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Effects of maternal hypothyroidism in the gastrointestinal system of male young offspring from Wistar rats

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Original Article

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Abstract

Alterations in the maternal environment may impact on the fetal development. The objective of this study was to investigate the gastrointestinal consequences of maternal hypothyroidism for the male offspring from Wistar rats. The pregnant rats were divided into three groups: control (*C* – received water), experimental 1 [*E*1 – received methimazole (MMI) solution] during gestation and lactation, and experimental 2 (*E*2 – received MMI solution) during gestation. Maternal parameters evaluated: free *T*3 and *T*4, bodyweight variation, and water/food intake. Offspring parameters evaluated: litter size, number of male/female, free *T*3 and *T*4, stomach area, gastric ulcer susceptibility, small intestine length and weight, small intestine and distal colon motility, the stomach and intestinal weight–body weight ratio (SW/BW–IW/BW), and the accumulation of intestinal fluid. Maternal *T*3 and *T*4 from *E*1 were decreased when compared to the other groups. There were no differences for maternal water/food intake and weight gain, litter size, and number of males and females. Regarding to offspring, free *T*3, SW/BW, IW/BW, and intestinal fluid accumulation were not different between the groups, but *T*4 was decreased in *E*1. However, 30-day-old pups from *E*1 and *E*2 were smaller with lower stomach and small intestine. Even more, *E*1 presented a lower ulcer index when compared to the *C*, while *E*2 had a higher distal colon transit. It can be concluded that maternal hypothyroidism impaired the total body development, as well as gastric and intestinal development, besides interfering with the susceptibility to the ulcer and intestinal transit of male offspring from Wistar rats.

Introduction

Adverse stimuli presented to mothers during gestation can permanently alter the structure and function of fetal tissues, a process known as fetal programming. The programming is based on the fact that alterations in the intrauterine environment can redirect developmental pathways during critical periods when tissues are still in proliferation and differentiation stages.[1-3] These changes are exemplified by maternal malnutrition, smoking, alcohol consumption, drug abuse, emotional stress,[4] and endocrine disorders.[5] As a result, exposure of the fetus or newborn to adverse environmental during gestation and/or throughout the lactation period, respectively, may trigger a number of diseases in adulthood.[6,7]

The thyroid secretes two major hormones, thyroxin (*T*4) and triiodothyronine (*T*3). Although *T*4 is produced in greater amounts, this hormone will be later converted by different tissues into another active hormone, *T*3. Since these hormones are able to increase the body metabolic rate, their deprivation promotes a significant fall in basal metabolism.[8,9] A deficiency of thyroid hormones is called hypothyroidism, a very common and condition which is most prevalent in women, the elderly, and in some ethnic groups. Among their symptoms are fatigue, bradycardia, weight gain, muscle weakness, and hypertension.[10,11] Consequences of the untreated hypothyroidism during pregnancy may be a miscarriage, preterm birth, preeclampsia, complications at birth, neonatal hypothyroidism, changes in fetal brain development, and growth, besides congenital hypothyroidism.[12,13] In this context, thyroid hormones have an important participation in early developmental processes, controlling cell growth, maturation, and the metabolism of virtually all tissues.[14]

Patients with hypothyroidism have alterations associated with gastrointestinal (GI) function, presenting symptoms such as constipation and flatulence.[15] Studies conducted in animals with hypothyroidism showed a reduction in the stomach, small intestine, and the motor activity in the colon,[16] an alteration which can lead to constipation, atony, and intestinal obstruction.[17,18] Albino male rats with hypothyroidism, for instance, have been shown to be more susceptible to

stress-induced gastritis and ulcers.[19] Since little is known about the long-term impact of maternal hypothyroidism on children's health or offspring from Wistar rats, and considering the effects of thyroid hormones on GI function, we test the hypothesis that maternal hypothyroidism during gestation and/or lactation could produce a long-lasting imprint on the offspring's GI tract.

Material and methods

Animals

The experiments were performed using male (300 g) and female (180 g) of Wistar rats. The animals were housed in a room at 21°C with 12 h light/dark cycles and were provided with free access to tap water and standard chow. All the protocols used were reviewed and approved by the Ethics Committee on Animal Use of the Federal University of Uberlândia (CEUA/UFU; process n° 069/17). Male animals ($n = 10$) were kept individually in their boxes at the end of the afternoon, and females were placed in the boxes with males in the proportion of two to three females for each male. Early morning of the following day, a vaginal smear was performed to detect any possible pregnancy, due to the presence of spermatozoa in the smear. Once pregnant, the females were placed in individual boxes, and after birth, the male pups were divided into the following groups:

Group C (Control). 30-day-old male offspring ($n = 77$) from dams ($n = 18$) that had free access to water normally during gestation until weaning, about 21 days after gestation.

