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Intrauterine exposure to a high-fat diet, with different levels of lipids, and its gastrointestinal repercussions: a model of fetal programming in rats

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Original Article

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Abstract

It is known that adverse stimuli, such as altered diets during pregnancy and lactation, can result in deleterious effects on the progeny. The aim of this study was to evaluate the possible gastrointestinal repercussions in the offspring of Wistar rats exposed to high-fat diets. Pregnant rats were divided into three groups: normolipidic diet (3.5% lipids), a diet containing 28% lipids, and a diet with 40% lipids. Body weight and food, water, daily caloric, and macronutrient intake were evaluated in the pregnant rats. Structural and functional gastrointestinal parameters were assessed in 30-day-old male pups. Depending on the lipid content of the maternal diet, the pups may exhibit gastric mucosal thickening, an increase in the relative weight of the small intestine, a reduction in the jejunal and ileal mucosa, and a decrease in the total thickness of the ileum. Additionally, there may be a reduction in the number of villi per area in these organs and a thinning of the muscular layer in the large intestine. The structural changes induced by the maternal high-fat diet seem to reduce the stomach's sensitivity to ethanol-induced ulcers, which is the only functional alteration observed. Therefore, the offspring of dams exposed to high-fat diets during pregnancy and lactation exhibits impaired gastrointestinal development, with alterations depending on dietary fat content and specific gastrointestinal regions. Structural changes did not always result in functional abnormalities and, in some cases, appeared protective. The long-term consequences of the observed morphological alterations require further investigation.

Introduction

The embryonic and fetal development during pregnancy depends entirely on the maternal environment to meet their nutritional and energy needs; these periods are critical due to rapid cell differentiation, organ growth, development, and maturation, making maternal nutrition decisive for physiological and metabolic functions throughout life. Fetal programming, a process resulting from adverse stimuli presented to mothers during pregnancy, involves a complex network of gene regulation that promotes permanent changes in fetal tissues, interfering with the functionality of target tissues and affecting their performance and adaptation to metabolic challenges.[6]

Studies in humans have identified a relationship between maternal hyperalimentation and an increased risk of obesity,[59] diabetes,[31,18] and other complications in children.[10,53] Animal models have similarly shown that offspring of rats exposed to modified diets with excess lipids during pregnancy exhibit accelerated growth, excess body adiposity, glucose intolerance, impaired insulin sensitivity, and liver dysfunction.[2,3,33,39,60] Furthermore, exposure to an obesogenic intrauterine environment can cause epigenetic changes, increasing susceptibility to type 2 diabetes[18] and systemic arterial hypertension in pups.[18,45]

Given the increasing rates of overweight and obesity among women of reproductive age and the high worldwide consumption of high-fat diets, it is evident that future generations will face greater risks of morbidities. Importantly, an unbalanced diet during pregnancy can have repercussions on offspring, irrespective of maternal obesity development.[63] This underscores the critical role of maternal nutrition not only in immediate health outcomes but also in shaping the long-term health trajectories of offspring. The gastrointestinal tract emerges as a pivotal player in this context, implicated in obesity through its influence on the production of incretins (such as glucagon-like peptides) and hormones that regulate appetite (e.g., ghrelin, cholecystokinin, and YY peptide). These substances impact postprandial responses and

nutrient absorption, ultimately contributing to a positive energy balance and obesity, and alterations in the microbiome and its metabolic products.[7] Consequently, gastroenterologists have shown significant interest in studying obesity, given its potential to result in gastrointestinal abnormalities and disorders, including gastroesophageal reflux, gastroesophageal adenocarcinoma, gastric and intestinal cancer, colon diverticular disease, polyps, nonalcoholic fatty liver disease, pancreatitis, and biliary lithiasis, among others.[22]

However, it is important to highlight the limited availability of studies that establish a correlation between gastrointestinal symptoms observed in children or young animals, including structural and functional abnormalities such as motility or mucosal defense issues, and increased maternal food intake during pregnancy, particularly concerning specific nutrients like lipids. Based on the scarce literature and going further on this subject, we tested the hypothesis that maternal exposure to a high-fat diet, varying in lipid content, might alter both structural and functional aspects of the gastrointestinal tract in Wistar rat pups. Therefore, in addition to investigating intestinal motility and stomach susceptibility to injury, our study also evaluated structural features, including mucosal thickness, jejunal villi length, and the total length of the intestines, which are critical for nutrient absorption.

Materials and methods

Animals

The experiments were conducted using male (300 g) and female (180 g) Wistar rats. The animals were housed in a room maintained at 21°C with 12-h light/dark cycles and had free access to water and standard feed. All the protocols used were reviewed and approved by the Ethics Committee on Animal Use of the Federal University of Uberlândia (CEUA/UFU; protocol n° 054/18). Male animals ($n = 15$) were individually housed, while in the late afternoon, females ($n = 49$) were introduced into the males' cages in a ratio of two to three females per male. The next morning, a vaginal smear was performed to detect any potential pregnancies based on the presence of spermatozoa. Once pregnant, the females were placed in individual cages. After birth, male pups at 30-day-old were divided into the following groups: *control group (C: normal fat diet – 3.5%)* – pups from the dams had free access to water and commercial diet during gestation and lactation; *experimental group 1 (E1: high-fat diet – 28%)* – pups from the dams had free access to water and high-fat diet with 28% lipids content, during pregnancy and lactation; and *experimental group 2 (E2: high-fat diet – 40%)* – pups from the dams also had free access to water and high-fat diet with 40% lipids content, during pregnancy and lactation.

During treatment with the respective diets, maternal parameters were monitored, such as daily caloric and macronutrient consumption, water and food average daily intake, and the variation in body weight throughout pregnancy.

The food average daily intake and the volume of liquid ingested by the pregnant rats were monitored for 5 d, starting from the 8th day of gestation. From the average food intake, the consumption of each macronutrient was calculated based on the carbohydrate, protein, and lipid contents of each ingredient in the high-fat diets obtained through the food composition table.

The evaluation of the total weight gain of the pregnant rats was performed every 5 d, from the pre-gestational weight to the 20th day of gestation. Gestational success was calculated as a percentage taking into account the number of rats that gave birth in relation to the number of rats that were considered pregnant.

