OBSTETRICS

Vascular and metabolic profiles in offspring born to pregnant mice with metabolic syndrome treated with inositols

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BACKGROUND: Inositols (INOs) supplementation during pregnancy, specifically the combination of myo-inositol (MI) and D-chiro-inositol (DCI), has been reported to improve vascular parameters in women with gestational diabetes mellitus. We demonstrated previously that offspring born to pregnant mice lacking the endothelial nitric oxide synthase (*eNOS*+/−) gene have hypertension (HTN) as adults and, when fed a high-fat diet (HFD), develop a metabolic syndrome (MS) phenotype.

OBJECTIVE: Our aim was to evaluate whether INOs treatment in pregnancy complicated by MS improves the vascular and metabolic profile in mice offspring programmed in utero to develop HTN and MS.

MATERIALS AND METHODS: Heterozygous eNOS+/− mice fed an HFD manifest a MS phenotype. Female eNOS+/− mice with MS were bred with a wild-type (WT) male. On gestational day 1, pregnant females were randomly allocated to receive either a mixture of INOs (MI/DCI: 7.2/0.18 mg/mL) or water as placebo until delivery. The female offspring obtained were genotyped and categorized as: WT (genetically normal, with *eNOS* gene) and eNOS+/− offspring (genetically modified, heterozygous for *eNOS* gene). Both offspring developed in an abnormal uterine environment due to maternal MS. At 9−10 weeks of age, the offspring underwent a glucose tolerance test (GTT) and systolic blood pressure (SBP) measurement. The mice were then sacrificed, and the carotid arteries were isolated for evaluation of vascular responses. Responses to phenylephrine (PE), in the presence and absence of a nonspecific nitric oxide inhibitor (*N*-nitro-L-arginine methyl ester [L-NAME]), the vasodilator acetylcholine (ACh), and sodium nitroprusside (SNP) were assessed.

RESULTS: The GTT showed lower glucose levels in both eNOS+/−INOs ($P = .03$) and WT-INOs ($P = .05$) offspring born to MS dams on INOs supplementation compared to offspring born to untreated dams. SBP was higher in eNOS+/− offspring compared to WT (169 ± 7 vs 142 ± 9 mm Hg, respectively, $P = .04$) and INOs treatment decreased SBP in WT-INOs (110 ± 10 mm Hg, $P = .01$) but not in eNOS+/−INOs offspring. Maximal (%Max) contractile response to PE was higher in eNOS+/− offspring born to MS dams and was decreased in those born to MS dams treated with INOs (%Max, eNOS+/−, 123 ± 7 vs eNOS+/−INOs, 82 ± 11 mm Hg, $P = .007$). No differences were seen in PE contractile responses in WT offspring born to MS dams treated or not treated with INOs (WT, 92 ± 4 vs WT-INOs, 75 ± 7). The L-NAME response was decreased in eNOS+/−INOs and WT-INOs offspring compared to untreated ones. The ACh vasorelaxation was impaired in eNOS+/− and WT offspring born to MS dams, and maternal INOs treatment improved offspring vascular relaxation in both offspring ($P = .01$ and $P = .03$, respectively). No differences were seen in response to SNP.

CONCLUSION: Inositols supplementation improved glucose tolerance, SBP, and vascular responses in adult eNOS+/− and WT offspring born to dams with MS. Interestingly, WT born to MS dams show an altered vascular profile similar to eNOS+/− offspring and exhibit an improved response to INOs treatment. Our findings suggest that the benefits of INOs treatment are more pronounced in offspring exposed to environmental factors in utero, and less likely in those due to genetic factors.

Key words: blood pressure, inositol, insulin resistance, metabolic syndrome, obesity, pregnancy

Because of changes in lifestyle across different sectors of the population worldwide, the incidence of chronic cardiometabolic diseases has been constantly rising within the past years. Metabolic syndrome (MS) represents a major health problem, and is defined by the National Institutes of Health as a condition with at least 3 of the following: hypertension, elevated fasting plasma glucose, central obesity, elevated plasma triglycerides, and/or low high-density lipoprotein.[1−3] Several studies have shown clear evidence that genetic−environmental interactions play an important role in the manifestation of the adult disease phenotype.[4] It is well recognized that the fetus develops adaptations in response to the maternal and uterine environments, and that the exposure of the developing fetus to insults during critical periods of development can permanently reprogram normal fetal physiologic responses that have long-term consequences in adult life.[5,6]

Supporting these premises, several epidemiologic studies have shown the association between perturbation of fetal environment in utero and onset of cardiovascular disease, diabetes, and MS in adult life.[7−10] This has been called "the developmental origins of adult disease" by Barker.[5,6] Thus, the maternal contribution to the fetal development of disease in later life can be either through transmission of maternal genetic factors and/or development of the fetus in an abnormal uterine environment, which might exist in pregnancies complicated by diabetes, MS, small or large for gestational age fetus, hypertension, preeclampsia, hypoxia, oxidative stress, and other conditions.[11−13]

Unfortunately, the molecular mechanisms underlying fetal developmental

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AJOG at a Glance:

Why was this study conducted?

