

The effect of combined inositol supplementation on maternal metabolic profile in pregnancies complicated by metabolic syndrome and obesity



Francesca Ferrari, MD; Fabio Facchinetti, MD; Alejandra E. Ontiveros, MD; Robyn P. Roberts, MD; Mia M. Saade; Sean C. Blackwell, MD; Baha M. Sibai, MD; Jerrie S. Refuerzo, MD; Monica Longo, MD, PhD



BACKGROUND: Myoinositol and D-chiroinositol improve insulin resistance in women with obesity and gestational diabetes and in postmenopausal women with metabolic syndrome. We previously reported that offspring born to hypertensive dams lacking endothelial nitric oxide synthase and fed a high-fat diet develop metabolic-like syndrome phenotype.

OBJECTIVE: The objective of the study was to investigate the effect of a mixture of myoinositol/D-chiroinositol supplementation during pregnancy on the maternal metabolic profile in pregnancies complicated by the metabolic-like syndrome and obesity using a pregnant mouse model.

STUDY DESIGN: Female heterozygous endothelial nitric oxide synthase^{-/+} mice with moderate hypertension were placed on a high-fat diet for 4 weeks to induce a metabolic-like syndrome phenotype. Similarly, wild-type C57BL/6 mice were placed on a high-fat diet for 4 weeks to induce a murine obesity model. Mice were then bred with wild-type males. On gestational day 1, dams were randomly allocated to receive either a mixture of myoinositol/D-chiroinositol in water (7.2/0.18 mg/mL, respectively) or water as control (placebo). At term (gestational day 18), maternal weights, systolic blood pressure, and a glucose tolerance test were obtained. Dams were then killed; pups and placentas were weighed and maternal blood collected. Serum levels of metabolic biomarkers relevant to diabetes and obesity (ghrelin, gastric inhibitory peptide, glucagon-like peptide 1, glucagon, insulin, leptin, resistin) were measured by a multiplex enzyme-linked immunosorbent assay. Analysis was done comparing metabolic-like syndrome-myoinositol/D-chiroinositol-treated vs metabolic-like syndrome-nontreated mice and obese-myoinositol/D-chiroinositol-treated vs obese nontreated mice.

RESULTS: Mean systolic blood pressure was lower in metabolic-like syndrome pregnant mice treated with myoinositol/D-chiroinositol

compared with placebo ($P = .04$), whereas there was no difference in systolic blood pressure between treated and placebo-treated obese pregnant mice. Pregnant metabolic-like syndrome mice treated with myoinositol/D-chiroinositol showed lower glucose values during the glucose tolerance test and in the area under the curve (myoinositol/D-chiroinositol: 17512.5 ± 3984.4 vs placebo: 29687.14 ± 8258.7 ; $P = .003$), but no differences were seen in the obese pregnant mice. Leptin serum levels were lower in the metabolic-like syndrome-myoinositol/D-chiroinositol-treated mice compared with the placebo group (myoinositol/D-chiroinositol: 16985 ± 976.4 pg/dL vs placebo: 24181.9 ± 3128.2 pg/dL, $P = .045$). No other differences were seen in any of the remaining serum metabolic biomarkers studied in metabolic-like syndrome and in obese pregnant mice. Maternal weight gain was not different in the pregnant metabolic-like syndrome dams, whereas it was lower in the obese myoinositol/D-chiroinositol-treated dams compared with the placebo group (myoinositol/D-chiroinositol: 10.9 ± 0.5 g vs 12.6 ± 0.6 g, $P = .04$). Fetal and placental weights did not differ between myoinositol/D-chiroinositol-treated and nontreated pregnant dams with metabolic-like syndrome and obesity.

CONCLUSION: Combined inositol treatment during pregnancy improves blood pressure, glucose levels at the glucose tolerance test, and leptin levels in pregnant dams with metabolic-like syndrome phenotype but not in obese pregnant dams. In addition, inositol treatment was associated with lower gestational weight gain in the obese but not in the metabolic-like syndrome pregnant dams.

Key words: blood pressure, C57BL/6J mice, endothelial nitric oxide synthase inositol, insulin resistance, knockout mice metabolic syndrome, obesity, pregnancy

Metabolic syndrome and obesity are growing causes of morbidity and mortality worldwide. Metabolic syndrome is a major risk factor for cardiovascular disease and is defined by the National Institutes of Health by having at least 3 of the following conditions:

hypertension, elevated fasting plasma glucose, central obesity, elevated triglycerides, or low high-density lipoprotein cholesterol.^{1,2}

Metabolic syndrome etiopathogenesis and long-term consequences are still largely unknown, and preventive strategies have not yet been identified.³ The impact of metabolic syndrome and obesity during pregnancy is substantial: indeed, all metabolic changes that develop during pregnancy have well-known effects on not only maternal and fetal health during pregnancy, but they also act as a catalyst for future health throughout later life.^{4,5}

Epidemiological and animal studies have shown that pregnancies complicated by metabolic syndrome and obesity are at risk for premature cardiovascular disease, gestational diabetes, and preeclampsia and predispose the offspring to an increased risk of cardiovascular and metabolic disease later in life.⁶⁻⁸

Inositols are a family of simple carbohydrates naturally found in several foods. Inositols exist in 9 possible stereoisomers, 2 of which are predominantly found in eukaryotic cells: myoinositol and D-chiroinositol.⁹ The exact molecular mechanisms of action of

Cite this article as: Ferrari F, Facchinetti F, Ontiveros AE, et al. The effect of combined inositol supplementation on maternal metabolic profile in pregnancies complicated by metabolic syndrome and obesity. *Am J Obstet Gynecol* 2016;215:503.e1-8.

0002-9378/\$36.00
© 2016 Elsevier Inc. All rights reserved.
<http://dx.doi.org/10.1016/j.ajog.2016.05.038>

myoinositol and D-chiroinositol have not yet been fully elucidated; however, it is well established that myoinositol and D-chiroinositol have different roles as mediators of insulin, which lead to different functions within the cells.

