Preeclampsia

17-Hydroxyprogesterone Caproate Significantly Improves Clinical Characteristics of Preeclampsia in the Reduced Uterine Perfusion Pressure Rat Model

Lorena M. Amaral, Denise C. Cornelius, Ashlyn Harmon, Janae Moseley, James N. Martin Jr, Babbette LaMarca

Abstract—Preeclampsia is characterized by increased uterine artery resistance index, chronic immune activation, and decreased circulating nitric oxide levels. 17-α-Hydroxyprogesterone caproate (17-OHPC) is a synthetic metabolite of progesterone used for the prevention of recurrent preterm birth. We hypothesized that 17-OHPC could reduce mean arterial pressure by decreasing inflammation, whereas improving vasodilation by increasing nitric oxide bioavailability and uterine artery resistance index during late gestation in the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia. 17-OHPC (3.32 mg/kg) was intraperitoneally administered on gestation day 18 into RUPP rats, carotid catheters inserted, and mean arterial pressure, blood, and tissues were collected on day 19. Mean arterial pressure in normal pregnant (NP; n=13) was 92±2.0 and increased to 123±2.0 in RUPP (n=18; P<0.0001), which was improved to 116±1.5 mm Hg in RUPP+17-OHPC (n=10; P<0.05). Circulating CD4+ T cells were 1.19%±1.0% of gated cells in NP (n=7), which increased to 8.52%±2.4% in RUPP rats (n=10; P<0.05) but was reduced to 2.72%±0.87% (n=14; P<0.05) in RUPP+17-OHPC. Circulating nitrate/nitrite was 26.34±3.5 μmol/L in NP (n=12) but was reduced to 14.58±3.1 in RUPP rats (n=8; P=0.03) and increased to 22.69±1.62 in RUPP+17-OHPC (n=7; P=0.05). Endothelial nitric oxide synthase expression was 0.65±0.11 AU in NP (n=4), which decreased to 0.33±0.01 in RUPP rats (n=4; P=0.05) but increased to 0.57±0.01 in RUPP+17-OHPC (n=5; P=0.03). Uterine artery resistance index was 0.54±0.02 in NP (n=3), 0.78±0.03 in RUPP (n=4), and 0.63±0.038 in RUPP+17-OHPC (n=8; both P=0.05). Our findings demonstrate that even though modest, lowering blood pressure with 17-OHPC could be a viable treatment option for suppressing inflammation, uterine artery vasoconstriction while improving litter size. (Hypertension. 2015;65:225-231. DOI: 10.1161/HYPERTENSIONAHA.114.04484.) • Online Data Supplement

Key Words: hypertension ■ inflammation ■ nitric oxide ■ pregnancy ■ progesterone

Preeclampsia is a relatively common pregnancy disorder usually characterized by hypertension, abnormal amounts of protein in the urine, increased inflammatory cytokines, decreased vasodilators, such as nitric oxide (NO) and other systemic disturbances.1–7 This condition affects 5% to 8% of pregnancies, and despite being one of the leading causes of death in pregnant women, complete understanding of the mechanisms responsible for preeclampsia pathogenesis remains elusive.8,9

A major initiating event leading to the development of preeclampsia is thought to be reduced placental perfusion that leads to widespread dysfunction of the maternal vascular endothelium by mechanisms that remain to be determined.2,8,10 In addition, mediators of endothelial dysfunction, such as decreased production of the NO, increased production of the vasoconstrictor endothelin-1 (ET-1), and enhanced vascular reactivity to angiotensin II type 1 receptor autoantibodies, play a role in the development of hypertension during pregnancy.4,6,11–17

Currently, there is no effective treatment for preterm preeclampsia, except for early delivery of the fetus along with the placenta. Thus, preeclampsia continues into the 21st century as the primary global cause of prematurity and perinatal morbidity/mortality. Progesterone supplementation in the form of 17-α-hydroxyprogesterone caproate (17-OHPC) is currently used obstetrically to prevent recurrent preterm birth in patients with pregnancies not complicated by preeclampsia.16–20 We reported that patients with severe preeclampsia exhibit significantly lower serum progesterone concentrations than gestational age- and race-matched nonpreeclamptics.21 In addition, we have previously shown that supplementation of placental ischemic rats with 17-OHPC decreased blood pressure, inflammatory cytokines, and ET-1 within 24 hours of treatment.21–23 In addition, we have shown that progesterone inhibits TNF-α–induced ET-1 secretion within 6 hours of exposure of human umbilical venous endothelial cells to TNF-α in vitro.22 Furthermore, human umbilical venous...
endothelial cells secreted significantly greater ET-1 after exposure to preeclampsia serum than when exposed to NP serum. This response was blunted within 6 hours of exposure to progesterone.21