- Inositols (INOs) are a family of naturally carbohydrates found in common foods.
- Several INOs, in particular myo-inositol (MI) and D-chiro-inositol (DCI), have insulin-like metabolic effects and have already been shown clinically to delay onset of gestational diabetes in pregnancy.
- We conducted this murine study to evaluate the effects of maternal INOs supplementation on vascular and metabolic programming of offspring born to pregnant dams with metabolic syndrome induced by diet, in the presence or absence of a genetic predisposition.

Key findings

- INOs supplementation to pregnant dams with metabolic syndrome improved vascular and metabolic function in the offspring.
- The beneficial effect of maternal INOs on the offspring was more evident when metabolic syndrome was induced in the absence of a genetic predisposition.

What does this add to what is known?

- INOs supplementation of pregnant dams with metabolic syndrome prevents the long term metabolic dysfunction in the offspring later in life.

programming remain largely unknown, and are likely due to interactions between genetics and the intrauterine environment that can influence individual risk for later-life chronic disease.[14,15]

There are several known animal models to induce metabolic abnormalities. Many researchers use a straightforward high-fat diet (HFD) to induce obesity; however, some use additional stressors to model human diabetes. Furthermore, insulin tolerance progressively worsen with time; wild-type (WT) mice (C57Bl/6J) on an HFD have shown hyperinsulinemia after 11 weeks only. Thus, we have chosen heterozygous mice lacking the endothelial nitric oxide synthase (*eNOS*) gene to boost the disease severity and to model a more progressed stage of metabolic disease using the shortest time for an HFD to cause metabolic abnormalities.[16,17] We reported previously that the MS phenotype develops in heterozygous eNOS+/−mice when fed a high-fat diet as evidenced by increased body weight, higher blood pressure, elevated insulin and glucose, decreased adiponectin, and high-density lipoprotein (HDL) levels.[18] This transgenic mouse model of MS combines genetic factors and an abnormal uterine environment, featuring decreased nitric oxide (NO) production leading to preexisting maternal hypertension due to the lack of the *eNOS* gene and in utero environmental manipulation using HFD producing the MS syndrome phenotype.[18−20]

Epidemiologic and animal studies have shown that pregnancies complicated by MS and obesity confer risk of premature cardiovascular (CV) diseases, gestational diabetes and preeclampsia, and predispose the offspring to an increased risk of CV and metabolic disease later in life.[21−23] Therefore, understanding the contribution of the genetic versus intrauterine environment factors in relation to long-term health consequences in adult offspring is imperative and can result in developing effective interventions to help mitigate the current rise in obesity and CV and metabolic diseases.

Inositols (INOs) are a family of natural carbohydrates found in common foods. Several INOs, in particular myo-inositol (MI) and D-chiro-inositol (DCI), already have demonstrated insulin-like metabolic effects in diabetic animals, primates, and humans, by decreasing hyperglycemia and hyperlipidemia, which are key components in endothelial dysfunction.[24−29]

Earlier, we showed that INOs (MI/DCI) supplementation in pregnant mice with the MS phenotype improved maternal blood pressure, glucose tolerance and leptin levels.[30] We also demonstrated that the abnormal intrauterine environment caused by maternal MS leads to altered fetal metabolic programming in the offspring, which is worse depending on the genotype.[18] However, the effect of INOs supplementation on the prevention of CV and metabolic disease in adult offspring born to pregnancy complicated by MS is largely unknown, and is the central point of this study.

Our hypothesis is that INOs supplementation during pregnancy will improve vascular and metabolic profiles in adult offspring born to pregnant mice with the MS phenotype. To test this hypothesis, we used a well-characterized murine model of MS, a heterozygous eNOS+/− mouse fed an HFD.[18] This model was used to generate the offspring in this study to understand further the gene−environment relationship, and specifically the relative contribution of a hostile intrauterine environment (MS) and the fetal genotype on the fetal vascular and metabolic programing impact on adult long-term health.

