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Fetal programming of adult hypertension in female rat offspring exposed to androgens *in utero*

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Abstract

Aims—The influence of prenatal factors on the development of arterial hypertension has gained considerable interest in recent years. We examined the effects of prenatal testosterone treatment on blood pressure in adult female rats. Further, to define the mechanisms whereby blood pressure may be raised, we examined vascular endothelial function and nitric oxide synthesis.

Methods and Results—Testosterone propionate (0.5mg/kg/day;SC) or vehicle was administered to pregnant Sprague-Dawley rats from gestational day 15–19. Maternal feed intake and plasma levels of steroid hormones were measured in the dams. In the female offspring, birth weight, growth rate, blood pressure, vascular reactivity, eNOS expression, and nitric oxide production were examined. In the pregnant rats, testosterone-treatment increased plasma testosterone levels by 2-fold without any significant changes in 17β-estradiol, progesterone and corticosterone levels. Testosterone-treatment did not affect maternal feed intake. The pups born to testosterone mothers were smaller in size but exhibited catch-up growth. The blood pressure in the testosterone offspring at 6 months of age was significantly higher compared to controls. Endothelium-intact mesenteric arteries from testosterone group exhibited increased contractile responses to phenylephrine, decreased vasodilation to acetylcholine and unaltered responses to sodium nitroprusside in comparison to control rats. Testosterone rats demonstrated decreased expression for eNOS, and reduced nitric oxide production.

Conclusions—Our data show that elevated plasma maternal testosterone levels: (1) causes low birth weight followed by catch-up growth and hypertension in female offspring; (2) alters endothelium-dependent vascular responses. The endothelial dysfunction is associated with decreased activity/expression of eNOS.

1. Introduction

Intrauterine growth restriction (IUGR) occurs when fetal weight and size gain are below the 10th percentile for gestational age. A large proportion of pregnancies result in IUGR offspring, 3–32% worldwide (WHO Health Statistics 2008), and in the last decade

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awareness of the potential clinical significance of this condition to chronic diseases later in life has increased. A number of studies have demonstrated an association between low birth weight (surrogate marker of IUGR) and an increased mortality later in life [1]. In fact, there is a growing evidence suggesting that an adverse intrauterine environment during the critical period of fetal development cause long-term structural and functional effects in the developing fetus, predisposing it to increased risk for development of hypertension later in life [2]. Several animal studies have confirmed the observation that unfavorable fetal environment causing fetal malnutrition, induced by restriction of food, protein, or uteroplacental blood flow, leads not only to IUGR but also to hypertension in adult offspring [3].

Nevertheless, the relevance of undernutrition and low-protein diet to populations in industrial countries is limited. Recent attention has been focused on maternal androgen exposure because pregnant women with elevated circulating testosterone (T) levels, and their problems with low birth weight and adverse adult health consequences, are rapidly increasing. T is an important regulator of growth and differentiation during fetal development [4;5]. Human epidemiological evidence show that elevated maternal T levels is associated with IUGR [6]. Prenatal androgen excess in pregnant animals was consistently associated with IUGR [7–9] with postnatal catch-up growth that is known to amplify adult life outcomes [10]. In addition to its effect on fetal growth, elevated T levels during pregnancy has lasting effects in their offspring causing reproductive and endocrine disturbances in adult life [11;12]. In fact, exogenous hyperandrogenemia in pregnant rats was consistently associated with increased testosterone levels in the female offspring [13]. In female offspring, it has been reported that prenatal T treatment causes enlargement of the left cardiac ventricle and kidneys [12] and moderate increase in blood pressure [14] during adult life. However, the vascular mechanisms linking IUGR in response to elevated maternal androgens and the increased arterial pressure during adult life are unclear. Sex differences have been observed in animal models of fetal programming, and recent studies suggest that sex steroid hormones may modulate activity of blood pressure regulators, such as renin-angiotensin system (RAS) and nitric oxide (NO) leading to development of hypertension and vascular dysfunction, with more profound effects in growth restricted males than females [15]. Ojeda et al. [16] showed that testosterone contributes to hypertension by regulating RAS in adult growth-restricted male offspring. In this study, we examined the vascular effects in the adult females and the regulatory role of NO system.

Examining the underlying mechanisms are of clinical relevance since higher androgen levels are reported in several obstetric pathological conditions that lead to IUGR, such as preeclampsia [17–19], maternal PCOS [20;21], obesity [22;23], stress [24;25], and smoking [26–28]. In addition, pregnant African-American mothers have higher serum T levels with a greater frequency of low birth weight babies [29–31]. Moreover, the highest rates of adult CV dysfunction are also concentrated in these populations [29–31]. The rat model of maternal androgen excess is particularly valuable because of absence of zona reticularis in the rat adrenal and lack of 17-hydroxilase. Therefore such rat models are somewhat clean from adrenal contributions. Although rats may not truly represent primate or human biology, but may provide a useful model to study the adult life consequences of maternal androgen excess. We used female offspring from pregnant rats receiving either T injection or its vehicle during late pregnancy and investigated whether (1) the birth weight, growth rate and blood pressure is altered; (2) vascular contraction to phenylephrine (PE) is enhanced; (3) vascular relaxation to acetylcholine (ACh) is reduced; and (4) the changes in vascular contraction/relaxation in blood vessels of T rats, involve alterations in the endothelium-dependent NO pathway.

Methods

Pregnant Rats

Timed-pregnant (day 12) Sprague Dawley rats (Harlan, Houston, TX), were housed in a temperature-controlled (23°C) room, and provided with food and water *ad libitum* under a 12-hour light/dark cycle. The pregnant rats were assigned to two groups and administered either T propionate (n=6) in sesame oil (0.5 mg/kg/day; s/c; Cat No. T-1875; Sigma, St. Louis, MO) or vehicle (n=6) from gestational days (GDs) 15 to 19 between 09.00 and 10.00 AM each day. This dose of T (0.5 mg/kg/day) was chosen since it produces approximately a 2-fold increase in the maternal circulating T levels [32;33], comparable to those observed in complicated human pregnancies. Because an increase in T levels during the third trimester of pregnancy was shown to be associated with IUGR, we chose this time period (GD 15–19) for T treatment in rats. Daily maternal feed intake was monitored throughout the dosing period and until delivery. All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee at the University of Texas Medical Branch.

