

## Region-specific effects of antenatal/early postnatal hypothyroidism on endothelial NO-pathway activity in systemic circulation

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### ABSTRACT

**Background:** Antenatal/early postnatal hypothyroidism weakens NO-mediated anticontractile influence of endothelium in coronary arteries of adult rats, but it remains unclear whether this occurs in other vascular regions. We hypothesized that developmental thyroid deficiency is followed by region-specific changes in the endothelial NO-pathway activity in systemic vasculature. To explore this, we estimated the effects of antenatal/early postnatal hypothyroidism on NO-pathway activity and its potential local control mechanisms in rat mesenteric and skeletal muscle (sural) arteries.

**Methods:** Dams were treated with 6-propyl-2-thiouracil (PTU) in drinking water (0.0007%) during pregnancy and 2 weeks postpartum; control (CON) females received PTU-free water. Adult offspring (10–12-weeks) arteries were studied by wire myography, qPCR, and Western blotting.

**Results:** Endothelium removal or inhibition of NO-synthase with L-NNA augmented contractile responses to  $\alpha_1$ -adrenoceptor agonist methoxamine. In PTU compared to CON group, these effects were stronger in sural arteries, but did not differ in mesenteric arteries. The responses of both arteries to NO-donor DEA/NO were similar in CON and PTU rats. mRNA contents of deiodinase 2 and thyroid hormone receptor  $\alpha$  were similar in mesenteric arteries of two groups but were elevated in sural arteries of PTU group compared to CON. The abundance of eNOS protein was higher in sural arteries of PTU compared to CON rats.

**Conclusion:** Antenatal/early postnatal hypothyroidism is followed by an increase in NO-mediated anticontractile influence in sural, but not in mesenteric arteries of adult animals. The diversity of hypothyroidism effects may be due to different alterations of local T3 synthesis/reception in different vascular beds.

### 1. Introduction

Thyroid hormones are important for the development and functioning of the cardiovascular system (McAllister et al., 2005; Danzi and Klein, 2014). Insufficient thyroid hormone production by the thyroid gland, or hypothyroidism, is one of the most common endocrine disorders during pregnancy (Stagnaro-Green and Pearce, 2012; Stagnaro-Green, 2015). Hypothyroidism during pregnancy can be attributed to environmental challenges such as dietary iodine or iron deficiency (Ghanbari and Ghasemi, 2017; Teng et al., 2018), the influence of various stressors (e.g., COVID-19 pandemic (Hua et al., 2021)) and adverse effects of other factors (Stagnaro-Green and Pearce, 2012; Teng et al., 2013). Since the influence of maternal thyroid hormones is crucial

for foetal development (Forhead and Fowden, 2014), maternal hypothyroidism can have long-term effects on the offspring, including persistent cardiovascular disorders in adulthood (Liu et al., 2013; Rytter et al., 2016; Miao et al., 2021).

Mechanistic studies of developmental hypothyroidism effects involve different animal models, among which the model of supplementation with antithyroid drug propylthiouracil (PTU) is often used (Santos et al., 2012; Ghanbari et al., 2015; Yousefzadeh et al., 2016; Gaynullina et al., 2017, 2018b, 2018a). When female rats are treated by PTU during the whole gestation and 2 weeks postpartum, the offspring is hypothyroid at the age of 2 weeks (Gaynullina et al., 2017, 2018b), but becomes euthyroid since at least 6 weeks of age (Gaynullina et al., 2018a). Using such PTU-induced model of antenatal/early postnatal

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hypothyroidism in rats, we demonstrated that this challenge weakens the anticontractile influence of the endothelium in coronary arteries at 3–4-month age (Gaynullina et al., 2017, 2018a). This was attributed to the reduction in the anticontractile influence of nitric oxide (NO) (Gaynullina et al., 2017) – a key relaxing factor derived from the endothelium (Vanhoutte et al., 2016). Therefore, thyroid deficiency at the early developmental stages may induce long-term alterations of NO-pathway activity in the circulation of the heart. Of note, the decrease in the NO-mediated anticontractile influence in adult offspring with developmental hypothyroidism is complemented by the reduction in NO metabolites in cardiac tissue (Ghasemi et al., 2013) but not in blood (Ghasemi et al., 2013; Gaynullina et al., 2017). However, it remains unclear whether antenatal/early postnatal hypothyroidism influences the anticontractile effect of NO in vascular regions other than coronary circulation.

