

## Hemodynamic properties and arterial structure in male rat offspring with fetal hypothyroidism

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**Abstract.** Thyroid hormones (THs) play a crucial role in the development of different systems during fetal life; fetal hypothyroidism (FH) is associated with reduced cardiac function and dimensions in neonates. The aim of this study is to determine whether TH deficiency during fetal life is associated with arterial structural and hemodynamic changes during adulthood. Hypothyroidism was induced by adding 0.025% 6-propyl-2-thiouracil in drinking water throughout pregnancy, while controls consumed only tap water. Hemodynamic parameters, cross-sectional area, intima-media thickness (IMT), and density of nuclei of smooth muscle cells and endothelial cells (ECs) in the aorta and mesenteric arteries were measured. Compared to controls, in the FH group, baseline systolic blood pressure ( $105.7 \pm 3.1$  vs.  $87.9 \pm 3.3$  mm Hg,  $p < 0.01$ ), diastolic blood pressure ( $64.4 \pm 1.7$  vs.  $53.2 \pm 2.1$  mm Hg,  $p < 0.05$ ), and mean arterial pressure ( $80.9 \pm 2.1$  vs.  $67.1 \pm 2.1$  mm Hg,  $p < 0.01$ ) were significantly lower. In addition, in the FH group, intensity and latency of response to phenylephrine were significantly lower and longer, respectively, as were the IMT and density of ECs in the aorta and superior mesenteric arteries. In conclusion, this study showed that TH deficiency during fetal life can have long-lasting functional and histological effects, which can compromise cardiovascular function during adulthood.

**Key words:** Fetal hypothyroidism – Blood pressure – Phenylephrine – Arterial structure – Rat

**Abbreviations:** BP, blood pressure; DBP, diastolic blood pressure; ECs, endothelial cells; FH, fetal hypothyroidism; HR, heart rate; IMT, intima-media thickness; MAP, mean arterial pressure; PE, phenylephrine; PTU, 6-propyl-2-thiouracil; SBP, systolic blood pressure; SMCs, smooth muscle cells; THs, thyroid hormones; TT<sub>3</sub>, total triiodothyronine; TT<sub>4</sub>, total thyroxine.

### Introduction

It is well known that thyroid hormones (THs) directly affect the peripheral vascular system and heart (Gomberg-Maitland and Frishman 1998). In adulthood, hypothyroidism is

associated with decrease of cardiac output and contractility, as well as normal or decreased resting heart rate (HR), whereas the systemic vascular resistance is increased (Rhee and Pearce 2011).

Fetal life and intrauterine growth conditions are strongly related to the physiological function and risk of disease during adulthood (Langley-Evans 2006). Development of the fetus is primarily dependent on its genetic equipment (York et al. 2014), as well as on the nutritional, hormonal,

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and metabolic influence during intrauterine growth (Tzanetakou et al. 2011).

Maternal hypothyroidism is a common clinical condition, affecting 2–5% of pregnant women (Teng et al. 2013). The fetal thyroid gland is inactive until birth (Galton et al. 1999) and maternal THs are the only source of THs during fetal life (Santos et al. 2012). Adverse effects of TH deficiency during fetal development have been evaluated on various systems (Chattergoon et al. 2012; Karbalaei et al. 2013).

Few studies documented developmental effects of TH deficiency during fetal life on cardiovascular system function in adulthood, some show that TH deficiency during this period leads to lower HR with a reduced cardiac output and cardiac dimensions in infancy (Fouron et al. 1982). Animal studies have also shown that TH deficiency during fetal life up to 30 days after birth postpones the maturation of ventricular contractile proteins in rats (Chizzonite and Zak 1984); moreover, our previous study showed that HR, left ventricular developed pressure (LVDP) and the peak rates of positive and negative changes in left ventricular pressure ( $\pm dp/dt$ ) are lower in isolated rat hearts from adult fetal hypothyroid (FH) rats (Ghanbari et al. 2015). Apparently insufficiency of THs during fetal life leads to a diminution of cardiac function in infancy. Since no data are available concerning the long-lasting effects of TH deficiency during fetal period on hemodynamic parameters and arterial structure in adulthood, the purpose of this study was to evaluate the effects of deficiency of THs during pregnancy on hemodynamic parameters, and their responses to phenylephrine (PE), as well as aorta/mesenteric arteries wall histological structures in adult rat offspring.

## Materials and Methods

### Animals

All animals were obtained from the Research Institute for Endocrine Sciences (RIES) of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Animals were housed in polypropylene cages (42 cm  $\times$  28 cm  $\times$  15 cm) in a 12-h light/dark cycle at  $22 \pm 2^\circ\text{C}$  with food and water ad libitum. Experimental procedures were conducted in accordance with the local ethics committee rules for the care and use of laboratory animals of the RIES.

### Hypothyroidism induction

Virgin female Wistar rats ( $200 \pm 10$  g,  $n = 33$ ) at the ovulatory phase (determined by vaginal smears) were housed overnight with male rats ( $300 \pm 20$  g,  $n = 33$ ) for mating (one female and one male rat in each cage). Following pregnancy, female rats were randomly divided into the hy-

pothyroid and control groups. To induce hypothyroidism, 0.025% (250 ppm) 6-propyl-2-thiouracil (PTU) (Sigma-Aldrich, Hamburg, Germany) was added to the drinking water of hypothyroid rats from the first day of pregnancy until delivery day (Farahani et al. 2013), while the control group consumed only tap water. After birth, the weight of the neonates in both groups ( $n = 45$ – $31$ /groups) was measured weekly (A & D scale, EK-300i, Japan; sensitivity 0.01 g) from the first day of birth till the end of the third month.

### $TT_3$ and $TT_4$ measurements

To assess TH status, blood samples were obtained from both mothers (after delivery) and offspring (at birth and days 7, 14, 21, 28, and 90), centrifuged ( $3000 \times g$ , 10 min at  $4^\circ\text{C}$ ), and the sera were stored at  $-80^\circ\text{C}$  until the time of assay. Total triiodothyronine ( $TT_3$ ) and total thyroxine ( $TT_4$ ) levels were measured by ELISA kits (PishtaztebZaman Co., Iran). Intra- and interassay coefficients of variations were 3.7% and 4.3% for  $TT_3$  and 5.3% and 5.9% for  $TT_4$ , respectively.

