Gestational hypothyroidism elicits more pronounced lipid dysregulation in mice than pre-pregnant hypothyroidism

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Abstract. Thyroid hormone is crucial for regulating lipid and glucose metabolism, which plays essential role in maintaining the health of pregnant women and their offspring. However, the current literature is just focusing on the development of offspring born to the untreated mothers with hypothyroidism, rather than mothers themselves. Additionally, the interaction between hypothyroidism and pregnancy, and its impact on the women ‘s health are still elusive. Therefore, this study was designed to compare the metabolic differences in dams with hypothyroidism starting before pregnancy and after pregnancy. Pre-pregnant hypothyroidism was generated in 5-week-old female C57/BL/6J mice using iodine-deficient diet containing 0.15% propylthiouracil for 4 weeks, and the hypothyroidism was maintained until delivery. Gestational hypothyroidism was induced in dams after mating, using the same diet intervention until delivery. Compared with normal control, gestational hypothyroidism exhibited more prominent increase than pre-pregnant hypothyroidism in plasma total cholesterol and low-density lipoprotein cholesterol, and caused hepatic triglycerides accumulation. Similarly, more significant elevations of protein expressions of SREBP1c and p-ACL, while more dramatic inhibition of CPT1A and LDL-R levels were also observed in murine livers with gestational hypothyroidism than those with pre-pregnant hypothyroidism. Moreover, the murine hepatic levels of total cholesterol and gluconeogenesis were dramatically and equally enhanced in two hypothyroid groups, while plasma triglycerides and protein expressions of p-AKT, p-FoxO1 and APOC3 were reduced substantially in two hypothyroid groups. Taken together, our current study illuminated that gestational hypothyroidism may elicit more pronounced lipid dysregulation in dams than dose the pre-pregnant hypothyroidism.

Key words: Thyroid hormone, Lipid dysregulation, Glucose metabolism, Insulin signaling

HYPOTHYROIDISM is a metabolic disease which is characterized by low level of thyroid hormone (TH) and high level of thyroid-stimulating hormone in serum [1]. TH plays an important role in regulating lipid and glucose metabolism and insulin signaling [2-4]. According to previous literature, TH stimulates cholesterol and other lipid synthesis, increases mobilization of plasma cholesterol and triglycerides (TG) [5-7]. Recent researches indicated that hypothyroidism is closely associated with non-alcoholic fatty liver disease (NAFLD) independently, which may involve in altering intracellular TH action [8, 9]. Furthermore, thyroid dysfunction is commonly related to changes in plasma lipid levels [10]. Patients with hypothyroidism are frequently accompanied by hypercholesterolemia and a remarkable increase in low-density lipoproteins (LDL-C), which may result

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Abbreviations: SREBP1c, Sterol regulatory element binding protein-1c; p-ACL, phosphorylated ATP citrate lyase; CPT1A, Carnitine palmitoyltransferase A; LDL-R, low-density lipoprotein cholesterol receptor; p- AKT, phosphorylated protein kinase B; p-FoxO1, phosphorylated forkhead box transcription factor O1; APOC3, Apolipoprotein CⅢ.
from the decrease in the number and activity of LDL receptors (LDL-R) in liver [11, 12]. Experimental hypothyroidism in rats treated with propylthiouracil (PTU) showed an increase in plasma LDL-C, and decrease in plasma TG [13], which can be normalized by substitution therapy with TH [14].

The prevalence of hypothyroidism in women at reproductive age is relatively high, which is connected with hyperprolactinemia, menstrual abnormalities and anovulation in some cases [15]. Hence, pregnancy has been regarded as infrequent in untreated women with hypothyroidism [16]. However, it has been reported that some hypothyroid women with insufficient or no treatment were still able to carry pregnancy to full terms [17, 18].