Materials and Methods

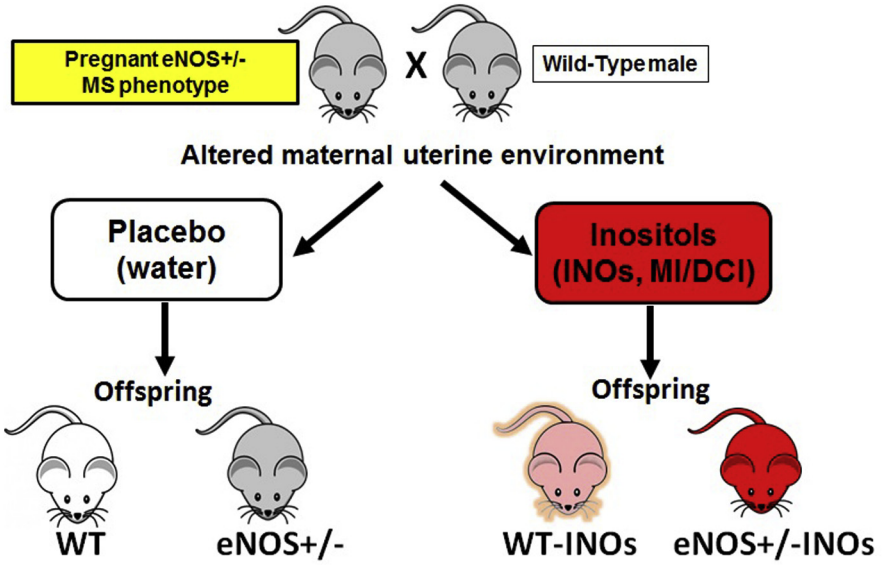
Animals

Female mice homozygous for disruption of the *eNOS* gene (eNOS-KO$^{-/-}$, strain B6.129P2, stock no. 002684) and their age-matched male WT controls (strain C57BL/6J, stock no. 000664) were purchased from Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. The study was approved by the Animal Welfare Committee (AWC-16-0166) of the University of Texas Health Science Center at Houston (UTHSC-H). The mice were housed separately in temperature- and humidity-controlled quarters with constant 12:12-hour light−dark cycles in the animal care facility at the UTHSC-H.

Metabolic syndrome mouse model

To generate the MS mouse model, females homozygous eNOS−/− mice were bred with WT males to obtained heterozygous females eNOS+/−, which were fed an obesogenic HFD (D12492, 60% of fat) for 4 consecutive weeks to induce the MS phenotype as shown previously by

FIGURE 1
Scheme of female WT and eNOS +/− offspring born to dams with MS treated with and without INOs mixture



Female eNOSD+/− heterozygous with MS phenotype were bred with WT males. MS dams were allocated to receive water as placebo (control) or Inositols (INOs, red). Offspring were genotyped and divided: non-treated WT (n = 8) and eNOSD+/− heterozygous (n = 8); INOs treated, WT-INOs (n = 10) and eNOSD+/−INOs offspring (n = 8).

eNOS, endothelial nitric oxide synthase; *INOs,* inositols; *MS,* metabolic syndrome; *WT,* wild-type.

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our group. At 7−8 weeks of age, they were bred with WT males.[18,30] We obtained 5−6 pregnant eNOS+/− heterozygous dams and at gestational day (GD) 1 of pregnancy, the MS dams were randomly allocated to receive either a mixture of INOs (MI/DCI, 7.2/0.18 mg/mL, respectively) dissolved in water, or alternatively plain water as placebo (control group).[30,31] The HFD was maintained during the whole pregnancy and offspring weaning period. After offspring birth, on postnatal day 2, the litter size was reduced to 6 if necessary to ensure an adequate and standardized supply of milk to all pups. Then 2−3 female offspring after genotype were used from each litter from the different dams to achieve the final number of offspring.

Female offspring genotype

Offspring obtained were either heterozygous eNOS+/− or WT. They were maintained on a regular diet until 9−10 weeks of age, the time of sacrifice. Those offspring had a different fetal genotype but were born to the same dams, eNOS+/− heterozygous on HFD, with an altered uterine environment due to MS and receiving either the INOs mixture or placebo. Female offspring were genotyped at weaning (3 weeks of age) and divided into the following groups: non-treated WT offspring, with *eNOS* gene (WT, n = 8); eNOS+/− heterozygous, lacking one *eNOS* gene (eNOS+/−, n = 8); INOs-treated WT offspring, WT-INOs (n = 10); and eNOS+/−INOs offspring (n = 8) (Figure 1). At 9−10 weeks of age, mice were weighed, and after the glucose tolerance test (GTT) and systolic blood pressure (SBP) assessments, they were sacrificed for the vascular reactivity experiments.

In vivo experiments

Glucose tolerance test

After 6 hours of fasting, at 9 weeks of age, female offspring mice received 1.0 g/kg glucose intraperitoneally (I.P). for the glucose tolerance test (GTT). Plasma glucose levels were determined with the Accu-Chek Aviva Blood Glucose Meter System (Roche Diagnostics, Indianapolis, IN) via a tail nick at 0, 15, 30, 60, and 120 minutes after glucose administration.

Systolic blood pressure

At 10 weeks of age, systolic blood pressure (SBP) was measured using the noninvasive CODA tail cuff system (Kent Scientific Corp., Torrington, CT). The animals were placed in a nose cone holder and on a warming plate (37°C). A tail cuff and a pneumatic pulse transducer were applied at the tail base. The tail cuff was programmed to insufflate to a maximal pressure of 250 mm Hg. A rest period of 15 seconds was allowed between cycles. The mice underwent 10 acclimation cycles, followed by 20 cycles for data collection.[32,33]