Thyroid hormones are established regulators of endothelial NO-pathway activity (McAllister et al., 2005; Samuel et al., 2017; Selivanova and Tarasova, 2021). Endothelial NO-synthase (eNOS) expression and activity are controlled by thyroid hormone receptor  $\alpha$  (TR $\alpha$ ) (Hiroi et al., 2006; Suarez et al., 2014; Geist et al., 2021) for which T3 has a greater affinity than T4 (Schroeder et al., 2014). Thuswise, T3 may be considered as a more active regulator of arterial vasomotor responses mediated by eNOS. T3 availability largely depends on local T4 to T3 conversion catalyzed by deiodinases 1 and 2 (Williams and Bassett, 2011). Of note, deiodinase 2 is the main deiodinase in the vasculature (Mizuma et al., 2001; Yasuzawa-Amano et al., 2004; Aoki et al., 2015) and its expression can vary significantly among different regions of the circulatory system (Sofronova et al., 2017; Jambusaria et al., 2020). In general, deiodinase 2 expression and activity are negatively regulated by thyroid hormones (Gereben et al., 2008). It was shown that in some tissues, e.g., in rat hypothalamus and brown adipose tissue, hypothyroidism led to an increase in the expression and activity of deiodinase 2 (Croteau et al., 1996; Burmeister et al., 1997) which may provide local compensation for the systemic deficiency of thyroid hormones. However, the effects of antenatal/early postnatal hypothyroidism on deiodinase 2 expression in the vasculature of adult offspring have not yet been studied.

According to our hypothesis, antenatal/early postnatal hypothyroidism is followed by region-specific alterations in the activity of the endothelial NO pathway in systemic vascular beds. To explore this, we utilized the model of antenatal/early postnatal hypothyroidism in rats and studied in adult offspring the responses of mesenteric and skeletal muscle arteries. In these vascular beds, which substantially contribute to the control of the systemic arterial pressure level, we estimated the effects of antenatal/early postnatal hypothyroidism on NO-pathway activity and potential mechanisms of its local thyroid control.

## 2. Materials and methods

### 2.1. Animals

All experimental procedures used in this work were approved by the Biomedical Ethics Committee of the Russian Federation State Research Center - Institute for Biomedical Problems, Russian Academy of Sciences (protocol № 426), and conformed to the European Convention on the protection of animals used for scientific purposes (EU Directive, 2010/63/EU).

Adult female (230–280 g) and male (300–350 g) Wistar rats were delivered from the vivarium of the Institute of General Pathology and Pathophysiology (Moscow, Russia) and then bred in the laboratory animal unit of the Biological Faculty, Moscow State University. All animals were maintained on a 12/12 h light/dark cycle and fed with normal rodent chow *ad libitum*.

### 2.2. The model of antenatal/early postnatal hypothyroidism

PTU-induced model of antenatal/early postnatal hypothyroidism was described in detail previously (Gaynullina et al., 2017, 2018b). Briefly, females were mated with males overnight and the next day (at 7–8 a.m.) the first gestation day (GD1) was identified by the presence of sperm in the vaginal smear. Pregnant dams were randomly distributed between two groups. The first group (n = 5) was supplemented with PTU (6-propyl-2-thiouracil, 7 ppm or 0.0007%, w/v, Sigma) in the drinking water from GD1 until 14th day after delivery. The second group of dams (n = 5) drank water without PTU and served as control (CON). Male progeny of the dams (CON and PTU groups) was grown until the age of 10–12 weeks.

### 2.3. Wire myography

The rats were killed by decapitation under CO<sub>2</sub> anesthesia to collect mesenteric (1–2 order branches of the superior mesenteric artery) and gastrocnemius muscle feed (sural) arteries. Arterial segments with the length of 2 mm were placed in wire myograph (DMT, Denmark, models 410A or 620) for isometric force recording. The preparations were heated and kept at 37 °C in physiological salt solution containing (in mM): 120 NaCl, 26 NaHCO<sub>3</sub>, 4.5 KCl, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 1.0 MgSO<sub>4</sub>, 1.6 CaCl<sub>2</sub>, 5.5 D-glucose, 0.025 EDTA, 5 HEPES, equilibrated with gas mixture 95% O<sub>2</sub> + 5% CO<sub>2</sub>. The isometric force was continuously recorded at 10 Hz sampling rate using an analog-to-digital data converter (E14-140, L-CARD, Russia) and PowerGraph 3.3 software (DISoft, Russia). The segments were stretched to 0.9 d<sub>100</sub>, where d<sub>100</sub> is the inner diameter which the vessel with relaxed smooth muscle would have if rounded and subjected to the transmural pressure of 100 mm Hg (Molvany and Halpern, 1977). The arterial preparations were activated with noradrenaline (10  $\mu$ M) and then with methoxamine (MX, selective agonist of  $\alpha_1$ -adrenoceptors, 10  $\mu$ M). The functional integrity of endothelium was checked by application of acetylcholine (10  $\mu$ M) on top of MX-induced contraction (3  $\mu$ M).

