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# Intrauterine exposure to metformin: Evaluation of endothelial and perivascular adipose tissue function in abdominal aorta of adult offspring

Camila B. Vidigal[a], Daniella R.B.S. Novi[a], Kawane F. Moura[a], Rafaela Picinin[a], Bruno G. Montagnini[a], Renée de N. O. da Silva[b], Matheus D.V. da Silva[c], Fábio G. de Andrade[c], Eliana H. Akamine[b], Daniela C.C. Gerardin[a], Graziela S. Ceravolo[a,*]

[a] Department of Physiological Sciences, Biological Sciences Center, State University of Londrina, Brazil
[b] Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil
[c] Department of Histology, State University of Londrina, Londrina, Brazil

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ABSTRACT

The biguanide metformin (MET) has been used during pregnancy for treatment of polycystic ovary syndrome and gestational diabetes. MET crosses the placenta and maternal treatment can expose the progeny to this drug during important phases of body development. Direct vascular protective effects have been described with the treatment of metformin. Nevertheless, it is unclear whether intrauterine exposure to metformin is safe for the vascular system of offspring. Thus, the present study aimed to investigate the intrinsic effects of metformin exposure *in utero* in the offspring abdominal aorta reactivity, in the presence and absence of perivascular adipose tissue (PVAT) and endothelium. For this, Wistar rats were treated with metformin 293 mg/kg/day (MET) or water (CTR) by gavage during the gestational period. The abdominal aorta reactivity to phenylephrine, acetylcholine, and sodium nitroprusside was evaluated in male adult offspring. It was observed that abdominal aorta relaxation was similar between MET and CTR groups in the presence or absence of PVAT. In addition, the contraction to phenylephrine was similar between MET and CTR groups in the presence and absence of PVAT and endothelium. Therefore, metformin exposure during pregnancy had no intrinsic effect on the offspring abdominal aorta PVAT and endothelial function, demonstrating it to be safe to the vascular system of the offspring.

## 1. Introduction

Metformin is an anti-hyperglycaemic biguanide that reduces glycemia without stimulating insulin secretion [1,2]. The anti-hyperglycemic effect of metformin is due to reduction in hepatic gluconeogenesis [2] and improvement in glucose uptake in the peripheral tissues [3,4], through activation of the AMP-activated protein kinase (AMPK), a serine/threonine protein kinase involved in the regulation of cellular energy [5,6].

Metformin is applied for the treatment of gestational diabetes mellitus (GDM) and polycystic ovary syndrome (PCOS). The Food and Drug Administration (FDA) consider this drug as category B during gestation, whereby although there are insufficient studies in humans, no teratogenic effects have been demonstrated in animals [7–10]. Metformin freely crosses the placenta and has been detected in the umbilical cord at similar concentrations to maternal blood [1,11,12]. Thus, maternal treatment with metformin can expose the progeny to this drug during important phases of body development.

The aorta is an essential functional unit of the vascular system [13]. There are biomechanical, histological, and functional differences between the regions of the aorta. The thoracic aorta presents greater diameter, higher mechanical strength, and elasticity (greater amount of elastic fibers) than the abdominal aorta [14]. Moreover, along the aorta there are phenotype differences in the perivascular adipose tissue (PVAT) [15,16].

The PVAT has been reported to be composed of both white adipose tissue (WAT) and brown adipose tissue (BAT) with different ratios depending on the location [17]. Abdominal PVAT has been proposed to have characteristics mostly resembling WAT, whereas thoracic PVAT has more characteristics of BAT [18,19]. WAT stores energy, whereas BAT generates heat and maintains body temperature. The thermogenesis in BAT is dependent on adrenergic receptor expression, intracellular lipolysis, and the presence of uncoupling protein 1 (UCP-1) [18]. Interestingly, it has been shown that beige adipocytes reside in

* Corresponding author at: Departamento de Ciências Fisiológicas, CCB-UEL-Campus Universitário, 86051-980 Londrina, PR, Brazil.
*E-mail address:* gsceravolo@uel.br (G.S. Ceravolo).

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**Table 1**
Maternal and offspring parameters.

| | Weight gain variation of dams (g) | Birth weight of pups (g) | Number of animals/litter | Adult male weight (g) |
|---|---|---|---|---|
| CTR | 34.22 ± 2.24 [11] | 1.85 ± 0.02 [11] | 12.45 ± 0.76 [11] | 357.52 ± 8.80 [11] |
| MET | 31.93 ± 3.04 [12] | 1.83 ± 0.02 [12] | 11.75 ± 0.57 [12] | 369.33 ± 12.40 [12] |

Data are means ± SEM. Numbers in brackets represent the number of animals/group. Student-*t*-test, $p > 0.05$. CTR: offspring exposed to water during gestational period; MET: offspring exposed to metformin during gestational period.

