Comparison of two models of intrauterine growth restriction for early catch-up growth and later development of glucose intolerance and obesity in rats

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INTRAUTERINE GROWTH RESTRICTION (IUGR), resulting in low birth weight and subsequent rapid catch-up growth, is considered to be an important risk factor for later development of chronic noncommunicable, metabolic diseases. Several large epidemiological studies have indeed shown a link between the incidence of poor fetal growth, rapid catch-up growth, and susceptibility to the development of type 2 diabetes, hypertension, cardiovascular diseases, and obesity later in life (10–11, 13, 16, 18–19, 23–24, 27). Barker et al. (1) have proposed that the origin of adult metabolic diseases is during fetal life and that suboptimal environmental factors, which hinder growth in the uterus, lead to long-lasting alterations in the structure and function of developing tissues, as well as changes in the neuroendocrine system. According to this hypothesis, such fetal “programming,” though beneficial for survival in a poor nutritional environment, may lead to higher risks for chronic diseases during improved nutrition and catch-up growth later in life.

To investigate the mechanisms by which intrauterine growth restriction (IUGR) predisposes to later development of metabolic diseases, different animal models have been developed and widely used during the last two decades, in particular, those based upon uterine artery ligation, prenatal food or protein restriction and exposure to specific hormones or to hypoxia during gestation (4, 15, 22). The choice of the animal model is often based upon the investigator’s experience and practical aspects of model development. However, it is uncertain to what extent these different IUGR models mimic human fetal growth restriction and how they might influence later catch-up growth, which is believed to be a central component in the link between IUGR and the later development of metabolic diseases in humans.

Indeed, a recent large-scale study has shown that uterine artery ligation in rats (one of the most frequently used animal model for IUGR investigations) does not necessarily lead to IUGR nor to neonatal catch-up growth in the offspring, and hence raises questions about the suitability of this model for providing insights into the mechanisms linking IUGR to later diseases in humans (25).

Other animal models of IUGR based upon maternal food restriction and prenatal hormonal stress are reported to induce IUGR, subsequent catch-up growth, and later health consequences. For example, in mice, food restriction by 50% during the last week of pregnancy reduces birth weight by 23% and results in permanent pancreatic β-cell dysfunction. This is followed by weight normalization after birth through catch-up growth and these offspring subsequently develop severe glucose intolerance by 6 mo of age (20). In rats, the induction of IUGR and low birth weight by prenatal food restriction or by maternal dexamethasone exposure is also reported to result in later glucose intolerance and obesity in the offspring (7, 17, 26, 32). However, although these two models of IUGR (maternal food restriction and glucocorticoid exposure) are commonly used in research, a direct comparison between these two models pertaining to the link between small birth weights, the kinetics of catch-up growth and the development of obesity and glucose intolerance are still lacking.

In this study, we have directly compared these two rat models of IUGR (maternal food restriction and dexamethasone exposure) for early catch-up growth and later glucose intolerance and obesity. To this end, we have followed their growth from birth until the age of 6 mo, and assessed body composition, blood glucose, and insulin concentrations at baseline, as well as during a test of glucose tolerance at 8, 16, and 22 wk of age. Pancreas insulin content, liver triglycerides, and plasma corticosterone were also determined at the end of the study (wk 22).

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MATERIALS AND METHODS

Animals and experimental design. The study was approved by the “Office Vétérinaire Cantonal Vaudois,” Lausanne, Switzerland. Programmed mated female Sprague-Dawley (SD) rats were purchased from Charles River (France). Virgin rats (225–250 g) were single coupled for 12 h and those with expelled vaginal plugs and four nonpregnant rats were selected and sent to our research center. After arrival, the animals were caged individually in a room at 23°C, with 55% relative humidity and a 12:12-h light-dark cycle. Animals had free access to water and a commercial chow rat diet with energy composition of 25% protein, 13% fat and 62% carbohydrate (Kliba 3434; Provimi, CH-4303 Kaiaeraugt, Switzerland) during the study unless otherwise indicated.

On postcoupling day 10, animals were randomly assigned to one of the three study groups: food restriction, dexamethasone, and control (FR, DEX, and CON, respectively; n = 6–7 per group), so that body weights and body fat contents were similar. The food intake of animals in Group FR was restricted to 50% intake of nonpregnant rats of similar body weight during last 11 days of gestation.