Myoinositol is the precursor of inositol triphosphate, a second messenger, responsible for the regulation of many hormones such as insulin, thyroid-stimulating hormone, and follicle-stimulating hormone. The activation of phospholipid-containing myoinositol by insulin activates glucose transporters, such as glucose transporter-4, and increases the cell membrane permeability to glucose, which gets into the cell and is immediately available as substrate.¹⁰

D-chiroinositol is the conversion product of myoinositol, by an epimerization reaction, which is unidirectional and insulin dependent.¹¹ D-chiroinositol, different from myoinositol, is able to determine the intracellular accumulation of glucose (ie, glycogen synthesis).

So both stereoisomers are considered to play a key role in the insulin pathway, acting synergistically as insulin-sensitizing agents through the enhancement of glucose peripheral tissue uptake and glycogen synthesis.¹² In vivo animal^{13,14} and human studies¹⁵⁻¹⁹ demonstrated that inositol supplementation during pregnancy improve the glucose profile and reduces the adverse effects of hyperglycemia. Moreover, myoinositol supplementation was proven to reduce insulin resistance in postmenopausal women with metabolic syndrome and in women with polycystic ovary syndrome, a metabolic and endocrine disorder associated with insulin resistance.¹²

However, to the best of our knowledge, the inositol supplementation effect has never been investigated in pregnancies complicated by metabolic syndrome. Moreover, most of the studies in pregnant women focus on the effect of myoinositol supplementation alone, whereas mounting evidence in polycystic ovary syndrome studies suggest that the administration of combined myoinositol/D-chiroinositol at the physiological plasma ratio (40:1) ensures

better clinical results, such as the reduction of insulin resistance and cardiovascular risk parameters.²⁰

Thus, our aim was to evaluate the effect of natural compounds, such as myoinositol and D-chiroinositol, used as a mixture, to treat pregnancies complicated by obesity and metabolic syndrome, which represent different degrees of a similar metabolic disorder. We hypothesized that myoinositol/D-chiroinositol treatment improves the abnormal maternal metabolic profile in a pregnant mouse model of either metabolic-like syndrome or obesity. The metabolic profile improvement seen with myoinositol/D-chiroinositol treatment could also translate into positive long-term maternal and fetal health.

To achieve our aim, we used well-characterized mice models of obesity and metabolic syndrome that will allow identifying the best target population for a myoinositol/D-chiroinositol treatment in pregnancies with metabolic abnormalities. We previously reported that heterozygous mice lacking endothelial nitric oxide synthase gene and born to hypertensive mothers,²¹ endothelial nitric oxide synthase^{-/+}, when fed a high-fat diet for at least 4 weeks, develop metabolic-like syndrome phenotype such as obesity, glucose intolerance, elevated systolic blood pressure, low high-density lipoprotein, high insulin, and low adiponectin levels compared with their counterpart fed a control diet.²² Furthermore, wild-type C57BL/6J female mice fed an obesogenic diet from weaning for 4 consecutive weeks show diet-induced obesity.²³

Materials and Methods

Animals

Female and male mice, homozygous for disruption of the *eNOS* gene (endothelial nitric oxide synthase-knockout^{-/-}, strain B6.129P2, stock number 002684) and their age-matched wild-type controls (strain C57BL/6J, stock number 000664) were purchased from Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. The study was approved by the Animal Welfare Committee (AWC) of the University of Texas Health Science Center at Houston. 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 University of Texas Health Science Center at Houston.

Metabolic-like syndrome mouse model

Endothelial nitric oxide synthase-knockout^{-/-} females were bred with wild-type males to obtain endothelial nitric oxide synthase^{+/-} heterozygous females. Then a metabolic-like syndrome mouse model was achieved by using endothelial nitric oxide synthase^{-/+} heterozygous offspring manifesting a moderate hypertension phenotype^{21,24} and fed an obesogenic diet for 4 consecutive weeks after weaning (Figure 1).²²

At 7–8 weeks of age, nonpregnant endothelial nitric oxide synthase^{-/+} females were bred with wild-type males. At gestational day 1 of pregnancy, determined by the presence of a vaginal plug after overnight exposure to male breeders, metabolic-like syndrome dams were randomly allocated to receive either a mixture of myoinositol/D-chiroinositol dissolved in water (see next paragraph for concentrations) or plain water, as placebo (control group) (Figure 1).

Obesity mouse model

To induce the obesity phenotype (Figure 1), wild-type—C57BL/6J female mice were fed an obesogenic diet from weaning for 4 consecutive weeks.²³ Then at 7–8 weeks of age, obese females were bred with wild-type males and starting from gestational day 1, obese mice were randomly allocated to receive either a mixture of myoinositol/D-chiroinositol dissolved in water (see next paragraph for concentrations) or plain water, as placebo (control group).

Obesogenic diet

Both models, endothelial nitric oxide synthase^{-/+}—heterozygous and wild-type females offspring, were allocated to an obesogenic diet, high-fat diet (D12492, 60% of fat) until 7–8 weeks of life to induce the metabolic-like

syndrome and obesity phenotypes, respectively. The high-fat diet was maintained during the whole pregnancy until animals were killed at term, on gestational day 18. Daily food consumption was estimated by subtracting the total amount of food left on the grid from the initial weight of food supplied.