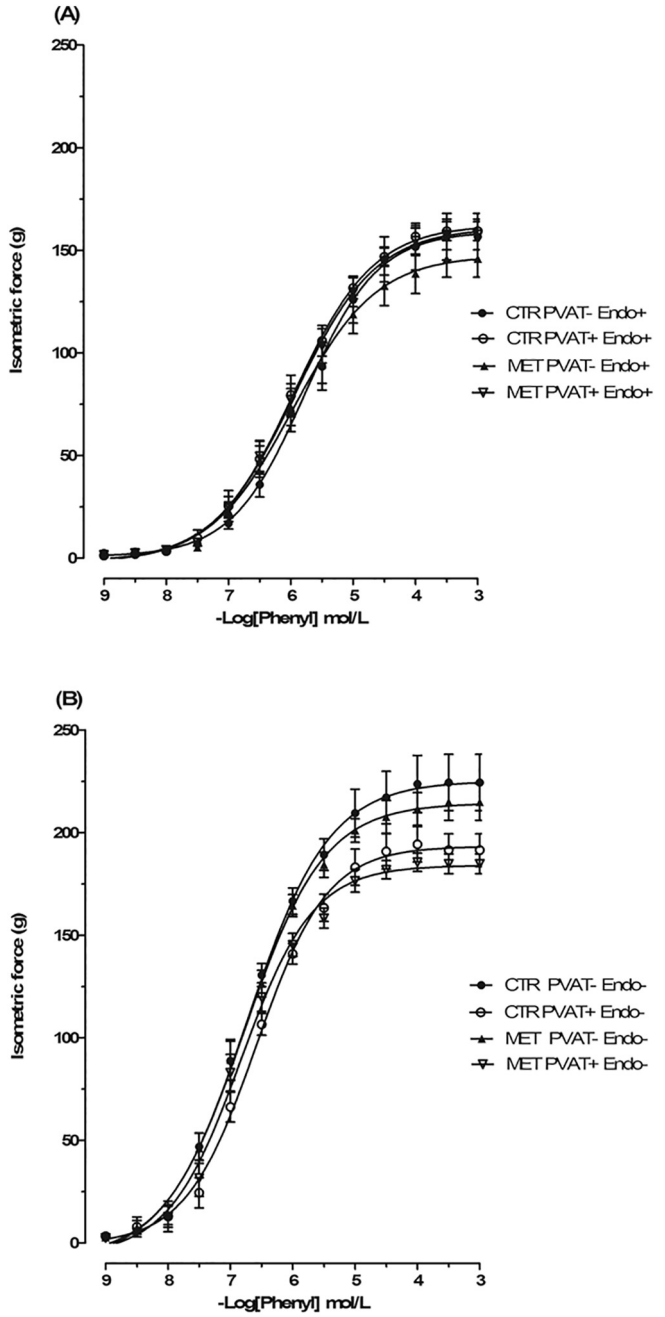


**Fig. 1.** Cumulative concentration-effect curves to phenylephrine (Phenyl) in aortic rings with (Endo+; A) and without (Endo−; B) endothelium, in the presence (PVAT+) and absence (PVAT−) of perivascular adipose tissue isolated from male adult offspring exposed to metformin (MET) or water (CTR) during gestational period. Data are reported as means ± SEM, $n = 9–12$. Two-way ANOVA, $p > 0.05$.

WAT; these adipocytes are brown-like cells with very low basal but highly inducible UCP-1 expression [18,20]. Thus, inherent properties are involved in the susceptibility to injury along the aorta, and aortic atherosclerotic lesion and aneurysm are predominant in the abdominal rather than the thoracic aorta [15].

Metformin was associated with increased nitric oxide (NO) bioavailability and vascular endothelial growth factor up-regulation in a genetic model of spontaneously hypertensive insulin-resistant rats [21]. This biguanide also corrects endothelial dysfunction in high-fat fed diabetic Goto-Kakizaki rats and in non-diabetic obese rats by enhancing vascular NO bioavailability and reducing oxidative stress and inflammation [22]. It has also been demonstrated that in fructose-feeding rats metformin prevented the dysregulation of adipocytokine expression in PVAT [23]. On the other hand, as far as we know, there is no information about the long-term effects of intrauterine exposure to metformin in the PVAT and endothelial function of abdominal aorta of exposed offspring. Considering the beneficial effects of metformin treatment during adulthood, we hypothesized that intrauterine exposure to this drug might be safe for the abdominal aorta reactivity of the offspring. Therefore, the present study aimed to evaluate the intrinsic effects of metformin exposure *in utero* on the abdominal aorta reactivity of offspring in the presence and absence of PVAT and endothelium.

## 2. Material and methods

### 2.1. Animals and treatment

Wistar rats were obtained from the breeding stock of the Center of Biological Sciences of the State University of Londrina. Male ($n = 10$) and female ($n = 23$) naive Wistar rats were mated (two females and one male per cage) overnight. The gestational day 0 (GD0) was determined if in the morning of the following day, estrus phase cells and sperm were found in vaginal smears. Dams were individually housed and randomly divided into two groups (11–12 dams/group): Control (CTR), daily gavage with tap water (the vehicle used for metformin) from GD0 to GD21; Metformin (MET), daily gavage with 293 mg/kg metformin (Glifage ®, MERCK S.A Laboratory, Brazil) from GD0 to GD21.

The recommended dose of metformin during pregnancy is 500 mg/day [24,25], equivalent to 7.14 mg/kg/day for a 70 kg adult. Applying the $BW^{3/4}$ scaling [26], the value of 29.26 mg/kg/day was obtained and considering the interspecies variation, a rat's metabolism is ten times faster than a human's, the dose of 293 mg/kg/day was chosen [27]. Dam weights were obtained every three days throughout pregnancy for dose adjustment and described as weight gain variation ($\Delta$ = PND1 − GD0). The day of birth was denominated post-natal day (PND) 0. On PND 1, pups were identified by sex, and on PND 4 the litters were culled to ten pups (six males and four females). Pups were weighed on PNDs 1, 4, 7, 14, and 21 when they were weaned and housed in groups (5 male animals per cage).

Male offspring were evaluated on PNDs 75–80, (9–11 rats in the control group and 10–12 rats in the metformin group). Different littermates from each litter were used for each evaluation conducted. All animals had free access to water and regular laboratory chow (Nuvital, Curitiba, Brazil). The animals were maintained at 21 ± 2 °C on a 12:12 h light–dark cycle (lights on at 06:00 AM). All experimental