



Nutritional programming of physical development and bone growth of offspring by a maternal high-fat diet

Programação nutricional do desenvolvimento físico e do crescimento ósseo da prole por dieta materna hiperlipídica

Programación nutricional del desarrollo físico y crecimiento óseo de la descendencia mediante una dieta materna rica en grasas

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ABSTRACT

Objective: Investigate the influence of a maternal extended high-fat diet (HFD) consumption, an animal model for the human Western diet pattern, combined to preconceptional obesity on offspring development and growth. **Methods:** Female Wistar rats were subjected to HFD (52% energy from fat - primarily sourced from lard) or a control diet (12% energy from fat) from 37 days of life until the end of lactation. The dams in the HFD group were obese at the time of mating. Post-weaning, all offspring consumed a standardized diet. Somatic growth, physical maturation, neurobehavioral assessments, locomotor performance, and linear cephalometric measurements of the mandible (anteroposterior and alveolar lengths) and skull (diastema and alveolar lengths) were evaluated in both groups of offspring. **Results:** The offspring in the HFD group exhibited prolonged latency in the negative geotaxis reflex, lateness mandibular incisive eruption, and notable reductions in somatic growth and all linear cephalometric measurements when compared to the control group. **Conclusion:** The long-lasting maternal HFD exposition and preconceptional obesity leads to a delay in offspring physical development and bone growth.

Keywords: Western diet, Animal model, Maternal exposure, Growth and development.

RESUMO

Objetivo: Investigar a influência do consumo materno prolongado de dieta hiperlipídica (DH), modelo animal para o padrão alimentar ocidental humano, combinado à obesidade pré-concepcional no desenvolvimento e crescimento da prole. **Métodos:** ratas Wistar foram submetidas à DH (52% de energia proveniente de gordura - principalmente de banha de porco) ou dieta controle (12% de energia proveniente de gordura) dos 37 dias de vida até o final da lactação. As mães do grupo DH apresentavam obesidade ao acasalamento. Após o

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desmame, todos os filhotes consumiram dieta padrão de laboratório. O crescimento somático, a maturação física, as avaliações neurocomportamentais, o desempenho locomotor e as medidas cefalométricas lineares da mandíbula (anteroposterior e comprimentos alveolares) e do crânio (diastema e comprimentos alveolares) foram avaliados em ambos os grupos de proles. **Resultados:** As proles do grupo DH demonstraram latência prolongada no reflexo de geotaxia negativa, erupção incisiva mandibular tardia e reduções notáveis no crescimento somático e em todas as medidas cefalométricas lineares quando comparadas ao grupo controle. **Conclusão:** A exposição materna prolongada à HD e a obesidade preconcepcional resultaram em atraso do desenvolvimento físico e do crescimento ósseo da prole.

Palavras-chave: Dieta ocidental, Modelo animal, Exposição materna, Crescimento e desenvolvimento.

RESUMEN

Objetivo: Investigar la influencia del consumo materno prolongado de dieta alta en grasas (DAG), un modelo animal del patrón de dieta occidental humano, en combinación con obesidad preconcepcional en el desarrollo y crecimiento de la descendencia. **Métodos:** Ratas hembra Wistar fueron expuestas a DAG (52 % de energía procedente de grasa, principalmente manteca de cerdo) o a dieta de control (12 % de energía procedente de grasa) desde los 37 días de vida hasta el final de la lactancia. Las madres en el grupo DAG estaban obesas en el apareamiento. Después del destete, todas las descendencias consumieron dieta estandarizada. Crecimiento somático, maduración física, evaluaciones neuroconductuales, rendimiento locomotor y mediciones cefalométricas lineales de la mandíbula (anteroposterior y longitudes alveolares) y del cráneo (diastema y longitudes alveolares) fueron evaluados en ambos grupos de descendencias. **Resultados:** La descendencia en el grupo DAG exhibió latencia prolongada en el reflejo negativo de geotaxis, erupción tardía de los incisivos mandibulares y reducciones notables en el crecimiento somático y en todas las mediciones cefalométricas lineales en comparación con el grupo control. **Conclusión:** La exposición materna prolongada a la DAG y la obesidad preconcepcional conducen a retraso en el desarrollo físico y el crecimiento óseo de la descendencia.

Palabras clave: Dieta occidental, Modelo animal, Exposición materna, Crecimiento y desarrollo.

INTRODUCTION

The Western diet pattern has severe health consequences, including obesity, type 2 diabetes mellitus (T2DM), cardiovascular disease, metabolic syndrome, and osteoarthritis (POPKIN BM and NG SW, 2022). Women of childbearing age and during pregnancy and lactation who face an obesogenic environment due to this diet pattern have increased risk of overweight/obesity (BOWERS K, et al., 2012). Moreover, maternal diet and nutritional status significantly influence offspring development and should be considered during the mother-baby relationship phases, such as preconception, gestation, and breastfeeding (BARKER M, et al., 2017; CERF ME, 2018). According to the theory of developmental origins of health and disease (DOHAD) stimuli and insults from prenatal to early postnatal stages can permanently alter offspring physiology, metabolism, and epigenetics, even in later life (BARKER M, et al., 2017; SUTTON EF, et al., 2016).