Two experimental protocols were used. The first protocol was performed on endothelium-intact preparations and included two concentration-response relationships (CRR) to MX (0.01  $\mu$ M – 100  $\mu$ M). The second CRR to MX was obtained in the presence of NO-synthase (NOS) inhibitor L-NNA (100  $\mu$ M) or a similar volume of vehicle (H<sub>2</sub>O, 50  $\mu$ L).

The second protocol was carried out on endothelium-denuded arterial preparations (endothelium was denuded mechanically with a rat whisker) and consisted of one CRR to MX (0.01  $\mu$ M – 100  $\mu$ M), followed by CRR to NO donor DEA/NO (0.001  $\mu$ M to 10  $\mu$ M) against the background of MX-induced precontraction (70–80% of the maximum active force). DEA/NO was dissolved in 0.01 M NaOH.

Myography results were analyzed as previously described (Gaynullina et al., 2017, 2018b). All active force values were calculated by subtracting the passive force values (recorded in the preparations with fully relaxed smooth muscle) from values recorded before the first and at each MX concentration. Passive force level was determined by exposing arterial segments to Ca<sup>2+</sup>-free physiological salt solution supplemented with EGTA (0.1 mM) and DEA/NO (1  $\mu$ M). The obtained values of active force were expressed as a percentage of the maximum active force value recorded in the first CRR to MX. Peak relaxatory responses to DEA/NO were calculated as a percentage of the precontraction level. Additionally, CRR were fitted to a sigmoidal function with variable slope using GraphPad Prism 8.0 Software.

### 2.4. qPCR

mRNA contents of deiodinase 1 (*Dio1*), deiodinase 2 (*Dio2*), deiodinase 3 (*Dio3*) and thyroid hormone receptor  $\alpha$  (TR $\alpha$ , *Thra*) in mesenteric and sural arteries were evaluated by qPCR as described earlier (Sofronova et al., 2015). In brief, freshly isolated arterial samples were fixed in

RNA-later (Qiagen) and thereafter kept at  $-80^{\circ}\text{C}$ . RNA was isolated using RNeasy Mini Kit (Qiagen) and processed with DNase I (Fermentas, 1000 U/ml). cDNA synthesis was performed using MMLV RT reverse transcription kit (Evrogen, Moscow, Russia). qPCR was performed in Rotor Gene 6000 (Corbett Research, Australia) using qPCRmix-HS SYBR Master Mix (Evrogen). *Rn18s*, *Rplp0* and *Gapdh* were used as house-keeping genes. The primers were synthesized by Evrogen (Moscow, Russia), sequences are listed in Table 1. Protocol of amplification consisted of 10-min heating at  $95^{\circ}\text{C}$ , followed by 40 cycles (30 s at  $95^{\circ}\text{C}$ , 30 s at  $60^{\circ}\text{C}$  and 60 s at  $72^{\circ}\text{C}$ ) and final step at  $72^{\circ}\text{C}$  for 10 min.

qPCR experiments were analyzed in Rotor Gene 6000 Software (Corbett Research, Australia). Gene expression levels were calculated as  $1/E^{C_t}$ , where E – primer efficiency,  $C_t$  – cycle number on which the curve for product accumulation is crossing the fluorescence detection threshold. The values for mRNA of interest were normalized to the geometric mean of the three housekeeping genes in the same sample and shown as a percentage of the mean value for mesenteric artery in CON group.

### 2.5. Western blotting

Sural arteries were homogenized in SDS-buffer (0.0625 mol/L Tris-HCl (pH 6.8), 2.5% SDS, 10% water-free glycerin, 2.47% dithiothreitol, 0.002% bromophenol blue) supplemented with protease and phosphatase inhibitors (aprotinin 50 mg/ml, leupeptin 100 mg/ml, pepstatin 30 mg/mL, NaF 2 mg/mL,  $\text{Na}_3\text{VO}_4$  180 mg/mL), centrifuged at 14000 g for 2 min and heated at  $99^{\circ}\text{C}$  for 2 min; supernatant was stored at  $-20^{\circ}\text{C}$ . Samples were subjected to SDS-PAGE to separate proteins. Immediately after SDS-PAGE the transfer to nitrocellulose membrane (Santa Cruz) was performed using a Trans-Blot Turbo transfer system (BioRad). The protein transfer was confirmed by Ponceau S staining and the membrane was cut into two fragments at the level of approximately 70 kDa protein marker (Abcam). Both fragments of membrane were blocked with 5% nonfat milk (Applichem, Germany) in TBS (20 mmol/l Tris-HCl, pH 7.6; 150 mmol/l NaCl) with 0.1% Tween 20 (TBSt). Then the lower membrane fragment was incubated with antibodies against GAPDH (Abcam, mouse, 1:2000 in TBSt with 5% milk, overnight). The upper membrane fragment was incubated with antibodies against eNOS (BD Transduction, mouse, 1:2000 in TBSt with 5% milk, overnight). Afterwards, both membrane fragments were incubated with anti-mouse secondary antibodies (Cell Signaling, 1:5000 in 5% milk, 1 h) and visualized with Super Signal West Dura Substrate (Thermo Scientific) using ChemiDoc (BioRad). The results of the experiments were evaluated in ImageLab Software (BioRad). eNOS to GAPDH ratio was calculated for each sample, and then the average ratio in CON group was taken as 100%.