On the other hand, hypothyroidism is also the most common diseases driven by pregnancy, affecting 3–5% of all pregnant women [19]. It’s worth mentioning that both women with hypothyroidism before pregnancy and those diagnosed to have hypothyroidism during pregnancy have experienced the short and long-term adverse consequences for themselves and their offspring [20]. Untreated mothers with hypothyroidism might have the increased risks of anemia, miscarriage, premature delivery, placental abortion, gestation-induced hypertension and pre-eclampsia, gestational diabetes, postpartum hemorrhage and adverse offspring outcomes such as, low birth weight, stunted growth, and mental retardation [21-23]. Nevertheless, not all studies have found an increased risk of adverse outcomes for mothers with hypothyroidism or for their offspring [22]. Meanwhile, previous researches suggested that untreated pregnant women with hypothyroidism may develop hypothyroidism and diabetes in the years after pregnancy [24, 25]. Considerable research efforts have been devoted to untreated experimentally-induced maternal hypothyroidism that might have serious consequences for offspring development like behavior, skin, brain [26-28]. However, few systematic studies have concerned the interaction between hypothyroidism and gestation. Therefore, the objective of the current study was to compare the differences of hepatic protein expression, insulin signaling, lipid and glucose metabolism between the pre-pregnant hypothyroidism and gestational hypothyroidism in dams after delivery. Taken together, our current data demonstrated that gestational hypothyroidism may elicit more obvious lipid dysregulation in dams after delivery than the pre-pregnant hypothyroidism.

**Materials and Methods**

**Animals and experimental design**

Animal procedures were approved by the Animal Care and Use Committee, faculty of science, Anhui Medical University, in accordance with the international guiding principles for biomedical research involving animals of CIOMS.

Male and female mice of the C57BL/6J were purchased at 4 weeks of age from Changzhou Cavens, China. All mice were raised in the specific-pathogen-free (SPF) laboratory under conditions of controlled temperature (23 ± 2°C) and relative humidity (50–60%) with a 12 h light/dark cycle. After one week of acclimatization, female mice (16.8 ± 0.8 g) were randomly divided into pre-pregnant hypothyroidism group (PH), gestational hypothyroidism group (GH) and normal control group (NC). The mice in PH group received iodine-deficient diet containing 0.15% propylthiouracil [29] (IDP diet, cat. #D18503, Jiangsu Xietong Organism Co, Jiangsu, China) and normal drinking water for 4 weeks before pregnancy, while standard irradiated diet (cat. #D10001, Jiangsu Xietong Organism Co, Jiangsu, China) and water were available ad libitum to the mice in GH and NC groups, simultaneously. The murine body weights and fasting plasma glucose (FPG) levels were measured each week. Serum samples were obtained (n = 6/group) after four weeks of treatment for detecting free triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH). At age of 10 weeks, female mice were placed together with males at a ratio of 2:1. The presence of a vaginal mucous plug in next morning after mating was considered as an index of pregnancy and this day was defined as the start of gestational days (GD0). The mice in PH and NC groups during pregnancy continued to receive the diet and water identical to pre-pregnancy, but the mice in GH group started to receive the IDP diet from GD0. Pregnant mouse’s body weights and FPG levels were detected at GD0, GD3, GD6, GD9, GD12, GD15, GD18 respectively, in three groups. By the end of delivery, after 8 h fasting, dams (n = 10/group) were euthanized with 10% chloral hydrate and then killed by cervical dislocation.

**Blood and Tissue Sample Collection**

Before the execution of the mice in different groups, whole blood was collected via orbital venous plexus at the indicated time points and then allowed it to clot. Serum samples were obtained by centrifuging at 3,000 rpm, 4°C for 10 minutes, then kept at –80°C. Thyroid, pancreas and liver tissues were harvested from the sacrificed mice after dissection. Major portions of thyroid and pancreas tissues were fixed in 4% paraformaldehyde solution for histological examination. Liver tissues were washed with saline and immediately frozen in liquid nitrogen, then stored at –80°C for western blotting and biochemical analysis.
Glucose tolerance test
To perform glucose tolerance test (GTT), mice were fasted 8 h after delivery \( (n = 8/\text{group}) \) and then the blood glucose from tail vein was measured by glucometer \( (t = 0) \) (ACCU-CHEK Performa, Roche, USA). At this point, glucose (2 g/kg of body weight) was injected intraperitoneally. Blood glucose samples were tested at 30, 60, 90, 120 and 150 min, respectively. Area-under-curve (AUC) was calculated by the trapezoid rule.