Maternal T, 17β-estradiol, progesterone and corticosterone level

Dams of control and T group were sacrificed on GD 19, 2 h after dosing and blood was collected by cardiac puncture from control (n=6) and T dams (n=5). Plasma levels of T, 17β-estradiol, and progesterone were determined by radioimmunoassay and corticosterone by enzyme-immunoassay (DSL, Webster, TX) as described in our previous studies [34]. Intra- and interassay coefficients of variation were 2% and 4% for progesterone, 3% and 2% for oestradiol, 3% and 5% for testosterone, and 2% and 4% for corticosterone, respectively. Sensitivity of the assay was 0.02 ng ml^{-1} for progesterone, 0.8 pg ml^{-1} for oestradiol, 0.04 ng ml^{-1} for testosterone and 0.5 ng ml^{-1} for corticosterone.

Offspring rats

The dams were allowed to deliver at term (on day 22). Pups were weighed and litter sizes were adjusted to 10 pups per dam. To ensure homogeneity of subjects, litters of less than 10 or more than 14 pups were not included in the study. Pups were sexed at birth and weaned from their dams at 21st day of age and only female offspring were used for this study. The rat offspring were fed with standard rat chow. The postnatal growth of all female offspring was monitored by weighing the rats at each month.

Offspring Blood Pressure

Progressive changes in the mean blood pressure (BP) of the control (n=6) and T group (n=6) were measured once a month starting from 2 to 6 months of age. CODA computerized noninvasive BP system (Kent Scientific, Litchfield, CT), which assesses tail BP by means of volume pressure recording was used as described in our previous studies [35;36]. Rats were held in a preheated restrainer with the tail exposed and both an occlusion cuff and a volume pressure–recording cuff were placed close to the base of the tail. Signals were recorded and analyzed using Kent Scientific software. To minimize stress-induced variations in BP, all measurements were taken by the same person in the same peaceful environment and at the same time of the day.

Offspring Mean Arterial Pressure

At 6 months of age; the changes in the BP measured by non invasive method were confirmed by direct mean arterial pressure (MAP) using an indwelling carotid arterial catheters in conscious rats [34;37]. A PE-50 catheter was placed in the carotid artery under anesthesia (ketamine-45 mg/kg; xylazine-5 mg/kg; Burns Veterinary Supply, Westbury, NY). After a 1-day recovery period, the catheter was connected to a pressure transducer, and

MAP in conscious rats (n=6 in each group) was recorded using a DBP001 direct BP system (Kent Scientific). Offspring from different litters were chosen, and MAP was monitored continuously for 30 min to determine the baseline MAP.

Vascular reactivity

The rat offspring were sacrificed at 6 month of age and the mesenteric arcade was excised in to Krebs solution, cleaned, and segments of third-order artery (200 μm diameter) were cut into 2 mm rings. For endothelium-intact rings, care was taken to avoid injury to the endothelium. In some rings, the endothelium was removed by gently rubbing the vessel interior with tungsten wire. Removal of the endothelium was verified by the absence of ACh-induced relaxation in tissues precontracted with a submaximal concentration of PE.

The rings were mounted on a myograph (DMT, Aarhus, Denmark) for isometric tension recording using Labchart software (ADInstruments, Colorado Springs, CO). The passive tension-internal circumference relationship (IC_{100}) was determined by incremental increases in tension to achieve an internal circumference equivalent to a transmural pressure of 100 mmHg (using the Laplace relationship) and the arteries were set to a diameter equivalent to $0.9 \times IC_{100}$ using normalization software (Labchart). Functional integrity of the vessels was assessed with 80 mM KCl Krebs solution. Rings were equilibrated for at least 1 h, and then concentration-contraction curves with increasing concentrations of PE were constructed (n=6–7 in each group). In other tissues, submaximal PE (10^{-6} mol/L) contraction was elicited, increasing concentrations of ACh or sodium nitroprusside (SNP) were added, and vascular relaxation was measured (n=6–7 in each group). In some experiments, the tissues were pretreated for 30 minutes with N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} mol/L) to inhibit NO synthase (NOS), and the effects on PE contraction and ACh-induced relaxation of PE contraction were measured.

Nitrite/nitrate production

Nitrite/nitrate (NO_x) production from endothelium-intact mesenteric rings was measured as described in our previous studies [34]. Briefly, the rings from control (n=5) and T offspring (n=5) were incubated with Krebs solution for 30 min and samples for basal accumulation of nitrite (NO_2) formed from released NO were first taken. Then arterial rings were stimulated with ACh (10^{-7} or 10^{-5}) for 30 min and the media collected. The stable end product of NO, NO_2^- was assayed in a 96-well microtiter plate using 100 μl of the Griess reagent (Sigma, St. Louis, MO). The concentration of NO_2^- was calculated from the standard curve constructed with known concentrations of $NaNO_2$.

eNOS Expression

Proteins were isolated from endothelium-intact aorta from control (n=5) and T rats (n=5) using a 2-mL tight-fitting homogenizer (Power Gen125 from Fisher) at 4°C. Protein-matched samples were electrophoresed on 8% SDS polyacrylamide gels then transferred to nitrocellulose membranes and processed for immunoblotting using standard procedure. Monoclonal anti-eNOS antibody (1:1000; BD Transduction Laboratory) was used a primary antibody with horseradish peroxidase-conjugated anti-mouse IgG as secondary antibody. Rabbit anti β-actin antibody (1:5000; Cell Signaling, Danvers, MA) was used for normalization. The amount of eNOS was expressed as the ratio of the eNOS/β-actin signals

Solutions, Drugs, and Chemicals

Normal Krebs solution contained (in mM) NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.17; $NaHCO_3$, 25; KH_2PO_4, 1.18; EDTA, 0.026; and d-glucose, 5.5 (pH 7.4). 80 mM KCl Krebs was prepared by replacing NaCl with equimolar concentrations of KCl. Stock solutions of

PE, ACh, and SNP (Sigma) were prepared in distilled water. All other chemicals were of reagent grade or better.