Longitudinal studies have shown that a Western diet during pregnancy can cause in children lower whole-body bone area, bone mineral content, and bone mineral density and increased risk of fracture when compared with children born to mothers who had a low-fat diet during pregnancy (JENSES KH, et al., 2020). While there is a need for studies in this field, ethical concerns and the challenge of isolating dietary factors and pathological conditions in clinical research have limited such investigations in humans (GRØFTE T, et al., 1997).

Therefore, preclinical studies represent an option to simulate human Western diet consumption and provide insight into the phenotypes developed by offspring after maternal high-fat diet (HFD) exposition. To accomplish this, it is necessary to expose the animals to an HFD comprising 35-60% of energy from fat for at least three weeks (HINTZE KJ, et al., 2018), preferably starting from preconception and continuing through lactation until weaning, with the primary fat source being lard (TELLECHEA ML, et al., 2017).

Considering the importance of maternal nutrition in program offspring development, the influence of maternal HFD on bone growth has been studied (ZHENG J, et al., 2018). Preclinical studies have revealed delays in skeletal development, including shorter craniofacial lengths, in offspring exposed to maternal HFD from preconception to gestation (CHEN J, et al., 2012; CHEN J, et al., 2018; LIANG C, et al., 2009). Additionally, maternal HFD during lactation led to delayed somatic growth (GIRIKO CÁ, et al., 2013), while maternal HFD during gestation and lactation resulted in decreased femur length in adulthood (KUSHWAHA P, et al., 2021). Environmental and nutritional factors can impact the structure of flat and irregular bones, including skull development (ANDREU-ARASA VC, et al., 2018). However, the impact of maternal HFD on the offspring growth of flat and irregular bones, such the skull and mandible, remains unexplored, and the physical development between birth and puberty needs further investigation.

In this sense, we hypothesized that a maternal HFD comprising 40-60% energy from fat, mainly lard, during peripubertal age through the nursing period, could significantly impact physical development and bone growth, even if a balanced diet is consumed after weaning. To confirm this hypothesis, our investigation aimed to assess neurobehavioral evaluations, morphometric measurements, locomotor performance, and linear cephalometric measurements of the mandible and the skull among offspring submitted to a control and high-fat maternal diet.

METHODS

Animals

Virgin female ($n = 10$) and male ($n = 10$) albino Wistar rats (*Rattus norvegicus*) were taken from the Central Animal House of the Federal University of Pelotas (Campus Capão do Leão). The animals were maintained in pairs in open, wire-top, polypropylene cages in a room at 22 ± 1 °C with lights on from 6:00 a.m. to 6:00 p.m. and free access to water. The experimental procedures followed the international norms for the handling, care of, and experimentation with animals that were established by the International Guiding Principles for Biomedical Research Involving Animals and approved by the Commission of Ethics in Animal Experimentation of the Federal University of Pelotas, Pelotas, RS, Brazil (protocol number: 23110.029720/2018-42). All efforts were taken to minimize the number of animals used and their suffering. The study conformed with the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines for preclinical animal studies. The method of determining sample size was based in the tradition or common sense, considering the low number of animals to allow discovering the effect sizes in different equations according to one of the main outcomes (cephalometric measurements of the mandible) (FESTING MFW, 2018).

Diet composition

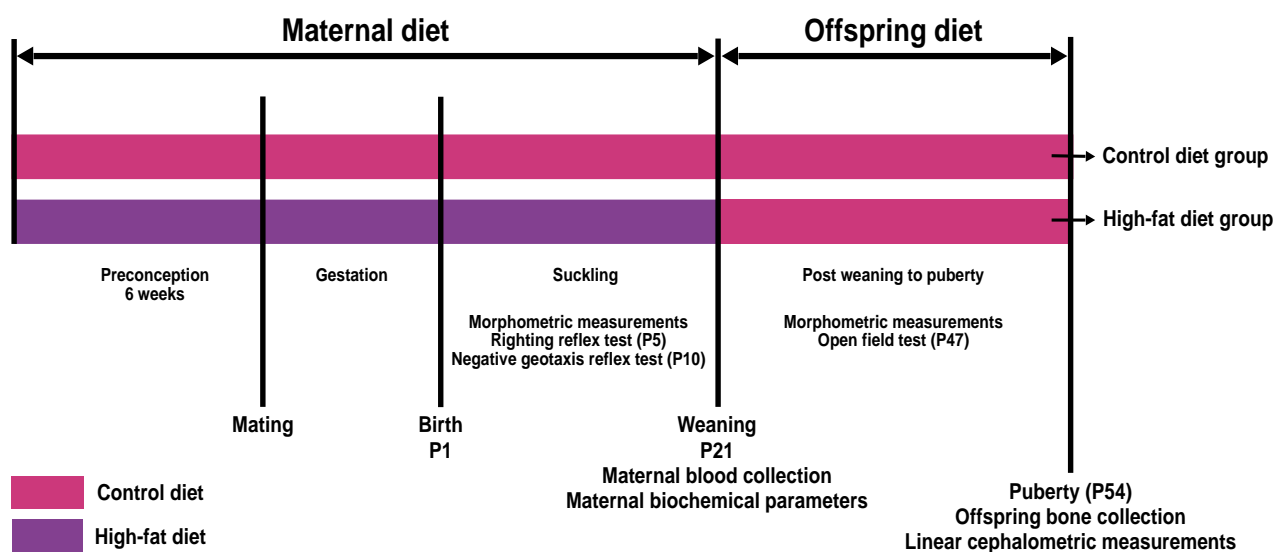
The animals were submitted to a normocaloric and normolipidic Nuvilab CR-1 irradiated feed produced by Quimtia® (Curitiba, Brazil) composed by 12% of energy from fat in 3,5 kcal/g or an hypercaloric and hyperlipidic diet manufactured with powdered standard chow enriched with lard, resulting in 52% of energy from fat in 4,9 kcal/g (Supplementary material table S1).