Group DEX received a subcutaneous injection of dexamethasone (100 μg/kg body wt/day, dissolved in 4% ethanol-0.9% saline) during the last week of gestation (days 15–21) of gestation. The choice for differential timing for start and duration of maternal manipulation by DEX and FR exposures (i.e., during the last 7 and 11 days of gestation, respectively) was based upon the common practice of utilizing DEX exposure during the last week of gestation (15, 26) in showing birth weight reduction and later hyperglycemia in the offspring, and 2) while taking into account other rat studies indicating that maternal FR has an impact on later impairment of glucose homeostasis in the offspring only when the FR procedure intervention during the last 11 days of gestation or longer (15, 17), but not when applied only during the last week of gestation (3).

All dams gave birth on postmating day 22 within a 20-h interval (from 10:00 AM through the night). After birth, the body weights of the pups were recorded within 12 h (referred to as birth weight), and only dams with 10–14 pups per litter were selected to continue the study (2 or 3 dams/group). The number of pups was reduced to 8 per litter during first day of birth, and they suckled milk from their own mothers until 21 days of age. Male pups were then separated from their mothers (n = 12, 10, and 7 in groups FR, DEX, and CON, respectively), caged individually, and fed ad libitum with chow diet until the end of the study.

Food intake of gestating and nonpregnant rats was measured daily during days 10 to 22 postmating, and the mean values were 18.6, 26.8, 9.3, and 24.6 g/day in nongestating females, CON, FR, and DEX groups, respectively. Thus, the food intake of FR dams was 50% lower relative to nongestating females and 65% lower relative to CON-gestating dams, while that of DEX dams was 8% lower than CON dams. Body weight and food intake of each pup were measured 2 or 3 times per week throughout the study, except during the suckling period. Body composition (body fat, lean mass, and body water content) was measured with NMR, using EchoMRI 2004 (Echo Medical Systems, Houston, TX) at 7, 16, 20, and 22 wk of age, and the lean dry mass (a proxy for protein mass) was calculated.

Animals were killed by decapitation at 22 wk, after 6-h daytime food deprivation (from 7:30 AM to 1:30 PM). Blood samples were collected into tubes containing EDTA. Plasma was separated (10 min centrifugation at 2,200 rpm), frozen in dry ice, and kept at −80°C until analysis. Different organs (pancreas, liver, and kidney) were dissected, weighed, frozen in dry ice, and kept at −80°C until analysis.

Intraperitoneal glucose tolerance test (IPGTT) and biochemical analysis. The IPGTT was performed in all animals after NMR measurement following 6 h of daytime food deprivation (from 7:30 AM to 1:30 PM) at 8, 16, and 22 wk of age. Two baseline blood samples (50 μl) were taken from the tail vein, with at least 10 min (time – 10 and 0 of study), followed by an intraperitoneal injection of a glucose solution (1.67 mol/l) (o-glucose; Merck, Darmstadt, Germany) at dose of 2 g glucose/kg body wt. Six further blood samples were collected (50 μl) from the tail vein at 15, 30, 45, 60, 90, and 120 min after glucose administration.

Glucose during the IPGTT was measured in blood using a glucometer (Ascensia ELITE XL; Bayer, Mishawaka, IN). Insulin was measured in plasma and after extraction from pancreas by acid-alcohol solution (vol/vol: 75% ethanol, 1.5% of 37% HCl and 23.5% distilled water) (28) with an ELISA method using a kit from Crystal Chem (Downers Grove, IL). Liver triglycerides were extracted by hexane/isopropanol (3:2, vol/vol), as previously described (14) and measured by colorimetric method using enzymatic Biomérieux PAP 150 kit (Lyon, France) following saponification (with KOH in ethanol at 70°C for 1 h) and neutralization (with MgSO4). Plasma corticosterone was analyzed by enzyme immunoassay (EIA) method using a kit from Immunodiagnostic Systems (Frankfurt am Main, Germany).