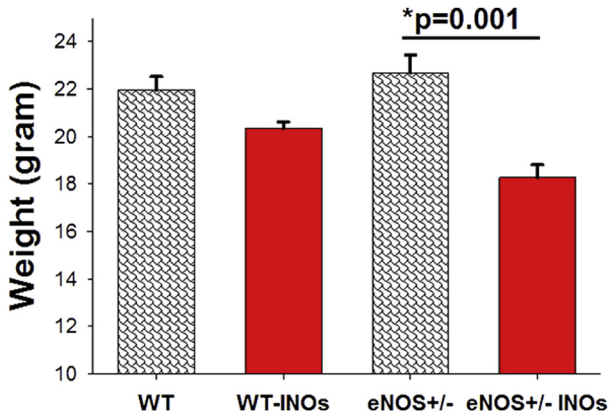
In vitro experiments

Vascular reactivity

At 10 weeks of age, the offspring were sacrificed and the carotid arteries were dissected. Two mm segments (2−4 segments per animal), were mounted on a wire-myograph system (model 410A, Danish Myo Technology, Aarhus, Denmark) using 25-μm tungsten wires. Arteries were bathed in Krebs solution, maintained at 37°C with a pH of ~7.4 and bubbled continuously with a mixture of 95% O_2 and 5% CO_2. The force was recorded by an isometric force transducer and analyzed with PowerLab data acquisition (ADInstruments, Castle Hill, NSW, Australia).

After stabilization of the vascular tone, the arteries were contracted twice with 60 mmol/L KCl for 30 minutes to stabilize vascular responsiveness. The second KCL contraction was used as a reference in the final calculations. After 1 hour of equilibration, contractile responses to cumulative concentrations of the α_1-adrenergic agonist phenylephrine (PE, 10^{-10}−10^{-5} mol/L) were assessed in the presence and absence of a nonselective nitric oxide synthase inhibitor (*N*-nitro-L-arginine methyl ester [L-NAME], 10^{-4} mol/L). Then, relaxant responses to the endothelium-dependent acetylcholine (Ach; 10^{-10}−10^{-5} mol/L) and the endothelium-independent sodium nitroprusside (SNP, 10^{-10}−10^{-5} mol/L)

FIGURE 2
Average weight in female offspring WT and eNOS+/− born to dams with MS with and without INOs mixture



At 10 weeks of age, weight gain was improved by INOs maternal supplementation in eNOSD+/−INOs, but not WT-INOs offspring. Data are shown as mean ± standard error of the mean. Significance is indicated on the figure (*$P = .001$).

eNOS, endothelial nitric oxide synthase; *INOs,* inositols; *MS,* metabolic syndrome; *WT,* wild-type.

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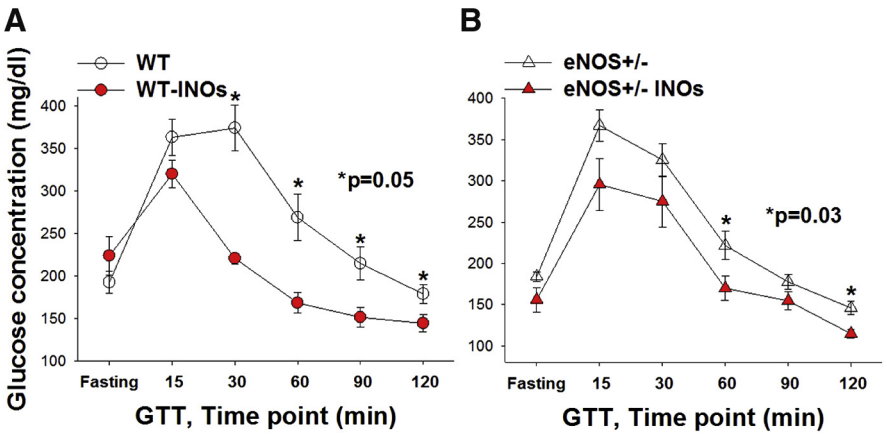
were evaluated after vascular precontraction with PE.

Drugs and solutions

The composition of Krebs solution was as follows: NaCl, 119 mmol/L, KCl, 4.7 mmol/L, NaH2PO4 1.2 mmol/L, NaHCO3 25 mmol/L, MgCl2 1.2 mmol/L, CaCl2 2.5 mmol/L, ethylenediaminetetraacetic acid (EDTA) 0.026 mmol/L, and glucose 11.5 mmol/L. All the components were purchasd from Sigma Chemical Company (St. Louis, MO) as well the Myo-inositol and D-chiro-inositol, PE, L-NAME, Ach, and SNP.

FIGURE 3
GTT in female offspring WT and eNOS+/− born to dams with MS treated with and without INOs mixture



A, Glucose levels (mg/dL) were lower in WTINOs offspring born to MS dams on INOs compared to WT offspring born to untreated dams (*$P = .05$). **B**, Levels were also lower in eNOSD+/−INOs offspring (*$P = .03$). Data are shown as mean ± standard error of the mean. Significance is indicated on the figure.

eNOS, endothelial nitric oxide synthase; *GTT,* glucose tolerance test; *INOs,* inositols; *MS,* metabolic syndrome.

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Statistical analysis

Sigma Plot 12 was used to analyze all the data: offspring weights, glucose levels at fasting and at each time point of the GTT, SBP, and vascular reactivity. Statistical tests were designed to compare the effects of genotype (WT vs eNOS+/−), the effects of INOs supplementation (maternal treatment vs no treatment), via 3-way analysis of variance. After interaction was verified, comparisons between groups were done using either the *t* test or 1-way analysis of variance followed by the Neuman−Keuls multiple comparisons test. In addition, for the vascular reactivity, the percent maximal effect (% Max) of the dose−response curve to each agent was calculated. The data are reported as mean ± standard error of the mean. A 2-tailed *P* value of $<.05$ was considered statistically significant.