Statistical Analysis

Data are presented as means ± SEM. Statistical analysis was performed using a two-way ANOVA followed by the Bonferroni post hoc test on all dose-response curves and the unpaired Student's t test for comparison of single observation between the control and T groups. Where more than one animal from any litter was studied, data from animals within the litter were averaged and analysis performed only on numbers of litters. The developed force was expressed as active stress (mN/mm). Contraction responses to PE were also calculated as percent of its maximal contraction. Relaxant responses to ACh were calculated as percent inhibition of the PE-induced contraction. Half maximal effective concentrations were determined by regression analysis (GraphPad Software, San Diego, CA) and expressed as negative log-molar concentration. Data from several vascular rings of the same rat were averaged and presented as the datum for 1 rat, with the n value representing the number of rats. Differences were considered statistically significant at $P<0.05$.

Results

During pregnancy, maternal feed intake was monitored daily and there were no significant differences in feed intake between control and T dams (Fig. 1). Administration of T to pregnant rats from GD 15–19 resulted in 2-fold increase in plasma T levels 2 h after the last dosing on GD19 ($p<0.05$; n=5–6; Fig 2A). There were no significant changes in the levels of 17β-estradiol, progesterone and corticosterone in the T dams compared to controls (Fig 2B–D; n=5–6). The length of gestation was not significantly affected by T treatment (control: 22 ± 0.3; T: 22 ± 0.7 days) and no significant differences were noted in the mean litter size between control (12.4 ± 1.6) and T (11.8 ± 1.4) groups.

The birth weight in offspring of control dams was 6.0±0.1 g. The birth weight was significantly reduced ($P<0.05$; n = 5–6 litters; females) in T group (4.7±0.1 g) compared to controls. Both normal and T offspring showed significant increases in body weight with age. However, the body weights were consistently lower in T compared with control offspring up to 4 months of age but the difference disappeared at 5 and 6 months of age (Fig. 3A).

There was no significant difference in mean BP between control and T exposed offspring up to 4 months of age but the BP was significantly greater in T offspring at 5 and 6 months of age compared to controls (Fig. 3B; n=6). Mean BP measured by CODA system correlated well with the MAP at 6 months of age. MAP was significantly greater ($P<0.05$) in T offspring (108 ± 3.2 mmHg; n=6) when compared to control offspring (93 ± 2.4 mmHg; n=6; Table 1).

In endothelium-intact mesenteric arterial rings, PE caused concentration-dependent increases in active stress (Fig 4A; n=6–7). The maximal PE (10^{-5} M)-induced vascular contraction in T offspring was significantly greater than in controls ($P<0.05$; Figure 4A and the Table 1; n=6–7). When the PE response was presented as the percentage of maximum PE contraction, T offspring exhibited a significant leftward shift (changes in the EC_{50}; $P<0.05$; Figure 4B and the Table 1; n=6–7) in comparison to those in control group. PE was significantly more potent in producing contractions in T than in control animals ($P<0.05$; Figure 4B and the Table 1; n=6–7). Removal of the endothelium greatly enhanced ($P<0.05$) the maximal PE-induced contraction in control group but was with no significant effects in T group (Fig 4A and Table 1; n=6–7). PE was significantly more potent in causing contractions in endothelium-denuded than in endothelium-intact rings of control offspring ($P<0.05$; Fig 4B and Table 1; n=6–7). In contrast, the potency of PE was not significantly

different between endothelium-denuded and endothelium-intact rings from T group (P=0.842; Fig 4B and Table 1; n=6).

In endothelium-intact arterial rings, pretreatment with *L*-NAME for 30 min to inhibit eNOS activity significantly enhanced the maximal PE-induced contraction in control rats (P<0.05) but was without any significant effects in T rats (Fig 5A and Table 1; n=6–7). Also, calculation of ED_{50} of PE showed that PE was more potent in causing contraction in *L*-NAME-pretreated than in non treated arterial rings of control rats (P<0.05; Fig 5B and Table 1; n=6–7). In contrast, PE potency was not significantly different in *L*-NAME-treated and non-treated vascular rings of T rats (Fig 5A and Table 1; n=6–7).

In endothelium-intact rings of control group, ACh caused concentration-dependent relaxation of PE-mediated (10^{-6} M) contraction (Fig 6A; n=6–7). The ACh-induced relaxation of the PE contraction was significantly less in T than in control animals (Fig 6A). Both the ED_{50} and the maximal relaxation were decreased in rings from T offspring compared to controls. Because the rings of T offspring showed greater vascular contraction to PE compared with those from controls, additional experiments were performed on rings of T offspring in which the initial PE concentration was lowered to 3×10^{-7} mol/L to produce a submaximal contraction that was roughly equal in magnitude to the contraction observed in rings of control offspring precontracted with 10^{-6} mol/L. These experiments showed that the ED_{50} of ACh in rings of T precontracted with 3×10^{-7} mol/L PE ($3.5\pm0.2\times10^{-7}$ mol/L) was not significantly different (P=0.111) from that in rings precontracted with 10^{-6} mol/L PE ($3.3\pm0.1\times10^{-7}$ mol/L). The relaxation response to ACh in L-NAME pretreated endothelium-intact rings was not significantly different between control and T rats (Fig 6A).

In endothelium-denuded rings, SNP, an exogenous NO donor, caused concentration-dependent relaxation of submaximal PE contractions. The SNP-induced relaxation to PE contractions was not significantly different in rings from control and T offspring (Fig 6B; n=6–7).

In endothelium-intact rings, the basal NO_x level showed a significant reduction in T compared with control offspring (P<0.05; Fig 7; n=5). ACh increased NO_x production in vascular rings from both groups of offspring but the proportion of increase was significantly lower in T compared with control groups (Fig 7). Western blot analysis showed that the optical density of eNOS was significantly reduced in T compared with control offspring (P<0.05; n=5).