Group E1 (Experimental Hypothyroid Gestation/Breastfeeding). 30-day-old male offspring ($n = 63$) from dams ($n = 13$) that had free access to 0.02% methimazole (MMI) in drinking water from the eighth day of gestation to the period of weaning, about 21 days after gestation.

Group E2 (Experimental Hypothyroid Gestation). 30-day-old male offspring ($n = 63$) from dams ($n = 12$) that had free access to 0.02% MMI in drinking water from the eighth to the last day of gestation.

All male pups from each dam were used in each study group, but only one to two male pups from each dam were randomly chosen and used in each type of analysis proposed to ensure maternal and offspring variability.

Drugs and reagents

For maternal hypothyroidism induction, a solution of 0.02% MMI (MMI diluted in drinking water) was used, from LEMMA Supply Solutions Ltda (São Paulo, SP, Brazil). The castor oil used was from Sigma-Aldrich Chemical Co. (San Louis, MO, USA), the activated carbon from Proquimios Comércio e Indústria Ltda (Rio de Janeiro, RJ, Brazil), and the sodium thiopental from Cristália Produtos Químicos e Farmacêuticos Ltda (Itapira, SP, Brazil). The other reagents were supplied by the Federal University of Uberlândia.

Analyses performed on dams

Water and food consumption and maternal body weight

The liquid and food intakes of the pregnant rats were carried out from the eighth day of gestation, for a period of 6 days. In addition, the bodyweight (BW) gain during gestation (from the 1st to the 12th day of pregnancy) was evaluated (control $n = 7$; *E*1 $n = 8$; *E*2 $n = 7$).

Hormone concentrations

Samples of 1 ml of tail blood were collected from dams (control $n = 7$; *E*1 $n = 8$; *E*2 $n = 7$) of three studied groups, 5 days postpartum, for the measurement of free *T*3 and *T*4 thyroid hormones to confirm the euthyroid state of dams from the *C* and *E*2 groups and the hypothyroid state of dams from the *E*1 group. The method used to measure the hormones was electrochemioluminescence. The kits used were Elecsys FT3 IIII and Elecsys FT4 III from Roche Diagnostics (Basel, Switzerland). In addition, for free *T*3, values below the white limit are indicated as <0.4 pmol/l and values above the measurement range as >50 pmol/l. For free *T*4, values below the detection limit are indicated as <0.5 pmol/l and values above the measurement range as >100 pmol/l.

After the weaning period, the dams were euthanized by overdose of anesthetics. The anesthetic used was sodium thiopental at a dose of 160 mg/kg of BW intraperitoneally.

Analyses performed on the pups

BW and number of pups

At 30 days old, the pups of all the groups were weighed and counted to determine the total litter and the proportion between the males and females.

Hormone concentrations

Samples of 1 ml of tail blood were collected from 30-day-old male pups of three studied groups (control $n = 11$; *E*1 $n = 9$; *E*2 $n = 10$), for the determination of free *T*3 and *T*4 thyroid hormones by the electrochemioluminescence method, and after collection, the animals were euthanized by cervical dislocation.

Organ analysis

In the 30-day-old male pups of the three groups studied, gastric analyses (control $n = 14$; *E*1 $n = 10$; *E*2 $n = 8$) were performed, such as total stomach area, BW, and the relationship between stomach weight (SW) and BW. In relation to the small intestine (control $n = 37$; *E*1 $n = 32$; *E*2 $n = 36$), we performed the analysis of the total length, organ weight, and the relationship between the intestinal weight and BW.