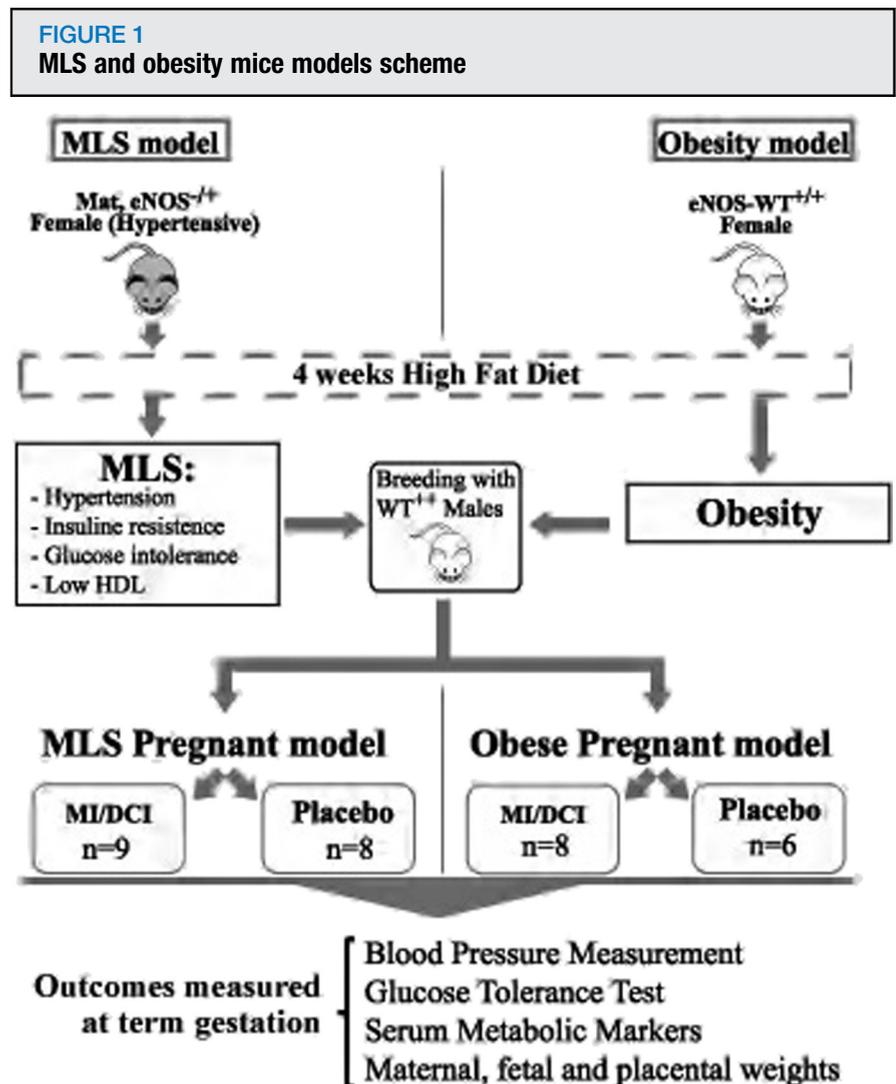
Inositol supplementation during pregnancy

The myoinositol/D-chiroinositol mixture corresponds to the physiological plasma ratio of myoinositol and D-chiroinositol equal to 40:1, which has been proven to be the most effective.²⁰ On gestational day 1, metabolic-like syndrome and obese mice were randomly allocated to receive either a mixture of myoinositol/D-chiroinositol dissolved in water (7.2/0.18 mg/mL, respectively, based on previous animals^{13,14} and human¹⁵⁻¹⁹ studies) or plain water as placebo.

Considering that blood volume during pregnancy physiologically shows a 20% increase compared with the nonpregnant status, we adjusted the myoinositol/D-chiroinositol doses by a 20% increase to the previously established doses used in a nonpregnant obese mouse model (myoinositol: 6 mg/mL).^{13,14} Myoinositol and D-chiroinositol were purchased from Sigma Chemicals (St Louis, MO).

The treatment was maintained until term gestation (gestational day 18 of pregnancy), when dams were killed. Pregnant mice were single housed to be able to carefully evaluate water intake and consequently the daily dose of myoinositol/D-chiroinositol. Daily water consumption was estimated by subtracting the total amount of water left in the bottle from the initial amount supplied. On average, pregnant mice drink 5 mL/day, so myoinositol/D-chiroinositol daily consumption was approximately 36/0.9 mg of, respectively, myoinositol/D-chiroinositol per day per mouse.

At term gestation, the metabolic profile of the metabolic-like syndrome and obese pregnant dams were obtained including the following: systolic blood



For the MLS mouse model, maternal (Mat; eNOS^{-/+}) heterozygous females (gray mouse), with mild hypertension, were placed on a high-fat diet for 4 weeks to develop an MLS phenotype. For the obesity mouse model, wild-type CB57 females (white mouse) were placed on a high-fat diet for 4 weeks to induce an obese phenotype. Both animal models were then bred with wild-type normotensive males and starting from gestational day 1 were allocated either to MI/DCI treatment or water as placebo until term gestation when the experiments were performed, as displayed in the scheme.

DCI, D-chiroinositol; eNOS, endothelial nitric oxide synthase; HDL, high-density lipoprotein; MI, myoinositol; MLS, metabolic-like syndrome; WT, wild type.

Ferrari et al. Inositols effect in pregnancies with metabolic syndrome and obesity. *Am J Obstet Gynecol* 2016.

pressure, a glucose tolerance test, maternal gestational weight gain, and serum levels of metabolic biomarkers relevant to diabetes and obesity. Dams were then killed and pups and placentas weights and numbers collected.