Results

Food and water intake

Daily food intake was similar between eNOS+/− and WT offspring born to MS dams treated with INOs mixture or placebo (eNOS+/−INOs, 8.1 ± 0.8 vs placebo, 8.6 ± 0.6 g) and (WT-INOs, 8.2 ± 0.5 vs placebo, 8.1 ± 0.9 g). During pregnancy, daily water intake was not different between MS pregnant dams receiving INOs supplementation or placebo (INOs, 5.0 ± 0.8 vs placebo, 5.1 ± 0.9 mL),

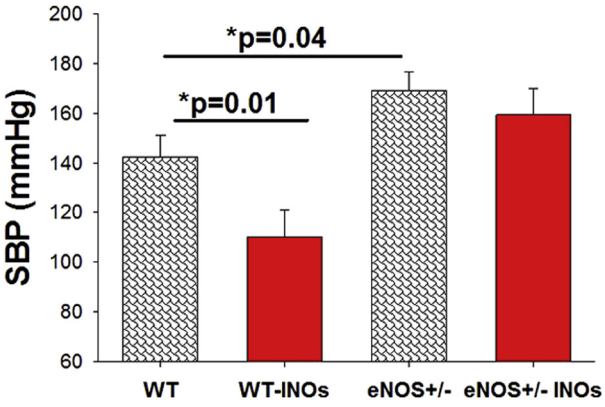
Offspring weight

Maternal INOs treatment did not significantly decrease weight gain in WT offspring (WT, 21.9 ± 0.5 g vs WT-INOs, 20.3 ± 0.2 g, $P = .07$). However, maternal INOs supplementation decreased the weight gain in eNOS+/− heterozygous offspring born to MS dams (eNOS+/−, 22.6 ± 0.8 g vs eNOS+/−INOs, 18.2 ± 0.5 g, $P = .0001$) (Figure 2).

Offspring glucose tolerance test

The GTT showed that glucose levels at 60, 90, and 120 minutes were lower in the WT-INOs vs WT offspring born to

FIGURE 4
Average SBP (mm Hg) in female offspring WT and eNOS+/− born to dams with MS treated with and without INOs mixture



SBP was higher in eNOSD+/− offspring compared to WT born to MS dams (*P = .04). Maternal treatment with INOs lowered SBP in WT-INOs offspring, but not in eNOSD+/−INOs offspring (*P = .01). Data are shown as mean ± standard error of the mean.

eNOS, endothelial nitric oxide synthase; *INOs,* inositols; *MS,* metabolic syndrome; *SBP,* systolic blood pressure; *WT,* wild-type.

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untreated dams with MS (P = .05) (Figure 3A). Similarly, lower glucose levels were noted at 60 and 120 minutes in the eNOS+/−INOs offspring vs eNOS+/− offspring born to untreated dams (P = .03) (Figure 3B).

Offspring systolic blood pressure

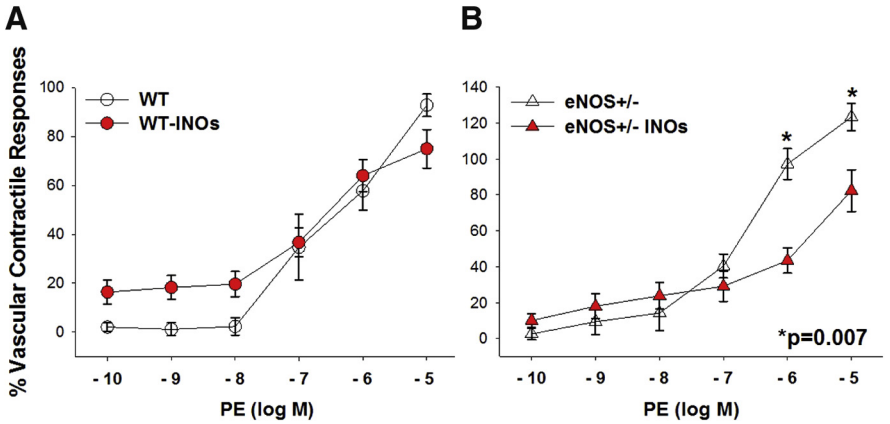
The average SBP was lower in WT versus eNOS+/− offspring born to dams with MS (WT, 142.34 ± 8.79 mm Hg vs eNOS+/− 169.05 ± 7.5 mm Hg, P = .04). Maternal treatment of MS with INOs decreased SBP in WT-INOs offspring (110.15 ± 10.8 mm Hg, P = .01) but not in eNOS+/−INOs offspring (159.24 ± 10.7 mm Hg) (Figure 4).