### 2.6. Statistical data analysis

Statistical analysis was performed in GraphPad Prism 8.0 (USA) using Student's t-test or Repeated Measures ANOVA with Geisser-Greenhouse correction. The differences were considered as statistical significant at  $P < 0.05$ . All data are given as mean  $\pm$  S.E.M.; n represents the number of animals.

**Table 1**  
Gene specific primers used in qPCR.

Gene	Protein	Forward primer (5'-3')	Reverse primer (5'-3')	Product length, bp
<i>Dio1</i>	Deiodinase 1	TCTGGGATTTCAATCAAGGC	TAGAGCCTCTCAGGCAGAGC	331
<i>Dio2</i>	Deiodinase 2	CTTTGAACGTGTGTCATCGT	TCTCCAGCCAACCTCGGACTT	100
<i>Dio3</i>	Deiodinase 3	GCTCGAACTGGCAACTTTGTC	GTGAGATGCTGGCGACTTATTG	105
<i>Thra</i>	TR $\alpha$	CAAGTGGAGTGTGGGTCAGA	CCCTGACATGCTGCTTTTCAG	133
<i>Gapdh</i>	GAPDH	CCATCAAGGACCCCTTCATT	CACCAGCATCACCCCATTT	157
<i>Rplp0</i>	RPLP0	AGGTCTCTGGCTTTGTCTGTGG	AGCTGCAGGAGCAGCAGTGG	134
<i>Rn18s</i>	-	CACGGGTGACGGGGAATCAG	CGGGTCGGGAGTGGGTAATTTG	105

## 3. Results

Inner diameter ( $d_{100}$ ) and maximum active force of either mesenteric or sural arteries did not differ between CON and PTU groups (Table 2).

The anticontractile effect of vascular endothelium was assessed by comparing the responses to MX of arteries with intact and removed endothelium. In mesenteric arteries endothelium denudation augmented contractile responses to MX to the same extent in CON (Fig. 1A) and PTU (Fig. 1B) rats. Similarly, inhibition of NO-synthase with L-NNA led to the comparable increase of contractile responses to MX in two experimental groups (Fig. 1C and D), indicating comparable anticontractile effects of endogenous NO. Importantly, the relaxatory responses to NO-donor DEA/NO did not differ between mesenteric arteries of CON and PTU rats (Fig. 1E).

In sural arteries, denudation of endothelium led to the increase of MX-induced contraction in either CON (Fig. 2A) or PTU (Fig. 2B) rats. However, the increase was more pronounced in the PTU group, as seen from the basal tone levels. The basal tone levels of endothelium-intact arterial preparations were not statistically different between CON and PTU groups ( $5.8 \pm 2.7\%$  and  $15.1 \pm 5.8\%$  of maximum active force,  $P > 0.05$ ), while in endothelium-denuded preparations the basal tone level was significantly higher in PTU group compared to CON group ( $56.3 \pm 5.7\%$  compared to  $28.5 \pm 6.9\%$  of maximum active force,  $P < 0.05$ ).