### Surgical procedures

Adult male offspring in the FH and control groups ( $n = 10$  each group) were assessed at age  $95 \pm 2$  days after birth. Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) (Sigma, Hamburg, Germany) which comparing to other anesthetics minimally affects the hemodynamic parameters (Bencze et al. 2013). The animals were heated to prevent the effect of temperature decline on cardiovascular parameters. Body temperature was monitored to maintain a constant rectal temperature. A flexible polyethylene cannula (PE-50) containing heparin solution (40 U/ml) (Ferreira et al. 2009) was inserted in the left femoral artery and connected to a pressure transducer (MLT844-Sweden). Hemodynamic parameters including systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and HR were then recorded with a power lab system (AD Instruments, ML866, Australia). The left femoral vein was also cannulated to provide venous access.

### Measurement of cardiovascular system response to PE

Baseline levels of hemodynamic parameters were recorded for a period of 20 minutes to allow their stabilization, after which SBP, DBP, MAP, and HR were measured. After documenting their baseline values, the PE (Sigma-USA) was injected in both groups intravenously in doses of 0.25, 0.5, 1, 2, 5, 8, and 10  $\mu\text{g}/\text{kg}$ , respectively (Nijsen et al. 2001; Loss Ide et al. 2007; Cannesson et al. 2012; Guimaraes et al. 2012) and the HR and blood pressure (BP) were recorded. Peak of hemodynamic changes was recorded for assessment

of response to PE. After the injection of each dose, and before the injection of next dose, the BP and HR levels were allowed to return to their baseline levels.

#### Measurement of heart weight

In both FH and control groups at the age of 7, 14, 21, 28 and ~ 95 days, the hearts of part of animals (5–6 animals at each age) were removed, their weights were measured by a digital scale (Sartorius, TE124S, d = 0.1 mg, Gottingen, Germany) and the heart to body weight ratio was calculated.

#### Histological study

To study the histology of the thoracic aorta and small mesenteric arteries, adult offspring at age ~ 95 days in both FH and control groups ( $n = 10$  in each group) were deeply anesthetized with sodium pentobarbital as described previously and perfused with 0.9% NaCl; specimens of the thoracic aorta (1 cm of the middle part) as well as superior mesenteric and small intestinal arteries were removed and fixed in 10% neutral formalin. Specimens were processed in paraffin and 6  $\mu\text{m}$  (for aorta) and 5  $\mu\text{m}$  (for mesenteric arteries) thick slices were then stained with hematoxylin and eosin. Four sections at equal intervals (1 slice every 30  $\mu\text{m}$ ) were selected in each sample (Morvan et al. 2013) and digitally photographed using a Nikon microscope (ECLIPSE-E200, Tokyo, Japan) at magnifications of 40 $\times$ , 400 $\times$ , and 1000 $\times$ . ImageJ software (1.44p.NIH.USA) was then used to calculate cross-sectional areas (CSA) in all slices.

A rectangular area with a size of 2200  $\mu\text{m}^2$  (for aorta), 1000  $\mu\text{m}^2$  (for superior mesenteric arteries) and 600  $\mu\text{m}^2$  (for intestinal mesenteric arteries), was selected in four fields in sections at angles 0 $^\circ$ , 90 $^\circ$ , 180 $^\circ$ , 270 $^\circ$  and then the intima-media thickness (IMT) and density of nuclei of smooth muscle cells (SMCs), expressed as a number of SMCs in this area, were measured in aorta and mesenteric arteries (Olivetti et al. 1982); an intimal segment with length

of 80  $\mu\text{m}$  (for aorta) and 180  $\mu\text{m}$  (for superior mesenteric arteries) was selected in the angles and density of nuclei of endothelial cells (ECs), defined as number of nuclei of the thin layer of cells that lines the selected part interior surface of vessels, was counted. In the intestinal mesenteric arteries, the numbers of nuclei of ECs in round cross-sections with equal diameter were counted. The average of the four regions was calculated for each section (Adijiang et al. 2008).

#### Statistical analysis

Values were expressed as mean  $\pm$  SEM. Data were analyzed by GraphPad Prism software (Version 5). Two-way ANOVA followed by Bonferroni test was used for comparing changes in offspring body weight and hemodynamic parameters between groups. Student's  $t$ -test was used to compare histological parameters, heart weight and THs between groups;  $p$  values < 0.05 were considered statistically significant.

## Results

#### Hormone measurements

Treatment with PTU was effective in inducing hypothyroidism; serum  $\text{TT}_4$  levels in mothers (at delivery day) and neonates (at birth and after 7, and 14 days), was significantly lower in FH group compared to controls; however, no significant difference was observed in offspring at ages of 21, 28, and 90 days;  $\text{TT}_3$  was also lower in mothers (at delivery day) and neonates (until 21 days) in the FH group, and the offspring became euthyroid at the age of 28 and 90 days (Table 1).

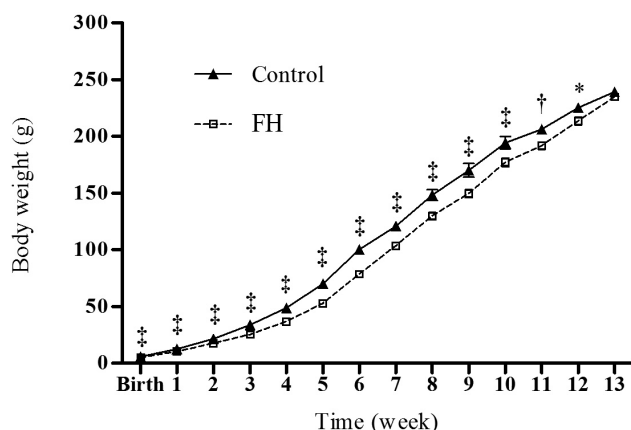
#### Effect of FH on heart, body weight and baseline hemodynamic parameters

Body weight in the FH group was significantly lower than in controls from birth until week 12, there was no difference at