Plasma biochemical parameters
The plasma levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and hepatic concentrations of TC, TG were determined \( (n = 10/\text{group}) \), respectively, according to the manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). The levels of TSH, FT3, FT4 and Insulin in plasma were detected by ELISA kits \( (n = 10/\text{group}) \) (Jianglai, Shanghai, China). The plasma glucose levels were analyzed using a glucose analyzer \( (n = 10/\text{group}) \). The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated using the following formula: fasting insulin levels (mIU/L) × fasting glucose levels (mmol/L)/22.5.

Histological analyses
Liver, thyroid and pancreas tissues were fixed in 4% paraformaldehyde solution and dehydrated in a graded series of ethanol, then embedded in paraffin and cut into 4 μm sections. The sections were deparaffinized with xylene, rehydrated in a descending graded series of ethanol and stained with hematoxylin and eosin (H&E) to assess morphological changes of the thyroid glands or pancreatic islets or hepatic cells, respectively.

Protein extraction and Western Blotting
Murine livers were homogenized in ice-cold RIPA lysis buffer containing 1% phenylmethanesulfonyl fluoride (PMSF) and 2% phosphatase inhibitor cocktail, followed by centrifugation at 15,000 rpm, 4°C for 15 min to harvest the protein lysates. Total protein concentrations were determined by bicinchoninic acid (BCA) method. The equal amounts of proteins for each lane were aliquoted, denatured and then frozen at –80°C before experiment. Electrophoresis was conducted to separate protein samples using SDS-PAGE, which were further transferred onto PVDF membranes. The membranes were blocked with 5% skim milk for 1.5 h at room temperature, and incubated with appropriate primary antibody at 4°C overnight. After being washed four times with TBST buffer for 8 min/each, the membranes were incubated with secondary antibodies at room temperature for 1–1.5 h, and then the signals were detected using enhanced chemiluminescence reagent and the densities of the bands were quantified by scanning densitometry using Image Software. Each sample was analyzed by an average of three independent tests involving different gels. Antibodies including protein kinase B (AKT), phospho-protein kinase B (Thr308) \( (p-\text{AKT} \ T308) \), forkhead box transcription factor O1 (FoxO1), phospho-forkhead box transcription factor O1 \( (p-\text{FoxO1}) \), ATP citrate lyase (ACL), phosphoenolpyruvate carboxykinase (PEPCK), glycogen synthase kinase 3 beta \( (\text{GSK3β}) \) and phospho- glycogen synthase kinase 3 beta \( (p-\text{GSK3β}) \) were purchased from Cell Signaling Technology Inc. Antibodies against \( p-\text{ACL} \), Sterol regulatory element binding protein-1c \( (\text{SREBP1c}) \), 3-hydroxy-3-methylglutaryl-CoA synthase 1 \( (\text{HMGS1}) \), Carnitine palmitoyl transferase 1A \( (\text{CPT1A}) \), LDL receptor \( (\text{LDL-R}) \), Apolipoprotein A1 \( (\text{ApoA1}) \), Apolipoprotein CIII \( (\text{APOC3}) \) were from Abcam. Glucose 6-phosphatase \( (\text{G6Pase}) \) was purchased from Thermo Scientific. Type I iodothyronine deiodinase (DIO1) was from Santa Cruz Biotechnology Inc.

Statistical analysis
Experiment data were analyzed using SPSS 16.0 software (IBM Corporation, Armonk, USA) and expressed as mean ± SEM. Comparisons among groups were performed using one-way or two-way ANOVA, followed by Student-Newman-Keuls (SNK) multiple range test or Tukey’s multiple comparison test. The pregnancy rates were statistically processed by chi-square test of four-fold table. A value of \( p < 0.05 \) indicated a statistically significant difference. Graphics were constructed using GraphPad Prism 6 (GraphPad Software, San Diego, CA).