Discussion

There is mounting evidence that elevated T levels during pregnancy may have adverse effects on fetal growth and thereby increase the potential for increased risk for CV diseases in adult life. Findings from our study indicated that in T-treated pregnant rats, the maternal plasma T levels were elevated without changes in 17β-estradiol, progesterone and corticosterone levels. T dams delivered smaller-sized pups compared to vehicle treated controls. Further, the female offspring born to T treated mothers exhibited catch-up growth and hypertension. In addition, the endothelium-dependent vascular relaxation was reduced in mesenteric arteries, and the reduced vascular relaxation and enhanced vascular contraction in T offspring appear to involve the endothelium-dependent NO pathway.

Previous studies have suggested a strong relation between low birth weight and the risk of hypertension during adult life [38]. Experimental studies also show that maternal protein restriction during pregnancy in the rat results in offspring that are hypertensive [34;35;39]. However, in the Western world, low-birth-weight as a result of maternal malnutrition

represents only a small proportion because of good perinatal care. Thus, a strong correlation between low birth weight and hypertension in well-nourished populations is more likely caused by fetal undernutrition and IUGR as a result of factors other than maternal malnutrition. Maternal androgen excess is one potential candidate since the proportion of pregnant women exposed to elevated T levels is rapidly increasing. The reported normal plasma T concentrations during late pregnancy range between 1.0 and 1.5 ng/mL in women [40–44] and 1.2–1.4 ng/mL in rats [45;46]. Higher T levels are observed in pregnant mothers with PCOS (1.4- to 2.7-fold) [20;21], preeclampsia (1.4- to 3.4-fold), [17–19] classical congenital adrenal hyperplasia (CAH; 1.7- to 5-fold) [47;48], obesity (1.3- to 1.4-fold) [49;50], or smoking (1.2- to 1.4-fold) [26–28], and they also deliver low-birth-weight babies compared to normal mothers. Furthermore, pregnant mothers can be inadvertently exposed to elevated testosterone levels via environmental pollutants and anabolic steroids. High androgenic activity is reported in water from kraft pulp and paper mills and concentrated animal feed operations in the United States and Europe [7]. Reports have shown that an androgenic growth promoter used in beef cattle, Trenbolone, has a half-life of greater than 260 days in animal by-products [7]. In the present study, a 2-fold increase in maternal plasma T levels in pregnant rats, which is similar in magnitude as reported in some of the abnormal clinical settings is associated with fetal growth restriction. T-induced low birth weight is consistent with previous reports in sheep [8;9]. Therefore, it is important to study the changes in vascular function associated with low birth weight in IUGR offspring produced in response to elevated maternal T, since CV dysfunction is still the leading cause of mortality and morbidity in the modern society.

T treatment in pregnant rats did not alter other steroid hormone levels such as progesterone, corticosterone or estradiol suggesting that the effects observed in T dams are direct effects of T and not a secondary consequence to alteration in maternal steroid hormones. Thus fetal growth restriction in T group appears to be mediated by androgenic actions of T. This was confirmed by using the non-aromatizable androgen, dihydrotestosterone, which also caused effects similar to T on birth weight (data not shown). Interestingly, the fetal growth restriction observed in T-administered pregnant rats was not due to reduced feed intake, thus differing from most nutrition restriction models of fetal programming. Alternatively, the growth restriction observed in nutrition restriction animals may be facilitated via increased T levels. For example, increases in plasma T were noted in the mothers after induction of hypoxia, stress, and protein restriction suggesting that developmental insults have the potential to cause significant increases in T[51–55]. Indeed, it has been reported that the low birth offspring born to protein restricted mothers have increased ano-genital distances compared to controls [56;57] suggesting that the fetuses are subjected to androgenic influences during intrauterine life.

In the present work, we focused on female offspring from T dams and detected differences in the magnitude of the increase in mean BP levels in these animals. We observed higher mean BP in T rats with consistent increase in MAP (measured through carotid catheters in conscious rats) compared to controls. A similar report of maternal T-treatment of pregnant sheep causing hypertension in female offspring is reported [14]. These observations suggest that elevated maternal androgen influences the CV system in their offspring. Studies have found that IUGR and postnatal catch-up growth pose threats to the well-being of the offspring, often leading to adverse postnatal health consequences [10]. Maternal T treatment from GDs 15–19, which resulted in growth restriction at birth and 0–2 months of age, exhibited catch-up growth between 2–4 months of age, with subsequent increase in BP between 4–6 months of age. Although development of hypertension appears to be a sequel to catch-up growth, further studies are essential to establish a definitive relationship. Postnatal catch-up growth is also observed in lambs born to T treated dams which is shown to amplify reproductive and metabolic dysfunctions [9;58;59]. Therefore, analysis of the role

of catch-up growth in the development of adult life health disorders should be carefully examined in future studies. However, whether the catch-up growth observed in this study in T rats is due to increased fat deposition or improved feed intake/efficiency is not known at this time.

We examined whether alterations in vascular reactivity could play a role in mediating the observed increase in arterial pressure in T group. For this we examined the responses of PE, a vasoconstrictor whose effects are modulated by the endothelium, ACh, an endothelium dependent vasodilator, and SNP, an endothelium-independent vasodilator. Our findings showed that the vascular contraction to PE is enhanced in T compared with control rats. In search for the possible mechanisms involved in the enhanced vascular contraction in the T rats, we found that removal of the endothelium enhanced the PE-induced contraction in control rats but not in T rats. Also, the ACh-induced relaxation was reduced in T compared with control rats. This suggest that function of endothelium is impaired in T rats compared to that in control rats.