**Table 1.** Serum levels of thyroid hormones and heart weight in control and hypothyroid rats

Groups	Total thyroxine ( $\mu\text{g}/\text{dl}$ )		Total triiodothyronine ( $\text{ng}/\text{dl}$ )		Heart weight (g)		Heart weight/Body weight	
	Control	Hypothyroid	Control	Hypothyroid	Control	Hypothyroid	Control	Hypothyroid
Mothers ( $n = 10$ )	2.4 $\pm$ 0.2	0.5 $\pm$ 0.1 <sup>‡</sup>	93.4 $\pm$ 3.8	51.7 $\pm$ 4.9 <sup>‡</sup>	–	–	–	–
Birth ( $n = 10$ )	0.7 $\pm$ 0.1	0.4 $\pm$ 0.1 <sup>‡</sup>	62.9 $\pm$ 3.4	39.5 $\pm$ 4.3 <sup>‡</sup>	–	–	–	–
7 days rats ( $n = 6$ )	5.2 $\pm$ 0.4	0.6 $\pm$ 0.2 <sup>‡</sup>	65.1 $\pm$ 4.4	27.6 $\pm$ 7.1 <sup>†</sup>	0.08 $\pm$ 0.004	0.05 $\pm$ 0.002 <sup>†</sup>	0.006 $\pm$ 0.0004	0.004 $\pm$ 0.0002 <sup>*</sup>
14 days rats ( $n = 6$ )	6.9 $\pm$ 0.7	2.1 $\pm$ 0.1 <sup>‡</sup>	86.4 $\pm$ 10.3	46.02 $\pm$ 9.2 <sup>†</sup>	0.14 $\pm$ 0.01	0.11 $\pm$ 0.01 <sup>*</sup>	0.005 $\pm$ 0.0003	0.01 $\pm$ 0.0005 <sup>†</sup>
21 days rats ( $n = 6$ )	5.8 $\pm$ 0.3	5.4 $\pm$ 0.3	98.1 $\pm$ 3.6	65.3 $\pm$ 7.8 <sup>†</sup>	0.15 $\pm$ 0.002	0.13 $\pm$ 0.01 <sup>*</sup>	0.004 $\pm$ 0.0001	0.006 $\pm$ 0.0004 <sup>†</sup>
28 days rats ( $n = 5$ )	5.7 $\pm$ 0.2	4.4 $\pm$ 0.7	108.9 $\pm$ 12.5	97.9 $\pm$ 10.5	0.26 $\pm$ 0.01	0.16 $\pm$ 0.003 <sup>‡</sup>	0.004 $\pm$ 0.0003	0.004 $\pm$ 0.0001
95 days rats ( $n = 10$ )	3.8 $\pm$ 0.1	3.4 $\pm$ 0.2	95.7 $\pm$ 4.3	87.7 $\pm$ 5.3	1.1 $\pm$ 0.1	0.9 $\pm$ 0.02 <sup>*</sup>	0.004 $\pm$ 0.0002	0.003 $\pm$ 0.00006

Each value is the mean  $\pm$  SEM; <sup>\*</sup>  $p < 0.05$ , <sup>†</sup>  $p < 0.01$ , <sup>‡</sup>  $p < 0.001$  vs. control.



**Figure 1.** Comparison of body weight of offspring with fetal hypothyroidism (FH) and control groups. Values are means  $\pm$  SEM ( $n = 45\text{--}31/\text{groups}$ ); repeated measure ANOVA followed by Bonferroni test was used for comparing changes; \*  $p < 0.05$ , †  $p < 0.01$ , ‡  $p < 0.001$  vs. control.

week 13 (Fig. 1). Compared to control offspring, heart weight in the FH group was significantly lower at days 7, 14, 21, 28 and  $\sim 95$ . The heart to body weight ratio was significantly lower at days 7, increased at days 14 and 21, and did not differ significantly at days 28 and  $\sim 95$  (Table 1). In adult offspring (day  $\sim 95$ ), basal levels of the MAP, SBP, and DBP

**Table 2.** Baseline values of hemodynamic parameters in control and fetal hypothyroidism rat offspring

	Adult rats (95 $\pm$ 2 days)	
	Control	FH
HR (bpm)	393.4 $\pm$ 10.6	364 $\pm$ 15.1
SBP (mm Hg)	105.7 $\pm$ 3.1	87.9 $\pm$ 3.8 <sup>†</sup>
DBP (mm Hg)	64.4 $\pm$ 1.7	53.2 $\pm$ 2.2 <sup>*</sup>
MAP (mm Hg)	80.9 $\pm$ 2.1	67.1 $\pm$ 2.1 <sup>†</sup>

FH, fetal hypothyroidism; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. Each value is the mean  $\pm$  SEM ( $n = 10$ ); \*  $p < 0.05$ , †  $p < 0.01$  vs. control.

**Table 3.** Quantitative evaluation of thoracic aorta and mesenteric arteries wall

	Thoracic aorta		Superior mesenteric artery		Intestinal mesenteric artery	
	Control	FH	Control	FH	Control	FH
CSA ( $\mu\text{m}^2$ )	1154904 $\pm$ 70851.5	1106914 $\pm$ 67160.2	109527.6 $\pm$ 42641.1	118580.1 $\pm$ 50583.8	6853.7 $\pm$ 445.3	5550.1 $\pm$ 496.1
IMT ( $\mu\text{m}$ )	90.8 $\pm$ 1.2	73.4 $\pm$ 2.3 <sup>‡</sup>	40.6 $\pm$ 1.2	29.8 $\pm$ 0.5 <sup>‡</sup>	23.4 $\pm$ 1.3	22.02 $\pm$ 1.04
NSMC	7.1 $\pm$ 0.2	7.2 $\pm$ 0.3	4.1 $\pm$ 0.1	4.2 $\pm$ 0.1	3.04 $\pm$ 0.13	3.4 $\pm$ 0.1
NEC	4.8 $\pm$ 0.1	3.2 $\pm$ 0.2 <sup>‡</sup>	11.2 $\pm$ 0.1	8.1 $\pm$ 0.4 <sup>‡</sup>	34.6 $\pm$ 1.1	37.9 $\pm$ 1.2

FH, fetal hypothyroidism; CSA, cross sectional area; IMT, intima-media thickness; NSMC, nuclei of smooth muscle cells; NEC, nuclei of endothelial cell. Each value is the mean  $\pm$  SEM ( $n = 10$ ); ‡  $p < 0.001$  vs. control.

in FH rats were lower than controls, whereas HR was not different (Table 2).

#### Response of the cardiovascular system to PE

In response to various doses of PE (0.25, 0.5, 1, 2, 5, 8 and 10 mg/kg/intravenous) increases in MAP, SBP, and DBP were significantly lower in adult offspring of hypothyroid mothers, compared to controls, the decrease in HR was, however, significantly higher than controls (Fig. 2). Latency of response to PE injection (the first dose) was significantly longer in the FH group (16.29  $\pm$  1.0 s) than in controls (12.7  $\pm$  0.9 s).