Experimental protocol

Female rats, divided into two randomized groups: the control diet group (CD; $n = 5$) and the HFD group (HFD; $n = 5$), were exposed to a normocaloric and normolipidic and a hypercaloric and hyperlipidic diet, respectively, from peripubertal age (37 days old) until the end of lactation period. Males (maintained in a control diet) and females (CD: body weight average 249 g; HFD: body weight average 203 g) were mated for five consecutive days approximately six weeks after the start of HFD exposure. There was no incidence of abortion or stillbirths. To minimize the potential effects of litters, two to three pups from each litter were used for each measurement (TOPPER LA, et al., 2015). The day of parturition was considered postnatal day 1 (P1). A litter was formed of a mean of 8 ± 2 pups (5 ± 2 male pups per litter). The litter size was not significantly different between groups. Male pups represented 57% of the total offspring. Pups were counted and sexed according to the anogenital distance at PD5 to limit possible early-life maternal separation stressors. The tails of each pup were marked for individual identification. Morphometric measurements were taken weekly throughout the

experiment. The offspring neurobehavioral was assessed by the evaluation of righting reflex test at P5 and negative geotaxis test at P10. After the weaning (P21), the litters were divided into males and females, identified according to maternal diet (offspring from CD group: OCD; offspring from HFD group: OHFD) and both groups received the normocaloric and normolipidic diet Nuvilab CR-1. The offspring locomotor activity and exploratory behavior were assessed by open field test at P47. At the end of suckling for the dams and at puberty for the offspring (P54), the animals were anesthetized with isoflurane inhalation (5%, 0.25 mL of liquid isoflurane per liter of chamber volume) followed by cardiac puncture after confirmed blockade of pain sensation and the offspring craniomandibular structures were collected for the linear cephalometric measurements. The maternal whole blood was collected, centrifuged at 3000 g (RCF) at 4° C for 5 minutes (Eppendorf™ Centrifuge 5427R), aliquoted in serum samples and stored at - 80° C for further analysis (Fig. 1).

Figure 1 - Experimental research design.



Subtitle: P1: postnatal day 1; P5: postnatal day 5; P10: postnatal day 10; P21: postnatal day 21; P47: postnatal day 47; P54: postnatal day 54. **Source:** Giorgi R, et al., 2024.

Maternal biochemical parameters and obesity index

The body weight was collected with an electronic scale (Geom, model BEL-00052, graduation of 1 gram (g)). The naso-anal length was measured using a plastic, non-extensible measuring tape with an accuracy of 0.1 centimeters (cm). Lee index [$\sqrt[3]{\text{body weight (g)/naso-anal length (cm)}}$] was used to assess obesity, classifying the animal as obese when the value was more than 0.300 plus a statistical difference between groups (BERNARDIS LL and PATTERSON BD, 1968). Serum total cholesterol (Cobas, Roche, Indianapolis, IN, USA; 03039773), high-density lipoprotein cholesterol (Cobas, Roche, Indianapolis, IN, USA; 04399803), triglycerides (Cobas, Roche, Indianapolis, IN, USA; 20767107) and glucose (Cobas, Roche, Indianapolis, IN, USA; 04404483) were analyzed by enzymatic methods assay kit according to the manufacturer's instructions (Roche Diagnostics).

Offspring somatic growth

The animals were gently immobilized through traction application and the body length (distance between the snout and the base of the tail) and the tail length (distance between the base and the end of the tail) were made using a plastic, nonextensible measuring tape, with an accuracy of 0.1 cm. The measures of mediolateral cranial axis (MLCA) (distance between the ear holes) and anteroposterior cranial axis (APCA) (distance between the snout and the head-neck articulation) were performed with digital caliper with a precision of 0.01 millimeters (GIRIKO CÁ, et al., 2013). The ratio between tail length and body length was calculated from the respectively morphometric measurements.