Additionally, there is evidence that progesterone beyond the anti-inflammatory effects may have vasodilatory effects and can improve NO availability.24,25 Interestingly, our previous study has shown that administration of 17-OHPC increased placental NO and decreased angiotensin II type 1 receptor autoantibodies, thus improving hypertension in the IL-6–induced hypertensive pregnant rats.26 Our previous studies examining an effect of 17-OHPC on pregnancy outcome in the reduced uterine perfusion pressure (RUPP) rat did not investigate the effect of 17-OHPC to decrease immune cells as potential source of lowered cytokines, nor did we examine the vasodilatory effects of 17-OHPC.

Although 17-OHPC is administered routinely for the prevention of recurrent of preterm labor, the addition of 17-OHPC for the management of preeclampsia has been debated, but the benefit of 17 OHPC in response to placental ischemia is still unclear. Although preeclampsia is associated with decreased circulating progesterone and increases in inflammatory cytokines, it remains unclear what role 17-OHPC may have in decreasing immune activation although improving vasodilation and hypertension in response to placental ischemia. Therefore, in the present study, we hypothesized that 17-OHPC could reduce mean arterial pressure, proinflammatory cytokines, and CD4+ T cells, although improving uterine artery resistance index (UARI) and increasing NO bioavailability in the hypertensive RUPP rat model of preeclampsia.

Materials and Methods

Animals and Treatment

This study complied with guidelines of the University of Mississippi Medical Center, and the animals were handled according to the guiding principles published in the National Institutes of Health Guide for the Care of Animals and the Institutional Animal Care and Use Committee. Sprague-Dawley rats purchased from Harlan Inc. (Indianapolis, IN) were used in the present study. Rats were housed in a temperature-controlled room (23°C) with a 12:12 hour light/dark cycle with free access to standard rat chow and water. Surgical procedures were performed under appropriate anesthesia, and analgesics were given postoperatively as needed. In general, inhalant anesthetics are safer than injectable anesthetics. Then, pregnant rats at gestation day 14 and day 18 were either exposed to 2.0% isoflurane. Inhalant anesthetics were given postoperatively as needed. In general, inhalant anesthetics are safer than injectable anesthetics. Therefore, in the present study, we hypothesized that 17-OHPC could reduce mean arterial pressure, proinflammatory cytokines, and CD4+ T cells, although improving uterine artery resistance index (UARI) and increasing NO bioavailability in the hypertensive RUPP rat model of preeclampsia.

Compounding Pharmacy, Jackson, MS) was diluted in normal saline and administered intraperitoneally as 0.5 cm3 solution of 3.32 mg/kg 17-OHPC to pregnant rats. We chose the 1-time 17-OHPC dose to be the weight equivalent of a typical human dose for the prevention of preterm labor and what was previously shown to be effective in RUPP rats.27

Please see the online-only Data Supplement for detailed methods regarding mean arterial blood pressure, UARI, circulating CD4+ T cells, Western blotting analysis of endothelial NO synthase (eNOS), determination of TNF-α levels, circulating nitrate/nitrite bioavailability, and determination of 8-isoprostane (8-isoPGF2α) concentrations.

Statistical Analysis

All data are expressed as mean±SEM. Comparisons between groups were assessed by 2-way analysis of variance. A Student’s t test was used for comparison of eNOS expression, nitrate/nitrite concentration, and TNF-α measurements between the groups. A value of P<0.05 was considered statistically significant.

Results

Administration of 17-OHPC Blunted Hypertension in RUPP Rats

As in previous studies with RUPP, mean arterial pressure in response to placental ischemia in RUPP rats was significantly increased compared with NP rats (p<0.0001; Figure 1). Administration of 17-OHPC significantly attenuated this increase in response to placental ischemia. Mean arterial pressure in NP rats was 92±2.0 (n=13); 123±2.0 in RUPP (n=18), and 116±1.5 mm Hg in RUPP+17-OHPC (n=10; P<0.05; Figure 1).

