Altered insulin-induced relaxation of aortic rings in a dihydrotestosterone-induced rodent model of polycystic ovary syndrome

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Objective: To clarify the effects of dihydrotestosterone (DHT)–induced polycystic ovary syndrome (PCOS) on the insulin-dependent vasodilatation of the thoracic aorta and the role of vitamin D in a rat model.
Design: Controlled experimental animal study.
Setting: Laboratory.
Animal(s): Thirty adolescent female Wistar rats.
Intervention(s): The PCOS model was induced by 10 weeks of DHT treatment (83 μg/d). One-half of the DHT-treated animals also received vitamin D (120 ng/kg/wk).
Main Outcome Measure(s): The aortic rings of the control, DHT, and DHT plus vitamin D–treated animals were isolated. The insulin-dependent vasodilation of the isolated aortic rings was compared in Krebs-Ringer solution and under blockade of nitric oxide (NO) synthase or cyclooxygenase.
Result(s): The insulin-dependent vasorelaxation decreased in both DHT-treated groups independently from the vitamin D treatment; NO-dependent and -independent relaxations were both impaired. In response to prostanoid, vasoconstriction was increased after DHT treatment. The NO-independent relaxation was partially improved by vitamin D treatment, which was neutralized by increased prostanoid-dependent vasoconstriction.
Conclusion(s): Previously, we found that vitamin D treatment prevented systemic insulin resistance; however, in this study, we did not detect any influence on the vascular insulin resistance of the aorta that was induced by DHT treatment. Consequently, controlling insulin resistance with vitamin D alone did not resolve the aortic endothelial dysfunction caused by the hyperandrogenic state. (Fertil Steril® 2013;99:573–8. ©2013 by American Society for Reproductive Medicine.)
Key Words: Rat, vascular insulin resistance, dihydrotestosterone, vitamin D, PCOS

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Certain metabolic changes, such as hyperinsulinemia, insulin resistance, and metabolic syndrome, may already be present before the onset of polycystic ovary syndrome (PCOS) and may in fact mediate the onset of PCOS (1, 2). Type 2 diabetes mellitus and the risk for cardiovascular diseases develop earlier in the majority of women with PCOS than in the general population (1, 2). In the present study, we examined early functional changes in large blood vessels, most

likely the earliest detectable difference, Manneras et al. developed a reliable experimental model to study PCOS (3). Chronic treatment of adolescent female rats with dihydrotestosterone (DHT) induces a PCOS-like condition, including impaired insulin sensitivity (3, 4).

The insulin resistance that occurs in PCOS can be reversed by insulin sensitizers such as metformin (5). Metformin decreases serum glucose and insulin levels. Furthermore, metformin has beneficial effects on the cardiovascular system. Agarwal et al. have proved that metformin reduces arterial stiffness and improves endothelial function in young women with PCOS (6). Palomba et al. and Naka et al. showed the enhancement of flow-mediated dilatation and of vascular endothelial function by metformin (7, 8). At the same time, vitamin D use has emerged as an adjuvant therapy in PCOS (9). Vitamin D therapy has positive effects on carbohydrate metabolism (10, 11) and has been suggested to prevent cardiovascular complications as well (12, 13). Therefore, we investigated the effects of protective doses of vitamin D in hyperandrogenic female (HAF) rats. A similar chronic vitamin D therapy prevented heart failure and left ventricular hypertrophy in adolescent heart failure–prone spontaneously hypertensive rats (SHR) (13). Previously, we reported that after 70 days of DHT administration, the insulin-induced vasorelaxation decreased in the small arteries of HAF rats (14). This effect was prevented by a concomitant weekly dosage of vitamin D. Because nitric oxide (NO)–dependent relaxation, which deteriorated with chronic DHT treatment, was not influenced by vitamin D, other mechanisms of compensation might be involved. In the present study, we aimed to clarify the effects of DHT on the insulin-dependent vasodilatation of the aortic rings of HAF rats and the possible modulatory role of a protective dose of vitamin D. To confirm these effects, we examined NO-dependent relaxation in the aortic rings as well as the possible role of prostanoids in the compensatory mechanism. In this study, we tested the two essential mechanisms regulating vascular tone: the relaxing capacity under pretreatment with L-N^G-nitroarginine methyl ester hydrochloride (L-NAME, i.e., NO synthase blocker) or indomethacin (i.e., cyclooxygenase blocker). Earlier, we demonstrated the positive effect of vitamin D on systemic insulin resistance (14). The DHT-dependent reduction of insulin-induced vasorelaxation found in small arteries was improved by parallel vitamin D administration (14). We sought to demonstrate a similar phenomenon in large vessels, such as the aorta, in a HAF rat model.