Blood pressure measurement

Systolic blood pressure measurements were always taken on gestational day 18

in the morning at the same time using a calibrated, 8-chamber, tail-cuff system (CODA; Kent Scientific, Torrington, CT). Dams were kept warm using a warming pad. Mice underwent an initial 10 cycles of acclimatization period; blood pressure was then monitored and recorded over 10 new cycles. Systolic blood pressure averaged of the last 10 cycles was used for the final blood

pressure measurement and utilized for data analysis.^{25,26}

Glucose tolerance test

Pregnant dams on gestational day 18 underwent a glucose tolerance test after being fasting for 6 hours. Mice received 1.0 g/kg of glucose intraperitoneally, and serial blood glucose levels, via a tail nick at 0, 15, 30, 60, and 120 minutes, were immediately determined with the Accu-Chek Aviva blood glucose meter system (Roche Diagnostics, Indianapolis, IN) after glucose administration. Glucose levels and the area under the curve were compared between the metabolic-like syndrome and obese pregnant mice treated with myoinositol/D-chiroinositol and identical nontreated mice.²⁷

Serum metabolic panel

Blood samples were obtained as soon as animals were killed by heart puncture. Serum levels of metabolic biomarkers relevant to diabetes and obesity such as glucagon, insulin, leptin, ghrelin, gastric inhibitory peptide, glucagon-like peptide 1, and resistin were

measured by a multiplex enzyme-linked immunosorbent assay (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis

Results are expressed as mean \pm SEM. All the data were analyzed using an unpaired *t* test with SigmaPlot 12. The analysis was done comparing metabolic-like syndrome myoinositol/D-chiroinositol-treated pregnant mice vs metabolic-like syndrome nontreated (placebo) pregnant mice and obese myoinositol/D-chiroinositol-treated pregnant vs obese nontreated (placebo) pregnant mice. Numbers of pregnant mice per group were as follows: metabolic-like syndrome myoinositol/D-chiroinositol treated, *n* = 9; metabolic-like syndrome placebo treated, *n* = 8; obese myoinositol/D-chiroinositol treated, *n* = 8; and obese placebo treated, *n* = 6. (Figure 1).

Results

Food and water intake

Daily food intake was not different between metabolic-like syndrome myoinositol/D-chiroinositol-treated and

metabolic-like syndrome—placebo mice (myoinositol/D-chiroinositol: 6.0 ± 0.9 g vs placebo: 6.5 ± 0.7 g) or between obese myoinositol/D-chiroinositol—treated and obese placebo-treated (myoinositol/D-chiroinositol: 6.3 ± 0.6 g vs placebo: 6.0 ± 0.7 g). Daily water intake was not different between metabolic-like syndrome—myoinositol/D-chiroinositol—treated and metabolic-like syndrome—placebo mice (myoinositol/D-chiroinositol: 5.1 ± 0.9 mL vs placebo: 4.9 ± 0.7 mL) or between obese myoinositol/D-chiroinositol—treated and obese placebo-treated (myoinositol/D-chiroinositol: 4.8 ± 0.9 g vs placebo: 5.0 ± 0.7 g).

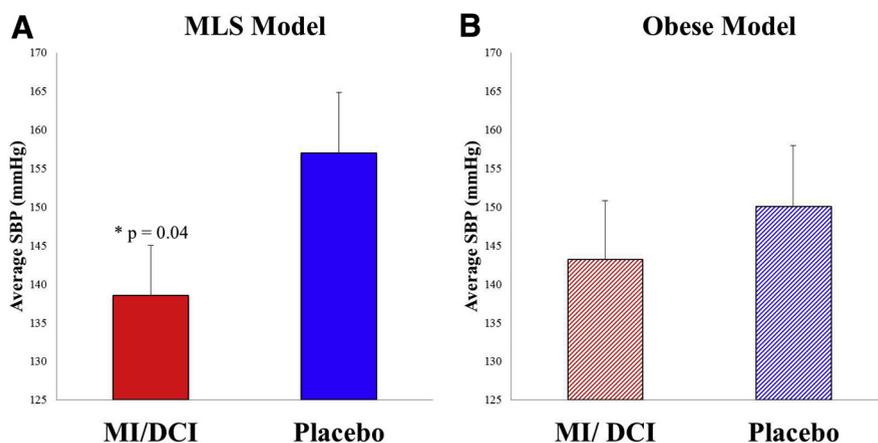
Systolic blood pressure measurement

At term gestation, mean systolic blood pressure was lower in metabolic-like syndrome pregnant mice treated with myoinositol/D-chiroinositol compared with the placebo group (myoinositol/D-chiroinositol: 138.52 ± 6.48 mm Hg vs placebo: 157.03 ± 7.79 mm Hg, *P* = .04) (Figure 2A). In the obese pregnant dams, there was no difference in systolic blood pressure between myoinositol/D-chiroinositol-treated and placebo dams (myoinositol/D-chiroinositol: 143.18 ± 7.6 vs placebo: 150.09 ± 7.9) (Figure 2B).

Glucose tolerance

The glucose levels in the glucose tolerance test were lower in the metabolic-like syndrome pregnant mice treated with myoinositol/D-chiroinositol compared with placebo-treated mice at all time periods except for the 15 minute value (Figure 3A). To confirm, the area under the curve for the glucose tolerance test was lower in metabolic-like syndrome mice myoinositol/D-chiroinositol—treated group (myoinositol/D-chiroinositol: 17512.5 ± 3984.4 vs placebo: 29687.14 ± 8258.7 ; *P* = .003). In contrast, in the obese pregnant dams, the glucose levels in the glucose tolerance test and the area under the curve were not different between myoinositol/D-chiroinositol—treated and placebo-treated dams (myoinositol/D-chiroinositol: 23573.6 ± 4758.2 vs placebo: 25410 ± 5764.4) (Figure 3B).

FIGURE 2
Average systolic blood pressure (mm Hg) in MLS and obese pregnant mice



Systolic blood pressure in MLS pregnant mice (A) and obese mice (B) at term gestation (gestational day 18) with (in red) and without (in blue) MI/DCl treatment. Data are shown as mean \pm SEM. Significance is indicated on the figure. Asterisk indicates a value of *P* = .04. Systolic blood pressure was higher in MLS mice treated with MI/DCl vs the nontreated dams; no difference in systolic blood pressure is seen in obese mice.

DCl, D-chiroinositol; MI, myoinositol; MLS, metabolic-like syndrome; SBP, systolic blood pressure.

Ferrari et al. Inositols effect in pregnancies with metabolic syndrome and obesity. *Am J Obstet Gynecol* 2016.