Administration of 17-OHPC Blunted CD4+ T Cells and TNF-α in RUPP Rats

To determine whether 17-OHPC could be a viable treatment for preeclampsia by decreasing inflammation (CD4+ T cells) in response to placental ischemia, we analyzed circulating CD4+ T cells in plasma from the whole blood collected on day 19 of gestation. Circulating CD4+ T cells were 1.19±1.0% of gated cells in NP (n=7), which increased to 8.52±2.4% in RUPP rats (n=10) but was significantly reduced to 2.72±0.87% (n=14) in RUPP+17-OHPC (p<0.05; Figure 2). In addition, as we have previously shown, 17-OHPC decreases circulating inflammatory cytokines. TNF-α was 65.8±17.7 pg/mL in RUPP rats (n=5), but was blunted to 17.24±3.9 in RUPP+17-OHPC (n=8).

![Figure 1](image-url) 17-α-Hydroxyprogesterone caproate (17-OHPC) supplementation blunts hypertension in reduced uterine perfusion pressure (RUPP) rats. Data are shown as means±SEM. (n=13-18/group). *P<0.05 vs normal pregnant (NP) group.

![Figure 2](image-url) Administration of 17-OHPC Blunted CD4+ T Cells and TNF-α in RUPP Rats.

![Figure 3](image-url) Administration of 17-OHPC Blunted CD4+ T Cells and TNF-α in RUPP Rats.

![Figure 4](image-url) Administration of 17-OHPC Blunted CD4+ T Cells and TNF-α in RUPP Rats.
Administration of 17-OHPC Increased eNOS Expression and Total Circulating Nitrate/Nitrite in RUPP Rats

To determine whether 17-OHPC improved vasodilation, we analyzed eNOS expression in protein isolated from the aorta collected on day 19 of gestation. Aortic eNOS expression was 0.65±0.11 AU in NP, which decreased to 0.33±0.01 in RUPP rats but increased to 0.57±0.01 in RUPP+17-OHPC (P<0.05; Figure 3A). Conduit vessels have a relative role in long-term regulation of arterial pressure. However, it is reasonable to expect that the vascular alterations reported here may well be found in small, resistance vessels, which are more relevant to control arterial pressure. In addition, we have measured the expression of eNOS and phosphorylated eNOS in placentas, and we have observed that 17-OHPC could increase eNOS expression compared with RUPP, but difference between the groups was not significant. Total circulating nitrate/nitrite was 26.34±3.5 µmol/L in NP (n=12); 14.58±3.1 in RUPP rats (n=8), and increased to 22.69±1.62 in RUPP+17-OHPC (n=7; P=0.05; Figure 3B).

Administration of 17-OHPC Improved Uterine Artery Resistance and Litter Size in RUPP Rats

Consistent with preeclampsia, we have shown the rise in UARI in response to induction of chronic placental ischemia to be associated with the RUPP rat. UARI was 0.54±0.02 in NP (n=3), which increased to 0.79±0.03 (n=8; p<0.05) in RUPP rats. Interestingly, the UARI was improved to 0.63±0.04 in RUPP rats treated with 17-OHPC (n=8) compared with untreated RUPP rats (p<0.05; Figure 4A). Pup weight from RUPP rats (1.87±0.06 g) was significantly lower than that of NP rats (2.31±0.12 g; p<0.05; Figure 4B). Importantly, 17-OHPC supplementation of RUPP rats improved pup weight; however, this did not reach significance (1.95±0.07 g). However, litter size from RUPP rats (10.10±1.40), which is significantly lower than that of NP rats (15.71±0.18; p<0.05; Figure 4C), was improved to 14.56±0.34, p<0.05, in RUPPS+17-OHPC.

Administration of 17-OHPC Improved Plasma 8-Isoprostane Concentrations

To determine whether 17-OHPC improved oxidative stress, we measured 8-isoprostane (8-isoPGF_2α) concentrations in plasma collect at day 19 of gestation. 8-isoprostane levels were increased in RUPP rats (n=6) compared with NP (n=3, P<0.05; Figure 5) but were reduced in RUPP+17-OHPC, however, this was not significantly different between the RUPP and RUPP+17 OHPC.

Discussion

Some clinical characteristics of preeclampsia are new-onset hypertension during pregnancy and increased UARI as measured by Doppler waveform sonography. In addition, many preeclamptic women are in a state of chronic inflammation, characterized by moderate increases in inflammatory cytokines.
Our current findings support previous conclusions that the hypertension associated with RUPP results in an increase of UARI. Determining increased UARI is instrumental in diagnosing placental deficiencies in pregnant women and is often used to identify those who may develop preeclampsia. Importantly, in this study, we demonstrated that administration of 17-OHPC improved UARI, hypertension, inflammation, and NO bioavailability in response to placental ischemia during pregnancy.