Offspring neurobehavioral tests

The righting reflex test involved placing the animals in a supine position and measuring the time it took for them to complete a 180° rotation to return to a prone position. Success was defined as achieving this rotation and making contact with all four paws on the surface within a 40-second observation window (KARALIS F, et al., 2011). The negative geotaxis test included placing the animals on a 35 cm-long inclined platform with a 45° slope, initially facing downhill. The expected response was a 180° turn to face uphill and ascending the incline, reaching the upper edge with their forepaws. Failure to complete this task within 60 seconds constituted a negative outcome (RUHELA RK, et al., 2019).

Offspring locomotor activity and exploratory behavior

The open-field apparatus was made of plywood and surrounded by 30 cm high walls. The floor of the apparatus (40 x 40 cm) was divided into 9 quadrants (3 x 3 cm). The animals were transferred to the testing room in their home cages and allowed to acclimatize to this room prior to testing. Each animal was gently placed in the center of the open field arena and left freely to explore for 5 minutes. After the apparatus was cleaned and disinfected with 70% ethyl alcohol after each trial. The sum of segments crossed with the four paws and the number of rears were recorded to measure for locomotor and exploratory activities (WALSH RN and CUMMINS RA, 1976).

Offspring linear cephalometric measurements

Nine cephalometric landmarks were selected for the linear measurements with digital caliper with a precision of 0.01 millimeters. The right side of the mandible and the right alveolus were used in the measurements. The fixed landmarks are mandibular condyle (Co), coronoid process of the mandible (Cor), angular process of the mandible (Go), alveolar process of the mandible (Id), upper incisors (Iu), lower molar alveoli (LMA), upper molar alveoli (UMA), nasal bone (H), and diastema – Dia (Supplementary material table S2; Supplementary material figure S1). The linear measurements of choice were fourth different lengths of the mandible (Cor-Id, Co-Id, Go-Id, LMAL) and three different lengths of the skull (UMAL, DiaL, H-Iu) (Supplementary material table S2; Supplementary material figure S1). Moreover, the ratio of Co-Id/Cor-Id and Co-Id/Go-Id were estimated to predict the proportionality of the mandible growth (KIM HJ, et al., 2018; ODIGIE AE, et al., 2018).

Statistical analysis

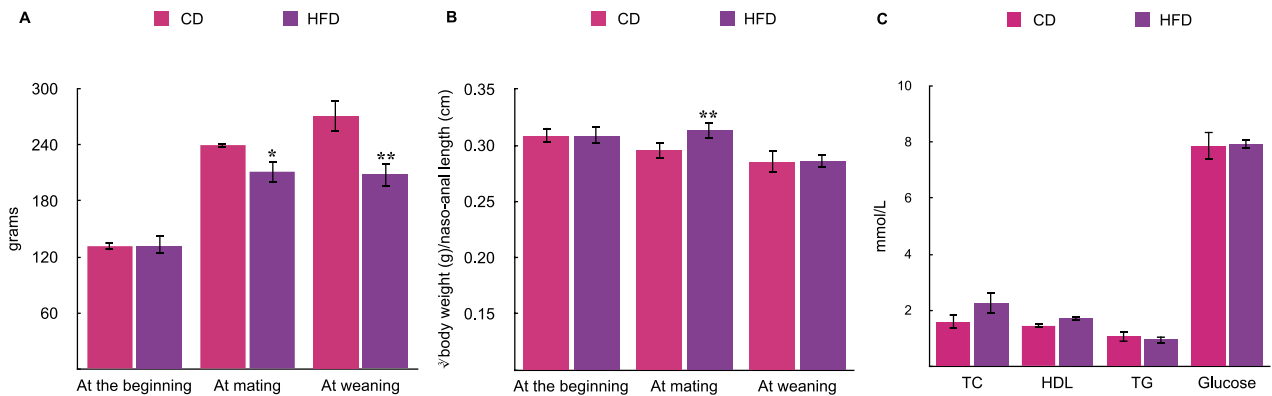
All measurement, determinations and calculations were made from each animal. The variables were entered, double-checked and the database was analyzed in R 3.1.2 software. For statistical analysis, Student's t-tests were used. Data were expressed as mean ± SD, and a significance level of $P < .05$ was considered statistically significant for all variables.

RESULTS

The dams of HFD group showed lower body weight when compared to CD group at mating and at (**Fig. 2A**). However, the Lee index was higher in the dams HFD group when compared to the CD group at mating, revealing maternal obesity due to HFD in prenatal period (**Fig. 2B**). The maternal serum biochemical parameters of total cholesterol, high-density lipoprotein cholesterol, triglycerides and glucose showed no statistic difference between the dams of the HFD group and CD group (**Fig. 2C**).