Results
Pre-pregnant hypothyroidism and gestational hypothyroidism exhibited equal thyroid dysfunction
The whole experimental scheme was demonstrated as Fig. 1A. As expected, we found that serum levels of FT3 and FT4 were significantly decreased in the female mice after 28 days of feeding the IDP diet compared to those with the standard diet \( (p < 0.05, \text{Fig. 1B}) \). Meanwhile, the plasma TSH levels in PH group were markedly higher than those in GH and NC groups \( (p < 0.05, \text{Fig. 1B}) \). To induce gestational hypothyroidism, the GH group was treated with IDP diet from day 1 of pregnancy. After delivery, plasma FT3, FT4, and TSH levels were measured to confirm the success of the model. Like pre-pregnant hypothyroidism, gestational hypothyroidism also observably lowered serum levels of FT3 and FT4 while enhanced TSH levels in the GH group by the end of pregnancy as compared with the NC group.
decreased mean size of follicular diameter as compared to normal controls, plasma TC and LDL-C levels as well as hepatic TG levels were markedly raised in PH group, whereas the levels in GH group were showed much more inhibition than those in PH group. Additionally, CPT1A protein levels were moderately suppressed in PH group compared to NC group, whereas the levels in GH group were showed much more inhibition than those in PH group. In comparison with GH group, the liver tissues from PH group exhibited dramatically elevated blood glucose levels starting from GD9, as compared with NC group at the same time, dams in PH groups displayed vacuoles of fat droplets at the periphery of hepatic lobule. In comparison with GH group, the liver tissues from PH group displayed vacuoles of fat droplets with decreased size and number. Plasma TG levels were vastly reduced in GH and PH groups compared with those in NC group, in which there was no significant difference between GH and PH groups. Upon comparison with normal controls, plasma TC and LDL-C levels as well as hepatic TG levels were markedly raised in PH group. Moreover, the three biochemical indexes in GH group were much more elevated than those in PH group, suggesting much more accumulation of hepatic fat in GH group. It should be pointed out that the hepatic ApoA1 protein levels were similar in all groups, which matches with normal controls, plasma TC and LDL-C levels as well as hepatic TG levels were markedly raised in PH group, whereas the levels in GH group were showed much more inhibition than those in PH group. Furthermore, CPT1A protein levels were moderately suppressed in PH group compared to NC group, whereas the levels in GH group were showed much more inhibition than those in PH group. In comparison with GH group, the liver tissues from PH group exhibited dramatically elevated blood glucose levels starting from GD9, as compared with NC group at the same time, dams in PH groups displayed vacuoles of fat droplets at the periphery of hepatic lobule. In comparison with GH group, the liver tissues from PH group displayed vacuoles of fat droplets with decreased size and number. Plasma TG levels were vastly reduced in GH and PH groups compared with those in NC group, in which there was no significant difference between GH and PH groups. Upon comparison with normal controls, plasma TC and LDL-C levels as well as hepatic TG levels were markedly raised in PH group. Moreover, the three biochemical indexes in GH group were much more elevated than those in PH group, suggesting much more accumulation of hepatic fat in GH group. It should be pointed out that the hepatic ApoA1 protein levels were similar in all groups, which matches
Fig. 1  Histopathological and hormonal examination of murine thyroid tissues revealed the hypothyroidism in postpartum dams