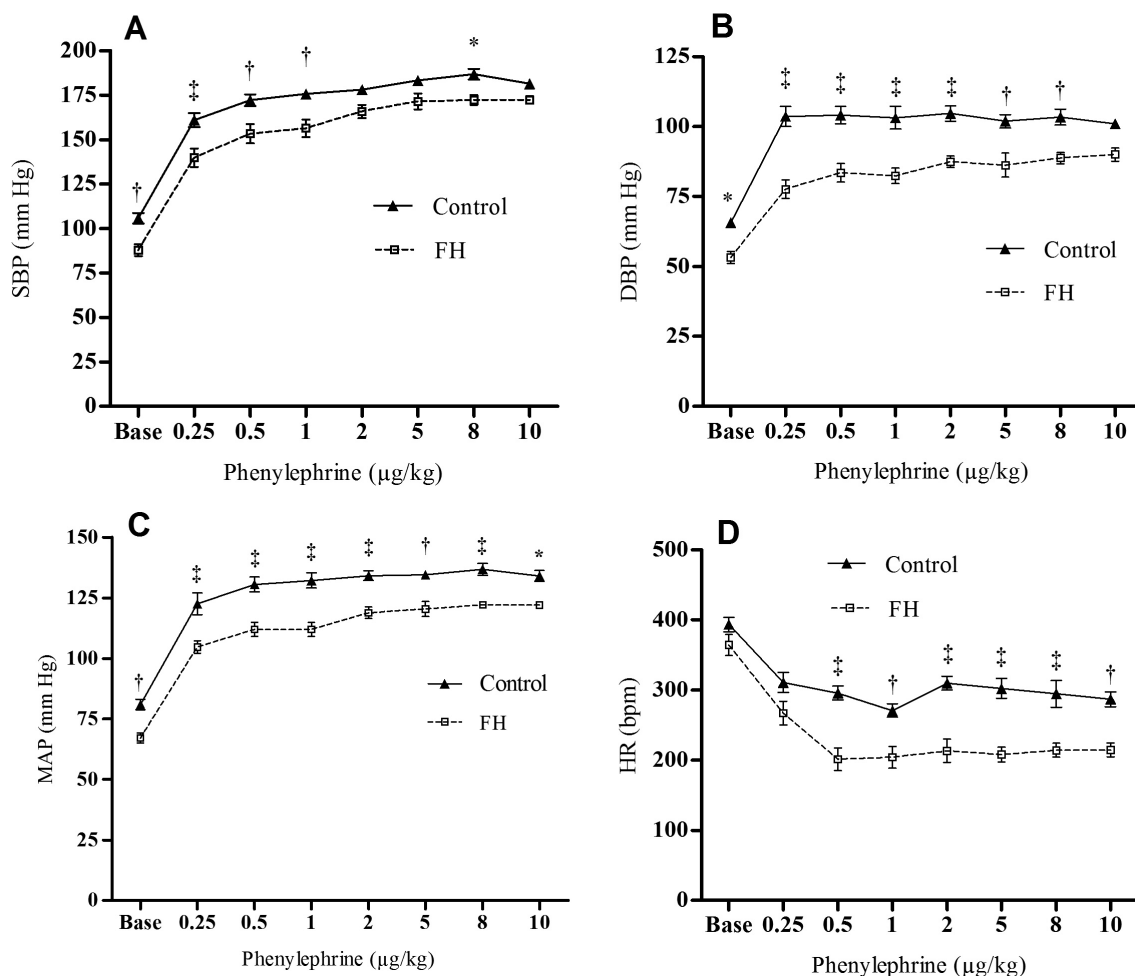
#### Histology of the arterial walls

The density of SMC nuclei and CSA for thoracic aorta, superior mesenteric, and small intestinal arteries did not differ significantly between the FH and control groups (Table 3). IMT and average ECs density in walls of the thoracic aorta (Fig. 3) and superior mesenteric artery but not small intestinal artery were, however, lower in FH rats (Fig. 3 and Fig. 4).

#### Discussion

This study showed that maternal hypothyroidism during pregnancy causes reduction of baseline hemodynamic parameters (SBP, DBP, and MAP) and decreases the intensity but raises latency of their response to PE in adult offspring; moreover it is accompanied by vascular structural changes. These findings highlight the fact that maternal hypothyroidism can disturb cardiovascular function during adulthood.

PTU administration induced hypothyroidism in mother rats during pregnancy, which affected TH status of their offspring. The offspring from hypothyroid mothers had significantly lower  $\text{TT}_4$  and  $\text{TT}_3$  levels, until the age of 21 and 28 days, respectively. These findings are in line with data indicating that maturation of TH activity continues up to 4 weeks of postnatal life in rats (Forhead and Fowden 2014).



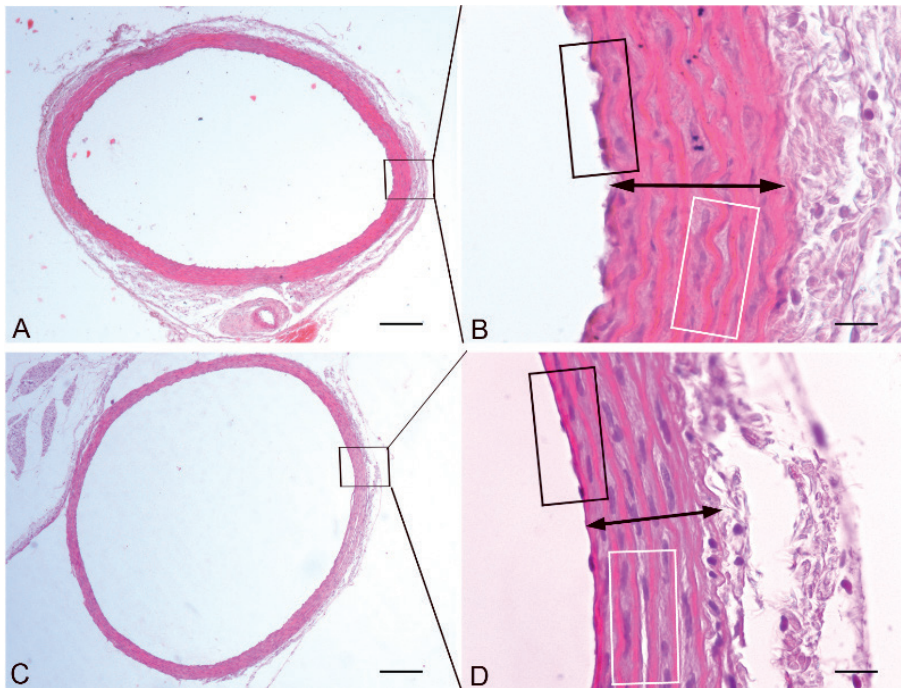
**Figure 2.** The effect of fetal hypothyroidism (FH) on response of the hemodynamic parameters to phenylephrine. Response of the hemodynamic parameters including, systolic blood pressure (SBP; A), diastolic arterial pressure (DBP; B), mean arterial pressure (MAP; C), and heart rate (HR; D) to phenylephrine were compared between offspring with FH and control groups. All values are means  $\pm$  SEM;  $n = 10$ ; repeated measure ANOVA followed by Bonferroni test was used for comparing between groups; all  $^* p < 0.05$ ,  $^\dagger p < 0.01$ ,  $^\ddagger p < 0.001$  vs. control.