The dams were exposed to each diet during pregnancy (21 d) and lactation (21 d). Thus, after weaning, the pups were separated from their respective dams and began consuming standard commercial diets until the date of euthanasia. At 30 d, male pups were used for experiments on the gastrointestinal system. To guarantee sample variability, a maximum of two pups from each dam were used for each experiment performed.

Composition of diets

The commercial diet (normal fat diet – Nuvilab – composition: ground whole corn, bran soybean, wheat bran, calcium carbonate, dicalcium phosphate, sodium chloride, vegetable oil, vitamin A, vitamin D3, vitamin E, vitamin K_3, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin, calcium pantothenate, folic acid, biotin, choline chloride, iron sulfate, iron monoxide, manganese, zinc oxide, copper sulfate, calcium iodate, sodium selenite, cobalt sulfate, lysine, methionine, BHT) for rats was used and contained by weight: 19% proteins, 56% carbohydrates, and 3.5% lipids, totaling 4.068 kcal/g (17.03 KJ/g).

The high-fat diet (28%) consisted of adding hypercaloric foods to 65 g of commercial feed (Nuvilab) in the following proportion: 12 g of roasted peanuts, 4 g of milk chocolate, 2 g of corn starch biscuit, and 17 g of lard so that the composition of the diet was 15% proteins, 42% carbohydrates, 28% lipids, totaling 5.25 kcal/g (21.96 KJ/g) (adapted from[16]).

The high-fat diet (40%) contained the same ingredients that were added to the commercial diet to obtain the high-fat diet (28%), but in different proportions. It used 48 g of commercial feed (Nuvilab), added to 18 g of roasted peanuts, 5 g of milk chocolate, 3 g of corn starch biscuit, and 26 g of lard were added. These ingredients were crushed, mixed, and offered in the form of pellets, containing by weight: 14% proteins, 35% carbohydrates, and 40% lipids, totaling 5.83 kcal/g (24.39 KJ/g).

Gastrointestinal function tests

In the 30-day-old male pups, gastric analyses were performed, including stomach total area, stomach relative weight, and susceptibility to ulcers (C: $n = 8$, E1: $n = 11$, E2: $n = 10$). Intestinal analyses were also conducted on the small intestine, evaluating parameters such as total length, organ relative weight, intestinal motility, and intestinal fluid accumulation (enteropooling). For the large intestine, the functional parameter evaluated was distal colonic motility. It is important to note that the animals were fasted for 12 h before all gastrointestinal function experiments.

Assessment of susceptibility to gastric ulcer

For gastric ulcer induction (C: $n = 8$, E1: $n = 11$, E2: $n = 10$), 0.8 ml/100 g of body weight of hydroalcoholic solution (0.3M HCl/60% ethanol) was administered intragastrically via gavage to 30-day-old male pups. After 1 h, the animals were euthanized by cervical dislocation following thiopental anesthesia (0.2 ml/100 g of body weight i.p.). A laparotomy was performed to remove the stomachs, which were opened along the great curvature, washed in saline solution, and flattened between two glass plates. The material was digitized on the HP Scanjet 2400 Scanner (Palo Alto, California, USA), and the stomach images were analyzed using the ImageJ software (https://imagej.nih.gov/ij/), with the results expressed in ulcer index (UI).

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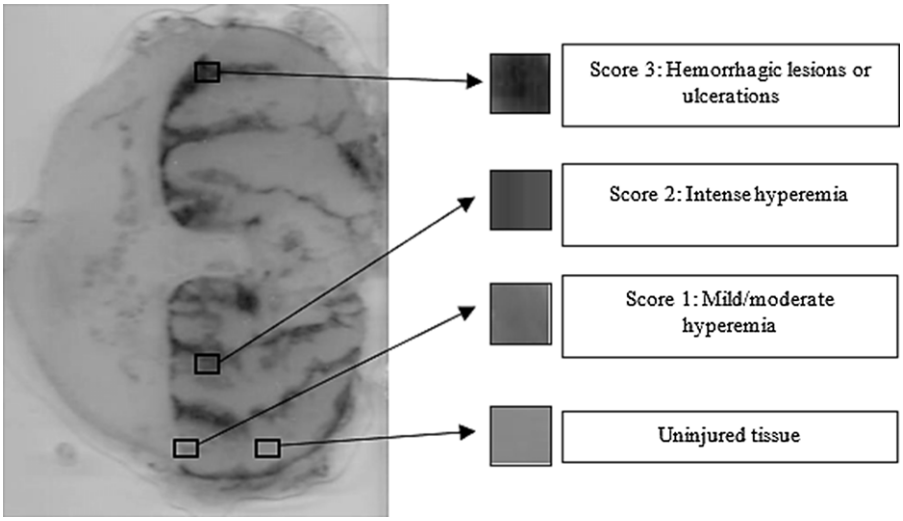

Figure 1. Classification in scores for gastric lesions.[42]

To calculate UI, gastric lesions were classified and received scores according to their severity, as follows: area of hemorrhagic lesions or ulceration itself (3); area of intense hyperemia (2) and area of mild/moderate hyperemia (1) (Fig. 1). UI was determined as previously described: UI = 3× area of hemorrhagic injury (mm[2]) + 2× area of intense hyperemia (mm[2]) + 1× area of mild/moderate hyperemia (mm[2]).[40]

Assessment of gastrointestinal motility using the charcoal meal model

The 30-day-old male pups received, via gavage 0.8 ml/100 g of body weight of 5% charcoal meal suspension prepared in 0.5% carboxymethylcellulose solution (C: $n = 7$, E1: $n = 9$, E2: $n = 10$). After 30 min of administration of this suspension, the animals were euthanized by cervical dislocation following anesthesia with thiopental (0.2 ml/100 g of body weight i.p.). Then the small intestine was then removed to determine the distance traveled by the charcoal meal suspension (expressed as % of the intestine length).[23,56]

Assessment of intestinal fluid accumulation (enteropooling) induced by castor oil

The 30-day-old male pups received castor oil or saline solution (0.8 ml/animal) by gavage (C saline: $n = 8$; C oil: $n = 8$; E1 saline: $n = 10$; E1 oil: $n = 10$; E2 saline: $n = 11$; E2 oil: $n = 11$).[15,43] After 30 min, the animals were anesthetized with thiopental (0.2 ml/100 g of body weight i.p.) and euthanized by cervical dislocation. A laparotomy was performed to isolate and extract the small intestine, which was weighed. The intestinal contents were then removed, and the intestine was weighed again. The difference between the weights of the full (FI) and empty (EI) intestine was used as an indication of the relative intestinal content, where enteropooling = (FI-EI)/animal weight (in mg/g ×1000).