The vascular endothelium is important in the control of vascular tone and the regulation of peripheral blood pressure [60]. Considering that NO is the main agonist responsible for endothelial-dependent relaxation, we characterized the relative contribution of NO in vasodilation of mesenteric arteries isolated from control and T rats. In both control and T rats ACh-induced vasorelaxation through NO-dependent mechanisms. This is evident in this study by the observation that majority of these relaxation responses are inhibited by *L*-NAME, although there was some NO-independent relaxation at higher doses of ACh. However, this *L*-NAME-resistant component was shown to contribute equally to vasodilation in both control and T rats, indicating that only the NO component may be impaired, thereby contributing to the observed difference in pressor response in these groups. In T rats, impaired NO function may be due to decrease in the synthesis/release of NO from endothelial cells or may reflect a change in the sensitivity of vascular smooth muscle to relaxation by NO. Absence of differences in relaxation responses of rings to SNP between T and control rats suggests that the decreased relaxation in the T rats is not due to decreased vascular smooth muscle vasodilating capacity but may be due to reduction in NO synthesis and/release. Our data supports that both the eNOS expression and NO_x production (basal and ACh-induced) were significantly reduced in vascular rings from the T compared with control rats.

It is essential to emphasize the following cautionary remarks regarding the aforesaid interpretations. First, although the decrease in endothelial cell function contributes to the observed elevation in arterial pressure, the endothelial dysfunction may also be secondary to arterial pressure alterations. Further analysis of longitudinal changes in vascular reactivity and BP are essential to establish a cause-effect relationship. Second, other factors such as neuronal, renal and hormonal mechanisms can also facilitate or contribute for CV dysfunction. The possible additional alterations in other systems represent important areas for future experiments.

In summary, elevated T levels during pregnancy causes fetal growth restriction and postnatal catch-up growth with development of hypertension in the adult lives. The decreased endothelium-dependent vascular relaxation pathway involving reduced release of NO from endothelial cells may play an important role to contribute at least in part for the increased vascular contraction and arterial pressure in T rats.

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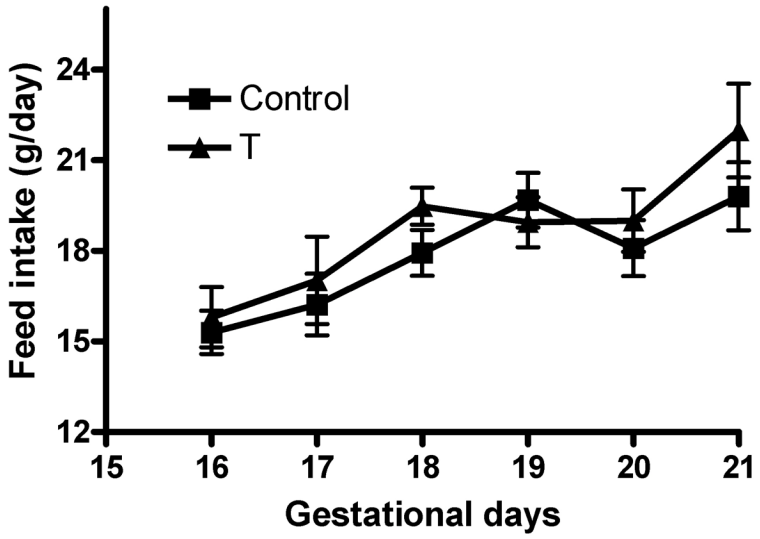

Figure 1.
Daily feed intake in pregnant rats quantified in g. Values are expressed as mean±SEM (n= 5).

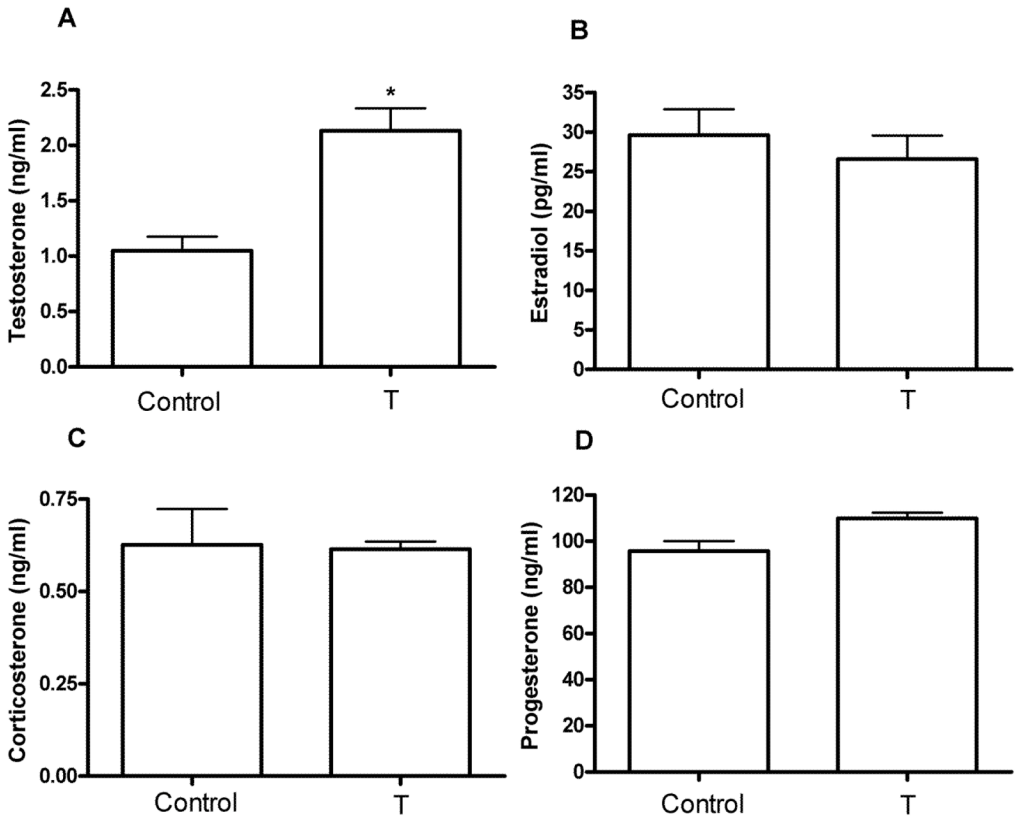

Figure 2.