Evaluation of ulcer susceptibility

The model of gastric lesions induced by hydroalcoholic solution was used to analyze the susceptibility to ulcers in 30-day-old male pups (control $n = 14$; *E*1 $n = 10$; *E*2 $n = 8$) from *Wistar* rats. The animals were fasted for 12 h prior to intragastric administration, and 0.8 ml/100 g of hydroalcoholic solution using a gavage needle was injected (0.3 M HCl/60% ethanol). After 1 h, the animals were euthanized by cervical dislocation after anesthesia with thiopental (0.2 ml/100 g i.p.). Then, a laparotomy was performed to remove the stomachs, which were opened through the greater curvature, emptied, weighed, washed in saline, and mounted between two glass plates. The material was scanned on the HP Scanjet 2400 Scanner, and images of the stomachs were analyzed in the Image J software (https://imagej.nih.gov/ij/), the results being expressed using the ulcer index (UI). To calculate the UI, the gastric lesions were classified and received scores according to their severity, as follows: area of the hemorrhagic lesions or ulceration proper (3); area of intense hyperemia (2); and mild/moderate hyperemia area (1). The UI was determined as previously described:[20] UI = 3*X* area of the hemorrhagic lesion (mm^2) + 2*X* area of intense hyperemia (mm^2) + 1*X* area of mild/moderate hyperemia (mm^2).

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Table 1. Maternal data: BW gain, food and water intake, and thyroid hormone levels (free *T*3 and *T*4) from control, experimental 1 and experimental 2 groups

	Control ($n = 7$)	Experimental 1 ($n = 8$)	Experimental 2 ($n = 7$)
BW (g)	109.50 ± 9.15	105.30 ± 6.37	97.33 ± 4.89
Food intake (g/day)	23.93 ± 0.80	21.23 ± 0.69	21.46 ± 1.22
Water intake (ml/day)	57.52 ± 1.62	49.40 ± 14.20	60.93 ± 2.41
Free *T*3 (pg/ml)	2.71 (2.64–2.97)	0.96 (0.47–1.12)$^{\#\#**}$	2.91 (2.56–3.52)
Free *T*4 (ng/ml)	1.69 (1.55–2.24)	0.14 (0.02–0.21)$^{\#\#**}$	1.83 (1.41–2.22)

Values are expressed as mean ± SEM for BW, food and water intake (one-way ANOVA with Tukey's post-test) or median with percentiles 25 and 75 for free *T*3 and *T*4 (Kruskal–Wallis with Dunn's post-test). The level of significance was set at $p < 0.05$. ##$p < 0.01$ versus control; **$p < 0.01$ versus experimental 2.

Evaluation of GI motility using the activated carbon transit model

The 30-day-old male pups (control $n = 15$; *E*1 $n = 16$; *E*2 $n = 16$) were fasted for 12 h and then received 0.8 ml/100 g of activated carbon suspension (5% in 0.5% carboxymethylcellulose solution, m/v) orally. Following 30 min of carbon administration, the animals were euthanized by cervical dislocation after thiopental anesthesia (0.2 ml/100 g i.p.). Then the small intestine was removed to determine the distance traveled by the activated carbon (expressed in % of intestine length).[21,22] After checking small intestine motility, the organ was emptied and weighed.

Evaluation of intestinal fluid (enteropooling) produced by castor oil

The 30-day-old male pups were fasted for 12 h and received (gavage) castor oil (control $n = 13$; *E*1 $n = 9$; *E*2 $n = 9$) and saline (0.8 ml/animal) (control $n = 12$; *E*1 $n = 11$; *E*2 $n = 10$) orally.[23,24] After 30 min, the animals were anesthetized with thiopental (0.2 ml/100 g i.p.) and euthanized by cervical dislocation. A laparotomy was performed followed by tying and insulation of the small intestine, which was weighed. The intestinal contents were removed and the intestine weighed again. The difference between the full intestinal (FI) and empty (EI) weights was used as an indicator of the relative intestinal contents, where enteropooling = (FI−EI)/animal weight (mg/g × 1000).

Study of distal colon motility

A 2-mm-diameter glass sphere was introduced into the distal colon (distance 2.5 cm) of the 30-day-old male pups (control $n = 14$; *E*1 $n = 10$; *E*2 $n = 14$) with the aid of a plastic probe. The time for the expulsion of the sphere was determined for each animal and served as a parameter to infer changes in distal colon motility.[25]

Statistical analysis

The statistical analysis was performed using GraphPad Prism Version 5.00 software (Trial; San Diego, CA, USA). The level of significance was set at $p < 0.05$. The tests used for each parameter studied are cited below and in the legend of each table/figure in the item Results.

(A) Maternal parameters
- BW, food and water intake: one-way analysis of variance (ANOVA) with Tukey's post-test.
- Free *T*3 and *T*4: Kruskal–Wallis with Dunn's post-test.