Study of distal colonic motility

After a 12-h fast, a plastic sphere measuring 2 mm in diameter was introduced into the distal colon (distance of 2.5 cm) of the 30-day-old male pups using a plastic probe (C: $n = 8$, E1: $n = 11$, E2: $n = 9$). The animals were observed by a designated observer, and the time of sphere expulsion was recorded for each animal. This served as a parameter to infer changes in colonic motility.[30]

Figure 2. Representation of intestinal portions referring to fragments collected for structural analysis: (1) duodenum, (2) jejunum, (3) ileum, (4) cecum, (5) colon, (6) rectum.[46]

Morphometric assessment of the gastrointestinal system

To evaluate the morphometric parameters, the 30-day-old male pups were anesthetized, euthanized, and had their organs collected, including the stomach and small intestine (divided into duodenum, jejunum, and ileum), as well as the large intestine (divided into colon, cecum, and rectum). The fragments obtained from the intestinal samples had an average length of 2 cm. After collecting the small intestine, the proximal portion was considered the duodenum, the intermediate portion was considered the jejunum, and the most distal portion was considered the ileum. For the large intestine, the fragments collected from the most proximal portion were considered the cecum, intermediate fractions were considered the colon, and fragments obtained from the distal portion were considered the rectum (Fig. 2).

The collected material was flushed with saline solution (0.9%) to remove the luminal contents completely, followed by immersion in Methacarn (60% methanol, 30% chloroform, and 10% glacial

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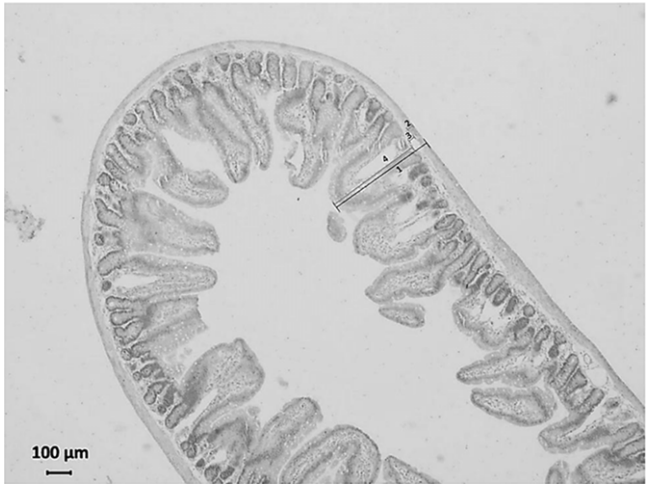

Figure 3. Schematic representation of the measurement of the layers' thickness from the small intestine wall. Hematoxylin and eosin staining. 4× increase. (1) Total thickness, (2) muscular layer thickness, (3) submucosa layer thickness, (4) mucosa layer thickness.

acetic acid) to ensure proper fixation. Subsequently, the material was stored in Methacarn for 24 h and then transferred to alcohol (70%) until the point of paraffin embedding. Finally, the tissue underwent sequential steps to prepare the histological slides (5 µm). The histological processing steps were performed sequentially. Identified tissues were placed in small cassettes, dehydrated through a graded series of alcohols in ascending concentrations (70%–100%), cleared by immersion in xylene (organic solvent, Synth), and then embedded in paraffin. The tissues were subsequently included in paraffin blocks and sectioned into 5 µm thick slices using a microtome. The sections were mounted on pre-gelatinized glass slides and kept in an oven at 60°C for 24 h to remove excess paraffin.

The slides were stained with hematoxylin and eosin to visualize the histological sections. These sections were then subjected to photographic image capture using digital optical microscopy with the OPTICAM software installed on the NIKON Eclipse Ti-S model microscope (Melville, NY, USA) at 4× magnification. Finally, using the ImageJ software, the average thickness (Fig. 3) of the external muscle, submucosa, and mucosa layers of the stomach (C: $n = 8$, E1: $n = 11$, E2: $n = 10$), small intestine (C: $n = 7$, E1: $n = 9$, E2: $n = 10$), and large intestine (C: $n = 8$, E1: $n = 7$, E2: $n = 9$) was calculated, as well as the total thickness of the stomach and intestinal segments, measuring the distance between the layer in four distinct regions of the cross-sections, chosen randomly. To achieve this, an imaginary line was drawn dividing each histological section into four quadrants, with a random point selected in each quadrant. Additionally, for the small intestine segments, the number and length of villi per mucosa area of each intestinal segment were also evaluated (adapted from[54,57]). To measure the mucosa area of the small intestine segments, the entire mucosa region was outlined, and the area was calculated using the software. Only the villi that continued with the intestinal wall were considered. Calibration was performed using an appropriate micrometer ruler. All structural parameters were evaluated from the same image and region.

Statistical analysis

When appropriate, data are reported as mean ± SEM or median with interquartile range (25th and 75th percentiles). Comparisons were made using one-way ANOVA followed by Tukey's test for normally distributed variables or the Kruskal–Wallis test with Dunn's post-test for non-normally distributed variables. Statistical significance was defined as $P < 0.05$. The statistical analysis was performed using GraphPad Prism Version 5.00 software (Trial) (Boston, MA, USA).

Results

Maternal data

The number of rats considered pregnant and that had their pups was not different between the groups studied (70.50% in C; 62.50% in E1 and 68.75% in E2), showing that the intake of a high-fat diet did not interfere with gestational success nor did it result in changes in the number of pups per litter or in the proportion between male and female pups. Likewise, maternal weight gain was also not different, showing that the intake of high-fat diets (28% and 40%) did not result in maternal obesity. Maternal consumption of liquid was similar between the studied groups, but food intake was lower in E2 when compared to E1, while the daily caloric intake of the dams from E1 was higher compared to the other groups, showing that the amount of food ingested decreased as the caloric supply increased. Taking into account the amount of food ingested, it was possible to calculate the consumption separately of each macronutrient; in this case, it was observed that the intake of carbohydrates and proteins by the dams was lower in E2 when compared to C and E1. The intake of lipids was higher in the two experimental groups, but there were no differences between them. These results were expected since the carbohydrate and protein content in experimental diets was lower than the lipid content (Table 1).