Steroid hormone levels in pregnant rats. Plasma **(A)** T, **(B)** 17β-estradiol, **(C)** corticosterone and (D) progesterone levels in control (n=6) and T (n=5) dams measured on gestational day 19 (2 h after last dosing) by radio/enzyme-immunoassay. Data are expressed as mean±SEM. **p*<0.05 vs controls.

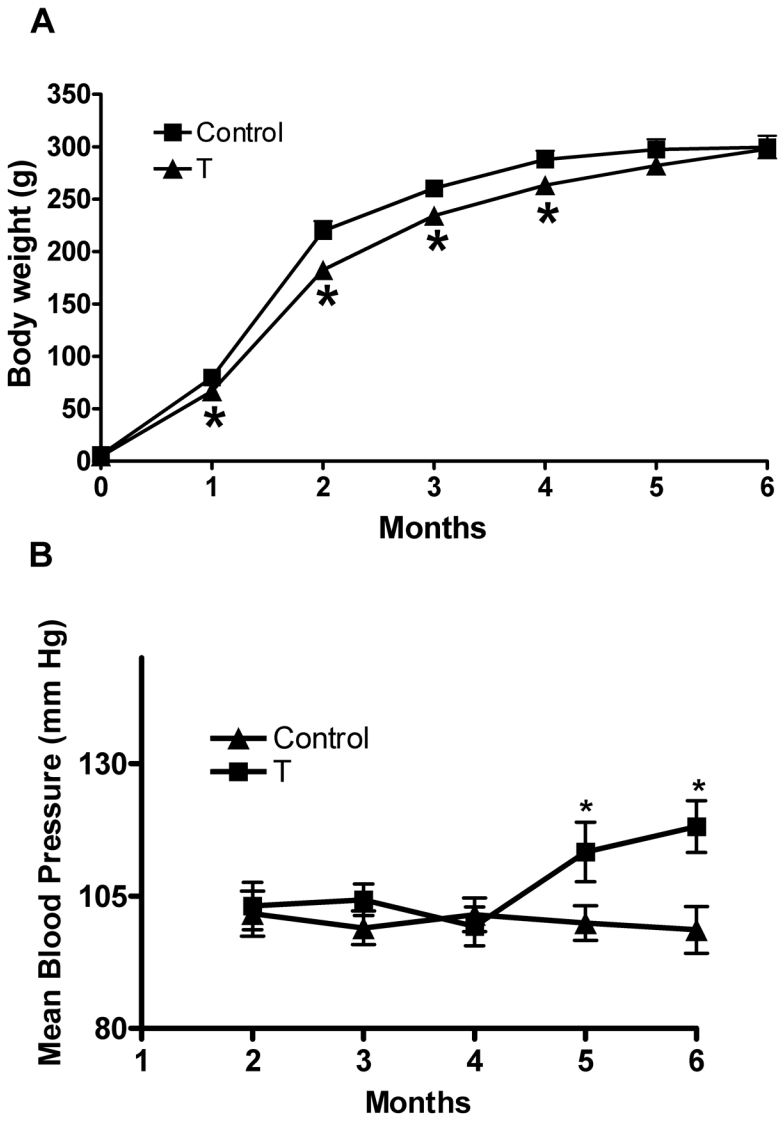

Figure 3.

Growth rate and blood pressure changes in control and T offspring. **(A)** Body weight from birth to 6 months of age (n= 10 in each group). (**B**) Mean blood pressure measured using non-invasive CODA system from 2 to 6 months of age. Values are expressed as mean ± SEM of 6 animals in each group. *p<0.05 compared to control group at respective month.

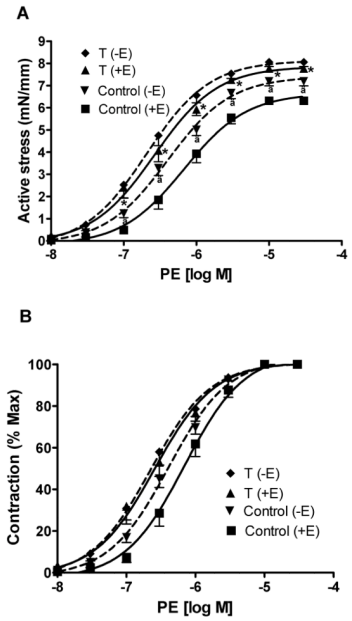

Figure 4.

PE-induced contraction in mesenteric arterial rings without endothelium (E−) and with endothelium (E+) in control and T offspring. Arterial rings were stimulated with increasing concentrations of PE and the responses were presented as **(A)** active stress or **(B)** percentage of maximum PE contraction (both semilog plots). Data points represent mean±SEM of measurements in 16 arterial rings from 6 to 7 rats of each group. *Measurements in T are significantly greater ($P<0.05$) than those in control rats. [a]Measurements in endothelium-denuded vessels are significantly greater ($P<0.05$) than those in endothelium-intact.