Statistical analysis. Results are expressed as means or medians and their SEs. Median and Rousseuw robust standard deviation Sn were used as robust estimators, when there were some discrepancies from normality in data distribution. In most of the cases, these deviations from normality were due to outliers. All data, except IPGTT results, were analyzed using Kruskal-Wallis test and Wilcoxon tests with Bonferroni alpha adjustment for the comparison FR vs. CON and DEX vs. CON. ANOVA and Dunnett’s tests were used for the IPGTT glucose data. For most of the parameters, the variability in Group DEX was higher than in the other two groups. To define at which age the body weights of pups from Group FR and DEX caught up with those of Group CON, with a 95% confidence interval, the Bootstrap method was used. On each subsample, catch-up time was calculated by linear interpolation between two consecutive time points. The level of significance was set at P < 0.05. The data were analyzed using the software SAS 8.2.

RESULTS

Body weight at birth and during the suckling period. Figure 1 illustrates body weight at birth. Both maternal interventions (food restriction and dexamethasone exposure) led to significantly lower pup birth weights than those in the control group. Relative to the control group, the median reduction in birth weight of pups of FR dams (18–22%) was similar to that of pups of DEX dams (20–26%), whether for both sexes considered together (n = 16–24/group), for sex analyzed separately.
(n = 6–15/group), or for male pups that continued the study (n = 7–11/group) (P < 0.0001, in all cases).

During the suckling period, the pattern of weight gain was different among the three groups. As shown in Fig. 2A, the rate of weight gain (median + SE) was significantly higher in Group FR than group CON. Weight gain over the 1st postnatal week (days 1–7) in FR and CON groups was 11.7 ± 0.4 vs. 10.2 ± 0.4 g (P = 0.007); at postnatal week 2 (days 1–15), it was 33.7 ± 0.4 vs. 28.9 ± 0.5 g (P = 0.0001); and at postnatal week 3 (days 1–21) it was 53.3 ± 0.8 vs. 46.7 ± 1.0 g (P = 0.008), respectively. These differential weight gains resulted in a significantly greater body weight at 15 days and 21 days in group FR than in group CON (P = 0.007 and P = 0.03, respectively) (Fig. 2B). For pups from Group DEX, the rate of weight gain (median ± SE) was slightly higher than that of Group CON at postnatal week 2 (29.6 ± 0.5 vs. 28.9 ± 0.5) and week 3 (47.6 ± 1.5 vs. 46.7 ± 1), but these differences were not statistically significant (Fig. 2A). This resulted in their absolute body weights remaining still significantly lower than that of Group CON at age of 7 days (P = 0.002) but not later at 15 and 21 days of age (Fig. 2B). Thus, although the offspring of both maternal interventions (Groups FR and DEX) demonstrated catch-up growth during the suckling period, this occurred much earlier in Group FR than in Group DEX. This is underscored by the analysis of data using the Bootstrap method (95% CI) showing differential values (means ± SE) corresponding to the age at which the body weights of pups from Group FR (6.1 ± 0.9 days old) and Group DEX (25.1 ± 6.6 days old) had caught up with those in Group CON.

Postweaning body weight, body composition, and food intake. Although the postweaning body weights of the three groups were not statistically different, but both the absolute final body weight and weight gain (median + SE) showed a tendency to be higher in Group FR (by 11%) and in Group DEX (by 9%) than in Group CON (587 ± 19, 570 ± 32 and 523 ± 10 g for body weight and 528 ± 19, 515 ± 31 and 470 ± 12 g for weight gain, respectively).

The data on dry body weight and body composition assessed at 7, 16, 20, and 22 wk of age are presented in Fig. 3. There are no differences in dry body weight and body fat mass at week 7 (Fig. 3, A and B, respectively). Over subsequent weeks, the data on dry body weight and body fat were consistently higher in Group FR than in either Groups DEX or CON; however, these differences did not reach statistical significance. Dry lean mass (Fig. 3C) was also consistently higher in Groups FR and DEX relative to the control group, CON, and this difference was found to be statistically significant only between FR and CON groups at 22 wk of age (P < 0.007) with a strong tendency at 7 wk (P = 0.052). Thus, the ratio of fat mass to lean body mass (Fig. 3D), an index of obesity, was consistently but not statistically lower in Group DEX relative to Group FR and the control group. These tendencies for differences in body composition across these three groups were observed with similar median food intakes during the postweaning period (3 to 22 wk) between groups FR and DEX (3,510 ± 84 and 3,590 ± 200 g, respectively) but slightly lower in the Group CON (3,310 ± 61 g). However, these 6–8% differences in food intake between the maternally treated groups and the control group were not statistically significant.

