effort was taken to minimize pain or discomfort in the animals.

First-time pregnant Wistar rats of the same age were obtained at 11 days of gestation (Harlan Laboratories B.V., Horst, The Netherlands) and housed individually in standard rat cages with free access to water. Rats were kept in the same room with constant temperature and humidity and on a controlled 12-hour light to dark cycle. A model of rat dams that were either normally fed or underwent 50% food restriction during pregnancy was used.

At 12 days of gestation the timed pregnant rats were randomized into one of the two following nutritional groups:

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1. Control diet Group: continued on an *ad libitum* diet of standard laboratory rodent food (4RF25, Mucedola, Milan, containing 22% protein, 3.5% fat and 50.5% carbohydrates, metabolizable energy 2789 kcal/kg)
2. Starved Group: receiving 50% food restricted diet that was determined by quantification of normal intake in the *ad libitum* fed rats.

The respective diets were given from 12 days of pregnancy to term and throughout the 25-day lactation period.

There was no significant difference of maternal body weight between two groups.

The Offspring

Rat dams gave birth normally on day 21; 24 hours after birth, the pups were culled to 8 (4 males and 4 females) per litter to normalize rearing. To differentiate the impact of prenatal food restriction and birth weight on postnatal heath, pups that were born from food restricted mothers were further divided into two subgroups:

i) FGR group: including prenatally starved neonates with mean body weight at birth < - 2SD of the mean body weight of the prenatal normally fed pups.
ii) 'non-FGR' group: prenatally starved neonates with mean body weight at birth > - 2SD of the mean body weight of the prenatal normally fed pups.

All neonates were cross-fostered to distinguish the effects of prenatal and postnatal food manipulation and to avoid bias caused by selective maternal deprivation stress. Therefore,

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we cross-fostered pups so that the offspring of mothers fed on a standard diet during pregnancy were suckled by normally fed and food restricted dams. The same cross-fostering procedure involved the offspring of food restricted mothers. Thus, 5 groups were studied:

(1) normally fed prenatally / normally fed postnatally (CONTROL/CONTROL),

(2) food restricted prenatally (FGR) / normally fed postnatally (FGR/CONTROL),

(3) food restricted prenatally (FGR) / food restricted postnatally (FGR/FR),

(4) food restricted prenatally (non-FGR) / normally fed postnatally (non-FGR /CONTROL),

(5) food restricted prenatally (non-FGR) / food restricted postnatally (non-FGR/FR).

Litters were left undisturbed until the 25th postnatal day. On postnatal day 26, offspring of all groups were assessed by DXA (Figure 1).

In this study we analyzed and discussed body composition data produced by the two types of postnatal food manipulation (control diet and food restriction) on the three groups produced by prenatal and postnatal lactation nutrition assignment (CONTROL, FGR, nonFGR). We further focused on the comparison of the impact of postnatal maternal food manipulation (control diet and food restriction) on the two subgroups of prenatally food restricted animals (FGR and nonFGR).

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Body composition assessment by Dual Energy X-ray Absorptiometry (DXA)

Animals were scanned using DXA (Lunar Prodigy, GE Healthcare, Diegem, Belgium). The rats were anaesthetized, ventrally positioned and scanned, with spine, pelvis, femur and tibia being the regions of interest (ROI) to determine the parameters of bone mineral density = BMD (g/cm2), bone mineral content = BMC (g), lean mass (g) and fat (%). Analysis was performed using the small-animal mode of the enCORE software (GE Healthcare, v. 13.40); the instrument was calibrated at each start.

Statistical analysis

Continuous variables are presented with mean and standard deviation (SD). For the comparison of DXA variables between the study groups analysis of variance (ANOVA) was used. In case of multiple comparisons, Bonferroni correction was used to control for type I errors. Log transformations were performed on non-normal variables. To investigate the independent effect of group (postnatal maternal food manipulation), FGR (prenatal events) and sex on DXA variables, multiple linear regression analyses were performed. Also, significant interactions were tested via regression analysis. Regression coefficients and standard errors were computed from the results of the linear regression analyses. All p values reported are two-tailed. Statistical significance was set at 0.05 and analyses were conducted using SPSS statistical software (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.).