Serum metabolic profile

Serum leptin levels were lower in the metabolic-like syndrome myoinositol/D-chiroinositol-treated mice compared with the placebo-treated group (myoinositol/D-chiroinositol: 16985 ± 976.4 pg/dL vs placebo: 24181.9 ± 3128.2 pg/dL, $P = .045$). No other differences were seen in the serum levels of the remaining metabolic biomarkers studied in the metabolic-like syndrome pregnant mice (Table). No difference was found between obese myoinositol/D-chiroinositol-treated and nontreated mice in any of the investigated metabolic biomarkers (Table).

Weights

Gestational weight gain was not different between metabolic-like syndrome—myoinositol/D-chiroinositol-treated and placebo mice (myoinositol/D-chiroinositol: 11.53 ± 0.9 g vs placebo: 11.6 ± 0.7 g) but was lower in the obese myoinositol/D-chiroinositol-treated dams compared with the placebo-treated mice (myoinositol/D-chiroinositol: 10.9 ± 0.5 g vs placebo: 12.6 ± 0.6 g, $P = .04$).

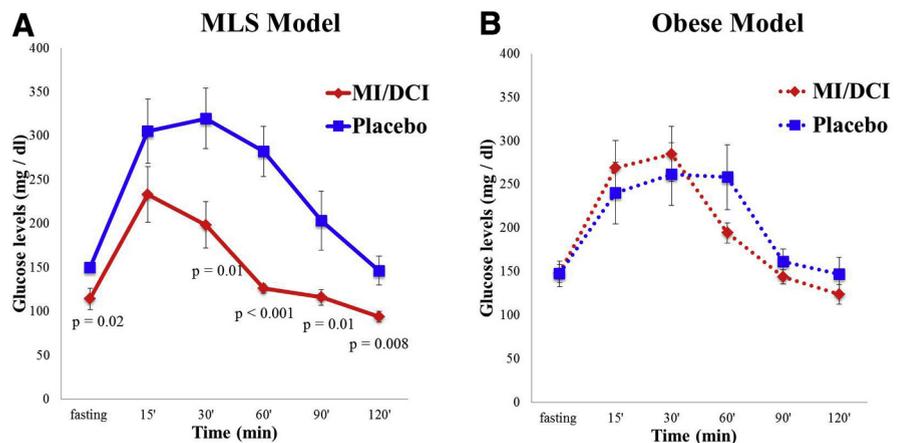
There were no differences between the pregnant metabolic-like syndrome myoinositol/D-chiroinositol-treated and placebo groups, respectively, in fetal weights and placental weights, as reported in the Table. Likewise, fetal and placental weights did not differ between myoinositol/D-chiroinositol-treated and placebo obese pregnant mice (Table). Furthermore, no differences were found regarding the number of pups born from dams with metabolic-like syndrome or obese either treated or nontreated myoinositol/D-chiroinositol.

A summary of all experiments results is reported in Figure 4.

Comment

We found that myoinositol/D-chiroinositol supplementation in pregnant mice with metabolic-like syndrome phenotype had improved blood pressure, glucose tolerance, and leptin levels. However, in obese pregnant mice, myoinositol/D-chiroinositol treatment prevented maternal increased weight gain associated with a high-fat diet but did not affect maternal glucose

FIGURE 3
Glucose Tolerance Test (mg/dL) in MLS and obese pregnant mice



Glucose levels in metabolic-like syndrome pregnant mice (A) and obese pregnant mice (B) at term gestation with and without MI/DCI treatment. Glucose levels were significantly lower in MI/DCI treated mice with MLS compared with nontreated animals at all time periods except for the 15 minutes. In obese mice the GTT was not different between MI/DCI-treated and nontreated animals. Significance is indicated on the figure. Asterisk indicates a value of $P < .05$.

DCI, D-chiroinositol; GTT, glucose tolerance test; MI, myoinositol; MLS, metabolic-like syndrome.

Ferrari et al. Inositols effect in pregnancies with metabolic syndrome and obesity. *Am J Obstet Gynecol* 2016.

tolerance, blood pressure, and the metabolic markers related to diabetes.

Our findings therefore support the rationale for the use of combined myoinositol/D-chiroinositol supplementation in pregnancies complicated by metabolic syndrome but not by obesity alone in these animal models. Inositols are Food and Drug Administration-approved supplements²⁸ with a potential role in improving metabolic profile in metabolic syndrome. It is well established that metabolic syndrome traits, such as obesity and insulin resistance, during pregnancy have a negative impact on the pregnancy and on the metabolic and cardiovascular future health of the mother and the offspring.^{4-7,29} Therefore, the potential of a new non-pharmacological approach as a preventive and therapeutic agent is clinically relevant because appropriate preventive strategies for metabolic syndrome in pregnancy have not yet been identified.

Previous human studies focused either on gestational diabetes prevention/therapy¹⁵⁻¹⁹ or on metabolic syndrome associated with menopause^{30,31} or polycystic ovary syndrome,³²⁻³⁴ whereas to the best of our knowledge,

this is the first study to investigate inositols' combination effect in a metabolic-like syndrome and obese pregnant mouse model. Four human studies reported on the efficacy of myoinositol supplementation during pregnancy to prevent gestational diabetes in patients at risk because of family history, obesity, or polycystic ovary syndrome.¹⁵⁻¹⁸

In addition, 1 prospective randomized trial demonstrated a therapeutic role of myoinositol in women with diagnosed gestational diabetes showing that myoinositol in second trimester reduced homeostatic model assessment resistance and adiponectin concentrations compared with controls.¹⁹ Similarly, in nonpregnant women with polycystic ovary syndrome³²⁻³⁴ and postmenopausal women,^{30,31} myoinositol was shown to reduce insulin resistance and improve cardiovascular risk parameters (reduction in blood pressure, total and low-density lipoprotein-cholesterol, triglycerides, and enhancement of the high-density lipoprotein-cholesterol). Our study reveals that inositol supplementation improved systolic blood pressure and glucose tolerance in pregnancies