(A) Schematic timeline and treatments of current study. Female mice (C57BL/6J) were fed with iodine-deficient diet containing 0.15% propylthiouracil or with standard diet for 4 weeks starting at the age of week 5. (B) Free triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH) levels were determined by ELISA kit respectively in the mice at week 9 \((n = 6/\text{group})\). After mating, the mice were either maintained the same diet feeding as pre-pregnancy (PH and NC groups) or switched to IDP diet feeding at day 1 of pregnancy (GH group) throughout the whole gestation. (C) The serum levels of FT3, FT4, and TSH in pregnant mice \((n = 10/\text{group})\) were analyzed by ELISA after delivery, respectively. (D) Paraffin-embedded murine thyroid tissues in various groups were stained with Hematoxylin and Eosin (H&E) dye. Morphological and pathological changes were observed by a microscope at ×200 in original magnification. Arrow: thyroid follicular cells. Scale bar 50 μm. (E) Hepatic protein expressions of DIO1 in dams in different groups were assayed by immunoblotting and quantified by actin levels. Data are presented as mean ± SEM, ** \(p < 0.01\), *** \(p < 0.001\). NC: normal control group, GH: gestational hypothyroidism group, PH: pre-pregnant hypothyroidism group.
Fig. 2  Both pre-pregnant hypothyroidism and gestational hypothyroidism induced slow weight gains and hyperglycemia during gestation. Murine body weights (A) and fasting plasma glucose levels (B) in each group (n = 10) were recorded once a week from week 5 to week 9. Maternal body weights (C) and fasting glucose concentrations (D) were measured once every three days from GD0 to GD18 in different groups (n = 10). (E) The 24 h-food intakes in three groups were measured once a week from week 5 to week 9. (F) The 24 h-food intakes were detected once every three days from GD0 to GD18 in different groups. (G) The pregnancy rate and litter size of each group. Data are presented as mean ± SEM. *p < 0.05, **p < 0.001, PH group vs. NC group; +p < 0.05, +++p < 0.001, GH group vs. NC group by two-way repeated-measure ANOVA with Tukey’s multiple comparison test. NC: normal control group, GH: gestational hypothyroidism group, PH: pre-pregnant hypothyroidism group.
up with plasma HDL-C data ($p < 0.05$, Fig. 4F). Additionally, the APOC3 protein levels have been observably restrained in two hypothyroid groups ($p < 0.05$, Fig. 4G), which was consistent with the data in Fig. 3A.

**Pre-pregnant hypothyroidism and gestational hypothyroidism elicited the same degree of insulin resistance in dams**

The serum insulin levels from the hypothyroid and euthyroid groups did not differ significantly, whereas the hypothyroid mice exhibited a significant increase in fasting plasma glucose, which led to higher HOMA-IR values than those in the control mice. Of note, the values of HOMA-IR were equally enhanced in two hypothyroid groups ($p < 0.05$, Fig. 5A-5C). As presented in Fig. 5D, the fasting plasma glucose levels were similar between GH and PH groups, which were, however, observably higher than those in normal control group. For the glucose tolerance tests, we can see that after administration of glucose in dams after delivery, both of the induced glucose disposals in PH and GH groups were sharply impaired as compared to normal controls. The dams from two hypothyroid groups displayed the same degree of increase in the AUC of blood glucose by the GTT as compared with NC mice ($p < 0.05$). By H&E staining, the murine islets in NC group appeared pale with stained rounded or oval areas inside the pancreatic lobules surrounded by the exocrine parenchyma (Fig. 5E). While pancreatic tissues from two groups of hypothyroid mice showed severe degeneration of islets, with the irregular shape and obvious reduction in the size and number, in which many of the islet cells demonstrated clear or vacuolated cytoplasm and pyknotic nuclei. Given the aforementioned changes of those characteristics, we examined the phosphorylated ($p$-) forms of AKT Thr308 and the FoxO1 to explore the intrahepatic mechanism of...
Fig. 4 Gestational hypothyroidism more effectively promoted hepatic fatty acid synthesis than did the pre-pregnant hypothyroidism in dams. (A) The murine hepatic protein levels of SREBP1c in NC, GH, PH groups were determined by immunoblotting and quantified by actin levels. (B) The protein levels of ACL and p-ACL were displayed by immunoblotting, and the phosphorylated ACL was quantified by total ACL. (C-G) Hepatic protein expressions of CPT1A, HMGCS1, LDL-R, ApoA1, APOC3 in different groups were assayed by immunoblotting and quantified by actin levels, respectively. Data are presented as the mean ± SEM from 3 independent experiments, *p < 0.05, **p < 0.01, ***p < 0.001. NC: normal control group, GH: gestational hypothyroidism group, PH: pre-pregnant hypothyroidism group.
insulin resistance. We found that the ratios of phosphorylated AKT (p-AKT T308) to total AKT indicated the effective inhibition of AKT activity elicited by the two hypothyroidism types as compared to that in the NC group. However, no difference of AKT activities was observed between two hypothyroidism groups (p < 0.05, Fig. 5F). The same pattern of effect on phosphorylated FoxO1 was also revealed in the mice from two hypothyroid groups. The ratios of p-FoxO1 to total FoxO1 were dramatically reduced in GH and PH groups, elucidating the similar impairment for hepatic insulin signaling in dams treated with IDP diet starting before and during pregnancy (p < 0.05, Fig. 5G).