Decreased activity of 5'-monodeiodinase in offspring with FH may partly explain the different times of  $TT_3$  and  $TT_4$  restoration to normal levels (Ahmed et al. 2010). Compared with adult offspring, neonates from control mothers had significantly higher  $TT_4$  findings similar to those of a previous report (David Kieffer et al. 1976). Although the reason for this difference remains unknown, sudden increase in TSH secretion and stimulation of  $TT_4$  secretion have, however, been reported in humans at birth (Williams et al. 2004).

In this study, TH deficiency during fetal life reduced body weight from birth until week 12; in addition, heart weight was lower in offspring of hypothyroid mothers. FH reduces the secretion of growth hormone and IGF-I leading possibly to reduced muscle mass (Fowden 1995), which may in turn cause body weight and heart weight loss.

Baseline levels of SBP, DBP, and MAP in adult offspring of the FH group were lower (16.8%, 17.4%, 17.1%, respectively) than in controls. These results are similar to those of Chen et al. (2005) showing thyroidectomy in sheep embryo caused a reduction of MAP. In addition, mutation in the TH receptor alpha 1 ( $TR\alpha_1$ ) decreased BP in other study (Bochukova et al. 2012). Blood pressure is determined by cardiac output and peripheral resistance, heart size and contractility could affect cardiac output (Brzezinski 1990). In our study, heart weight was lower in the FH group until adulthood; moreover we recently showed that also LVDP and  $\pm dp/dt_{max}$  (indices of contractility) were lower in the isolated heart of offspring with FH (Ghanbari et al. 2015), so the lower BP in FH group could hence be due to reduced cardiac output.

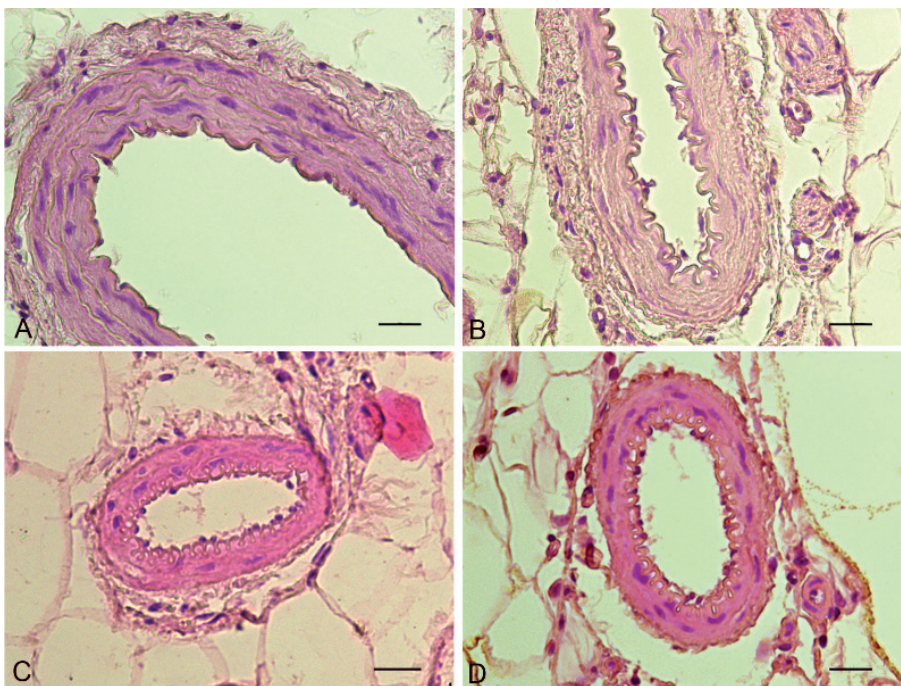




**Figure 3.** Histological changes in the thoracic aorta wall of rats with fetal hypothyroidism. Panels A and B show aorta section from control rats; the measured intima-media thickness (IMT) is illustrated with a black arrow. Nuclei of endothelial cell (ECs) were counted in cross sections in a length of 80  $\mu\text{m}$  (indicated by a black rectangle), and nuclei of smooth muscle cells (SMCs) were counted in cross sections in an area 2200  $\mu\text{m}^2$  (indicated by a white rectangle). Panels C and D show the IMT, SMCs and ECs in the fetal hypothyroidism group ( $n = 10$ ). Scale bars: 200  $\mu\text{m}$  in panels A and C; 20  $\mu\text{m}$  in panels B and D. Student's *t*-test was used to compare the two groups.

Contrary to the above mentioned results, Santos et al. (2012) report that FH induces hypertension. Although the reason for this discrepancy is not exactly clear, explanations include difference in types of antithyroid drugs used (PTU vs. methimazole), time of drug administration during pregnancy (first day of conception until delivery vs. day 9 until

delivery), and age of rats at time of BP measurement ( $\sim 95$  days vs. 60 days). Maternal hypothyroid status during initial or late gestation could affect outcome (Idris et al. 2005). Santos et al. in their study also combined the results obtained in male and female rats. The sex-associated differences in blood pressure, observed in humans, have also been documented



**Figure 4.** Histological changes in the mesenteric arteries wall of rats with fetal hypothyroidism. Panels A and B show superior mesenteric arteries section of both control and fetal hypothyroidism (FH) group, respectively; the intima-media thickness, smooth muscle cells and number of endothelial cell in FH offspring compared to the two groups. Panels C and D show these parameters in the intestinal arteries section in control and FH group, respectively ( $n = 10$ ). Scale bars: 20  $\mu\text{m}$  in all panels. Student's *t*-test was used to compare the two groups.

in animal models (Reckelhoff 2001). Finally, outcomes of impaired intrauterine growth may vary according to age of offspring (Roland et al. 2010; Karbalaie et al. 2013), results that justify both ours and those of Santos et al. (2012).

Following injection of PE, an  $\alpha_1$ -adrenergic agonist, SBP, DBP, and MAP were lower in the FH group than in controls. These findings demonstrate that FH decreases the vasoconstriction and inotropic effects of PE. It may be associated with decreased numbers of  $\alpha_1$  and  $\beta$  adrenergic receptors in the myocardium (Noguchi and Whitsett 1982), and decreased arterial response to PE due to impairment in L-type  $\text{Ca}^{2+}$  channel function (Sedaghat et al. 2015) found in hypothyroid offspring.