Offspring data

Body weight and stomach area were lower in E2 animals when compared to C and E1, whereas the relative weight of this organ was lower in E1 than in C. These data show that the high-fat diet of 40% impaired the body and stomach growth, while the high-fat diet of 28% caused changes only in the growth of the organ. Despite being smaller, it presented a greater total thickness, probably due to the increase in the mucosa layer, compared to C. The other layers (submucosa and muscle) were not different between groups. Regarding the gastric functional part, the UI was lower in the experimental groups compared to C. In other words, the pups from the dams submitted to a high-fat diet respond with less intensity to harmful stimuli to the stomach wall (Fig. 4).

The small intestine total length was not different between the studied groups. However, its relative weight was higher in E2, when compared to C and E1, demonstrating that there was an imbalance between body and organ growth since the animals from this group had lower body weight (Fig. 5).

The offering of diets with different lipids content to the dams from E1 and E2 did not change the thickness of the layers of the duodenal wall of the pups. In the jejunum, there was a reduction in the mucosa in E1, but without impact on the total thickness of this segment. In the ileum, the total thickness and mucosa thickness were lower in E2, when compared to C and E1 (Fig. 6).

Although the jejunal mucosa thickness was smaller in E1, the area of this layer was increased in this group in relation to C. The number of duodenal, jejunal, and ileal villi per area was not different between groups (Fig. 5), but the jejunal and ileal villi length per area was lower in E1 compared to C (Fig. 7).

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Table 1. Maternal data: food, liquid, daily caloric, carbohydrates, proteins and lipids intake, weight gain, and number of pups from control (C: normal fat diet – 3.5%), Experimental 1 (E1: high-fat diet – 28%), and Experimental 2 (E2: high-fat diet – 40%) groups

Parameters	Control (n = 7)	Experimental 1 (n = 8)	Experimental 2 (n = 8)
Food intake (g/day)	21.94 (19.93–23.06)	27.01 (20.17–29.40)	16.93 (13.64–19.94)^{**E1}
Liquid intake (ml/day)	66.40 ± 3.41	63.10 ± 3.35	61.85 ± 6.35
Daily caloric intake	87.81 ± 2.94	134.80 ± 9.06^{***C**E2}	98.54 ± 6.95
Carbohydrates	12.00 ± 0.47	10.85 ± 0.73	6.03 ± 0.43^{***C***E1}
Proteins	4.17 (3.79–4.38)	4.35 (3.12–4.92)	2.34 (1.88–2.75)^{**C**E1}
Lipids	0.76 ± 0.03	7.14 ± 0.48^{***C}	6.81 ± 0.48^{***C}
Weight gain	105.70 ± 8.13	122.50 ± 9.88	122.2 ± 12.81
Number of pups (M: male and F: female)	M: 4.09 ± 0.60 F: 4.64 ± 0.53	M: 4.80 ± 0.72 F: 4.90 ± 0.80	M: 5.18 ± 0.81 F: 5.09 ± 0.77

Values are expressed as median with percentiles 25 and 75 for food and proteins intake (Kruskal–Wallis with Dunn's post-test) or mean ± SEM for liquid, daily caloric, carbohydrates, lipids intake, weight gain, and number of pups (one-way ANOVA with Tukey's post-test). The level of significance was set at $p < 0.05$. ***C: $p < 0.001$ versus Control; ***E1: $p < 0.001$ versus Experimental 1; **E1: $p < 0.01$ versus Experimental 1; **E2: $p < 0.01$ versus Experimental 2.

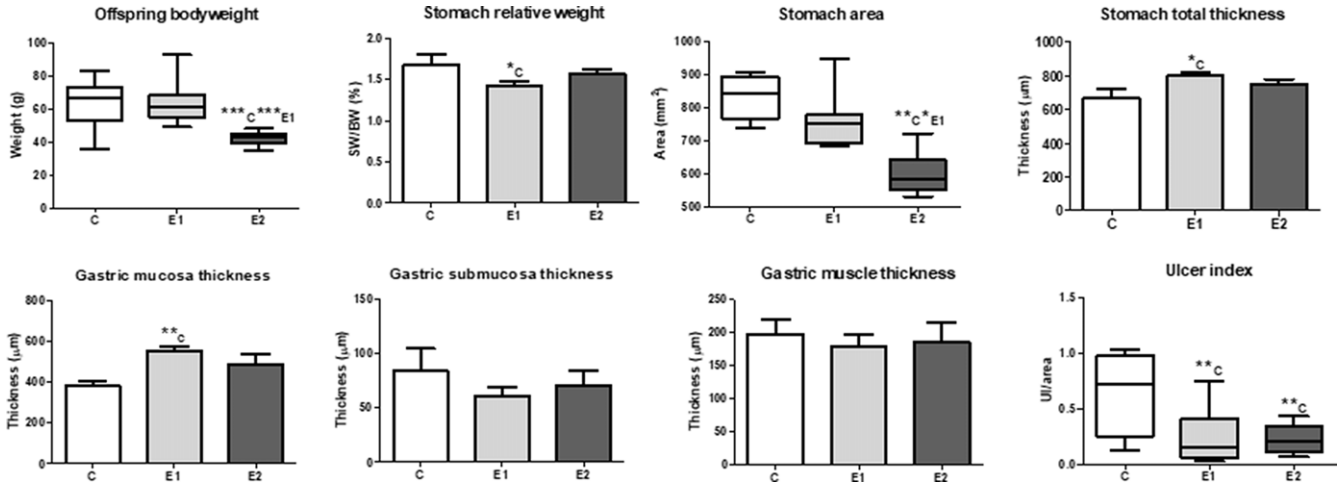

Figure 4. Offspring body weight and stomach structural and functional data from control (C: normal fat diet – 3.5%), Experimental 1 (E1: high-fat diet – 28%), and Experimental 2 (E2: high-fat diet – 40%) groups. Values are expressed as median with percentiles 25 and 75 for offspring body weight, stomach areas, and ulcer index (Kruskal–Wallis with Dunn's post-test) or mean: SEM for stomach relative weight, stomach total thickness, gastric submucosa thickness, and gastric muscle thickness (one-way ANOVA with Tukey's post-test). The level of significance was set at $p < 0.05$ ***C: $p < 0.001$ versus control; *C: $p < 0.05$ versus control, ***E1: $p < 0.001$ versus Experimental 1; *E1: $p < 0.05$ versus Experimental 1. SW/BW = stomach weight/body weight.