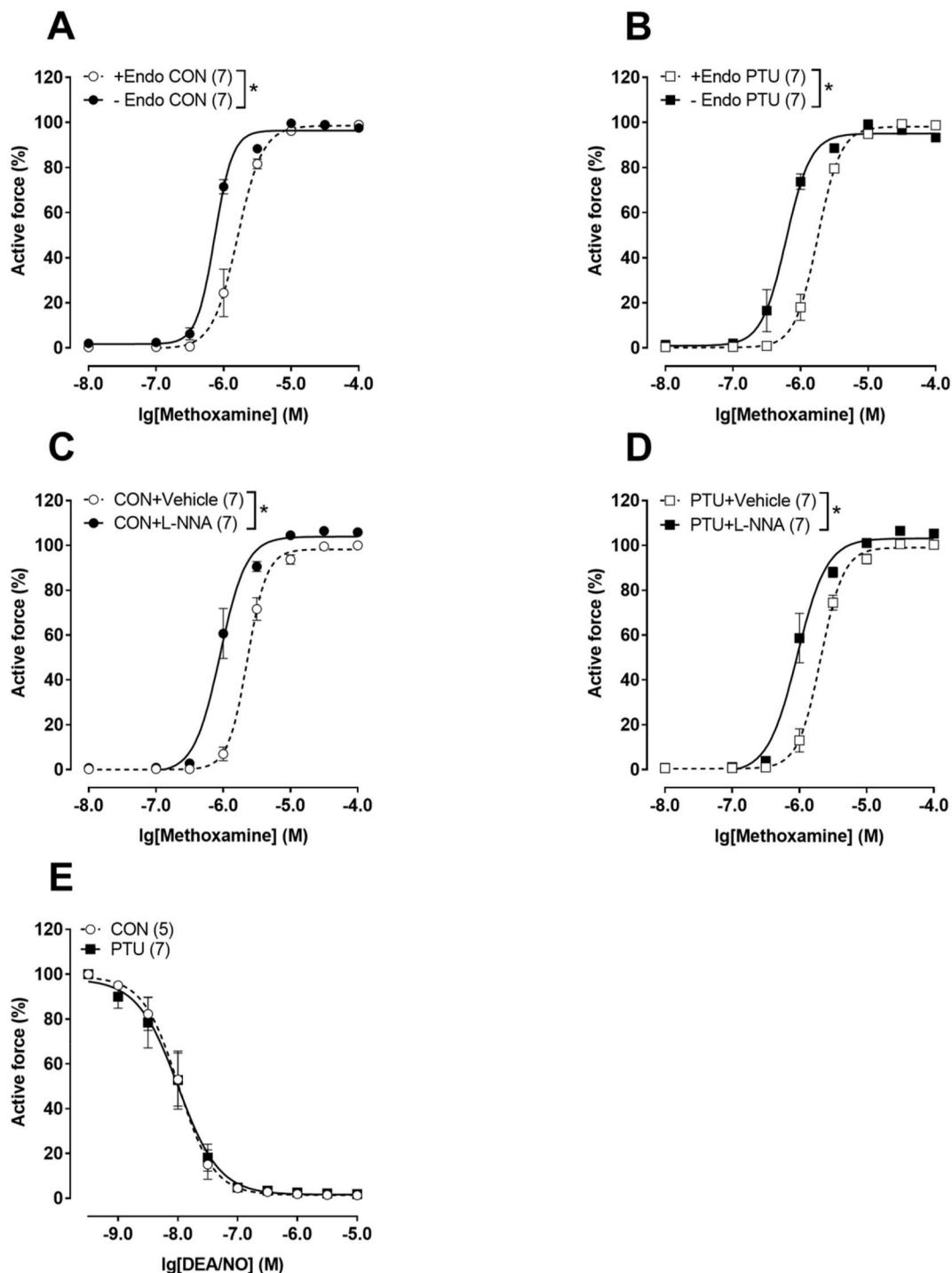
Inhibition of NO-synthase with L-NNA increased MX-induced contractile responses of sural arteries in both experimental groups (Fig. 2C and D). Again, this increase was stronger in PTU group compared to CON group, as indicated from the effect on basal tone levels. The basal tone levels of vehicle-treated arterial preparations were not statistically different between CON and PTU groups ( $13.4 \pm 5.3\%$  and  $20.2 \pm 8.2\%$  of maximum active force,  $P > 0.05$ ), while in L-NNA-treated preparations the basal tone level was significantly higher in PTU group compared to CON group ( $65.1 \pm 3.4\%$  compared to  $34.9 \pm 9.4\%$  of maximum active force,  $P < 0.05$ ). Importantly, the responses to NO-donor DEA/NO did not differ between CON and PTU rats (Fig. 2E). The abundance of eNOS protein was higher in the sural arteries of PTU rats compared to CON animals (Fig. 2F).

Finally, we evaluated the expression levels of deiodinases and TR $\alpha$  in mesenteric and sural arteries of CON and PTU rats. In mesenteric arteries, the mRNA expression levels of deiodinase 2 did not differ between CON and PTU rats (Fig. 3A), as well as the level of TR $\alpha$  (Fig. 3B). However, in sural arteries, the mRNA expression levels of both deiodinase 2 (Fig. 3A) and TR $\alpha$  (Fig. 3B) were higher in PTU group compared

**Table 2**  
Inner diameter ( $d_{100}$ ) and maximum active force of mesenteric and sural arteries in CON and PTU rats.

Parameters	Mesenteric arteries		Sural arteries	
	CON	PTU	CON	PTU
Inner diameter, $\mu\text{m}$ (n = 8; 7;11; 11)	367 $\pm$ 24	374 $\pm$ 27	272 $\pm$ 11	285 $\pm$ 13
Maximum active force, mN (n = 8; 7;11; 11)	19.7 $\pm$ 0.9	21.3 $\pm$ 1.5	18.5 $\pm$ 1.1	17.8 $\pm$ 0.9

Number in parentheses indicates the number of animals.