The IMT and density of ECs were lower in thoracic aorta and superior mesenteric arteries of adult offspring with FH, compared to controls, findings supported by data demonstrating decrease in IMT of the aortic wall in adult hypothyroid rats (Tousson et al. 2012). Presence of iodothyronine deiodinase enzymes in SMCs of the aorta in rats (Toyoda et al. 2009) and the expression of TH receptors in coronary ECs of mice (Makino et al. 2009) and bovine aorta (Hu et al. 1994) suggests that during adulthood, arteries are a target for THs. They bind to integrin  $\alpha_v\beta_3$  in the plasma membrane of the ECs (Bergh et al. 2005) and activate the mitogen-activated protein kinases cascade, increasing angiogenesis (Davis et al. 2004) and proliferation of ECs via the fibroblast growth factor (Al Hussein et al. 2013).

Arterial structural changes observed in our study, can affect cardiovascular system function. Blood pressure is known to have a direct association with IMT (Ciobanu et al. 2013); in addition ECs by releasing both vasoconstrictor and vasodilator agents, could affect hemodynamic parameters (Vane et al. 1990).

This is the first study addressing the effects of FH on the histological structure of arterial walls. Nevertheless, this study has its limitations which should be considered in the interpretation of data; first, TSH levels were not measured in this study, however, previous reports from our laboratory (Ghasemi et al. 2013) indicate that in this model of FH, serum TSH levels are comparable between control and FH groups during adulthood. Second, in this study, arterial wall structure was only assessed by IMT and SMC nuclei *per area*; both passive (elastic and collagenous connective tissue) and active (smooth muscle) component could, however, affect viscoelastic properties of the arterial wall and it has been reported that the passive component changes in hypothyroidism (Zaki and Youssef 2013). Third, we did not measure inflammatory markers, which are related to hypothyroidism and can lead to changes in the arterial wall (Dizdarevic-Bostandic et al. 2013).

One final word, PTU was used to induce hypothyroidism during pregnancy. Thioamide drugs such as PTU and methimazole have been categorized as class D drugs in pregnancy (Nambiar et al. 2014) and they are the treatment of choice for

thyrotoxicosis in pregnancy (Diav-Citrin and Ornoy 2002), when other treatments including radiotherapy and thyroidectomy are contraindicated (Taylor and Vaidya 2012). PTU and methimazole have similar placental transfer kinetics (Diav-Citrin et al. 2002). Due to the fetal teratogenicity associated with methimazole (Laurberg and Andersen 2014), PTU is considered the first-line in the treatment of Graves' disease during pregnancy (Chattaway and Klepser 2007). PTU could, however, induce hepatitis in fetus (Taylor and Vaidya 2012) and sometimes, changing from PTU therapy after first trimester to methimazole is considered to be the optimum treatment during pregnancy (Taylor and Vaidya 2012).

In conclusion, offspring born from hypothyroid mothers have lower values of hemodynamic parameters in adulthood; in addition, their response to  $\alpha_1$ -adrenergic agonist is lower than in controls. These changes may be associated with microstructural modifications including decreased IMT and ECs in arteries. THs could be a key factor in development of the cardiovascular system, and warrant more attention being paid to pregnant women with hypothyroidism.

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## References

- Adijiang A., Goto S., Uramoto S., Nishijima F., Niwa T. (2008): Indoxyl sulphate promotes aortic calcification with expression of osteoblast-specific proteins in hypertensive rats. *Nephrol. Dial. Transplant.* **23**, 1892–1901  
<http://dx.doi.org/10.1093/ndt/gfm861>
- Ahmed O., Abd El-Tawab S. A., Ahmed R. (2010): Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: I. The development of the thyroid hormones–neurotransmitters and adenosinergic system interactions. *Int. J. Dev. Neurosci.* **28**, 437–454  
<http://dx.doi.org/10.1016/j.ijdevneu.2010.06.007>
- Al Hussein A., Bagnato G., Farkas L., Gomez-Arroyo J., Farkas D., Mizuno S., Kraskauskas D., Abbate A., Van Tassel B., Voelkel N. F., Bogaard H. J. (2013): Thyroid hormone is highly permissive in angioproliferative pulmonary hypertension in rats. *Eur. Respir. J.* **41**, 104–114  
<http://dx.doi.org/10.1183/09031936.00196511>
- Bencze M., Behuliak M., Zicha J. (2013): The impact of four different classes of anesthetics on the mechanisms of blood pressure regulation in normotensive and spontaneously hypertensive rats. *Physiol. Res.* **62**, 471–478
- Bergh J. J., Lin H. Y., Lansing L., Mohamed S. N., Davis F. B., Mousa S., Davis P. J. (2005): Integrin  $\alpha_v\beta_3$  contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* **146**, 2864–2871