TABLE
Serum metabolic profile (pg/mL) and fetal and placental weights (g)

Variables	MLS model			Obese model		
	MI/DCI	Placebo	P value	MI/DCI	Placebo	P value
GIP	530.1 ± 49.3	674.6 ± 88.0	.18	412.5 ± 111.3	542.1 ± 70.8	.34
GLP-1	42.6 ± 4.2	45 ± 5.9	.74	34.9 ± 6.7	36.6 ± 4.6	.84
Glucagon	564.5 ± 83.0	461.1 ± 75.8	.37	400.8 ± 48.7	453.1 ± 64.5	.53
Insulin	11,79.3 ± 136.9	1146.7 ± 176.7	.88	999.7 ± 204.9	1042.8 ± 231.3	.89
Leptin	18,156.6 ± 1472.1 ^a	22,620.4 ± 3290.2	.045	21,998.2 ± 3525.4	18,914.4 ± 3809.1	.66
Ghrelin	15,016.1 ± 3459.7	22,676.6 ± 15492.8	.64	10,052.3 ± 5764.2	42,544.8 ± 26,978.8	.28
Mean fetal weight	0.9 ± 0.01	0.88 ± 0.01	.81	0.9 ± 0.01	0.87 ± 0.01	.88
Mean placental weight	0.11 ± 0.02	0.11 ± 0.01	.92	0.10 ± 0.01	0.10 ± 0.01	.99
Number of pups	7	6.7	.77	7.3	6.5	.63

All serum metabolic marker were measured in picograms per milliliter. Data are shown as mean ± SEM.

DCI, D-chiroinositol; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; MI, myoinositol; MLS, metabolic-like syndrome.

^a P = .045, MLS MI/DCI-treated pregnant mice vs MLS placebo pregnant mice and obese MI/DCI-treated pregnant mice vs obese placebo pregnant mice.

Ferrari et al. Inositols effect in pregnancies with metabolic syndrome and obesity. Am J Obstet Gynecol 2016.

complicated by metabolic syndrome but not in obese pregnancies.

The lack of effect in the obese pregnant mice could be due to the short period of high-fat diet exposure leading only to weight gain but not to metabolic abnormalities severe enough to reveal effects of myoinositol/D-chiroinositol supplementation. In fact, according to the literature, 4 weeks of high-fat diet exposure should be sufficient to develop

a diet-induced obesity phenotype²³; however, several animal studies reported a longer period of high-fat diet exposure, up to 12 weeks, to manifest metabolic abnormalities and an inositol effect.¹³ Moreover, the lack of effect in obese pregnant mice could be due to different mechanistic pathways involved.

We speculate that myoinositol/D-chiroinositol treatment improves glucose tolerance in metabolic-like

syndrome pregnant mice but not in the obese mice, possibly involving the nitric oxide nitric oxide pathway, which is known to be defective in our metabolic-like syndrome model, but not in the obese one.^{21,35} The exact molecular mechanisms of action of myoinositol and D-chiroinositol have not yet been fully elucidated. It has been shown that potential mechanisms by which the inositols might act is by improving endothelial function in decreasing radical oxidative stress, enhancing endothelial nitric oxide synthase and nitric oxide activity.³⁶

This endothelial nitric oxide synthase^{-/+} heterozygous mouse manifests metabolic-like syndrome, probably because of the lack of endothelial nitric oxide synthase associated with an obesogenic diet during pregnancy. The metabolic abnormalities seen in this model of metabolic-like syndrome might be a consequence of an endothelial dysfunction leading to hyperinsulinemia, insulin resistance, and increased blood pressure. Thus, this mouse model of metabolic-like syndrome seems very well suited to benefit from inositol supplementation, which aims at restoring endothelial function. Indeed, in our obese pregnant mouse model, an obesogenic diet alone and for

FIGURE 4
Results summary

	MLS PREGNANT MICE MI/DCI - Treated	OBESSE PREGNANT MICE MI/DCI - Treated
Blood pressure	↓	↔
GTT	↓	↔
Leptin	↓	↔
Other serum metabolic markers	↔	↔
Gestational weight gain	↔	↓
Fetal and placental weights	↔	↔

Pregnant mice with the MLS phenotype treated with MI/D-chiroinositol during pregnancy have improved blood pressure, glucose tolerance, and leptin levels compared with nontreated mice. In obese mice instead, MI/DCI treatment prevented maternal increased weight gain associated with a high-fat diet but did not affect maternal insulin resistance and blood pressure.

DCI, D-chiroinositol; GTT, glucose tolerance test; MI, myoinositol; MLS, metabolic-like syndrome.

Ferrari et al. Inositols effect in pregnancies with metabolic syndrome and obesity. Am J Obstet Gynecol 2016.

a short period of time does not seem to lead to such an endothelial dysfunction for which inositol supplementation could show a target effect.

In our study leptin levels were improved by myoinositol/D-chiroinositol treatment in metabolic-like syndrome pregnant mice, which is consistent with previous studies that showed decreased circulating leptin concentration after 16 weeks of myoinositol treatment in women with polycystic ovary syndrome.^{12,37} The exact mechanism by which myoinositol/D-chiroinositol reduces leptin level remains unclear. Leptin controls energy balance and body weight by regulating neuronal activity in the hypothalamus.³⁸

It is possible that myoinositol/D-chiroinositol improved leptin resistance through either reductions in circulating leptin levels or the up-regulations of receptors mediating leptin's action to the brain, which needs further investigation. Furthermore, in our study, maternal gestational weight gain was significantly reduced in the obese mice after myoinositol/D-chiroinositol treatment, in agreement with previous studies on gestational diabetes patients.^{15,39} In both obese and metabolic-like syndrome models, pups and placental weights did not differ between myoinositol/D-chiroinositol-treated and placebo animals.