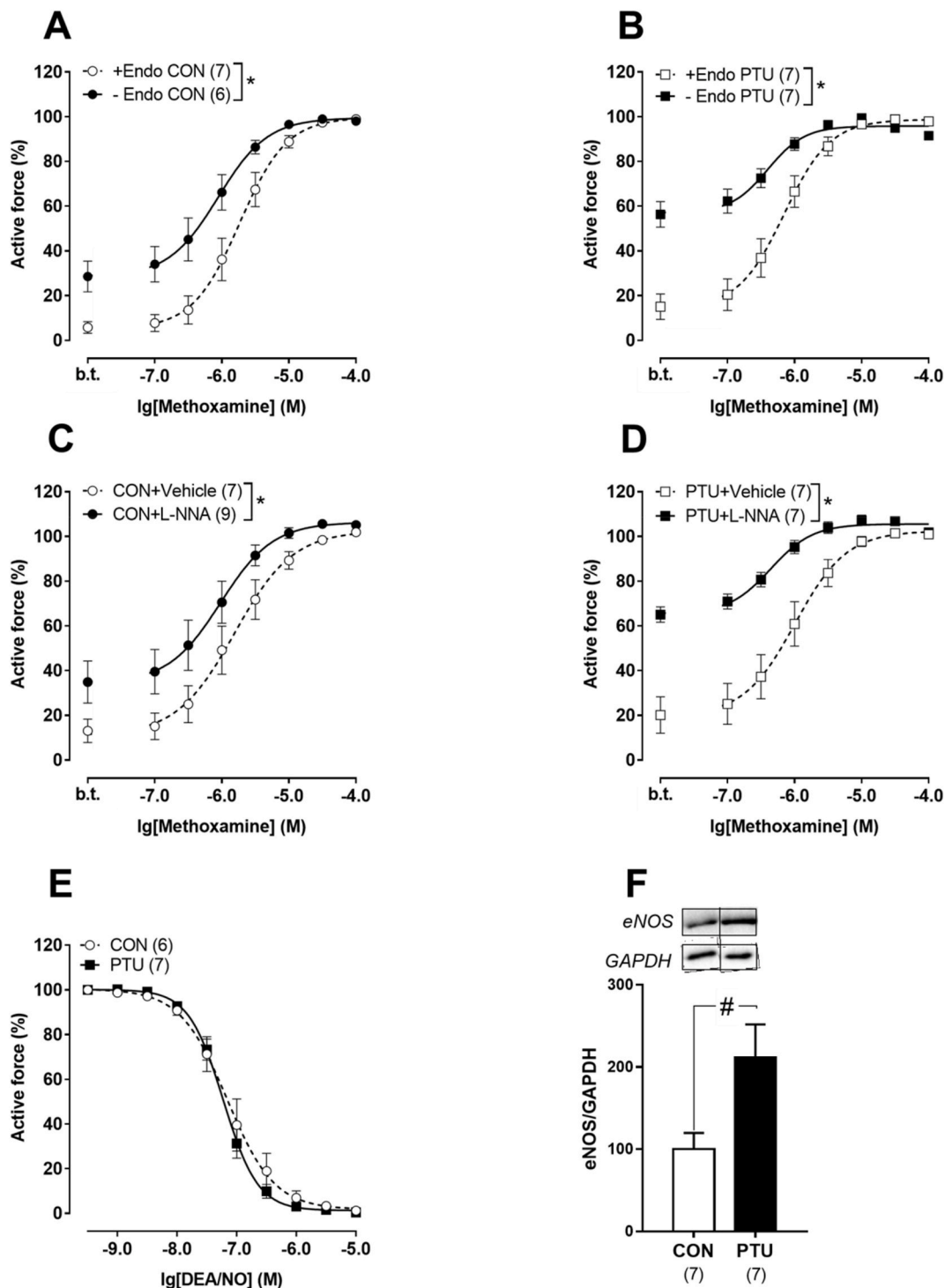
**Fig. 1.** Characteristics of mesenteric arteries of adult male offspring from CON and PTU groups. (A–B) CRR to methoxamine of endothelium-intact (+Endo) or endothelium-denuded (-Endo) arteries from CON (A) and PTU (B) groups. (C–D) CRR to methoxamine of endothelium-intact arteries from CON (C) or PTU (D) rats obtained in the presence of NOS inhibitor L-NNA (100  $\mu$ M) or vehicle ( $H_2O$ , 50  $\mu$ L). (E) CRR to DEA/NO of endothelium-denuded arteries from CON and PTU rats. Numbers in parenthesis indicate the number of animals. \* $P < 0.05$  (Repeated measures ANOVA).

to CON group. Expression of either deiodinase 1 mRNA or deiodinase 3 mRNA was not observed in any group of rats. Along with that, deiodinase 1 and deiodinase 3 mRNAs were detected in rat liver and rat heart, respectively, using the same primers and qPCR protocol.