**Pre-pregnant hypothyroidism and gestational hypothyroidism vastly and equally enhanced the hepatic gluconeogenesis**

Both PEPCK and G6Pase are key rate-limiting enzymes required for hepatic gluconeogenesis, which plays an important role in maintaining glucose homeostasis in fasting conditions, and which is also dramatically enhanced under some pathological conditions such as insulin resistance. Hence, we next examined the two protein expression levels in murine livers by western blotting to assess the effects of two different hypothyroidisms on dams. Fig. 6A and 6B suggest that the PEPCK and G6Pase protein levels have been dramatically elevated in GH and PH groups as compared with NC group (p < 0.05), but those levels in two hypothyroid groups did not differ statistically (p > 0.05). On the other hand, the ratios of p-GSK3β to total GSK3β were remarkably curbed in two hypothyroid groups, illustrating improved hepatic GSK3β activity or weakened hepatic glycogen synthesis in two hypothyroid mice (p < 0.05, Fig. 6C).

**Discussion**

Based on our present study, both pre-pregnant hypothyroidism and gestational hypothyroidism, as compared with euthyroid controls, led to decreased growth and hyperglycemia in mice. On the contrary, hypothyroidism is associated with weight gains elicited by reduced energy expenditure in humans. In spite of the difference, our current findings regarding the inhibited growth are supported by the previous studies in experimental rodents with hypothyroidism [29, 30]. In comparison with NC group, the female mice in hypothyroid groups displayed a significant decrease in food intake (Fig. 2E and 2F), it may explain the differences of body weights exerted by hypothyroidism between humans and rodents.

The patients with hypothyroidism have been associated with elevated serum TC and TG levels as well as NAFLD [31]. However, our current results showed that two hypothyroidism groups elicited significantly reduced plasma TG (Fig. 3A), which may be partly explained by both significantly increased serum pseudocholinesterase (PChE) and lipoprotein lipase (LPL) activity elicited by TH deficiency in animals, although the activities of both enzymes are reduced in hypothyroid patients [32]. Moreover, APOC3, an inhibitor of LPL activity, plays a crucial role in the catabolism of triglycerides, and the suppressed expression of the gene may be connected with the raised LPL activity and then the decrease in plasma TG level [33], which is consistent with our current result (Fig. 4G).

Unexpectedly, our findings indicated that gestational hypothyroidism may be more potent than pre-pregnant hypothyroidism to increase plasma TC and LDL-C levels in dams, which may be owing to intrahepatic mechanisms. The GH may be more effective to decrease the hepatic absorption of plasma cholesterol by reducing the number and activity of hepatic LDL-R, than does the PH, contributing to the difference of plasma TC, LDL-C levels in two hypothyroid groups.

In our current study, in addition to the decrease in serum TH, hepatic DIO1, a key enzyme that activates TH and converts thyroxine to triiodothyronine, is also downregulated in GH and PH groups, which matches up with previous study [34, 35]. The mechanism for the regulation of SREBP1c by TH has remained controversial. One experimental animal study reported that SREBP1c transcription was negatively regulated by TH via a putative negative thyroid hormone response element (nTRE) [36], but another study has shown that SREBP1c transcription is upregulated by non-genomic thyroid hormone signaling [37]. In our current study, the expressions of some hepatic TH-responsive proteins were sharply enhanced, such as SREBP1c, ACL [38], HMGCS1 [9], which are consistent with the promotions of fatty acid and cholesterol synthesis, respectively (Fig. 4A, 4B and 4D). Whereas CPT1A, another TH-responsive protein [39], was more greatly down-regulated in GH group than did in PH group (Fig. 4C), that matches with more accumulation of hepatic TG in GH mice. To date, no systematic researches have been conducted to explore the time effects of thyroid hormone on lipid dysregulation. Hence, why the GH group showed unexpected more vigorous elevation of hepatic lipids than PH group requires further investigation.