These findings are in contrast to others that found a reduction in hyperglycemia-related complications, such as fetal macrosomia, after myoinositol supplementation in women at risk for gestational diabetes for a family history of type 2 diabetes¹⁶ or elevating fasting glucose levels¹⁵; however, again this could be due to a difference in the duration of a high-fat diet or to the fact that we evaluated the average weights of litters and placentas independently from their genotype (endothelial nitric oxide synthase^{-/+} heterozygous, 25%, or wild type, 75%).

Study strengths are the following: (1) to the best of our knowledge, this is the first time that combined inositols (myoinositol/D-chiroinositol) have been evaluated as a mixture rather than single compounds during pregnancy and (2)

and furthermore being used in a metabolic-like syndrome mouse model during pregnancy. Indeed, diet-induced obesity could be considered a less severe degree of the same metabolic disease that ultimately leads to metabolic syndrome. Therefore, the thorough investigation of inositol in the presence of a spectrum of components of metabolic syndrome, rather than obesity alone, allows for the identification of the best target population for myoinositol/D-chiroinositol treatment. Moreover myoinositol and D-chiroinositol have shown different concentrations at the tissue level^{40,41} and different roles in the whole cell,^{11,20} leading to the conclusion that both of them should be used in a synergistic action for the purpose of better treatment efficacy in metabolic diseases.²⁰

Unfer and colleagues^{20,42} performed dose-response studies identifying the physiological plasma ratio of myoinositol and D-chiroinositol equal to 40:1, which was proven to be the most effective in polycystic ovary syndrome overweight patients with metabolic abnormalities, but it has never been studied in pregnancies with metabolic abnormalities.

The main weakness of our study is that we did not investigate the effect of myoinositol/D-chiroinositol on the lipid profile, which is an important feature of metabolic syndrome. We also did not evaluate the nitric oxide pathway as well as the inositol phosphoglycans intracellular mediators as possible mechanisms involved in the myoinositol/D-chiroinositol actions to elucidate our results. Moreover, in our study we did not plan to evaluate the combined myoinositol/D-chiroinositol effect in physiological uncomplicated pregnancies, nor did we plan to evaluate the effect of myoinositol or D-chiroinositol alone.

Future studies need to be performed to investigate the effect of myoinositol/D-chiroinositol treatment on long-term maternal health and in the offspring born to metabolic syndrome and obese pregnancies to identify a possible role of inositols on metabolic fetal programming. Undoubtedly, further studies are warranted to fully elucidate the molecular pathways triggered by myoinositol

and D-chiroinositol and to define the ideal timing (before conception, during pregnancy, and/or postpartum) dose and the combination of inositol stereoisomers of such a supplementation. ■

References

1. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735-52.
2. Kahn R, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal. *Diabetologia* 2005;48:1684-99.
3. Hutcheson R, Rocic P. The metabolic syndrome, oxidative stress, environment, and cardiovascular disease: the great exploration. *Exp Diabetes Res* 2012;2012:271028.
4. Catalano PM, Eherberg HM. The short and long-term implications of maternal obesity on the mother and her offspring. *BJOG* 2006;113:1126-33.
5. Li M, Sloboda DM, Vickers MH. Maternal obesity and developmental programming of metabolic disorders in offspring: evidence from animal models. *Exp Diabetes Res* 2011;2011:592408.
6. Malek A. The impact of metabolic disease associated with metabolic syndrome on human pregnancy. *Curr Pharm Biotechnol* 2014;15:3-12.
7. Chatzi L, Plana E, Pappas A, et al. The metabolic syndrome in early pregnancy and risk of gestational diabetes mellitus. *Diabetes Metab* 2009;35:490-4.
8. Vryonidou A, Paschou SA, Muscogiuri G, Orio F, Goulis DG. Mechanisms in endocrinology: metabolic syndrome through the female life cycle. *Eur J Endocrinol* 2015;173:R153-63.
9. Noventa M, Vitagliano A, Quaranta M, Borgato S, Abdulrahim B, Gizzo S. Preventive and therapeutic role of dietary inositol supplementation in periconceptional period and during pregnancy: a summary of evidences and future applications. *Reprod Sci* 2016;23:278-88.
10. Huang LC, Fonteles MC, Houston DB, et al. Chiroinositol deficiency and insulin resistance. III. Acute glycogenic and hypoglycemic effects of two inositol phosphoglycan insulin mediators in normal and streptozotocin-diabetic rats in vivo. *Endocrinology* 1993;132:652-7.
11. Lerner J. D-chiro-inositol—its functional role in insulin action and its deficit in insulin resistance. *Int J Exp Diabetes Res* 2002;3:47-60.
12. Croze ML, Soulage CO. Potential role and therapeutic interests of myo-inositol in metabolic diseases. *Biochimie* 2013;95:1811-27.
13. Croze ML, Gélouën A, Soulage CO. Abnormalities in myo-inositol metabolism associated with type 2 diabetes in mice fed a high fat diet: benefits of a dietary myo-inositol supplementation. *Br J Nutr* 2015;113:1862-75.