#### 4. Discussion

Here we report a novel finding that antenatal/early postnatal hypothyroidism is followed by a prominent increase of anticontractile

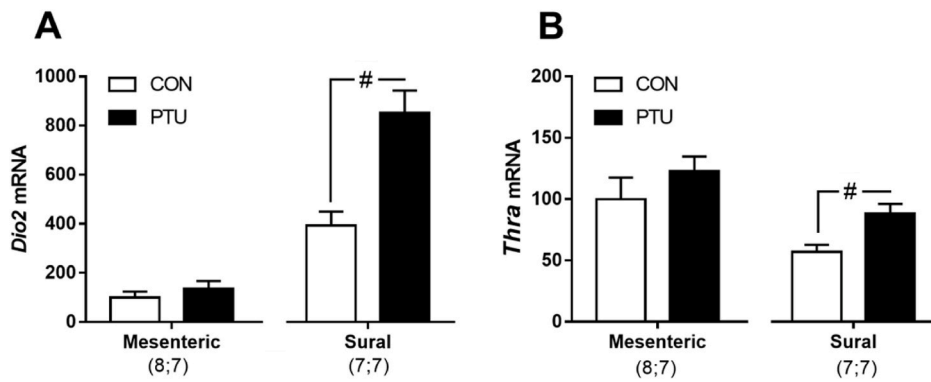
influence of endothelial NO in skeletal muscle arteries, but not in the intestinal circulation of adult rats. The changes in NO-mediated anticontractile influences correlate with the alterations in the local thyroid hormones signaling in different vascular beds of previously hypothyroid adult rats. We have demonstrated that antenatal/early postnatal hypothyroidism leads to the rise in the expression levels of deiodinase 2 and TR $\alpha$  in skeletal muscle arteries, but not in small intestine arteries, thereby creating the potential for the increased local thyroid hormone signaling in arteries of skeletal muscle.



**Fig. 2.** Characteristics of sural arteries of adult male offspring from CON and PTU groups. (A–B) CRR to methoxamine of endothelium-intact (+Endo) or endothelium-denuded (-Endo) arteries from CON (A) and PTU (B) groups. (C–D) CRR to methoxamine of endothelium-intact arteries from CON (C) or PTU (D) rats obtained in the presence of NOS inhibitor L-NNA (100  $\mu$ M) or vehicle ( $H_2O$ , 50  $\mu$ L). (E) CRR to DEA/NO of endothelium-denuded arteries of CON and PTU rats. (F) Relative eNOS protein abundance in arteries of CON and PTU rats. Data were normalized to GAPDH level in the same sample and average ratio in control group was taken as 100%. Numbers in parenthesis indicate the number of animals. \* $P < 0.05$  (Repeated measures ANOVA), # $P < 0.05$  (unpaired  $t$ -test). B.t. - basal tone level.

Importantly, our previous studies have shown that 10-12-week-old offspring of dams treated with PTU using the same protocol as in this work had normal levels of total and free T4 and T3, as well as TSH for at least a month (Gaynullina et al., 2017, 2018b). This is consistent with other studies of gestational hypothyroidism (Gilbert and Sui, 2006; Taylor et al., 2008; Ghanbari et al., 2015; Yousefzadeh et al., 2016). Moreover, adult rats with antenatal/early postnatal hypothyroidism

demonstrate normal blood levels of total cholesterol and triglycerides (Gaynullina et al., 2017), thus they had no lipid metabolism disorders which might be an independent factor influencing vascular function. Therefore, at the systemic level adult animals had euthyroid state and do not suffer from acute manifestations of hypothyroidism. This means that vascular changes observed in this study were not due to alterations in direct effects of thyroid hormone or TSH as well as to other factors



**Fig. 3.** mRNA expression levels of deiodinase 2 (*Dio2*, A) and thyroid receptor  $\alpha$  (*Thra*, B) in mesenteric and sural arteries of adult male offspring from CON and PTU groups. # $p < 0.05$  compared to respective artery in CON group (unpaired *t*-test). Data are normalized to the geometric mean of the three housekeeping genes (*Gapdh*, *Rn18s* and *Rplp0*) in the same tissue sample and shown as percentage of the mean value for mesenteric artery in CON group. Numbers in parenthesis indicate the number of animals.

associated with hypothyroid state.

Thyroid hormones were shown to exert programming effects on the development of organs and tissues (Fowden et al., 2006; Moog et al., 2017). This means that the transient deficiency of thyroid influence during prenatal and early postnatal development may have a long-term impact on body functions including predisposition to chronic disease in adult life. Present study addressed, for the first time, the influence of antenatal/early postnatal hypothyroidism on the anticontractile influence of endothelium in two hemodynamically important vascular regions, such as mesenteric and skeletal muscle vasculature. The anticontractile effect of NO was not changed in mesentery, but prominently increased in skeletal muscle. Along with that, NO-responsiveness of smooth muscle was not changed in either mesenteric or skeletal muscle vascular beds. Taken together, these observations allow us to conclude that antenatal/early postnatal hypothyroidism differently affects the activity of endothelial NO-pathway in these vascular regions.