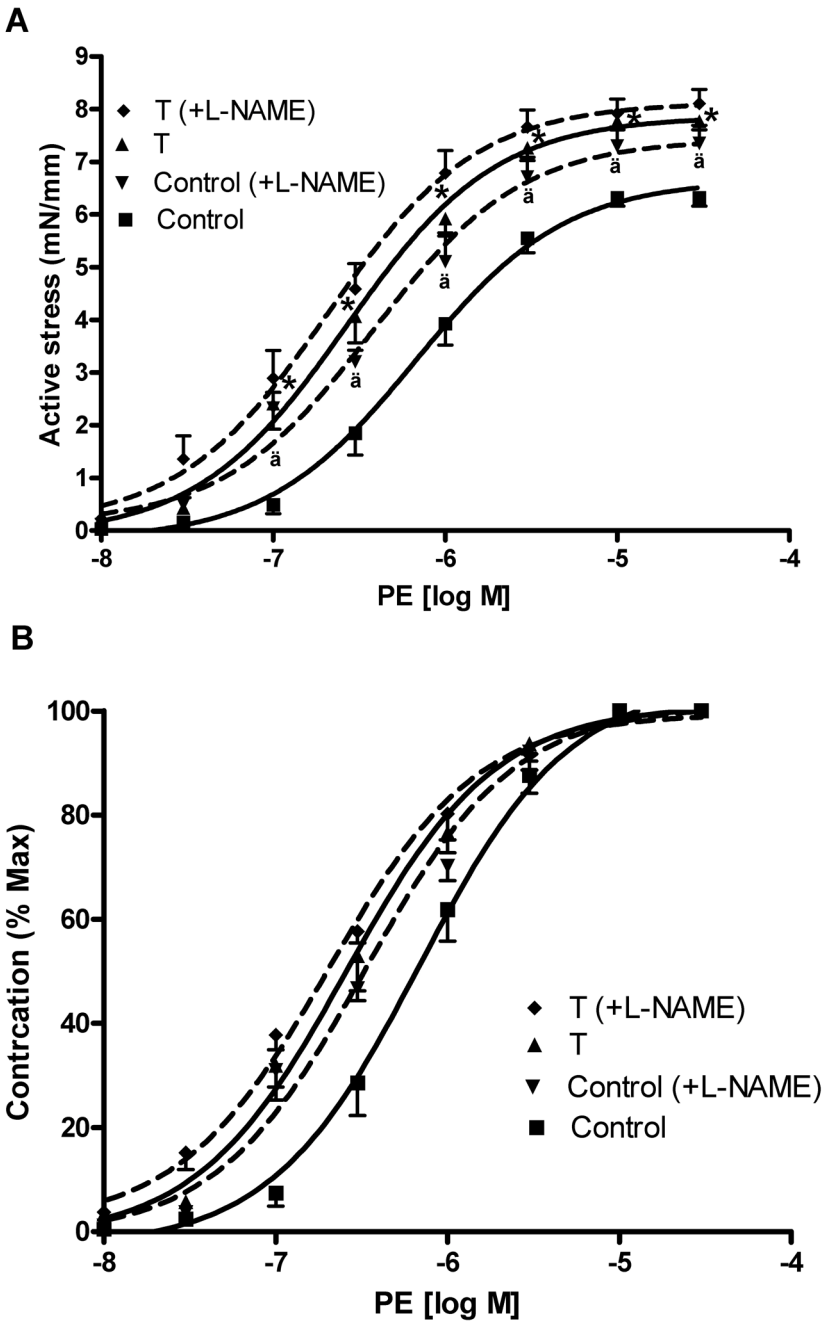

Fig. 5.

PE-induced contraction in endothelium-intact mesenteric arterial rings in presence or absence of L-NAME (semilog plots). Mesenteric arterial rings were incubated in the absence or presence of *L*-NAME (10^{-4} mol/l) for 30 min and then stimulated with increasing concentrations of PE. PE contraction is presented as **(A)** active stress or **(B)** percentage of maximum PE contraction (both semilog plots). Data points represent mean±SEM of measurements in 16 arterial rings from 6 to 7 rats of each group. *Measurements in T are significantly greater ($P<0.05$) than those in control rats. [a]Measurements in L-NAME pretreated rings are significantly greater ($P<0.05$) than in rings without L-NAME pretreatment.

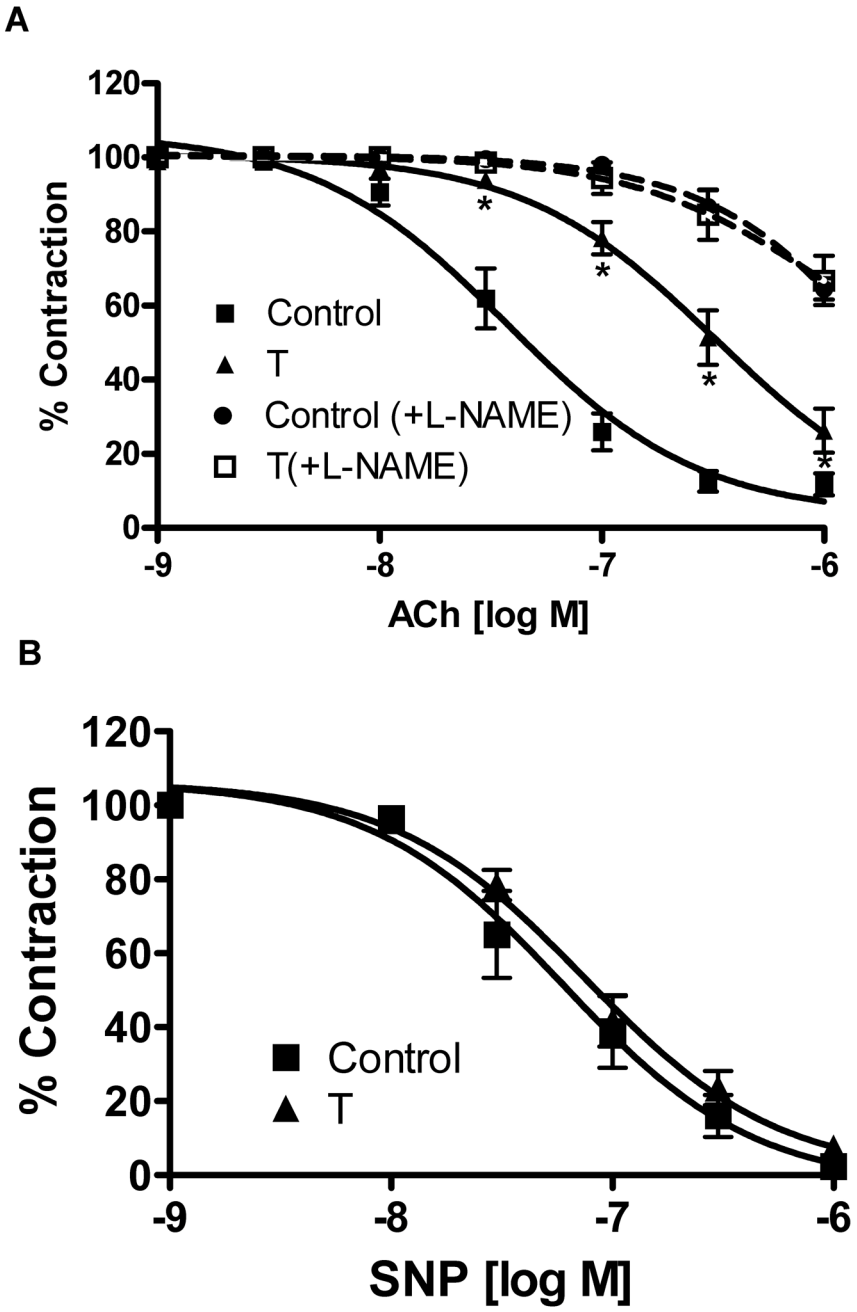

Figure 6.