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Table 1. Number of animals in each study group

	No of animals	Males	Females
A. CONTROL/CONTROL	16	7	9
B. FGR / CONTROL	16	7	9
C. non-FGR / CONTROL	20	10	10
D. FGR / FR	21	10	11
E. non-FGR / FR	27	15	12
Total	*100*	*49*	*51*

Table 2. Mean values for all DXA outcomes and comparisons between different study groups

	Control A Mean(SD)	FGR/Control B Mean(SD)	nonFGR/ Control C Mean(SD)	FGR/ FR D Mean(SD)	nonFGR/FR E Mean(SD)	P*	Multiple comparisons**
Weight (g)	129.9(16)	75.6(2.7)	105.3(23.7)	35.3(11.5)	36.8(14.2)	<0.001	A vs.B, A vs.C, A vs.D, A vs.E, B vs.C, B vs.D, B vs.E, C vs.D, C vs.E, D vs.E
% T. Fat [a]	0.3(2.1)	1.2(1.7)	7.2(12.6)	8.6(14.2)	4(14.4)	<0.001	A vs.D, A vs.E, B vs.C, B vs.D, B vs.E
tot. T.Mass (kg)	0.13(0.02)	0.07(0.01)	0.14(0.17)	0.03(0.01)	0.04(0.01)	<0.001	A vs.D, A vs.E, C vs.D, C vs.E
tot. Fat (g) [a]	0.4(2.8)	0.9(1.4)	5.1(9.7)	1.6(2.7)	0.01(4.8)	0.011	A vs.C, B vs.C
tot. Lean (g)	123.9(16.6)	71.2(5.6)	92.8(32)	31(13.5)	34.3(17.6)	<0.001	A vs.B, A vs.C, A vs.D, A vs.E, B vs.C, B vs.D, B vs.E, C vs.D, C vs.E,
tot. BMC (g)	2.3(0.3)	1.3(0.1)	2.2(0.6)	0.8(0.2)	0.9(0.3)	<0.001	A vs. B, A vs.D, A vs.E, B vs.C, B vs.D, B vs.E, C vs.D, C vs.E
Hd BMD (g/cm^2)	0.16(0.01)	0.13(0.03)	0.14(0.02)	0.11(0.01)	0.11(0.02)	<0.001	A vs.B, A vs.D, A vs.E, B vs.D, B vs.E, Cvs.D, C vs.E
Hd BMC (g)	0.9(0.1)	0.7(0)	0.8(0.1)	0.4(0.1)	0.4(0.1)	<0.001	A vs.B, A vs.D, A vs.E, B vs.C, B vs.D, B vs.E, C vs.D, C vs.E
Hd Area (cm^2)	5.4(0.5)	4.8(0.4)	5.9(0.8)	3.6(0.6)	3.7(0.6)	<0.001	A vs.B, A vs.D, A vs.E, B vs.C, B vs.D, B vs.E, C vs.D, C vs.E
Abd. BMD (g/cm^2)	0.1(0.01)	0.08(0.01)	0.08(0.04)	0.06(0.01)	0.05(0.02)	<0.001	A vs.C, A vs.D, A vs.E, B vs.D, B vs.E, C vs.D, C vs.E
Abd. BMC (g)	0.18(0.04)	0.11(0.03)	0.33(0.24)	0.13(0.07)	0.12(0.07)	<0.001	A vs.C, B vs.C, C vs.D, C vs.E
Abd. Area (cm^2)	1.7(0.4)	1.2(0.4)	5.6(4.7)	2.4(1.4)	2.5(1.3)	<0.001	A vs.C, B vs.C, C vs.D, C vs.E
Abd. %T.Fat[a]	3.1(3)	3.1(2.6)	10.6(21.5)	1.6(9)	0(14.7)	0.229	
Abd. T.Mass (g)	38.3(5.7)	20.3(2.5)	32.9(11.1)	10.9(4.7)	11.6(5.6)	<0.001	A vs.B, A vs.D, A vs.E, B vs.C, B vs.D, B vs.E, C vs.D, C vs.E
Abd. Fat (g)[a]	1.3(1.1)	-0.7(0.6)	2.3(5.1)	0(1.1)	0.6(1.8)	<0.001	B vs.C, C vs.D, C vs.E
Abd. Lean (g)	39.7(6)	20.9(2.4)	30.4(14.5)	10.9(5.3)	12.1(7.0)	<0.001	A vs.B, A vs.C, A vs.D, A vs.E, B vs.C, B vs.D, B vs.E, C vs.D, C vs.E

*p_value from ANOVA; **significant differences between groups after multiple comparisons with Bonferroni correction; [a] Comparisons were based on log-transformed values

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Table 3. Results from multiple linear regression models with dependent variables all DXA outcomes