14. Croze ML, Vella RE, Pillon NJ, et al. Chronic treatment with myo-inositol reduces white adipose tissue accretion and improves insulin sensitivity in female mice. *J Nutr Biochem* 2013;24:457-66.
15. Matarrelli B, Vitacolonna E, D'Angelo M, et al. Effect of dietary myo-inositol supplementation in pregnancy on the incidence of maternal gestational diabetes mellitus and fetal outcomes: a randomized controlled trial. *J Matern Fetal Neonatal Med* 2013;26:967-72.
16. D'Anna R, Scillipoti A, Giordano D, et al. Myo-Inositol supplementation and onset of gestational diabetes mellitus in pregnant women with a family history of type 2 diabetes: a prospective, randomized, placebo-controlled study. *Diabetes Care* 2013;36:854-7.
17. D'Anna R, Di Benedetto V, Rizzo P, et al. Myo-inositol may prevent gestational diabetes in PCOS women. *Gynecol Endocrinol* 2012;28:440-2.
18. D'Anna R, Di Benedetto A, Scillipoti A, et al. Myo-inositol supplementation for prevention of gestational diabetes in obese pregnant women: a randomized controlled trial. *Obstet Gynecol* 2015;126:310-5.
19. Corrado F, D'Anna R, Di Vieste G, et al. The effect of myoinositol supplementation on insulin resistance in patients with gestational diabetes. *Diabet Med* 2011;28:972-5.
20. Unfer V, Porcaro G. Updates on the myo-inositol plus D-chiro-inositol combined therapy in polycystic ovary syndrome. *Expert Rev Clin Pharmacol* 2014;7:623-31.
21. Longo M, Jain V, Vedernikov YP, et al. Fetal origins of adult vascular dysfunction in mice lacking endothelial nitric oxide synthase. *Am J Physiol Regul Integr Comp Physiol* 2005;288:1114-21.
22. Longo M, Refuerzo J, Mann L, et al. Effect of high fat diet on metabolic programming in offspring born to hypertensive mother. *Am J Obstet Gynecol* 2015;212:S32-3.
23. Chao-Yung Wang C, Liao JK. A mouse model of diet-induced obesity and insulin resistance. *Methods Mol Biol* 2012;821:421-33.
24. Chiossi G, Costantine MM, Tamayo E, et al. Effect of age and gender on the progression of adult vascular dysfunction in a mouse model of fetal programming lacking endothelial nitric oxide synthase. *Am J Physiol Heart Circ Physiol* 2011;301:H297-305.
25. Whitesall SE, Hoff JB, Vollmer AP, D'Alecy LG. Comparison of simultaneous measurement of mouse systolic arterial blood pressure by radiotelemetry and tail-cuff methods. *Am J Physiol Heart Circ Physiol* 2004;286:H2408-15.
26. Feng M, Whitesall S, Zhang Y, Beibel M, D'Alecy L, DiPetrillo K. Validation of volume-pressure recording tail-cuff blood pressure measurements. *Am J Hypertens* 2008;21:1288-91.
27. Barros RPA, Gabbi C, Morani A, Warner M, Gustafsson JA. Participation of ER α and ER β in glucose homeostasis in skeletal muscle and white adipose tissue. *Am J Physiol Endocrinol Metab* 2009;297:E124-33.
28. Carlomagno G, Unfer V. Inositol safety: clinical evidences. *Eur Rev Med Pharmacol Sci* 2011;15:931-6.
29. Kaul P, Savu A, Nerenberg KA, et al. Impact of gestational diabetes mellitus and high maternal weight on the development of diabetes, hypertension and cardiovascular disease: a population level analysis. *Diabet Med* 2015;32:164-73.
30. Santamaria A, Giordano D, Corrado F, et al. One-year effects of myo-inositol supplementation in postmenopausal women with metabolic syndrome. *Climacteric* 2012;15:490-5.
31. Giordano D, Corrado F, Santamaria A, et al. Effects of myo-inositol supplementation in postmenopausal women with metabolic syndrome: a perspective, randomized, placebo-controlled study. *Menopause* 2011;18:102-4.
32. Unfer V, Carlomagno G, Dante G, Facchinetti F. Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. *Gynecol Endocrinol* 2012;28:509-15.
33. Genazzani AD, Lanzoni C, Ricchieri F, Jasonni VM. Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. *Gynecol Endocrinol* 2008;24:139-44.
34. Facchinetti F, Bizzarri M, Benvenga S, et al. Results from the International Consensus Conference on Myo-Inositol and D-Chiro-Inositol in Obstetrics and Gynecology: the link between metabolic syndrome and PCOS. *Eur J Obstet Gynecol Reprod Biol* 2015;195:72-6.
35. Atochin Dmitriy N, Huang Paul L. Endothelial nitric oxide synthase transgenic models of endothelial dysfunction. *Pflugers Arch* 2010;460:965-74.
36. Nascimento NR, Lessa LM, Kerntopf MR, et al. Inositols prevent and reverse endothelial dysfunction in diabetic rat and rabbit vasculature metabolically and by scavenging superoxide. *Proc Natl Acad Sci USA* 2006;103:218-23.
37. Gerli S, Papaleo E, Ferrari A, Di Renzo GC. Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. *Eur Rev Med Pharmacol Sci* 2007;11:347-54.
38. de Git KC, Adan RA. Leptin resistance in diet-induced obesity: the role of hypothalamic inflammation. *Obes Rev* 2015;16:207-24.
39. Reece EA, Khandelwal M, Wu YK, Borenstein M. Dietary intake of myo-inositol and neural tube defects in offspring of diabetic rats. *Am J Obstet Gynecol* 1997;176:536-9.
40. Nestler JE, Jakubowicz DJ, Reamer P, et al. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. *N Engl J Med* 1999;340:1314-20.
41. Heimark D, McAllister J, Lamer J. Decreased myo-inositol to chiro-inositol (m/c) ratios and increased m/c epimerase activity in PCOS theca cells demonstrate increased insulin sensitivity compared to controls. *Endocr J* 2014;61:111-7.
42. Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. *Fertil Steril* 2011;95:2515-6.

Author and article information

From the Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Texas Health Science Center at Houston, Houston, Texas (Drs Ferrari, Ontiveros, Roberts, Blackwell, Sibai, Refuerzo, and Longo and Ms Saade); and Department of Obstetrics and Gynecology, University of Modena and Reggio Emilia, Modena, Italy (Drs Ferrari and Facchinetti).

Received March 5, 2016; revised May 18, 2016; accepted May 24, 2016.

Financial support: Partially supported by the Larry Gilstrap Center.

The authors report no conflict of interest.

Presented as oral presentation number 69 at the 36th annual meeting of the Society of Maternal-Fetal Medicine, Atlanta, GA, Feb. 1–6, 2016.

Corresponding author: Francesca Ferrari, MD. ferrari196@yahoo.com