TH is one of the key regulators of development, growth and metabolism in all tissues. It has been reported that lessened TH levels could have a role in progression of pancreatic β-cell dysfunction, resulting in the decrease in glucose stimulated insulin secretion [40, 41], which is consistent with the pathological changes of the
Fig. 5  Gestational hypothyroidism significantly impaired hepatic insulin sensitivity on dams, which was similar to the effect by pre-pregnant hypothyroidism

Murine plasma insulin levels (A) and blood glucose (B) were detected after 8 h fast (n = 10/group). (C) The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a screening index for insulin sensitivity and calculated via the values measured in (A) and (B). (D) Blood glucose levels during a glucose tolerance test (GTT, 2 g/kg glucose) were assayed after delivery (n = 8/group). (E) Pancreatic tissues from hypothyroid or normal mice were fixed in 4% paraformaldehyde for H&E staining. The results are shown at ×200 magnification. Arrow: pancreatic islet. Scale bar 50 μm. (F) Displayed are the protein levels of AKT, and p-AKT S473. The AKT Ser473 phosphorylation was quantified by total AKT. (G) Total and phosphorylated forms of FoxO1 were detected by immunoblotting, and the p-FoxO1 was quantified by total FoxO1. All data are presented as the mean ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.001, PH group vs. NC group; ' p < 0.05, ** p < 0.01, *** p < 0.001, GH group vs. NC group by two-way repeated-measure ANOVA with Tukey’s multiple comparison test. *** p < 0.001. NC: normal control group, GH: gestational hypothyroidism group, PH: pre-pregnant hypothyroidism group.
Due to the influence of many factors, our findings may suggest that two types of hypothyroidism equally and significantly impaired hepatic insulin sensitivity, and that these results were achieved by regulating the key proteins involved in the insulin signaling, such as AKT, FoxO1 (Fig. 5F and 5G). Meanwhile, enhanced fatty acids delivery to the liver increases hepatic acetyl-CoA (Ac-CoA) content, leading to allosteric activation of pyruvate carboxylase (PC) that promotes hepatic gluconeogenesis [42]. PEPCK and G6Pase are involved in the rate-limiting steps in gluconeogenesis, and it has been recently reported that TH is essential for PEPCK and G6Pase transcriptions [43]. In this study, the up-regulations...
of PEPCK and G6Pase were observed in hypothyroid mice, which might be mediated by the indirect action of TH (Fig. 6A and 6B). In addition, it is well accepted that the repression of AKT phosphorylation downregulates GSK3β phosphorylation [44]. Our current study manifested that hypothyroidism in two groups of mice elicited the same degree of promotion of GSK3β activity, thus resulting in the same inhibition of hepatic glycogen synthesis, which is consistent with our data that the FPG levels in hypothyroid mice were enhanced (Fig. 5B and 6C).

In conclusion, our current study illuminated that mild hypothyroidism may have an impact on insulin resistance and result in metabolic dysfunction of glucose and lipid in dams. More importantly, our present results revealed that gestational hypothyroidism may elicit much more significant lipid dysregulation in dams than do pre-pregnant hypothyroidism. These findings may shed a new light on future clinical practice.

Author Contributions

Keyang Chen and Zhengxuan Jiang designed the study, obtained research fund, supervised the study and reviewed/revised the manuscript. Jun Zhou and Xuan Dong performed the experiments, analyzed and interpreted data, and wrote the draft of manuscript. Yajing Liu, Yajing Jia and Yang Wang prepared reagents and other experimental materials. Ji Zhou validated the data. All authors read and approved the manuscript for publication.

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Competing Interests

All authors declare no conflict of interest.

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