Growing evidence indicates that altered immune mechanisms play an important role in the pathophysiology of preeclampsia. Furthermore, preeclampsia is associated with increased CD4+ T cells, inflammatory mediators, such as TNF-α and IL-6, and decreased regulatory mechanisms, such as T regulatory cells and IL-10. We have recently shown that T cells from RUPP rat model of preeclampsia causes hypertension, inflammation, and NO bioavailability in response to placental ischemia during pregnancy.

Although 17-OHPC is obstetrically safe and used for the attenuation of preterm labor and may have anti-inflammatory and vasodilatory actions, its utility in the treatment of preeclampsia is unclear. Interestingly, our unpublished data demonstrates from cultured placental explants in the presence of hypoxia and 1 uMole progesterone that IL-6, TNF-α, IL-17, and sFlt-1 were all reduced when compared with hypoxic cultures alone, thereby indicating an important anti-inflammatory role progesterone could play at the level of the placenta. In addition, our previous in vitro studies demonstrate a profound effect of progesterone to inhibit TNF-α-induced ET-1 secretion and to inhibit ET-1 secretion from human umbilical venous endothelial cells stimulated with preeclampsia serum within a short 6 hour period of progesterone treatment. These study demonstrate an important effect of progesterone on vascular cells. In the current study, we demonstrated that CD4+ T cells were increased in the circulation of RUPP rats and 17-OHPC administration significantly reduced these pro-inflammatory cells. In addition, concurring with previous data, we demonstrate that 17 OHPC significantly reduced TNF-α in response to placental ischemia. These data support a role for the anti-inflammatory properties of 17-OHPC to modulate decreases in maternal blood pressure. Although the reduction in blood pressure in RUPP rats treated with 17 OHPC may be considered modest, this decrease could improve maternal health and thereby increase time to delivery. This is important because furthering the pregnancy leads to overall preparedness of the baby for birth, such as increased fetal weight and fetal lung maturation. Small improvements in maternal health and well being could lead to ever larger improvements in the health of babies born to preeclampsia moms.

Another effect of 17-OHPC could be vasodilatory by improving NO synthesis. We found increased vascular eNOS expression and nitrate/nitrite levels with 17-OHPC administration in response to placental ischemia. These data suggest that 17-OHPC may affect the vascular function, which depends on the balanced production/bioavailability of NO, which is maintained by the normal activity of eNOS. Consistent with this suggestion, it is well known that hormones may affect production of NO both by rapid effects on the activity of eNOS through phosphorylation of the enzyme and longer term modulation through changes in amount of eNOS protein.

Previous studies have demonstrated that increased eNOS activity and expression have been shown to play an important role during normal pregnancy. Levels of eNOS and NO are
elevated in the uterine artery during pregnancy and higher circulating of hormone may be in part responsible for modulating these NO levels and uterine vasculature changes. In fact, in this study, the rise in UARI after the RUPP procedure may reflect a lack of autoregulation of uteroplacental blood flow. In addition to RUPP procedure, it is possible that release of vascular mediators, including NO, has been impaired. Although the mechanism of 17-OHPC is not completely understood, it is expected that 17-OHPC interacts with the progesterone receptors to increase NO production, which could cause relaxation of the uterus and slow contractions during preterm labor. Indeed, we demonstrated that 17-OHPC administration improved UARI in response to placental ischemia during pregnancy. This could be a mechanism of improved litter size in this group. Although 17-OHPC did not improve individual pup weight, it normalized the number of pups in RUPP rats to that seen in NP rats. These data indicate improved intrauterine growth restriction in response to placental ischemia. It could be that pup weight would have increased if 17-OHPC had been given at an earlier time point in gestation to RUPP rats; however, this has not yet been performed but will be the subject of future studies. In fact, future studies are under way to determine the effects of 17-OHPC administered on gestation day 15 in the RUPP rat model of preeclampsia to determine the beneficial effects of 17-OHPC on preterm preeclampsia. In addition, future studies will examine how 17-OHPC interacts with progesterone receptors in the systemic or uterine vasculature to allow for vasodilation. Interestingly, whether progesterone could reduce the incidence of preeclampsia and its complications still remains unclear. At present, progesterone is not being used for this purpose in clinical practice because there are insufficient data
to be able to say the benefits of progesterone for the mother and child. In fact, a previous review has shown that there is no good evidence showing that progesterone, both oral and vaginal, could help to reduce the incidence of preeclampsia.\(^4\)

Furthermore, there is little information about potential adverse outcomes. In fact, more studies will be necessary to obtain the better understanding about the effects of progesterone for prevention or management of this disease. In our present study, we have studied the effects of progesterone on late gestation, and further studies in patients are warranted to examine whether intervention, such as progesterone supplementation to enhance current management for affected patients, could be positive for mother and child.