The body length of the OHFD was smaller from the first to the seventh weeks after birth when compared to the OCD group (**Fig. 3A**). Moreover, tail length was also smaller from the first to the seventh weeks after birth when compared to the OCD group (**Fig. 3B**). However, the ratio between tail length and body length was only significantly different at first and second weeks after birth when compared to the OCD group (**Fig. 3C**). The physical feature of maturation regarding to mandibular incisive eruption was delayed in pups of the OHFD when compared to OCD (OCD: 11 ± 0 days, OHFD: 12.3 ± 0.58 days; $P < .02$).

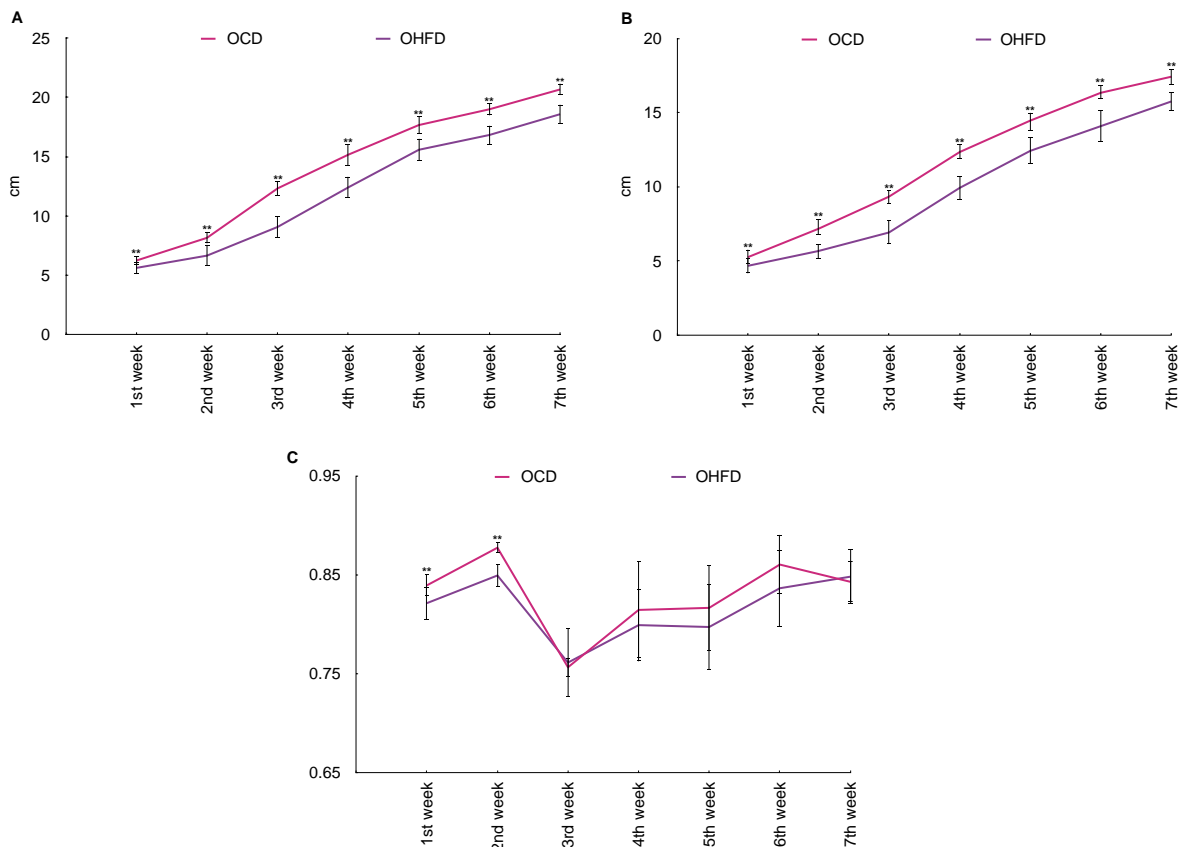
Figure 2 - Maternal physiological and biochemical parameters.



Subtitle: A) Body weight. **B)** Lee index. **C)** Biochemical parameters. Data represent mean \pm standard deviation, n CD = 4-5; n HFD = 4-5. TC: total cholesterol; HDL: high-density lipoprotein cholesterol; TG: triglycerides. (*) $P \leq .05$ and (**) $P \leq .01$ in the comparison between groups by Student's t-test. c: centimeters; g: grams; mmol/L: millimoles per liter.

Source: Giorgi R, et al., 2024.

Figure 3 - Morphometric measurements of the offspring in weeks after birth.