Blood glucose and insulin in plasma and pancreas. Figure 4, A–C illustrates blood glucose at baseline (after 6-h food deprivation) and in response to IPGTT, assessed at 8, 16, and 22 wk of age. Group FR had higher baseline glucose concentrations when compared with Group CON, at 8, 16, and 22 wk, with these differences being significantly different at 22 wk of age (P < 0.02). During the IPGTT, the 2-h glucose area under the curve (AUC; Fig. 4, A–C) was greater in Group FR than Group CON at all ages with these differences being significantly different at 8 and 22 wk of age (P = 0.02 and P = 0.04, respectively). This variable was lower in Group DEX and not significantly different from that of Group CON at all assessed ages. The peak values for glucose concentrations were not significantly different among groups at all assessed ages.

Baseline insulin concentrations, peaks of insulin, and 2-h insulin AUC in response to IPGTT had a large within-group variation, and they were not significantly different among groups at all assessed ages.

The insulin content of the pancreas was lower in both maternally treated groups than in Group CON (median + SE values of 192 ± 16, 150 ± 18, and 328 ± 82 µg/pancreas in FR, DEX, and CON groups, respectively), with this difference reaching statistical significance only between DEX and CON groups (P = 0.03) (Fig. 5B).

Fig. 2. Weight gain (A) and body weight (B) of male offspring of FR, DEX, and CON groups (3 or 4 pups from each litter/group) during the suckling period. Values are expressed as medians + SE. Within the same age, *FR vs. CON, P = 0.007 (day 7), P < 0.0001 (day 15), and P = 0.008 (day 21); †FR vs. CON, P = 0.0002 (day 1), P = 0.007 (day 15), and P = 0.03 (day 21); and ‡DEX vs. CON, P = 0.0003 (day 1), P = 0.002 (day 7).
Organ weights, liver triglycerides, and plasma corticosterone. Figure 5A illustrates that at the end of study (age 22 wk), the FR group had significantly heavier liver relative to CON group ($P < 0.01$), but the liver triglycerides content was not significantly different between all groups (Fig. 5C). The weight of other assessed organs (heart, pancreas, spleen, and kidney) and fat pads (retroperitoneal and epididymal) were similar in all groups (Fig. 5A). The plasma corticosterone concentration had a large within-group variation and was not significantly different among groups (median $\pm$ SE of 84.7 $\pm$ 24, 79.4 $\pm$ 19, and 130.6 $\pm$ 34 ng/ml in groups FR, DEX, and CON, respectively) at the end of study (age 22 wk).

**DISCUSSION**

The results of the present study indicate that prenatal food restriction is a more sensitive model than gestational dexamethasone exposure for investigating the consequence of IUGR and rapid catch-up growth on glucose tolerance in SD rats: only gestational exposure to food restriction, but not to dexamethasone, led to rapid postnatal catch-up growth and...
later development of glucose intolerance at age of 22 wk. There are conflicting reports on the development of adult hyperphagia after fetal growth retardation (5, 7–8, 21, 32) with reports showing that the hyperphagia is more pronounced in response to postnatal hypercaloric diet in some animal studies (7, 32), but not in others (5). In the study reported here, we observed no significant increase in food intake nor in body composition in both IUGR groups relative to control.

Both types of prenatal interventions resulted in significantly lower birth weights of pups (Fig. 1). However, only the offspring exposed to prenatal food-restriction demonstrated consistent and significant impairments in glucose homeostasis later in life, namely, low glucose tolerance (postprandial hyperglycemia) at 8 and 22 wk of age (Fig. 4, A and C), and higher baseline blood glucose (hyperglycemia) at 22 wk of age (Fig. 4C). The observed different responses in glucose homeostasis between the two IUGR models used in this study cannot be explained by degree of IUGR. In fact, the reduction in birth weight in the male offspring of the food-restricted group (23% less than controls) was similar to that observed in those whose mothers were exposed to dexamethasone (25% less than controls) (Fig. 1). The reduction in birth weight observed in the present study is in agreement with that observed in other studies using animal models of IUGR (7, 9, 20). Furthermore, the differential responses in glucose homeostasis cannot be attributed to differences in plasma insulin at baseline or in response to IPGTT, postscucking food intake, body weight, and body composition. In fact, these parameters, which are known to influence insulin sensitivity (6), were not significantly different between the offspring of the maternally treated groups when compared with the control group at the time points when the IPGTT was performed later in adult life. In addition, the plasma corticosterone concentrations in offspring of FR and DEX groups were similar and not significantly different from that of CON group at the end of study (age 22 wk).