Vasodilatory effects of ACh and SNP in mesenteric arterial rings of control and T offspring. **(A)** ACh-induced relaxation of PE contraction in endothelium-intact mesenteric arterial rings of control and T rats in the absence or presence of *L*-NAME **(B)** SNP-induced relaxation of PE contraction in endothelium-denuded mesenteric arterial rings of control and T rats. Submaximal PE contraction was elicited, ACh or SNP was added, and then the percentage of relaxation to PE contraction was measured. Data points represent mean±SEM of measurements in 16 arterial rings from 6 to 7 rats of each group. *Measurements in T are significantly different ($P<0.05$) from those in control rats.

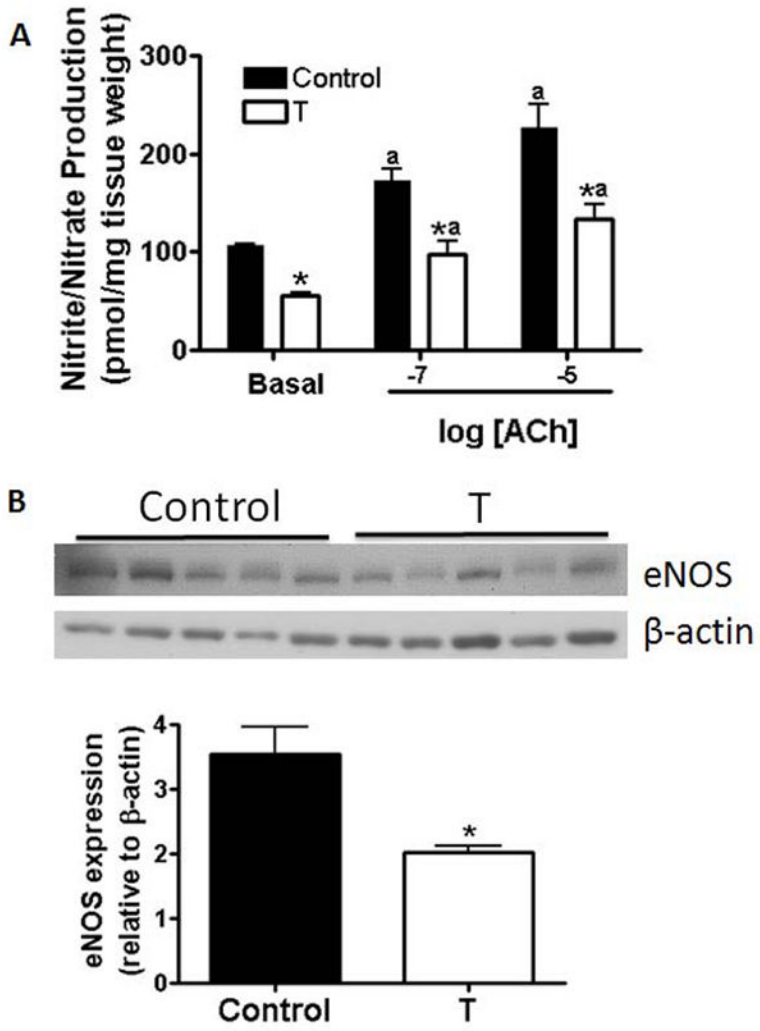

Figure 7.

Nitrate/nitrite production and eNOS expression in control and T offspring. (**A**) Basal and ACh-induced NO_x production in endothelium-intact mesenteric arteries of control and T rats. Data points represent mean±SEM of measurements in mesenteric arterial rings from 5 rats of each group. *p<0.05 compared with respective treatment in control group. ^{a}p<0.05 compared to basal level in respective group. (**B**) Western blot analysis of eNOS using anti eNOS antibody (1:1000; Transduction Laboratory) as normalized to β-actin. Data points represent the mean±SEM of measurements in 5 rats of each group. *Measurements in T rats are significantly different (P<0.05) from in control rats.

Table 1

MAP, Maximal PE (10^{-5} M)-Induced Active Stress, and PE ED_{50} in Mesenteric Arterial rings of Control and T Rats

Variable	Control	T
MAP, mm Hg	93 ± 2.4	108 ± 3.2*
PE, 10^{-5} M, Active stress (mN/mm)		
+endo	6.32 ±0.16	7.77±0.16*
-Endo	7.19 ±0.21†	8.05 ±0.19*
L-NAME	7.34±0.29a	8.10±0.30a
PE ED_{50}, [−log M]		
+Endo	6.12 ±0.07	6.59±0.08*
-Endo	6.39 ±0.05†	6.65 ±0.08
L-NAME	6.48±0.04a	6.69±0.06
ACh (+Endo)		
ED_{50}, [−log M]	7.43 ±0.9	6.48 ±0.13*
E_{max},(10^{-6} M, % Relax)	88.19±3.0	73.71±5.95*
SNP(−Endo)		
ED_{50}, [−log M]	7.22 ±0.15	7.12 ±0.09

Endo indicates endothelium. Relax indicates relaxation

All other abbreviations are as defined in text. Data represent mean±SEM of measurements in 16 arterial rings from 6 to 7 rats of each group.

*Measurements in T are significantly different ($P<0.05$) from control.

†Measurements in −Endo are significantly different ($P<0.05$) from +Endo.

aMeasurements in +L-NAME are significantly different ($P<0.05$) from corresponding +Endo rings