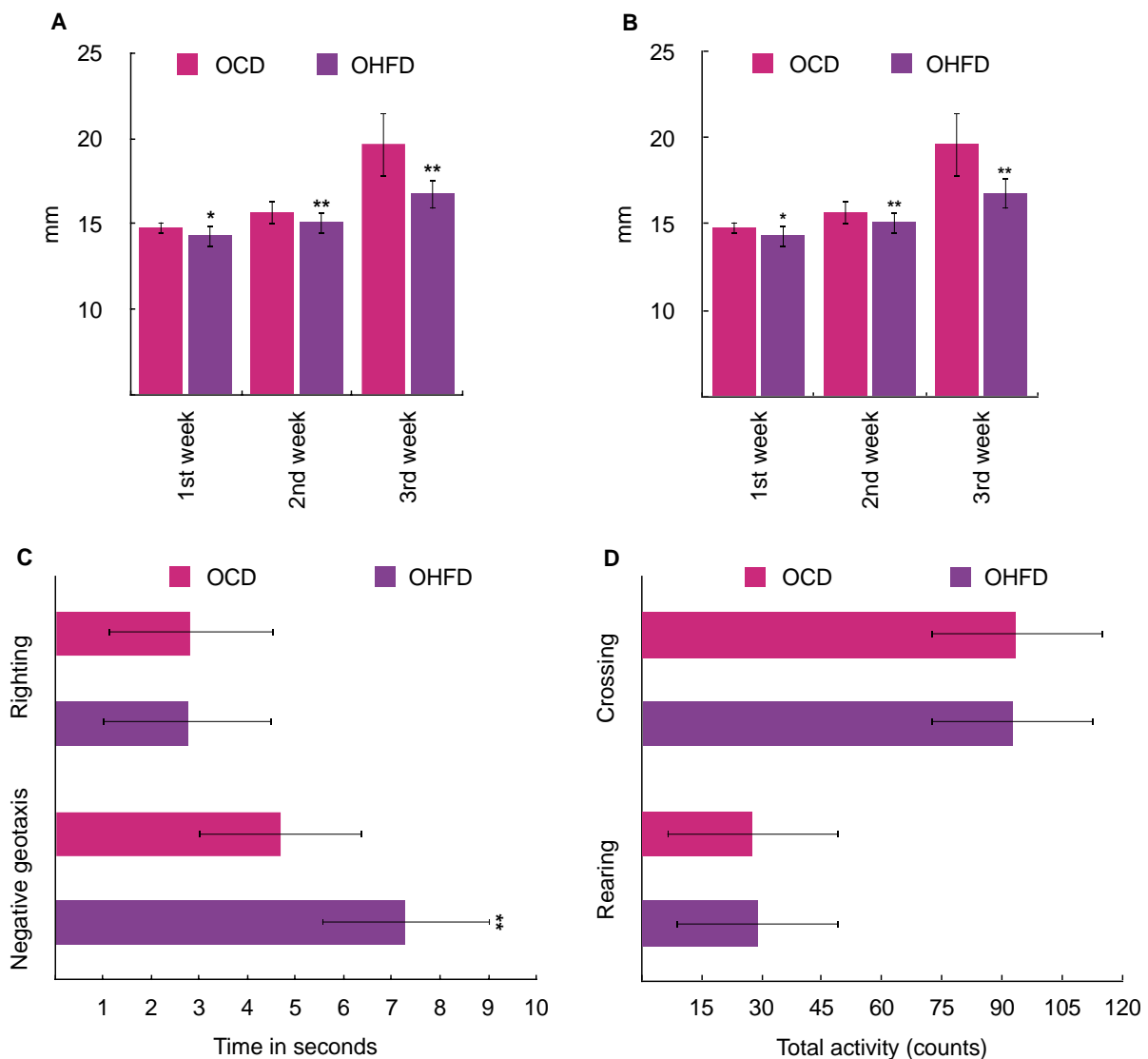


Subtitle: A) Body length. **B)** Tail length. **C)** Tail/body length ratio. Data represent mean \pm standard deviation, n OCD = 12; n OHFD = 12. (*) $P \leq .05$ and (**) $P \leq .01$ in the comparison between groups by Student's t-test. mm: millimeters.

Source: Giorgi R, et al., 2024.

The cranial axes were reduced at all times evaluated. The MLCA was smaller from the first to third weeks after birth when compared to the OCD group (**Fig. 4A**). The APCA was smaller from the first to third weeks after birth when compared to the OCD group (**Fig. 4B**). There was no statistical difference between the OHFD and OCD groups regarding righting reflex test, however, the OHFD showed a delay in latency of negative geotaxis reflex test (**Fig. 4C**). The locomotor activity and exploratory behavior evaluated by the open field test did not show statistical differences between offspring groups concerning both analyzed parameters, crossing and rearing (**Fig. 4D**).

Figure 4 - Cranial axes of the offspring in weeks after birth, neurobehavioral assessment and open filed test. Data represent mean \pm standard deviation, n OCD = 12; n OHFD = 12. (*) $P \leq .05$ and (**) $P \leq .01$ in the comparison groups by Student's t-test. mm: millimeters.



Subtitle: **A)** Mediolateral cranial axis. **B)** Anteroposterior cranial axis. **C)** Latency in righting and negative geotaxis reflex. **D)** Number of counts of crossing and rearing of the open filed test. Data represent mean \pm standard deviation, n OCD = 12; n OHFD = 12. (*) $P \leq .05$ and (**) $P \leq .01$ in the comparison groups by Student's t-test. mm: millimeters.

Source: Giorgi R, et al., 2024.