(B) Offspring parameters
- BW, number of pups and free *T*4, small intestine weight: BW ratio (SIW:BW ratio), UI, enteropooling and small intestine and distal colon motility: Kruskal–Wallis with Dunn's post-test.
- Free *T*3, stomach area, SW, SW:BW ratio, SIW, and length: one-way ANOVA with Tukey's post-test.

Results

There were no differences between the groups for maternal weight gain, water, and food intake during gestation. The evaluation of maternal thyroid hormones, five days after delivery, showed that free *T*3 and *T*4 plasma levels were lower in the *E*1 group when compared to the *C* and *E*2 groups, confirming the postpartum hypothyroid state only of the dams from the *E*1 group (Table 1).

The total number of pups and the proportion between the males and females per litter were evaluated, and there was no difference between the groups. The 30-day-old pups from the *E*1 and *E*2 groups presented a significant reduction in BW when compared to the controls. However, weight reduction was higher in pups from *E*1 than in *E*2. The evaluation of the plasma levels of thyroid hormones from 30-day-old pups showed that there was no significant difference between the groups for free *T*3. However, free *T*4 levels were significantly lower in the *E*1 group when compared to the *C* and *E*2 groups (Table 2).

With regard to the gastric development of the offspring, it was observed that the stomach area of the 30-day-old pups was smaller in the *E*1 and *E*2 groups when compared to the *C* group (Fig. 1a), but the SW was lower only in the *E*1 group than in the other groups (Fig. 1b) and the SW:BW ratio was not different between the groups studied (Fig. 1c).

Regarding the development of the small intestine, it was observed that pups from the *E*1 and *E*2 groups presented with a lower intestinal weight when compared to group *C*. However, the intestinal weight in *E*1 was lower than in *E*2 (Fig. 2a). The intestinal length (Fig. 2b), as well as the intestinal weight:BW ratio (Fig. 2c), was not different between the groups.

In response to the administration of the hydroalcoholic solution, the susceptibility to an ulcer was lower in group *E*1 when compared to groups *C* and *E*2 (Fig. 3a). In all the groups studied, intestinal fluid accumulation was greater in animals receiving castor oil than in those receiving the saline solution. There were no differences between the experimental groups and the control group for this parameter (Fig. 3b). Regarding intestinal motility, the transit of the small intestine was smaller in the experimental groups in relation to the control (Fig. 3c), and on the other hand, the distal colonic transit was increased only in *E*2 compared to the control (Fig. 3d).

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Table 2. Offspring data: BW, total number of pups, and thyroid hormone levels (free *T*3 and *T*4) from control, experimental 1 and experimental 2 groups

	Control	Experimental 1	Experimental 2
BW (g) (*C* $n = 77$; *E*1 $n = 63$; *E*2 $n = 63$)	68.80 (63.60–78.20)	37.10 (28.81–44.60)$^{\#\#\#\star}$	46.45 (36.46–57.78)$^{\#\#\#}$
Number of pups	11.50 (7.75–12.00)	9.00 (8.00–12.50)	11.50 (8.25–13.00)
Free *T*3 (pg/ml) (*C* $n = 11$; *E*1 $n = 9$; *E*2 $n = 10$)	3.23 ± 0.14	3.52 ± 0.17	3.34 ± 0.16
Free *T*4 (ng/ml) (*C* $n = 11$; *E*1 $n = 9$; *E*2 $n = 10$)	1.65 (1.41–1.97)	0.76 (0.51–0.93)$^{\#\#\star}$	1.69 (1.42–1.87)

Values are expressed as median with percentiles 25 and 75 for BW, number of pups, and free *T*4 (Kruskal–Wallis with Dunn's post-test) or mean ± SEM for free *T*3 (one-way ANOVA with Tukey's post-test). The level of significance was set at $p < 0.05$. ##$p < 0.01$ versus control; ###$p < 0.001$ versus Control; *$p < 0.05$ versus experimental 2.