METHODS

Drugs and Chemicals

The composition of the normal Krebs-Ringer (nKR) solution used in the in vitro studies was (in mmol/L) 119 NaCl, 4.7 KCl, 2.5 $CaCl_2 \cdot 2H_2O$, 1.17 $MgSO_4 \cdot 7H_2O$, 20 $NaHCO_3$, 1.18 KH_2PO_4, 0.027 EDTA, and 11 glucose (Sigma Aldrich). The solution was maintained at 37°C and aerated with 5% CO_2 and 95% O_2, which stabilized the pH at 7.4.

Norepinephrine, acetylcholine chloride, 17β-estradiol, L-NAME, and indomethacin were obtained from Sigma-Aldrich. Human recombinant insulin (100 NE/mL Actrapid Penfill) was obtained from Novo Nordisk. The drugs were freshly prepared in nKR solution on the day of the experiment.

Animals

Thirty adolescent 21–28-day-old female Wistar rats weighing 100–140 g were used (Semmelweis University Animal Colony, Budapest, Hungary; originated from Charles River). Experimental polycystic ovary syndrome was achieved as described by Manneras et al. (3), with the use of 90-day continuous-release pellets containing 7.5 mg DHT (Innovative Research of America; daily dose 83 μg). Ten animals underwent sham operations (control group). The rats were anesthetized with pentobarbital (Nembutal; Phylaxia-Sanofi) during surgical interventions. Following chronic surgical interventions, 20 mg amoxicillin + 4 mg clavulanic acid (Augmentin; Glaxo Smith Kline) dissolved in 0.2 mL saline solution was administered intramuscularly to prevent infections. Ten DHT-treated animals received 120 ng/100 g body wt./wk of 1,25$(OH)_2$-vitamin D_3 (DHT+D_3 group). The vitamin D was given subcutaneously as previously described by Przybilski et al. We administered a weekly—for 10 weeks—instead of daily dosage (13, 14) of vitamin D to reduce the stress to the animals. 1,25$(OH)_2$-vitamin D_3 was chosen (injectable Calcijex, 2 μg/mL; Abbott Laboratories) because this is the active form of vitamin D and the action of other forms depends on liver and renal function (13, 14). The control group and ten of the DHT-treated animals received vitamin D vehicle (saline solution) subcutaneously. As described earlier, after 8 weeks of treatment, the oral glucose tolerance test (OGTT) was performed in short ether narcosis to assess glucose homeostasis (fasting and 120-minute blood glucose and plasma insulin levels were measured after the administration of 20 mg glucose/100 g body weight, in gauge) (14). The fasting glucose and insulin levels were similar in all groups. At the 120-minute time point of the OGTT, the insulin level measured in the DHT-treated rats was three times higher than in both the control and the DHT+D_3-treated animals (14). A significant difference between the fasting and the 120-minute insulin levels was found only in the DHT group (14), which developed insulin resistance after DHT treatment (14). Vitamin D treatment prevented insulin elevation (14). The serum fructoseamine level was similar in all groups and within the reference range, indicating that the animals did not have diabetes or elevated blood glucose (14). The ovaries of the animals were collected and immediately fixed for histologic examinations to determine whether polycystic morphology was present as described earlier (14). No medical or surgical complications were observed. Conventional rat chow (S8106-S011 SMR/M-Z+H, with physiologic vitamin D content; Spezialdiäten) and tap water were provided ad libitum. The study conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health and was approved by the Institutional Animal Care Commission (Institutional Review Board approval 22.1/2960/003/2009).

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