The transit in the small intestine was evaluated by the distance traveled by the suspension of charcoal meal along the organ. No differences were observed between the groups for this parameter, nor for the evaluation of enteropooling (Fig. 7), a parameter that allows evaluation of the accumulation of intestinal liquid, resulting from the secretory and absorptive capacities of the small intestine as a whole. In all groups, the response to castor oil was greater than the response to saline, which was expected, but there were no differences between them.

The distal colonic transit, evaluated by the time of the expulsion of a plastic sphere introduced in the rectum of the animals, was quite variable between the groups, and no differences were found between them. However, it was possible to observe a reduction in the total thickness in the regions of the cecum and colon of the large intestine of E2 pups. Both in the cecum and in the colon, this reduction can be attributed to the thickness of the mucous and muscular layers. Although the total thickness of the rectum was smaller in group E1 compared to C, no significant changes were identified in the other regions of this segment (Fig. 8).

Discussion

The gastrointestinal system plays a crucial role in providing nutrients to the body. Its early development is complex, involving growth, an increase in the mass, number, and size of cells, as well as maturations and structural and functional changes in the cells. Thus, the embryonic, fetal, and postnatal development of the gastrointestinal tract must be adequately managed to ensure it can perform its functions effectively. Although research on fetal metabolic programming has intensified in recent years, highlighting that developmental changes can become permanent and predispose individuals to health issues, few studies have focused on the impact of fetal programming on the structure and function of the gastrointestinal system.[6,11,27,32,36,48,49]

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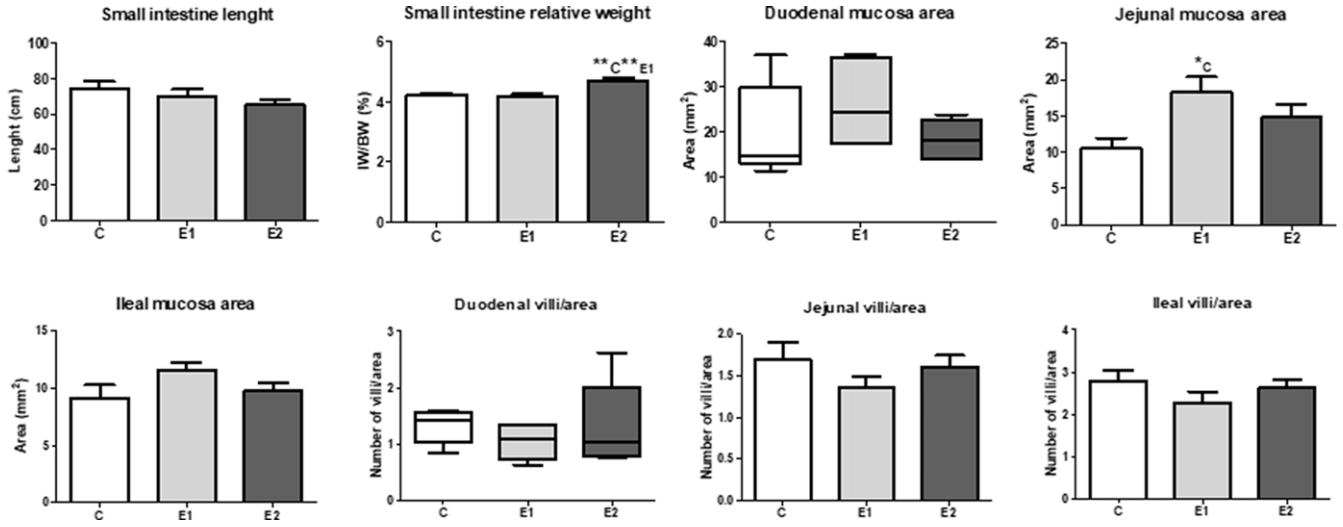

Figure 5. Small intestine length and relative weight and duodenal, jejunal, ileal area and villi/area from control (C: normal fat diet – 3.5%), Experimental 1 (E1: high-fat diet – 28%), and Experimental 2 (E2: high-fat diet – 40%) groups. Values are expressed as median with percentiles 25 and 75 for duodenal mucosa area and villi area (Kruskal–Wallis with Dunn's post-test) or mean=SEM for small intestine length and relative weight, jejunal and ileal mucosa area, jejunal and ileal villi area (one-way ANOVA with Tukey's post-test). The level of significance was set at $p < 0.05$ **C: $p < 0.01$ versus control; *C: $p < 0.05$ versus control, **E1: $p < 0.01$ versus Experimental 1. IW/BW = intestine weight/body weight.