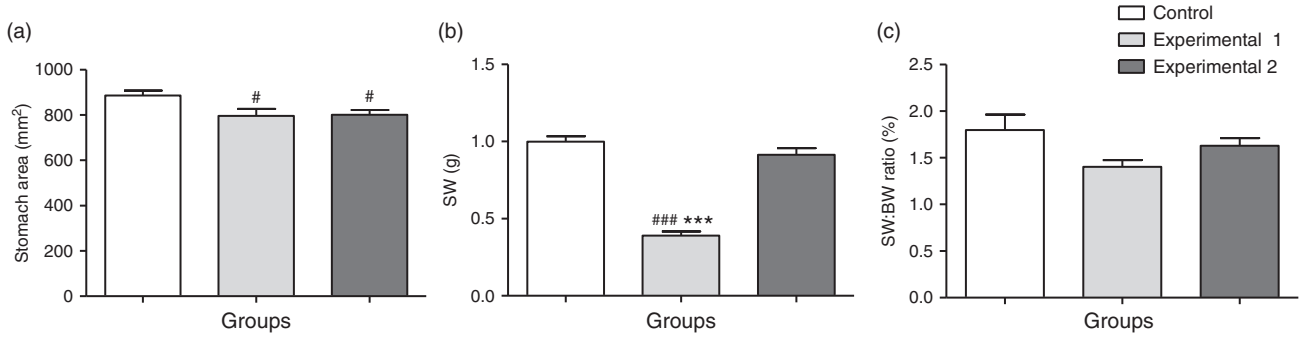

Fig. 1. Stomach area and weight and SW:BW ratio of offspring from control, experimental 1, and experimental 2 groups.
Values are expressed as mean ± SEM for stomach area and weight and BW:BW ratio (SW:BW ratio) (*C*: $n = 14$; *E*1: $n = 10$; *E*2: $n = 8$) (one-way ANOVA with Tukey's post-test). The level of significance was set at $p < 0.05$. #$p < 0.05$ versus control; ###$p < 0.001$ versus control; ***$p < 0.001$ versus experimental 2.

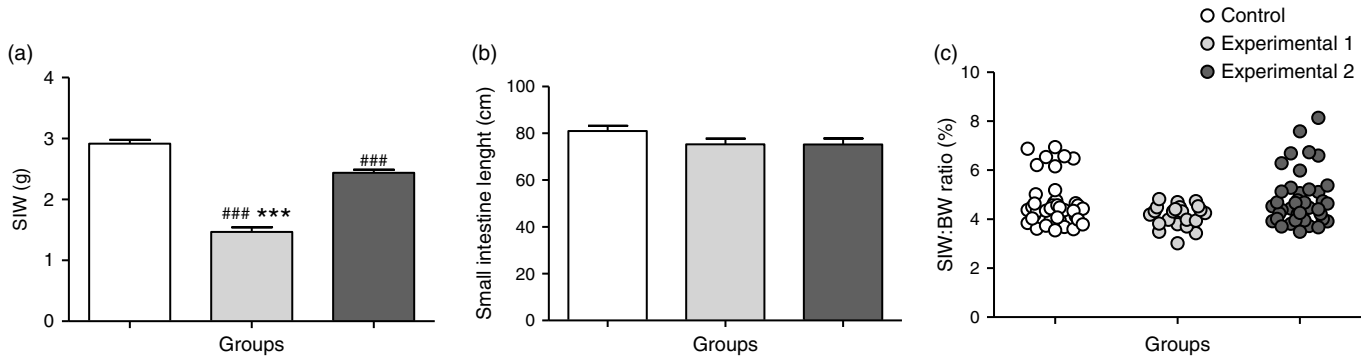

Fig. 2. Small intestine area and weight and SIW:BW ratio of offspring from control, experimental 1, and experimental 2 groups.
Values are expressed as mean ± SEM for SIW (*C*: $n = 37$; *E*1: $n = 32$; *E*2: $n = 36$) and length (*C*: $n = 15$; *E*1: $n = 16$; *E*2: $n = 16$) (one-way ANOVA with Tukey's post-test) or individual values for SIW:BW ratio (SIW:BW ratio) (*C*: $n = 37$; *E*1: $n = 32$; *E*2: $n = 36$) (Kruskall–Wallis with Dunn's post-test). The level of significance was set at $p < 0.05$. ###$p < 0.001$ versus control; ***$p < 0.001$ versus experimental 2.

Discussion

In the present study, we aimed to evaluate any possible GI changes in the young offspring of mothers that ingested MMI during pregnancy and lactation, or only during the gestational period. The MMI is a thioamide drug that inhibits a key enzyme involved in thyroid hormones synthesis, the thyroid peroxidases.[26] Indeed, effective maternal hypothyroidism was induced by MMI administration, as suggested by reduced plasma levels of *T*3 and *T*4 observed in the dams of the *E*1 group. MMI administration was discontinued as soon as the *E*2 pups were born and 5 days postpartum, *E*2 dams were euthyroid, as indicated by *T*3 and *T*4 plasmatic levels normalization.