Moreover, to promote a wide view of this broad reduction of bone growth in the OHFD group we demonstrated in percentages how much smaller the offspring was at different evaluated times (**Table 2**). We identified similar reductions in the first week of life and puberty and a more pronounced reduction at weaning, which indicates intense effect of indirect exposition to maternal HFD during lactation.

Table 1 - Linear cephalometric measurements of skull and mandible of the OCD and OHFD groups. Data represent mean ± standard deviation (SD). *P*-values marked with bold indicate statistically significant differences between the groups.

| Variable | OCD (n = 12) | | OHFD (n = 12) | | P value |
|---------------------|--------------|--------|---------------|--------|------------------|
| | Mean | SD | Mean | SD | |
| H-Iu (mm) | 12.29 | ± 0.20 | 11.43 | ± 0.38 | < .001 |
| Cor-Id (mm) | 22.41 | ± 0.26 | 20.77 | ± 0.71 | < .001 |
| Co-Id (mm)* | 18.90 | ± 0.13 | 17.04 | ± 0.51 | < .001 |
| Go-Id (mm) | 21.59 | ± 0.28 | 20.25 | ± 0.69 | < .001 |
| Cor-Id/Co-Id ratio* | 1.18 | ± 0.01 | 1.22 | ± 0.04 | .02 |
| Cor-Id/Go-Id ratio | 1.04 | ± 0.01 | 1.03 | ± 0.01 | .02 |
| DiaL (mm) | 10.49 | ± 0.19 | 8.90 | ± 0.16 | < .001 |
| UMAL (mm) | 7.47 | ± 0.22 | 6.96 | ± 0.19 | < .001 |
| LMAL (mm) | 7.27 | ± 0.14 | 6.76 | ± 0.21 | < .001 |

Subtitle: OCD: offspring of control diet group; OHFD: offspring of high-fat diet group; *: n = 10, mm: millimeters.

Source: Giorgi R, et al., 2024.

Table 2 - Percentage of morphometric and linear cephalometric measurements reduction of the OHFD in comparison with OCD group.

| Variable | 1st week | Weaning | Puberty |
|----------|----------|---------|---------|
| BL | 10% | 26% | 10% |
| TL | 11% | 26% | 10% |
| MLHA | 3% | 15% | - |
| APHA | 5% | 12% | - |
| Cor-Id | - | - | 10% |
| Co-Id | - | - | 7% |
| Go-Id | - | - | 6% |
| DiaL | - | - | 15% |
| LMAL | - | - | 7% |
| UMAL | - | - | 7% |
| H-Iu | - | - | 7% |

Subtitle: OCD: offspring of control diet group (n = 10-12); OHFD: offspring of high-fat diet group (n = 10-12); %: percent; -: measurement not evaluated. **Source:** Giorgi R, et al., 2024.

DISCUSSION

Our study provided an expanded view of the effects of maternal HFD on offspring's physical development, with a special focus on craniofacial growth. We demonstrated that maternal consumption of a HFD induced a significant reduction in all morphometric measurements. Furthermore, delays in mandibular incisor eruption and negative geotaxis reflex were observed in offspring from the maternal HFD group, indicating a delay in physical maturation. The only difference in exposure was the maternal diet, either high-fat or control, as both offspring groups consumed a control diet after weaning, and no further interventions were performed. Therefore, our results indicate that growth dysfunction could not be restored after normalization of the diet, confirming the hypothesis proposed it was accepted.

The timing of maternal diet manipulation is crucial in determining which offspring cell populations may be affected. Various maternal metabolic disorders can arise based on exposure during preconception, gestation, lactation, or a combination of these periods. Long-term diet exposure can result in insults that trigger programming during sensitive developmental phases in offspring, including the germ cell, early embryo, fetus, and neonate (KUSHWAHA P, et al., 2021). Additionally, maternal obesity before conception contributes to outcome variability due to alterations in dam body metabolism, oocyte metabolism, morphology, and maturation (REYNOLDS KA, et al., 2015). Factors such as the maternal age at HFD exposure, duration of dietary manipulation, maternal age, and metabolic status at conception can disrupt offspring's skeletal formation through various pathways, including precursor cell differentiation, cellular senescence, and longitudinal growth, depending on specific exposure windows (KUSHWAHA P, et al., 2021; BUCKELS EJ, et al., 2021)

Studies in rodents evaluating maternal HFD with fat intake ranging from 45% to 60% of calories during the dietary intervention period, spanning 4 to 8 weeks before mating and throughout pregnancy, revealed decreased total bone volume, mineralization, bone formation, ossification, and body/long bone lengths (ANDREU-ARASA VC, et al., 2018; CHEN J, et al., 2012; LIANG C, et al., 2009). LIANG C, et al. (2009) specifically investigated maternal HFD from 4 weeks before conception to gestational day 19, observing in fetus shorter crown-rump lengths as a general indicator for fetal axial skeletal development. Additionally, the study revealed significant reductions in mandibular and maxillary lengths in the fetuses of HFD-fed dams compared to controls, along with notable retardation of intramembranous and endochondral ossification (LIANG C, et al., 2009), indicating clear nutritional programming effects on craniofacial development by maternal HFD.