Offspring vascular reactivity

The dose−response curve to PE was similar in WT offspring carotid arteries independent of INOs maternal supplementation (Figure 5A, Table 1). The vascular contractile responses to PE were decreased in eNOS+/−INOs compared to eNOS+/− offspring born to untreated MS dams (P = .007) (Figure 5B, Table 1). After incubation of the carotid arteries with L-NAME, a nonspecific NO synthase inhibitor, the contractile responses to PE were decreased in WT-INOs and eNOS+/−INOs heterozygous offspring born to MS dams treated with INOs compared to offspring born to untreated dams (P = .03, P = .01, respectively) (Figure 6A and 6B, Table 1). This result suggested that the INOs mixture works on mechanisms different from the NO pathway in regulating vascular responses.

The dose−response curve to the vasorelaxant ACh demonstrated that carotid artery vasorelaxation was altered in WT mice born to MS untreated dams and was improved in the WT-INOs offspring (P = .03) (Figure 7A, Table 1). Similarly, ACh vasorelaxation, as expected, was abolished in eNOS+/− offspring and was improved in the eNOS+/−INOs offspring born to MS dams on INOs supplementation (P = .01) (Figure 7B, Table 1). No changes were observed in any offspring group for any genotyped considered in response to SNP (Table 1).

FIGURE 5
Effect of PE on the contractile responses in carotid artery of female offspring WT and eNOS+/− born to dams with MS treated with and without INOs



A, No contractile differences were seen between WT offspring born to either INOs-treated or untreated dams with MS (WT and WT-INO). **B**, PE contraction was lower in eNOSD+/−INOs offspring born to INOs treated dams vs untreated ones (*P = .007). Data are shown as mean ± standard error of the mean. Significance is indicated in the figure.

eNOS, endothelial nitric oxide synthase; *INOs,* inositols; *MS,* metabolic syndrome; *PE,* phenylephrine; *WT,* wild-type.

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Comment

Our results demonstrated that at 10 weeks of age, eNOS+/− offspring compared to WT, both born to dams with MS, showed higher weight gain, higher glucose levels in response to GTT (lower glucose tolerance), higher SBP, and altered contractile as well as vasorelaxant responses. Maternal treatment of MS dams with the INOs mixture improved eNOS+/−INOs offspring weight, glucose tolerance, and vascular reactivity, and the WT-INOs offspring displayed better glucose tolerance, lower SBP, and improved responses to

TABLE 1
Percent maximal effect in response to vascular contractile and relaxant agents

Drugs	WT	WT-INO	eNOS+/−	eNOS+/−INOs
PE (10^{-10}–10^{-5})	92.8 ± 4.6	75 ± 7.8	123.4 ± 7.5	82.4 ± 7.5*
L-NAME (10^{-4})	203.7 ± 13.7	157.8 ± 17.7#	217.4 ± 12.2	160.5 ± 21.4*
Ach (10^{-10}–10^{-5})	77.4 ± 8.1	98.2 ± 7.6#	49 ± 6.3	66.8 ± 4.9*
SNP (10^{-10}–10^{-5})	112.1 ± 4.7	103.5 ± 2.9	108.5 ± 7.8	112.9 ± 6.6

Data are shown as mean ± SEM. % Max PE contraction was lower in eNOS+/−INOs compared to control eNOS+/− (*$P = .007$); L-NAME increased % Max PE response, and INOs maternal treatment decrease it in eNOS+/−INOs (*$P = .03$) and WT-INOs ($P = .01$). ACh vasodilation was impaired in eNOS+/− offspring, and WT and was improved in WT-INOs ($P = .03$) and eNOS+/− INOs ($P = .03$) offspring born to dams with MS treated with INOs supplementation. No differences were seen in SNP responses. *ACh,* acetylcholine; *eNOS,* endothelial nitric oxide synthase; *INOs,* inositols; *L-NAME,* *N*-nitro-L-arginine methyl ester; *PE,* phenylephrine; *SNP,* sodium nitroprusside; *WT,* wild-type.

* Refers to eNOS+/−; # Refers to WT.

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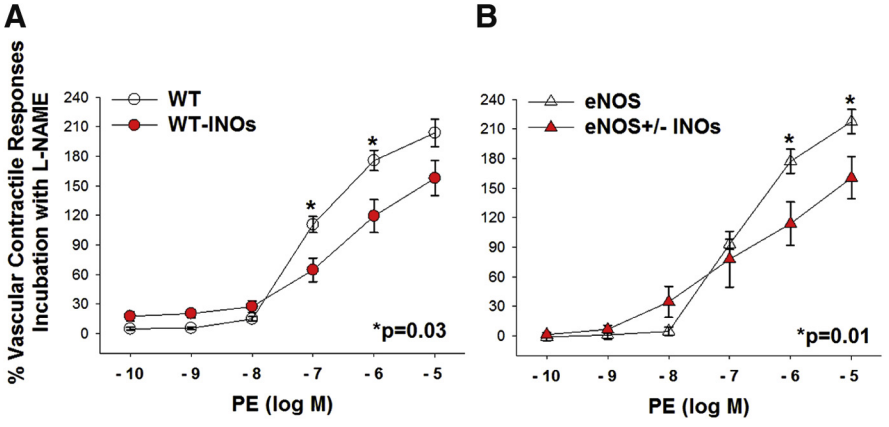
contractile and vasorelaxant agents (Table 2).