It is well established that MMI treatment induces weight loss in rats.[27,28] Since the literature has long considered the pivotal role of *T*3 in the control of body growth, not only through direct and specific effects, but also as a mediator that potentiates growth hormone and insulin-like growth factor 1 activity,[28] the slowed weight gain associated to hypothyroidism in rodents was simply attributed to *T*3 deficiency. However, this is not the only possible explanation. Indeed, it has shown that MMI-induced weight gain reduction can occur with no change in food consumed by the animals, observations that raised the question about what has happened with energy ingested but not built into the body mass.[27] A proposed mechanism is that the energy was released owing to an increase in the brown

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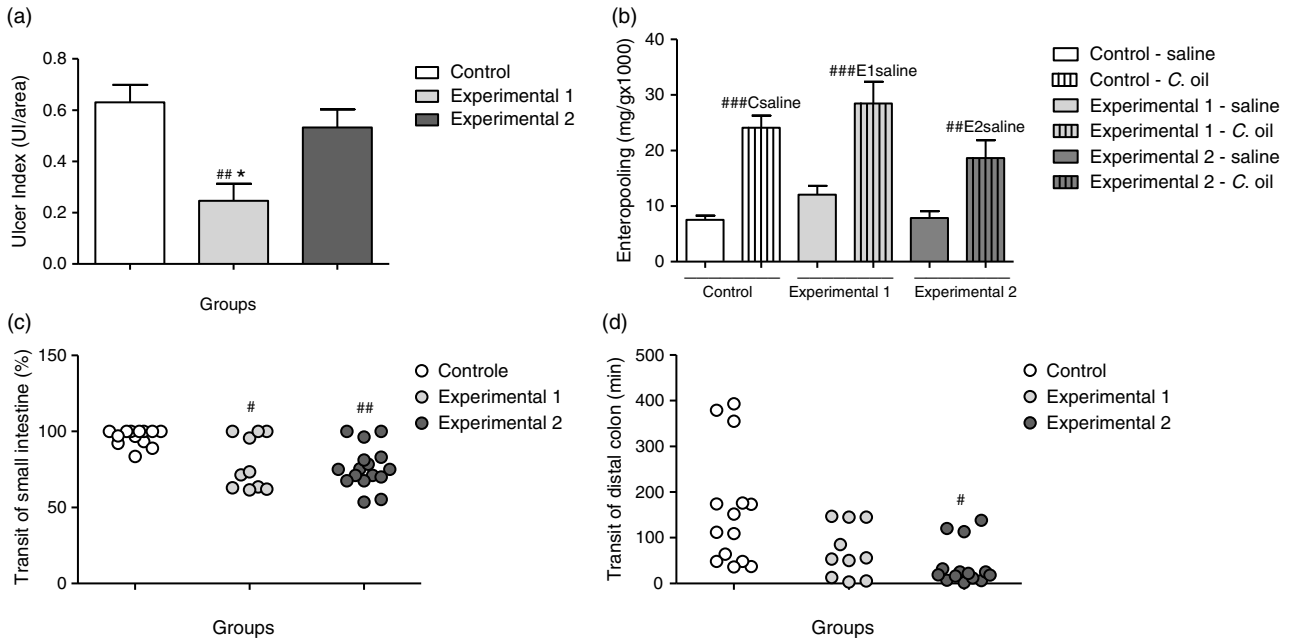

Fig. 3. UI, transit of small intestine and distal colon and enteropooling of offspring from control, experimental 1, and experimental 2 groups.
Values are expressed as mean ± SEM for UI (*C*: $n = 14$; *E*1: $n = 10$; *E*2: $n = 8$) and enteropooling (*C*: saline $n = 12$ e castor oil $n = 13$; *E*1: saline $n = 11$ e castor oil $n = 9$; *E*2: saline $n = 10$ e castor oil $n = 9$) (one-way ANOVA with Tukey's post-test) or individual values for transit of small intestine (*C*: $n = 15$; *E*1: $n = 16$; *E*2: $n = 16$) and distal colon (*C*: $n = 14$; *E*1: $n = 10$; *E*2: $n = 14$) (Kruskall–Wallis with Dunn's post-test). The level of significance was set at $p < 0.05$. #$p < 0.05$ versus control; ##$p < 0.01$ versus control; *$p < 0.05$ versus experimental 2.