- <http://dx.doi.org/10.1210/en.2005-0102>
- Bochukova E., Schoenmakers N., Agostini M., Schoenmakers E., Rajanayagam O., Keogh J. M., Henning E., Reinemund J., Gevers E., Sarri M. (2012): A mutation in the thyroid hormone receptor alpha gene. *N. Engl. J. Med.* **366**, 243–249  
<http://dx.doi.org/10.1056/NEJMoa1110296>
- Brzezinski W. A. (1990): Blood pressure. In: *Clinical Methods: The History, Physical, and Laboratory Examinations.* (Eds.: Walker H. K., Hall W. D., Hurst J. W.) 3rd ed., Boston
- Cannesson M., Jian Z., Chen G., Vu T. Q., Hatib F. (2012): Effects of phenylephrine on cardiac output and venous return depend on the position of the heart on the Frank-Starling relationship. *J. Appl. Physiol.* **113**, 281–289  
<http://dx.doi.org/10.1152/jappphysiol.00126.2012>
- Chattaway J. M., Klepser T. B. (2007): Propylthiouracil versus methimazole in treatment of Graves' disease during pregnancy. *Ann. Pharmacother.* **41**, 1018–1022  
<http://dx.doi.org/10.1345/aph.1H535>
- Chattergoon N. N., Giraud G. D., Louey S., Stork P., Fowden A. L., Thornburg K. L. (2012): Thyroid hormone drives fetal cardiomyocyte maturation. *FASEB J.* **26**, 397–408  
<http://dx.doi.org/10.1096/fj.10-179895>
- Chen K., Carey L. C., Valego N. K., Liu J., Rose J. C. (2005): Thyroid hormone modulates renin and ANG II receptor expression in fetal sheep. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **289**, R1006–1014  
<http://dx.doi.org/10.1152/ajpregu.00046.2005>
- Chizzonite R. A., Zak R. (1984): Regulation of myosin isoenzyme composition in fetal and neonatal rat ventricle by endogenous thyroid hormones. *J. Biol. Chem.* **259**, 12628–12632
- Ciobanu A. O., Gherghinescu C. L., Dulgheru R., Magda S., Dragoi Galrinho R., Florescu M., Guberna S., Cinteza M., Vinereanu D. (2013): The impact of blood pressure variability on subclinical ventricular, renal and vascular dysfunction, in patients with hypertension and diabetes. *Maedica (Buchar)* **8**, 129–136
- David Kieffer J., Morver H., Federico P., Maloof F. (1976): Pituitary-thyroid axis in neonatal and adult rats: comparison of the sexes. *Endocrinology* **98**, 295–304  
<http://dx.doi.org/10.1210/endo-98-2-295>
- Davis F. B., Mousa S. A., O'connor L., Mohamed S., Lin H. Y., Cao H. J., Davis P. J. (2004): Proangiogenic action of thyroid hormone is fibroblast growth factor-dependent and is initiated at the cell surface. *Circ. Res.* **94**, 1500–1506  
<http://dx.doi.org/10.1161/01.RES.0000130784.90237.4a>
- Diav-Citrin O., Ornoy A. (2002): Teratogen update: antithyroid drugs – methimazole, carbimazole, and propylthiouracil. *Teratology* **65**, 38–44  
<http://dx.doi.org/10.1002/tera.1096>
- Dizdarevic-Bostandic A., Burekovic A., Velija-Asimi Z., Godinjak A. (2013): Inflammatory markers in patients with hypothyroidism and diabetes mellitus type 1. *Med. Arh.* **67**, 160–161  
<http://dx.doi.org/10.5455/medarh.2013.67.160-161>
- Farahani H., Ghasemi A., Roghani M., Zahediasl S. (2013): Effect of neonatal hypothyroidism on carbohydrate metabolism, insulin secretion, and pancreatic islets morphology of adult male offspring in rats. *J. Endocrinol. Invest.* **36**, 44–49
- Ferreira A. J., Shenoy V., Yamazato Y., Sriramula S., Francis J., Yuan L., Castellano R. K., Ostrov D. A., Oh S. P., Katovich M. J., Raizada M. K. (2009): Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **179**, 1048–1054  
<http://dx.doi.org/10.1164/rccm.200811-1678OC>
- Forhead A. J., Fowden A. L. (2014): Thyroid hormones in fetal growth and prepartum maturation. *J. Endocrinol.* **221**, R87–103  
<http://dx.doi.org/10.1530/JOE-14-0025>
- Fouron J. C., Bourgin J. H., Letarte J., Dussault J. H., Ducharme G., Davignon A. (1982): Cardiac dimensions and myocardial function of infants with congenital hypothyroidism. An echocardiographic study. *Br. Heart J.* **47**, 584–587  
<http://dx.doi.org/10.1136/hrt.47.6.584>
- Fowden A. L. (1995): Endocrine regulation of fetal growth. *Reprod. Fertil. Dev.* **7**, 351–363  
<http://dx.doi.org/10.1071/RD9950351>
- Galton V. A., Martinez E., Hernandez A., Germain E. S., Bates J. M., Germain D. L. S. (1999): Pregnant rat uterus expresses high levels of the type 3 iodothyronine deiodinase. *J. Clin. Invest.* **103**, 979–987  
<http://dx.doi.org/10.1172/JCI6073>
- Ghanbari M., Jeddi S., Bagheripour F., Ghasemi A. (2015): The effect of maternal hypothyroidism on cardiac function and tolerance to ischemia–reperfusion injury in offspring male and female rats. *J. Endocrinol. Invest.* **38**, 915–922  
<http://dx.doi.org/10.1007/s40618-015-0267-x>
- Ghasemi A., Mehrazin F., Zahediasl S. (2013): Effect of nitrate and L-arginine therapy on nitric oxide levels in serum, heart, and aorta of fetal hypothyroid rats. *J. Physiol. Biochem.* **69**, 751–759  
<http://dx.doi.org/10.1007/s13105-013-0251-x>
- Gomberg-Maitland M., Frishman W. H. (1998): Thyroid hormone and cardiovascular disease. *Am. Heart J.* **135**, 187–196  
[http://dx.doi.org/10.1016/S0002-8703\(98\)70081-X](http://dx.doi.org/10.1016/S0002-8703(98)70081-X)
- Guimaraes D. D., Carvalho C. C., Braga V.A. (2012): Scavenging of NADPH oxidase-derived superoxide anions improves depressed baroreflex sensitivity in spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* **39**, 373–378  
<http://dx.doi.org/10.1111/j.1440-1681.2012.05679.x>
- Hu R. M., Wu L. M., Frank H. J., Pedram A., Levin E. R. (1994): Insulin stimulates thyroid hormone receptor alpha gene expression in cultured bovine aortic endothelial cells. *Mol. Cell. Endocrinol.* **103**, 65–71  
[http://dx.doi.org/10.1016/0303-7207\(94\)90070-1](http://dx.doi.org/10.1016/0303-7207(94)90070-1)
- Idris I., Srinivasan R., Simm A., Page R. C. (2005): Maternal hypothyroidism in early and late gestation: effects on neonatal and obstetric outcome. *Clin. Endocrinology* **63**, 560–565  
<http://dx.doi.org/10.1111/j.1365-2265.2005.02382.x>
- Karbalaee N., Ghasemi A., Faraji F., Zahediasl S. (2013): Comparison of the effect of maternal hypothyroidism on carbohydrate metabolism in young and aged male offspring in rats. *Scand. J. Clin. Lab. Invest.* **73**, 87–94  
<http://dx.doi.org/10.3109/00365513.2012.743164>
- Langley-Evans S. C. (2006): Developmental programming of health and disease. *Proc. Nutr. Soc.* **65**, 97–105  
<http://dx.doi.org/10.1079/PNS2005478>
- Laurberg P., Andersson S. L. (2014): Therapy of endocrine disease: antithyroid drug use in early pregnancy and birth defects: time windows of relative safety and high risk? *Eur. J. Endocrinol.* **171**, R13–20