INOs supplementation decreased the weight gain in the eNOS+/−INOs offspring but not in the WT-INOs mice. The eNOS+/− dams, which exhibited MS during pregnancy, had an altered uterine environment due to genetically impaired NO production and an HFD. In metabolic disorders, NO synthesis and stability are reduced.[34,35] Hence, NO is a key regulator of vascular and metabolic homeostasis. In the eNOS+/− heterozygous offspring compared to WT, NO production is lower, which can lead to further metabolic imbalance, resulting in a greater weight gain in adulthood without differences in their food consumption. The decrease in NO production leads to increased expression of proinflammatory cytokines and macrophages recruitment in adipose tissue,[36,37] and INOs supplementation during pregnancy seems to prevent those derangements, avoiding the increase in weight gain in eNOS+/− heterozygous offspring.

The glucose responses in the GTT were altered, being higher in WT offspring, similar to the eNOS+/− heterozygous offspring. This demonstrates that the insulin resistance seen in the eNOS+/− offspring also is present in WT when developing in an abnormal uterine environment as in dams with MS. An HFD has been shown to impair NO production, and to induce insulin resistance by altering the glucose transport pathway.[38–40] Thus, even the normal fetus with normal NO levels, developing in MS dams, might have altered NO production, leading to increased susceptibility to inflammation and altered insulin signaling in the adipose tissue and glucose homeostasis, which seems to be partially restored by INOs mixture supplementation during pregnancy.[37,41] However, INOs treatment ameliorates glucose tolerance in the WT-INOs offspring, but not as much in the eNOS+/−INOs offspring. NO also is known to increase glucose transport, in part by increasing the cell membrane fraction of Glut 4, the active transporter of glucose.[39,40] In eNOS knockout mice, studies have shown that lower NO levels lead to decreased insulin-stimulated glucose uptake.[38] This observation theorized that the eNOS+/− offspring genotype contribute to the altered glucose responses, which cannot completely be re-established in the eNOS+/−INOs offspring in the presence of lower level of NO production due to partial lack of the *eNOS* gene.

FIGURE 6
Effect of L-NAME on PE contraction in carotid artery of female offspring WT and eNOS+/− born to dams with MS treated with and without INOs



PE response in the presence of the nonspecific NO synthase inhibitor L-NAME (10^{-4}). **A**, WT-INOs had lower contractile response to PE compared to WT (*$P = .03$) offspring. **B**, Contractile PE response was lower in eNOSD+/−INOs offspring compared to eNOSD+/− offspring born to untreated dams (*$P = .01$). Data are shown as mean ± standard error of the mean. Significance is indicated in the figure.

eNOS, endothelial nitric oxide synthase; INOs, inositols; *L-NAME,* *N*-nitro-L-arginine methyl ester; *MS,* metabolic syndrome; *PE,* phenylephrine; *WT,* wild-type.

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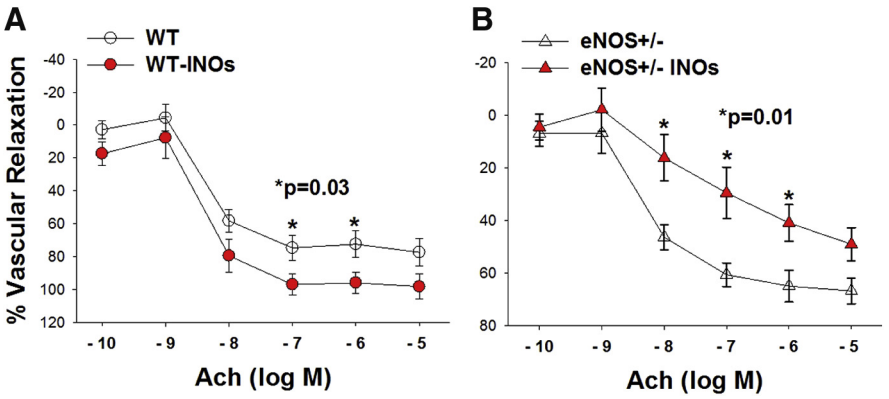
Vascular function

SBP was elevated in eNOS+/− and WT offspring born to untreated MS dams compared to those born to INOs-treated dams. INOs supplementation lowered SBP in WT-INOs, but not in eNOS+/−INOs offspring.

FIGURE 7
Effect of ACh on the carotid artery of female offspring WT and eNOS+/− born to dams with MS treated with and without INOs



A, ACh vasodilatatory response was altered in WT offspring born to MS dams and was re-established in WT-INOs offspring born to treated dams (*$P = .03$). **B**, ACh vasorelaxation was impaired in eNOSD+/− offspring born to MS dams and was restored in eNOSD+/−INOs offspring born to treated dams (*$P = .01$). Data are shown as mean ± standard error of the mean. Significance is indicated in the figure.

ACh, acetylcholine; *eNOS,* endothelial nitric oxide synthase; *INOs,* inositols; *MS,* metabolic syndrome; *WT,* wild-type.