In early postnatal rat mandible development, a growth center formed by a cartilaginous bar in the symphyseal region has a close relationship with alveolar incisor development, influencing incisor eruption (BERNICK S and PATEK PQ, 1969). The delay in mandibular incisor eruption observed in the OHFD group is probably a reflex of retarded mandible formation due to nutritional programming by maternal HFD. Considering that mandibular incisors typically erupt around the middle of the suckling period, we can hypothesize that lactation under maternal HFD may enhance mandible growth programming, contributing to craniofacial growth retardation. This delay in craniofacial growth, exacerbated by maternal HFD during lactation, is evident in the analysis of cranial axes of the OHFD group, with retardation increasing from 3% of MLHA and 5% of APHA in the 1st week of life to 12% and 15%, respectively, at weaning when compared to measurements of the OCD group. Smaller somatic growth in both offspring's cranial axes under maternal HFD, particularly in MLHA, has been reported, with lactational programming having a significant effect (GIRILO CÁ, et al., 2013; MENDES-DA-SILVA C, et al., 2013)

The magnification of the effects of maternal HFD through suckling is similarly perceived by the increase in latency of the negative geotaxis reflex, also performed around the middle of lactation, which was not detected in righting reflex tested on the 5th day of life. An analogous observation is valid for the body and tail lengths, which showed more pronounced shortness at weaning, with both lengths of OHFD reaching more than a 25%. Following the same logic, the evaluation of the body and tail lengths at puberty, with 10% reduction in both lengths, allows to hypothesize that the exposition to a CD after weaning diminished the deleterious effect of developmental programming, without, however, prevent the groups from being statistically different.

Despite significant morphometric differences at puberty, locomotor and exploratory behaviors were similar for both groups, suggesting no loss of function in the OHFD group. Our findings align with CADENA-BURBANO et al. (2019), who observed no differences in offspring's locomotor activity when dams were exposed to HFD (52% energy from fat) from gestation to weaning. While literature on body and tail lengths is limited, studies of maternal HFD during suckling by various authors have reported reduced sizes in offspring of the HFD groups (MENDES-DA-SILVA C, et al., 2013; SANTILLAN ME, et al., 2010; OLIVEIRA TR dos P, et al., 2018). Notwithstanding the reduction in body and tail lengths, our examination of growth curves and tail length/body length ratio from the first to seventh week before birth revealed proportional development in OHFD compared to the ODC group.

Lastly, our study is the first to show that maternal HFD from peripubertal age to weaning can cause bone growth retardation of the skull and mandible in puberty offspring. The delay in craniofacial growth can be identified from early perinatal to puberty due to changes in physical maturation and bone growth, corroborating with several studies that revealed bone formation changes at fetal and postnatal stages (LIANG C, et al., 2009; SANTILLÁN ME, et al., 2010; CHEN J, et al., 2012; KUSHWAHA P, et al., 2021). Nonetheless, at least until now, the research into the mechanisms of action related to the bone phenotype modifications promoted by a maternal HFD is still unclear.

The ratio between the mandibular lengths and the percentual reduction of the skull and mandibular measurements of the OHFD group was disproportional when compared to the OCD group and deviant from age-appropriate (KIM HJ, et al., 2018). Some linear cephalometric measurements of OHFD showed proportional reductions in comparison to OCD, as those related to teeth (LMAL, UMAL, and H-lu), and others were more substantial, as DiaL. The mandible is essential to the masticatory function and facial morphology, being an important factor in total facial development (KIM HJ, et al., 2018). Thereby, a difference in the ratios of their length is an alert for further research since there is a possibility link between maternal HFD and inadequate craniofacial formation. On the hand, the diastema separates spatially and functionally the incisors from the molar, which may also cause damage to skull growth if it doesn't develop properly (ESSELSTYN JA, et al., 2012).

The current study had a few limitations that should be taken into consideration. Firstly, although we observed changes in dams body composition at mating that can alter gestational conditions, we did not examine the composition of the mother's milk. Knowing the nutrient content in terms of macronutrients, vitamins, and minerals may have helped to understand the more severe underlying changes observed at the time of weaning. Second, the microarchitecture and morphological changes in the mandible and skull were not examined. These could help determine if the craniofacial bones are just shorter or have ossification and mineralization alterations.

CONCLUSION

The maternal HFD from peripuberty to suckling can induce a delay of physical development and retard bone growth, which is not reversed with the diet normalization after weaning due to the persistence of the altered phenotype until puberty. Based on our findings we point out the need for further studies including female offspring, to observe the effect of developmental programming on both sexes. Moreover, future investigations should evaluate other bone health markers and conduct morphological and micro-tomography analyses of mandibular and skull bones to elucidate the signaling pathways involved in the process that led to the dysfunction of these important skeletal structures.

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