We hypothesized that the non-uniform influence of antenatal/early postnatal hypothyroidism on NO-pathway activity in different vascular regions may be associated with the differences in local T4 to T3 conversion or in T3 reception in the vascular wall. Accordingly, expression levels of deiodinase 2, the main isoform of deiodinase in peripheral arteries (Mizuma et al., 2001; Yasuzawa-Amano et al., 2004; Sofronova et al., 2017) and the main vascular receptor TR $\alpha$  (Diekman et al., 2001; Hiroi et al., 2006) would differ in two studied vascular beds. This assumption turned out to be correct. Adult rats with antenatal/early postnatal hypothyroidism as compared to control animals had unchanged mRNA levels of deiodinase 2 and TR $\alpha$  in mesenteric arteries, and the level of NO-mediated anticontractile influence of endothelium also did not change. However, in sural arteries antenatal/early postnatal hypothyroidism led to the prominent increase of deiodinase 2 and TR $\alpha$  mRNA expression levels and this was associated with a considerable increase of anticontractile influence of NO.

The question arises about the change of local thyroid hormone signaling in the heart, the vasculature of which demonstrated a prominent decrease in the anticontractile effect of NO (Gaynullina et al., 2017). Therefore, we compared deiodinase 2 mRNA expression levels in the left ventricle myocardium of adult previously hypothyroid and control rats. According to our expectations, the myocardial content of deiodinase 2 mRNA in previously hypothyroid rats was almost two-fold lower than in control animals (Fig. S1). Thus, according to our data, there is a correlation between the anticontractile effect of NO and the content of deiodinase 2 mRNA in different vascular regions. It should be noted that our suggestion about the functional link between deiodinase 2 mRNA content and activity of NO signaling pathway is limited as we did not measure the activity of deiodinase 2. However, the data on deiodinase 2 mRNA level are also relevant since many factors, including T3 and other hormones regulate deiodinase 2 expression at the transcriptional level (Mizuma et al., 2001; Gereben et al., 2008; Toyoda et al., 2009; Awad and Alrefaie, 2014).

However, the question of why the content of deiodinase 2 in different

vascular regions changes differently due to antenatal/early postnatal hypothyroidism remains open and requires further studies. Nevertheless, the phenomenon of tissue-specific changes of deiodinase 2 expression in response to the systemic thyroid hormone deficiency was demonstrated in several studies. For instance, deiodinase 2 mRNA content was increased in brown adipose tissue and pituitary gland but not cerebral cortex and cerebellum of hypothyroid rats (Croteau et al., 1996). Different T3/T4 ratio in deiodinase 2-containing tissues such as brain, heart and white adipose tissue in hypothyroid rats also presume tissue-specific regulation of deiodinase 2 expression and activity (Donzelli et al., 2016). Presumably, deiodinase 2 expression in different vascular beds is influenced by changes in the activity of respective organs. However, the mechanistic link between organ activity and vascular deiodinase 2 expression remains elusive. Specific alterations of intestine, hind limb skeletal muscle and heart in rats with antenatal/early postnatal hypothyroidism have not been explored as well and can be addressed in future studies.

In conclusion, in the present study we have shown that antenatal/early postnatal hypothyroidism is accompanied by the prominent increase of anticontractile influence of NO in skeletal muscle arteries, but not in mesenteric arteries of adult animals. The diversity of long-term hypothyroidism effects may be due to different alterations of the local thyroid hormone signaling in different vascular beds. Potential increase of local T3 synthesis in sural arteries due to elevated level of deiodinase 2 stimulates eNOS expression and therefore NO-mediated anticontractile influence of endothelium in adult rats with antenatal/early postnatal hypothyroidism.

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## CRediT authorship contribution statement

**Dina K. Gaynullina:** Conceptualization, Investigation, Data curation, Writing – original draft, all authors approved the final version of the manuscript. **Svetlana I. Sofronova:** Conceptualization, Investigation, all authors approved the final version of the manuscript. **Ekaterina K. Selivanova:** Investigation, Writing – original draft, all authors approved the final version of the manuscript. **Anastasia A. Shvetsova:** Investigation, all authors approved the final version of the manuscript. **Anna A. Borzykh:** Investigation, Data curation, all authors approved the final version of the manuscript. **Olga S. Tarasova:** Conceptualization, Investigation, Data curation, Writing – original draft, all authors approved the final version of the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crphys.2021.12.002>.

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