The mechanism of IUGR programming of adult metabolic disease is not known. However, it has been associated with pancreas beta cell development and function (1, 12, 20, 29), as well as with insulin resistance linked to postnatal catch-up growth (2). Although a reduction in fetal beta cell mass is reported in different IUGR models, the underlying cellular and molecular mechanisms are not the same in these various IUGR models (9, 29, 30). For example, the deficit in beta cell mass in IUGR models of food restriction and dexamethasone is proposed to be due to the downregulation of pancreatic and duodenal homeobox-1 (Pdx1) and other transcription factors involve in beta cell differentiation, to early alteration of beta cell neogenesis (as a consequence of elevated glucocorticoid), or to the proapoptotic effect of dexamethasone (9, 29, 30, 31). In the protein-restriction model, by contrast, the mechanisms of programming have been associated with increased beta cell proliferation, low islet vascularization, and apoptosis (9, 29, 30). These parameters were not assessed in our study and could have an impact on the differential glucose homeostasis responses observed between our two IUGR models.

We observed a lower pancreatic insulin content in the DEX group (54% lower than control, \( P < 0.05 \)) at age of 22 wks (Fig. 5B), but without consequences on plasma insulin at baseline or in response to a glucose load during IPGTT. Although a reduction in pancreatic insulin content was also observed in the food-restricted group (41% lower than in controls), this difference was less pronounced, statistically insignificant and hence cannot explain the findings here of an impairment in glucose tolerance observed only in the offspring of the food-restricted group but not in those of DEX group.

In contrast, we observed a significant difference in dynamics of body weight gain during the first few weeks of life (Fig. 2A). The offspring exposed to gestational undernutrition demonstrated the most rapid catch-up growth during the first week of life and attained the body weight of the controls by 6 days of age (range of 4.1–7.7 days; 95% CI) (Fig. 2B). On the other hand, offspring of dams exposed to gestational dexamethasone treatment exhibited much slower catch-up growth and only attained the body weight of the controls much later—by 25 days of age (range 19.4–45.3 days; 95% CI). The catch-up growth is likely to be due to higher milk intake of both IUGR groups relative to the CON group during lactation. However, the mechanisms that lead to differential dynamics of catch-up growth between offspring of FR and DEX groups are not clear and may be due to different effect of gestational interventions on milk quantity and/or milk quality (hormonal or compositional impact).

Perspectives and Significance

Our study indicates that programming for later impairment in glucose homeostasis is associated with early and rapid catch-up growth during suckling period. This association is demonstrated to occur even in the absence of a high-fat diet (since the animals consumed a low-fat chow diet) and furthermore without excess adiposity in adult life. Indeed, the conse-
sequence of IUGR and rapid catch-up growth on the development of obesity has been reported when animals were challenged with a high-calorie diet during the post-suckling period (5, 21, 32) or in a longer-term study of 9-mo duration (7). For example, in a study using Wistar rats, offspring of nutrient-restricted dams, demonstrated amplified obesity development, when challenged with a high-calorie postnatal diet (5). Consequently, our prenatal food restriction model of IUGR, which shows programming for impaired glucose homeostasis, even on a low-fat diet (13% fat E) and in the absence of excess adiposity, may be particularly susceptible to insulin resistance and obesity in adult life if challenged with a more energy-dense (high-fat) diet. Overall, the results reported here suggest that maternal food restriction during gestation is a more sensitive model than gestational hormonal (dexamethasone) intervention for rat studies aimed at investigating the link between low birth weight, rapid postnatal catch-up growth and later development of glucose intolerance.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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