adipose tissue mass and activity observed in rats treated with MMI.[27] Similar to that described by Kobayashi *et al.* [29] concerning the BW of rats exposed to propiltiuracil (PTU, another drug used to induce experimental hypothyroidism) during gestation and lactation, we observed no alteration in BW of dams treated with MMI, although they were hypothyroid and their food consumption was unchanged. Therefore, some maternal physiological adaption altered the effect of MMI concerning the BW loss. One possible explanation is that pregnancy may change the reported increases in brown adipose mass and activity associated to the experimental hypothyroidism. Corroborating this hypothesis, it has shown that brown adipose tissue thermogenesis is inhibited during late pregnancy and lactation in rats.[30,31] An adaption that might explain the unchanged weight gain in dams from *E*1 and *E*2 groups reported in the present study. Alternatively or in addition, it is possible that more time is required for changes in weight gain to become evident after hypothyroidism induction. Indeed, it has shown that food intake decreased only 14 days post PTU treatment in both pregnant and male rats,[32,33] while weight loss was observed only 3 weeks after hypothyroidism induction.

Thyroid hormones affect growth,[34] and their effects during critical periods of cell proliferation and fetal development are evident in the reduced pups' BW from dams of the *E*1 and *E*2 groups. A variety of mechanisms can contribute to the growth retardation associated with hypothyroidism, such as abnormalities on growth factor secretion, decreases in insulin-like growth factor I synthesis, and also a direct (but insufficient) action of low *T*3 levels on growth plate and skeletal growth.[35] Despite these mechanisms might be responsible for the general growth failure in pups from both the *E*1 and *E*2 groups, they cannot explain the reduced BW of pups from the *E*1 group compared to those from the *E*2 group. Indeed, pups from dams that received MMI during gestation and lactation (*E*1) and those from dams that were treated with MMI only throughout the gestational period (*E*2) were not hypothyroid, as suggested by their normal *T*3 plasma levels (the active hormone). The literature has reported that pregnant rats with hypothyroidism present reduced prolactin plasma levels, decreased milk production and ejection, and also changes in milk composition.[36,37] Therefore, since the reduced BW observed in pups from the *E*1 group compared to those of pups from the *C* and *E*2 groups appears to be unrelated to the actions of the thyroid hormones, a malnutrition-like state in pups from the *E*1 group associated to alterations in the feeding processes (owing to the abnormalities described above) appears to be a reasonable explanation. In this regard, it is important to mention that postnatal growth is related to the size of the litter in rats[38]; however, maternal hypothyroidism did not affect the number of pups per litter nor the proportion between the male and female birth disproving, in both the *E*1 and *E*2 groups, that changes in feeding processes may be related to pups competition for milk. Concerning free *T*4 plasma levels (acting mainly as a prohormone), they were decreased only in pups that had fed in dams with hypothyroidism, probably reflecting the action of MMI in the sons' thyroid due to its excretion in the milk.[39,40] Altogether, *T*3 and *T*4 plasma levels observed in pups from the *E*1 group suggest a compensatory mechanism related to increases in *T*4 to *T*3 conversion and/or to decreases in *T*3 metabolism in this group, allowing the maintenance of the euthyroidism state.

As described above, thyroid hormones are important for normal development and hypothyroidism greatly impairs GI physiology in human and experimental animals; thus, we hypothesized that maternal hypothyroidism (during gestation and lactation) might be responsible for alterations in the offspring's GI development and function. Despite pups from the dams treated with MMI presented with decreases in the stomach and SIW, in the *E*1 and *E*1/*E*2 groups, respectively, no alterations in the organ:BW ratio (relative weight) were observed. Moreover, maternal hypothyroidism decreased the stomach area in both the *E*1 and *E*2 groups without a change in the small intestine length. These data suggest that the fetus exposure to the intrauterine environment provided by

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maternal hypothyroidism caused a general decrease in body development and that the alterations in the absolute weight of the small intestine and in the stomach area reflect this general change. However, the postnatal development of the stomach concerning the organ mass seems to have occurred normally in offspring fed by euthyroid dams but was impaired in puppies from dams with hypothyroidism. Since the offspring's BW from hypothyroid dams (i.e., *E*1) was smaller than that from euthyroid ones (i.e., *C* and *E*2 groups), the observed alteration in the SW of the *E*1 group probably reflects the postnatal impairment on the pups' growth associated with the feeding processes, as argued above. In this respect, the only truly morphological alterations associated with maternal hypothyroidism observed in the present study appear to be the stomach mass thickness, that was seen to be smaller in the *E*1 and biggest in the *E*2 group, and the ratio between the small intestine mass and small intestine length that was smaller in both the *E*1 and *E*2 groups. A lighter small intestine that has a normal length strongly suggests a reduced amount of contractile elements throughout its length. This would possibly explain, at least partially, the decreased intestinal transit observed in the present study in both the experimental groups. Impairment in the excitation–contraction coupling mechanism in the smooth muscle of small intestine unrelated to the number of contractile elements should also be considered and needs further investigation. Interestingly, the motor activity of distal colon was increased in pups that had fed in dams with hypothyroidism only during the gestational period. This alteration could represent an adaptive response to deal with the decrease in the small intestine activity discussed above; moreover, to be fed in dams with hypothyroidism appears to delay the expression of this response, since the distal colon activity of the *E*1 group was not different from that of control. The mechanism responsible for this functional alteration still to be determined.