- <http://dx.doi.org/10.1530/EJE-14-0135>
- Loss Ide O., Fernandes L. G., Martins C. D., Cardoso L. M., Silva M. E., Dias-Da-Silva V. J., Moraes M. F., Chianca D. A., Jr. (2007): Baroreflex dysfunction in rats submitted to protein restriction. *Life Sci.* **81**, 944–950  
<http://dx.doi.org/10.1016/j.lfs.2007.08.005>
- Makino A., Suarez J., Wang H., Belke D. D., Scott B. T., Dillmann W. H. (2009): Thyroid hormone receptor-beta is associated with coronary angiogenesis during pathological cardiac hypertrophy. *Endocrinology* **150**, 2008–2015  
<http://dx.doi.org/10.1210/en.2008-0634>
- Morvan E., Lima N. E., Machi J. F., Mostarda C., De Angelis K., Irigoyen M. C., Wich R. B., Rodrigues B., Maifrino L. B. (2013): Metabolic, hemodynamic and structural adjustments to low intensity exercise training in a metabolic syndrome model. *Cardiovasc. Diabetol.* **12**, 89  
<http://dx.doi.org/10.1186/1475-2840-12-89>
- Nambiar P. R., Palanisamy G. S., Okerberg C., Wolford A., Walters K., Buckbinder L., Reagan W. J. (2014): Toxicities associated with 1-month treatment with propylthiouracil (PTU) and methimazole (MMI) in male rats. *Toxicol. Pathol.* **42**, 970–983  
<http://dx.doi.org/10.1177/0192623313502708>
- Nijssen M. J., De Ruiter G. J., Kasbergen C. M., De Wildt D. J. (2001): Effect of gamma-melanocyte-stimulating hormones on baroreflex sensitivity and cerebral blood flow autoregulation in rats. *Cardiovasc. Res.* **49**, 226–233  
[http://dx.doi.org/10.1016/S0008-6363\(00\)00204-2](http://dx.doi.org/10.1016/S0008-6363(00)00204-2)
- Noguchi A., Whitsett J. A. (1982): Ontogeny of alpha 1-adrenergic receptors in the rat myocardium: effects of hypothyroidism. *Eur. J. Pharmacol.* **86**, 43–50  
[http://dx.doi.org/10.1016/0014-2999\(82\)90394-6](http://dx.doi.org/10.1016/0014-2999(82)90394-6)
- Olivetti G., Melissari M., Marchetti G., Anversa P. (1982): Quantitative structural changes of the rat thoracic aorta in early spontaneous hypertension. Tissue composition, and hypertrophy and hyperplasia of smooth muscle cells. *Circ. Res.* **51**, 19–26  
<http://dx.doi.org/10.1161/01.RES.51.1.19>
- Reckelhoff J. F. (2001): Gender differences in the regulation of blood pressure. *Hypertension* **37**, 1199–1208  
<http://dx.doi.org/10.1161/01.HYP.37.5.1199>
- Rhee S. S., Pearce E. N. (2011): The endocrine system and the heart: a review. *Revista Espa-ola de Cardiología (English Edition)* **64**, 220–231  
<http://dx.doi.org/10.1016/j.rec.2010.10.016>
- Roland A. V., Nunemaker C. S., Keller S. R., Moenter S. M. (2010): Prenatal androgen exposure programs metabolic dysfunction in female mice. *J. Endocrinol.* **207**, 213–223  
<http://dx.doi.org/10.1677/JOE-10-0217>
- Santos S. O., Loureiro S. M., Alves I. G., Jesus C. S., Santos P. R., Santos M. R., Dias D. P., Santana-Filho V. J., Badaue-Passos D., Jr. (2012): Experimental gestational hypothyroidism evokes hypertension in adult offspring rats. *Auton. Neurosci.* **170**, 36–41  
<http://dx.doi.org/10.1016/j.autneu.2012.07.004>
- Sedaghat K., Zahedi S., Ghasemi A. (2015): Gestational hypothyroidism-induced changes in L-type calcium channels of rat aorta smooth muscle and their impact on the responses to vasoconstrictors. *Iran. J. Basic Med. Sci.* **18**, 172–179
- Taylor P. N., Vaidya B. (2012): Side effects of anti-thyroid drugs and their impact on the choice of treatment for thyrotoxicosis in pregnancy. *Eur. Thyroid. J.* **1**, 176–185  
<http://dx.doi.org/10.1159/000342920>
- Teng W., Shan Z., Patil-Sisodia K., Cooper D. S. (2013): Hypothyroidism in pregnancy. *The Lancet Diabetes Endocrinol.* **1**, 228–237  
[http://dx.doi.org/10.1016/S2213-8587\(13\)70109-8](http://dx.doi.org/10.1016/S2213-8587(13)70109-8)
- Tousson E., Ali E. M., Ibrahim W., Mansour M. A. (2012): Treatment with folic acid ameliorated the histopathological alterations caused by propylthiouracil-induced hypothyroid rat testes. *Toxicol. Ind. Health* **28**, 566–576  
<http://dx.doi.org/10.1177/0748233711420469>
- Toyoda N., Yasuzawa-Amano S., Nomura E., Yamauchi A., Nishimura K., Ukita C., Morimoto S., Kosaki A., Iwasaka T., Harney J. W., Larsen P. R., Nishikawa M. (2009): Thyroid hormone activation in vascular smooth muscle cells is negatively regulated by glucocorticoid. *Thyroid.* **19**, 755–763  
<http://dx.doi.org/10.1089/thy.2009.0044>
- Tzanetakou I. P., Mikhailidis D. P., Perrea D. N. (2011): Nutrition during pregnancy and the effect of carbohydrates on the offspring's metabolic profile: In search of the „perfect maternal diet“. *Open Cardiovasc. Med. J.* **5**, 103–109  
<http://dx.doi.org/10.2174/1874192401105010103>
- Vane J. R., Anggard E. E., Botting R. M. (1990): Regulatory functions of the vascular endothelium. *N. Engl. J. Med.* **323**, 27–36  
<http://dx.doi.org/10.1056/NEJM199007053230106>
- Williams F. L., Simpson J., Delahunty C., Ogston S. A., Bongers-Schokking J. J., Murphy N., Van Toor H., Wu S.-Y., Visser T. J., Hume R. (2004): Developmental trends in cord and postpartum serum thyroid hormones in preterm infants. *J. Clin. Endocrinol. Metab.* **89**, 5314–5320  
<http://dx.doi.org/10.1210/jc.2004-0869>
- York T. P., Eaves L. J., Neale M. C., Strauss J. F. (2014): The contribution of genetic and environmental factors to the duration of pregnancy. *Am. J. Obstet. Gynecol.* **210**, 398–405  
<http://dx.doi.org/10.1016/j.ajog.2013.10.001>
- Zaki S., Youssef M. (2013): Thyroid hormone dysfunctions affect the structure of rat thoracic aorta: a histological and morphometric study. *Folia Morphol.* **72**, 333–339  
<http://dx.doi.org/10.5603/FM.2013.0056>

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