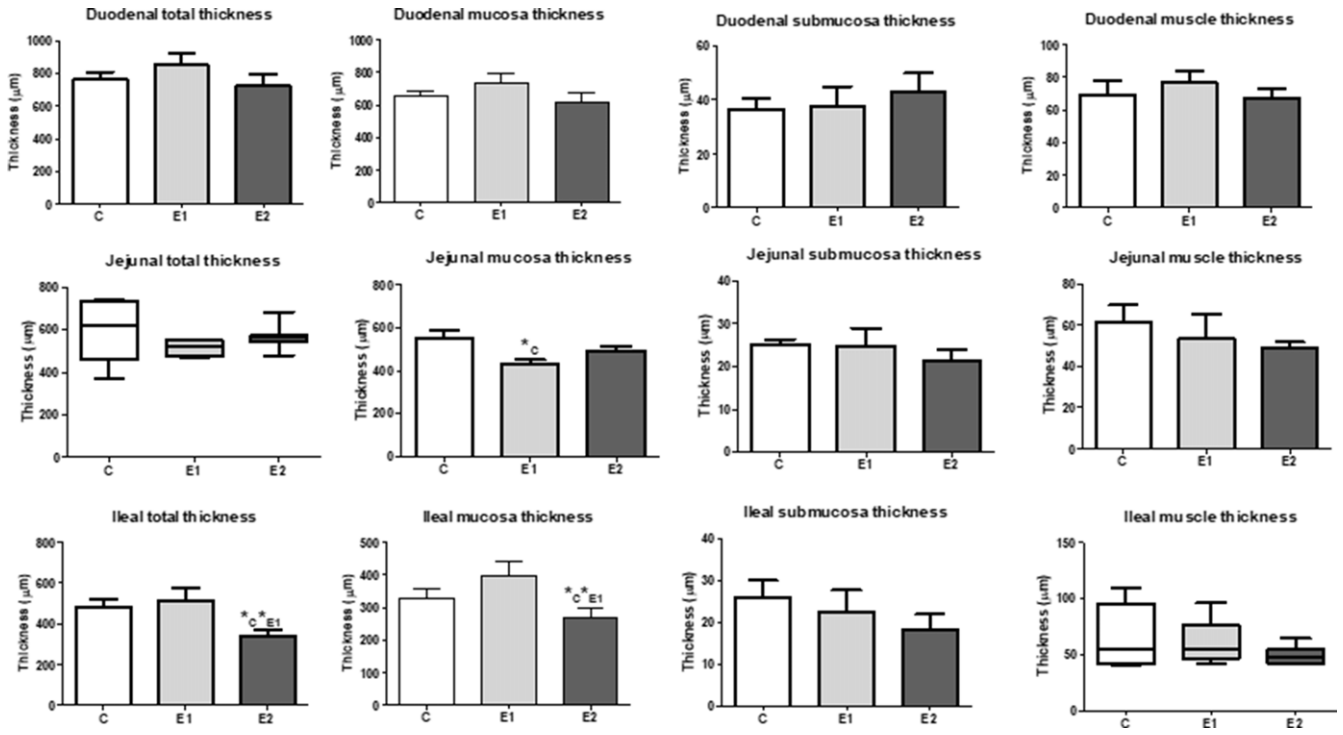

Figure 6. Morphometric data total, mucosal, submucosal, and muscle thickness of duodenum, jejunum, and ileum of small intestine from control (C; normal fat diet 3.5%), Experimental 1 (E1: high fat diet – 28%), and Experimental 2 (E2: high-fat diet – 40%) groups. Values are expressed as median with percentiles 25 and 75 for jejunal total thickness and ileal muscle thickness (Kruskal–Wallis with Dunn's post-test) or mean=SEM for duodenal total, mucosal, submucosal, and muscle thickness, jejunal mucosal, submucosal, and muscle thickness, and ileal total, mucosal, and submucosal thickness (one-way ANOVA with Tukey's post-test). The level of significance was set at $p < 0.05$ *C: $p < 0.05$ versus control; *E1: $p < 0.05$ versus Experimental 1.

In the present study, maternal high-fat diets impaired the stomach growth of pups, inducing structural alterations that made the organ less sensitive to ethanol-induced ulcers. Indeed, pups from the dams that consumed the diet with 28% lipids showed evidence of gastric mucosal thickening. This structural alteration may have resulted in an increase in the number of mucous surface cells, ultimately enhancing the protective functions of the mucosal layer through increased mucous production.[28,52] However, the gastroprotective effect can also be related to functional changes, since both diets produced positive outcomes for the pups, but the diet with 40% lipids was not associated with mucosal thickening. To our knowledge, this is the first report in the literature showing an apparent gastroprotective effect resulting from fetal programming induced by a high-fat diet. While negative health outcomes

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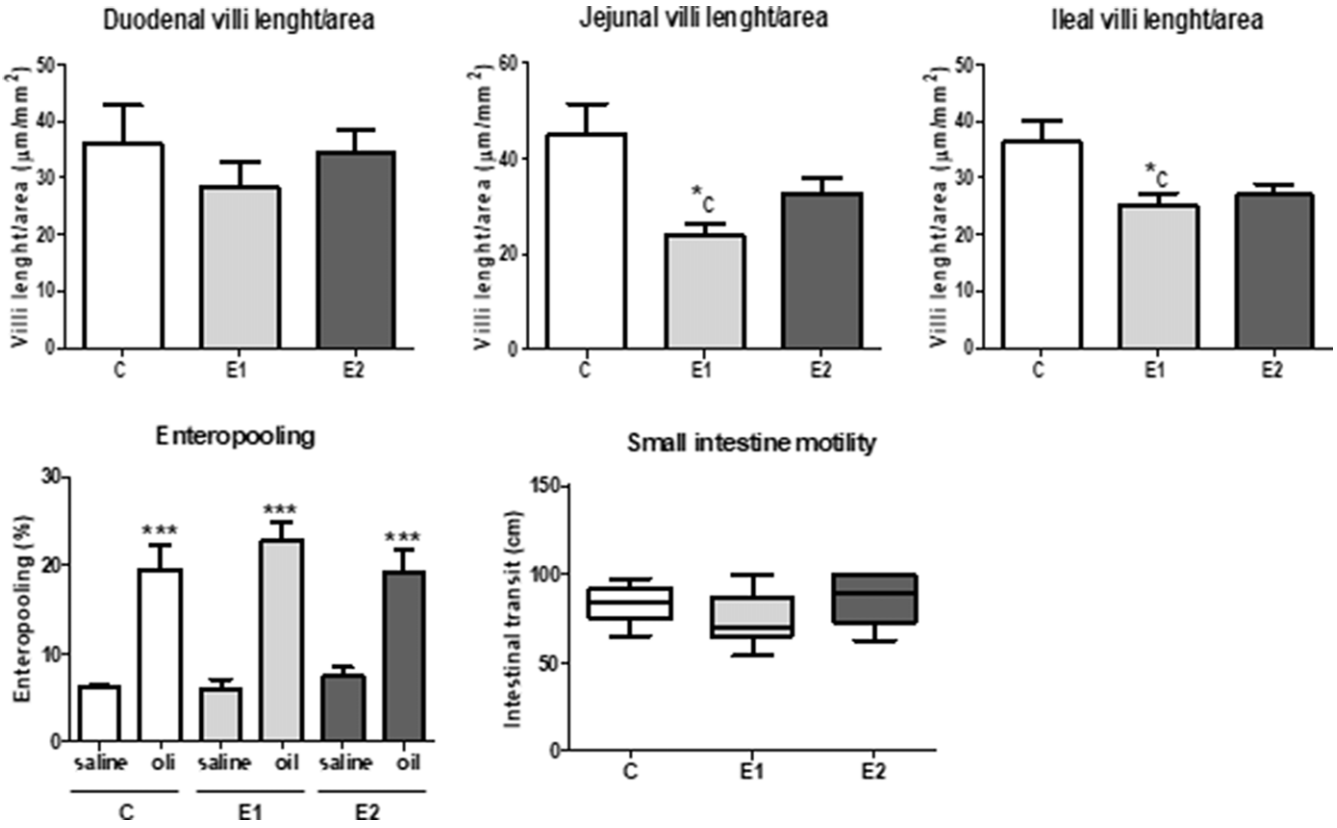