	β(SE)[a] groups based on postnatal diet (control or FR)	β(SE)[b] groups based on division to FGR and non FGR	β(SE)[c] sex	β(SE) interaction of postnatal diet groups with FGR / non FGR
Weight(g)	68.91(4.38) †	57.45(10.85) †	7.88(3.25)*	28.31(6.59) †
% T. Fat[d]	0.55(0.15)**	0.24(0.15)	0.27(0.16)	
tot. T.Mass (kg)	0.08(0.02) †	0.03(0.02)	0.03(0.02)	
tot. Fat (g)[d]	0.32(0.09)**	0.28(0.09)**	0.16(0.09)	5.82(2.51)*
tot. Lean (g)	58.95(5.73) †	39.4(14.18)**	9.63(4.25)*	18.44(8.61)*
tot. BMC (g)	1.35(0.1) †	1.75(0.24) †	0.02(0.07)	0.87(0.15) †
Hd BMD (g/cm^2)	0.03(0.01) †	0(0.01)	0(0.01)	
Hd BMC (g)	0.45(0.03) †	0.36(0.07) †	0.01(0.02)	0.17(0.04) †
Hd Area (cm^2)	2.24(0.19) †	2.2(0.46) †	0.19(0.14)	1.06(0.28) †
Abd. BMD (g/cm^2)	0.03(0.01) †	0.0(0.01)	0.01(0.01)	
Abd. BMC (g)	0.2(0.04) †	0.45(0.1) †	0.03(0.03)	0.23(0.06) †
Abd. Area (cm^2)	3.04(0.73) †	8.77(1.81) †	0.79(0.54)	4.31(1.1) †
Abd. %T.Fat [d]	3.57(3.18)	5.43(3.18)	5.61(3.16)	
Abd. T.Mass (g)	21.36(1.97) †	24.36(4.89) †	2.56(1.46)	11.92(2.97) †
Abd. Fat (g) [d]	0.36(0.10)**	0.94(0.28)**	0.08(0.08)	0.44(0.17)*
Abd. Lean (g)	18.48(2.5) †	17.49(6.19)**	3.68(1.85)*	8.31(3.76)*

[a] Regression coefficient (Standard Error) for FR/ FR group vs. FR/Control group; [b] regression coefficient (Standard Error) for FGR vs. non FGR; [c] regression coefficient (Standard Error) for females vs. males; [d] analyses were based on log-transformed values; *p<0.05; **p<0.01; †p<0.001

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Figure legend

Figure1. Experimental design of the study.

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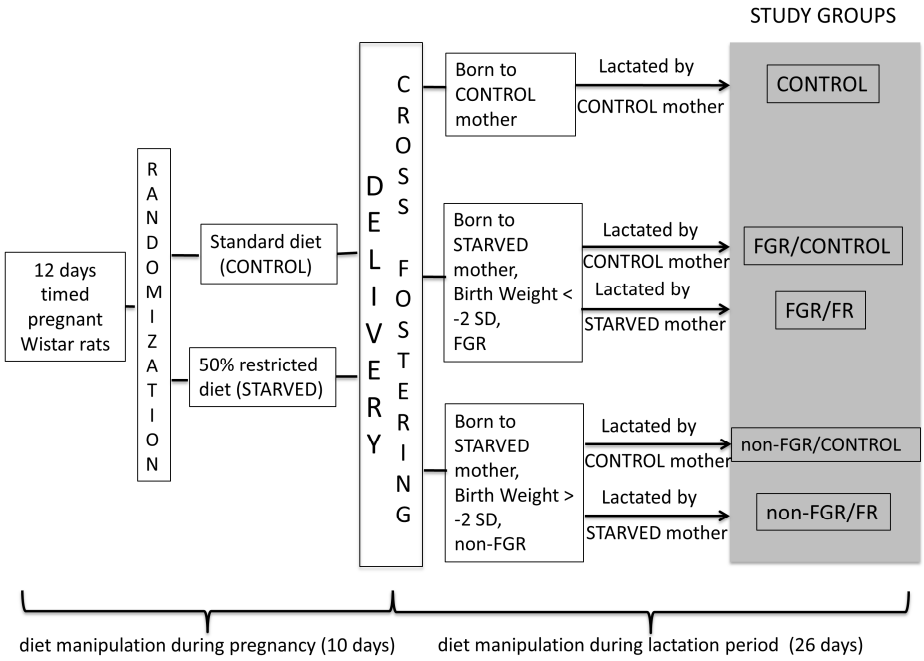
Figure 1



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