An ethanol-induced ulcer is a multifaceted process that involves, among other mechanisms, polymorphonuclear leukocytes migration into the inflammation zones, as suggested by increases in myeloperoxidase activity (MPO; a marker enzyme of leukocytes) in those areas; these cells appear to be responsible for the production of reactive oxygen species that damage the stomach mucosa, an action related to MPO activity.[41] Moreover, pretreatment with MMI has been shown to protect the intestinal mucosa from injuries produced by trinitrobenzene sulfonic acid administration, an effect associated with decreases in inflammation and MPO activity.[42] Finally, neutrophil chemotaxis was impaired in children with protein-energy malnutrition.[43] In our experimental conditions, the offspring from the *E*1 group appeared to have malnutrition, judging by their reduced body mass, and they also had ingested milk with MMI in an effective dose, as suggested by their low *T*4 plasma levels. Therefore, these two facts, along with the apparent small stomach mass thickness, can explain the decreased susceptibility to ethanol-induced ulcers in pups from the *E*1 group.

It is known that ricinoleic acid, released after hydrolysis of castor oil by lipases, is a laxative that decreases fluid absorption, increases electrolyte secretion, and produces changes in intestinal motility.[44,45] Indeed, castor oil administration increased intestinal fluid accumulation (i.e., produced enteropooling) in offspring, an effect unaltered by maternal hypothyroidism during gestation or gestation and lactation periods. These observations suggest that intestinal fluid absorption and/or the electrolyte secretion process are sensitive to ricinoleic acid, which is preserved in pups from hypothyroid dams.

Finally, pregnancy is associated with several physiological adaptations such as increased maternal thyroid gland activity due to increased thyroxine-binding globulins, altered maternal thyroid hormones peripheral metabolism, and increased chorionic gonadotropin levels. Thus, a new state of thyroid balance is achieved and maintained until the end of pregnancy, but this state may be compromised in women who develop hypothyroidism in pregnancy or were already hypothyroid when they became pregnant.[46] The consequences of untreated maternal hypothyroidism during pregnancy range from preeclampsia to complications such as perinatal death, premature birth, among others.[12,13,47] In our study, pups from hypothyroid mothers (*E*1 and *E*2) had lower weight at 30 days old compared to controls, as it reinforces the importance of maternal thyroid hormones for the proper development of offspring. In this context, from human studies, fetal sex also appears to interfere with intrauterine development, so that male fetuses are most exposed to macrosomia, whereas female fetuses more predisposed to intrauterine growth restriction.[48,49] Regarding maternal thyroid function, a human study showed that increased thyroid activity during pregnancy was more associated with low birth weight in male newborns, whereas subclinical maternal hypothyroidism was more related with higher weight in newborn from the same sex.[50] It is possible that maternal hypothyroidism may result in different GI changes or different intensities between male and female pups from Wistar rats, but this was not the objective of our study.

Conclusion

Maternal hypothyroidism impaired the total body development, as well as gastric and intestinal development, and interfered with the susceptibility to the ulcer of the offspring. Furthermore, reduced small intestine motility; this alteration appears to be associated to an adaptive increase in the distal colon motility. Thus, the reduced GI motility in euthyroid adults may be a consequence of intrauterine exposure to low thyroid hormone levels and of this exposure during breastfeeding in hypothyroid dams. The present study adds to our general understanding of GI disorders pointing to pup's small intestine and stomach as pivotal sites associated to maternal hypothyroidism.

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Conflicts of Interest. None.

Ethical Standards. The authors assert that all procedures contributing to this work comply with the ethical standards of relevant national guides on the care and use of laboratory animals and has been approved by the institutional committee of UFU (069/17).

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