Figure 7. Duodenal, jejunal, and ileal villi length/area, enteropooling and small intestinal motility from control (C: normal fat diet – 3.5%). Experimental 1 (E1: high-fat diet – 28%) and Experimental 2 (E2 high-fat diet – 40%) groups. Values are expressed as median with percentiles 25 and 75 for small intestine motility (Kruskal–Wallis with Dunn's post-test) or mean=SEM for duodenal, jejunal, ileal villi length/area and enteropooling (one-way ANOVA with Tukey's post-test). The level of significance was set at $p < 0.05$ ***C: $p < 0.001$ versus saline of the same group, *C: $p < 0.05$ versus control.

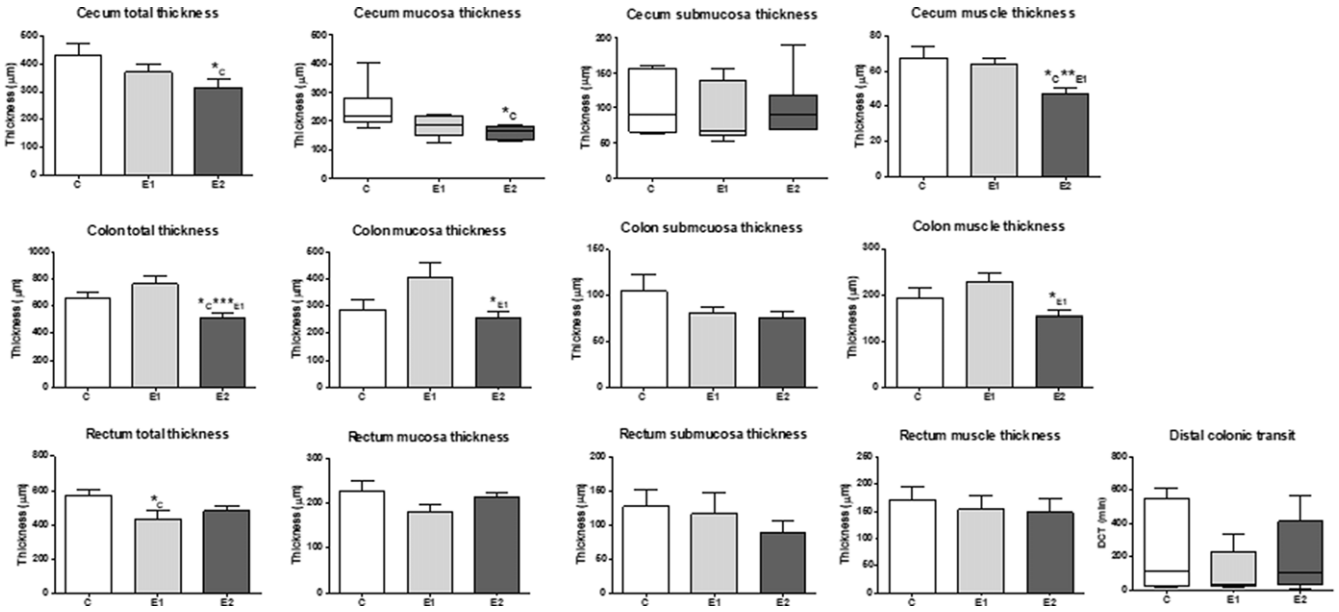

Figure 8. Morphometric data: total, mucosal, submucosal, and muscle thickness of cecum, colon, and rectum of large intestine from control (C normal fat diet – 3.5%), Experimental 1 (E1: high-fat diet – 28%), and Experimental 2 (E2: high-fat diet – 40%) groups. Values are expressed as median with percentiles 25 and 75 for cecum mucosal and submucosal thickness and distal colonic transit (Kruskal–Wallis with Dunn's post-test) or mean=SEM for cecum total and muscle thickness, colon total, mucosal, submucosal and muscle thickness, rectum total, mucosal, submucosal, and muscle thickness (one-way ANOVA with Tukey's post-test). The level of significance was set at $p < 0.05$. *C: $p < 0.05$ versus control, ***E:1 $p < 0.001$ versus Experimental 1; *E1: $p < 0.05$ versus Experimental 1.

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are more commonly associated with fetal programming, it is important to mention that possible positive outcomes have also been reported.[42]

Interestingly, while the diet with 40% lipids seems to impair stomach growth due to an overall body growth impairment, as indicated by the unaltered relative weight of the stomach and the lower body weight of the animals, it caused an imbalance between body and intestinal growth, evidenced by an increase in the relative weight of the intestine. However, consistent with previous reports, including studies on animals of similar age,[32,49] the maternal high-fat diet did not affect the pups' small intestine length. Our results not only reinforce the concept that the gastrointestinal tract of rodents begins its formation during the prenatal period and is not fully mature until weaning[49] but also that the development of digestive organs is affected by maternal nutrition.[36]

Our data suggest that the effects of maternal nutrition on gastrointestinal organ development are not uniform. Specifically, the thickness of different layers of the small intestine in pups was generally unaffected by the maternal high-fat diet, except for reductions in the jejunal mucosa thickness in pups from the 28% lipid diet group and both the ileal mucosa thickness and total ileal thickness in pups from the 40% lipid diet group. Since the ileum is the intestinal segment where most dietary lipids are processed and absorbed,[37] alterations in the ileal mucosa may have compromised nutrient absorption, contributing to the weight deficit observed in pups from the 40% lipid group. Although no alterations were observed in the duodenum, previous studies have documented changes in the muscle layer thickness of this intestinal segment in the offspring of rats fed a high-fat diet.[49] While we did not explore the molecular mechanisms involved in this aspect of intestinal growth impairment caused by the maternal high-fat diet, it has been suggested by[24] that intrauterine exposure to an excess of fatty acids, particularly saturated ones, may activate epigenetic mechanisms related to micro-RNAs. These micro-RNAs act as gene expression modulators, capable of silencing mRNAs.[21,45] Furthermore, considering the significance of insulin-like growth factor (IGF-1) and its receptor in intestinal development and function establishment,[51] it is proposed that a high-fat diet induces epigenetic changes involving the action of micro-RNAs that silence mRNAs encoding IGF-1, ultimately resulting in the observed impairment of intestinal growth in our study. This hypothesis warrants further investigation.