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These data confirm that the hostile intrauterine environment (maternal MS) can alter fetal vascular programming regardless of offspring genotype (WT and eNOS+/−). Oxidative damage has been proved to have a pivotal role in the development of diabetic complications.[42,43] INOs improved SBP in WT-INOs, probably by decreasing radical oxidative species, enhancing endothelial NOS and NO bioactivity.[44,45] The INOs effect became negligible in the eNOS+/−INOs offspring due to the combination of the following: (1) eNOS deficiency, leading to lower basal levels of NO production compared to WT; and 2) an altered uterine environment due to maternal MS, causing damage in endothelial function, hyperinsulinemia, and further impairment in vascular NO synthesis.[46] Vascular contractile responses to PE were higher in eNOS+/− offspring, and treatment with INOs decreased the contractile effect similar to that in WT offspring. It is known that lack of eNOS can lead to increased vascular responsiveness to adrenergic agonist.[47] The eNOS inhibitor L-NAME induced even higher PE contraction in both offspring, which was decreased by maternal treatment with INOs, and clearly this effect was independent of the NO pathway. A reduction in eNOS activity is associated with an increased susceptibility to fat-induced changes in gene expression that promote adipogenesis.[47,48] Namely, adiponectin, is an adipose tissue−specific protein that has been shown to improve insulin sensitivity and to exert anti-atherogenic effects by increasing NO production and preventing NO degradation by reducing superoxide anion production by endothelial cells.[49−51] In support of adiponectin vasoprotective properties, studies have shown that adiponectin-deficient mice display impaired endothelium-dependent vasodilation.[48] Thus, maternal MS seems to increase offspring susceptibility to changes in gene expression that alter adipogenesis and glucose homeostasis, which is worsened by the offspring genotype lacking in the *eNOS* gene.

Our findings suggested that the beneficial effects of INOs supplementation to dams with MS have been seen when testing the vascular function in response to ACh. Vascular relaxation in response to ACh was decreased in WT and eNOS+/− offspring to MS dams, and this effect was ameliorated by maternal INOs supplementation. These data suggest that INOs enhances offspring tissue sensitivity through NO-independent pathways.[52,53] The relaxation to SNP, an endothelium-independent agonist, was not affected by maternal MS or by INOs treatment and offspring genotype. This was expected, as SNP is a NO donor, and the maximal relaxation of the arteries had already been achieved.

In MS, there is an increase in free radicals, which contributes to enhanced basal vascular responses, macrophage infiltration, and impaired endothelium-dependent relaxation.[42,43,54,55] Our findings suggested that maternal INOs treatment improved offspring endothelial function by reducing free radical levels in endothelial cells.

TABLE 2
Results summary

	WT-INOs	eNOS+/−INOs
Weight	↔	↓
Glucose levels (GTT)	↓	↓
Systolic blood pressure	↓	↔
Vascular contraction	↔	↓
Vascular relaxation	↑	↑

Offspring WT-INOs as well eNOS+/−INOs born to dams with MS treated during pregnancy with INOs supplementation showed improvement in several parameter of MS. WT-INOs, displayed lower glucose level and SBP and improved response to vasorelaxant agent. The eNOS+/−INOs offspring showed improved in weight, lower glucose level, and parameters of vascular function.

eNOS, endothelial nitric oxide synthase; *GTT,* glucose tolerance test; *INOs,* inositols; *WT,* wild-type.

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Study strengths are as follows. First, to the best of our knowledge, this is the first report evaluating the metabolic long-term effect of maternal inositols supplementation on offspring born to MS dams. Second, this model allows evaluation of the intrauterine environment vs the solely genetic phenotype. The main weaknesses of our study are the following. First, we did not investigate the various pathways (NO, inositol mediators, reactive oxygen species) that might be involved in the improved metabolic programming seen in adult offspring. Second, it is clear that the timing and length of maternal diet intervention differentially affect the offspring phenotype, and changing the HFD to a normal diet in the offspring weaning period could positively affect the offspring metabolic programming. Third, as we know, there are sex-specific effects of programming; however, the study was limited to female offspring only. We acknowledge that different programming effects may be present in males, and we would seek to include animals of both sexes in any future study.

In conclusion, a hostile maternal uterine environment (maternal MS) can alter fetal vascular and metabolic programming, and this effect is genotype dependent. In this murine model of MS, maternal INOs treatment improved the altered fetal vascular−metabolic programming in normal WT offspring; however, INOs supplementation only partially restored the altered vascular−metabolic programming in the eNOS+/− offspring. These data confirm the following: first, the contribution of the uterine environment to the developing fetus; second, that maternal INOs treatment positively affected more offspring exposed to environmental factors in utero than those due to genetic factors; and third, that INOs effects on the offspring could occur directly on the fetus or could ameliorate the maternal intrauterine environment. These data suggest a role of INOs as therapeutic agents for preventing and treating metabolic syndrome, by virtue of their capability to reduce radicals, enhance NO action, and improve insulin signals.

Further studies are needed to better characterize the target actions of INOs and its mechanisms. However, considering that several clinical trials have shown the efficacy and safety of INOs periconceptional supplementation,[28,29,56] an INOs mixture seems a promising natural compound for pregnant women with MS to improve their vascular−metabolic profile and, consequently, the long-term consequences to their offspring. ■

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