Alterations in the morphology of intestinal villi in fetuses or offspring from animals exposed to a high-fat diet have shown conflicting results in various studies. Some have reported no changes,[35] while others observed a tendency toward increases,[11] transitory changes,[32] or significant increases.[49] These discrepancies may be attributed to the varying amounts of lipids added to the dams' diet, the species analyzed, and the time point and specific intestinal segment used to investigate these potential alterations. Our data support the notion that the proportion of lipids in the diet is a crucial factor and that the impact of these lipids varies depending on the specific segment of the gastrointestinal tract under investigation, as previously discussed. Specifically, we found that only the diet with 28% lipids led to a significant decrease in the villi length/area ratio in the ileum and jejunum. Since the mucosa thickness in the jejunum was smaller compared to control animals, the observed increase in its area could be linked to the thickening of the jejunal villi (an analysis not performed in this study).

Pups born to the dams who consumed high-fat diets apparently exhibit decreases in cellular components in the small intestine, as indicated by the observed reduction in mucosa thickness in both experimental groups across various segments of the small intestine. To assess the potential functional implications of this morphological change on the secretory and/or absorptive capacities of the small intestine, the animals were administered castor oil. Castor oil undergoes hydrolysis by intestinal lipases, releasing ricinoleic acid,[58] known for its laxative effects due to fluid and electrolyte secretion,[41] which leads to fluid accumulation (enteropooling). Additionally, ricinoleic acid directly stimulates intestinal motility, contributing to castor-oil-induced diarrhea.[55] No differences were observed among the groups in terms of enteropooling induced by castor oil. This finding suggests, for the first time in the literature, that the fluid and electrolyte absorptive functions of the small intestine, as well as the cellular and molecular components underlying intestinal fluid and electrolyte secretion sensitive to ricinoleic acid, remain intact in pups from the dams exposed to high-fat diets during pregnancy and lactation, at least at this 30-d stage of development.

Slower intestinal transit,[4] potentially resulting from decreased viability of enteric neurons,[38] reduced enterochromaffin cell populations, and altered neurotransmitter levels,[5] contribute to gastrointestinal dysmotility in animals subjected high-fat diets. Furthermore, fetal exposure to high-fat diets impacts the development of the enteric nervous system, leading to glial proliferation and loss of inhibitory neurons, which may compromise intestinal motility later in life.[34] In our study, we examined the muscular layer of the intestinal tract in animals exposed to high-fat diets during fetal development and the breastfeeding period, assessing the potential functional implications of the observed morphological changes. Our findings again highlight the heterogeneity of the morphological alterations, demonstrating that structural changes do not invariably lead to functional abnormalities. For instance, the reduction in the colonic muscular layer observed only in offspring from the dams on a high-fat diet with 40% lipids was not associated with altered colonic motility, suggesting a compensatory mechanism in the colon of these animals at least within the first 30 d post-birth. However, potential functional impacts later in life cannot be excluded. Moreover, the motility of the pups' small intestine was unaffected by any maternal nutritional intervention, which is consistent with the absence of morphological changes in the small intestinal muscular layer across the groups.

Pups from the dams on a high-fat diet had inconsistent body weight outcomes across studies.[8,9,14,64] We observed that the weight of 30-day-old pups from the dams on a high-fat diet was either unaffected (28% lipids) or reduced (40% lipids). Therefore, although literature shows a relationship between diet-induced obesity and changes in gastrointestinal motility,[13,17,61] overweight is not an explanation for the alterations described here.

Furthermore, although high-fat diets typically promote body weight gain in animals,[12,19,26,32,49,50] the pregnant rats on a high-fat diet in our study gained weight similarly to the controls. This discrepancy may be due to differences in diet duration, diet composition, and the species of animals used.[32] Moreover, possibly due to the high energy density of their diet, rats on the 40% lipid diet consumed less food, resulting in lower protein intake and reduced nutrient absorption. This likely affected their pups' growth, as suggested by studies using intrauterine growth restriction models.[62]

Although the specific composition of each fatty acid in the diets investigated in the present study has not been assessed, the saturated fat content surpasses that of mono- and polyunsaturated fatty acids, including omega-6, primarily because lard is the main

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contributor to the increased lipid content. Researchers have highlighted that omega-6 plays a crucial role in the survival and proliferation of crypt cells and in the permeability of the large intestine.[1,25,29,47] Additionally, maternal dietary fatty acids have been reported to influence the composition of the lipid bilayer of the colon membranes in newborns.[27] These findings underscore that not only the quantity but also the quality of the fats in the diets may have affected the growth of different layers of the large intestine, colon, cecum, and rectum described in our study. Finally, increasing the lipid content in the diet alters the composition of other macronutrients, typically reducing carbohydrates and proteins to accommodate the rise in fatty acids. Thus, although the high-fat diets used in the present study are not classified as low-protein (as this would require a protein content of approximately 8%),[36] the observed changes may also be partially attributed to the altered macronutrient proportions, as previously discussed in the context of intrauterine growth restriction models.[62]

Conclusion

The present study supports the concept that offspring of the dams exposed to high-fat diets during pregnancy and lactation exhibit impaired gastrointestinal development. The alterations appear to depend on the fat content in the diet and on the specific gastrointestinal region. Additionally, the structural changes do not invariably lead to functional abnormalities and may, in some cases, result in protective outcomes. However, this does not imply that the consumption of high-fat diets should be encouraged, given their well-known deleterious effects on general health and the potential for negative impacts of the observed alteration later in life. Further experiments are necessary to investigate this suggestion as well as the molecular mechanism involved in the positive and negative outcomes described in the present study.

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Competing interests. None.

Ethical standard. All experimental protocols necessary for the development of this work were approved by the Ethics Committee on the Use of Animals under the protocol CEUA/UFU n° 054/18.

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