In conclusion, our findings may have important clinical implications because they suggest that attenuation of CD4\(^+\) T cells and proinflammatory cytokines accompany an increase of NO bioavailability and an improvement of UARI, litter size, and intrauterine growth restriction and hypertension in response to placental ischemia with 17-OHPC administration, which could profoundly affect pregnancy outcomes during preeclampsia. Thus, we think that 17-OHPC should be considered further for addition to the clinical management of preeclampsia.

**Perspective**

The discovery of mechanisms that are relevant to the pathophysiology of preeclampsia may offer new therapeutic targets. Our findings suggest that 17-OHPC administration into RUPP rats may regulate inflammation and NO bioavailability, resulting in decreased blood pressure, inflammation, and increased uterine artery resistance and intrauterine growth restriction in response to placental ischemia during pregnancy. Thus, an intervention such as 17-OHPC supplementation to enhance current management for affected patients could be positive for mother and child.

**Sources of Funding**

This work was supported by National Institutes of Health grants RO1HD067541, T32HL105324, and HL51971 and the Department of Pharmacology, University of Mississippi Medical Center.

**Disclosures**

None.

**References**


### Novelty and Significance

**What Is New?**

- 17α-Hydroxyprogesterone caproate (17-OHPC) administered on day 18 attenuated hypertension by suppressing proinflammatory cells although improving uterine artery resistance index and nitric oxide bioavailability and litter size in response to placental ischemia during pregnancy.

**What Is Relevant?**

- The suppressed CD4+ cells population and an upregulation of endothelial nitric oxide synthase with 17-OHPC supplementation may have a clinical role to improve factors implicated in mediating the pathophysiology associated with preeclampsia.

### Summary

Progesterone supplementation in the form of 17-OHPC is currently used obstetrically to reduce the risk of recurrent preterm labor if the prior preterm birth was not undertaken because of preeclampsia. The role for 17-OHPC in the treatment of preeclampsia is debated. Our study illustrates that 17-OHPC could be a viable addition to the treatment of preeclampsia demonstrated by its effect to decrease blood pressure and inflammation and improve uterine artery resistance index and nitric oxide bioavailability in response to placental ischemia during pregnancy.
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Hypertension. 2015;65:225-231; originally published online November 3, 2014;
doi: 10.1161/HYPERTENSIONAHA.114.04484

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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17-HYDROXYPROGESTERONE CAPROATE SIGNIFICANTLY IMPROVES CLINICAL CHARACTERISTICS OF PREECLAMPSIA IN THE RUPP RAT MODEL

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17-OHPC blunts hypertension in RUPP rats

Manuscript Number: HYPE201404484D

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Expanded method:

**Measurement of arterial blood pressure**

On day 19 of gestation, arterial blood pressure was analyzed after placing the rats in individual restraining cages. In order to avoid clotting within catheters instrumental for blood pressure measurements, pregnant rats were catheterized on day 18 of gestation under anesthesia using isoflurane (Webster, Sterling MA) delivered by an anesthesia apparatus (Vaporizer for Forane Anesthetic, Ohio Medical Products, Madison, WI). Mean arterial blood pressure (MAP) was recorded continuously for one hour after a one hour stabilization as described previously.

**Uterine artery resistance index**

On day 18 of gestation, to evaluate uteroplacental circulation, Power Doppler velocimetry measurements were performed on anesthetized pregnant dams at an imaging station with a Vevo 770 unit (Visual sonics) using a 30 Hz transducer and an insonating angle <30° as previously described. The peak systolic flow velocity (PSV) and end diastolic flow velocity (EDV) were recorded using the uterine artery Doppler waveform. The uterine artery resistance index was calculated using the following formula: UARI = (PSV-EDV)/PSV. Uterine artery resistance index was determined for the uterine artery bilaterally at three levels and the mean was calculated for NP group (n=3), control RUPPs group and RUPPs+17OHP group (n=6-8/group).

**Determination of circulating CD4+ T cells**

Flow cytometric analysis was used to detect CD4+ T cells. Plasma was collected and peripheral blood leukocytes were isolated from plasma by centrifugation on a cushion of Ficoll-Hypaque (Lymphoprep; Accurate Chemical & Scientific Corp, Westbury, NY) according to the manufactures instructions. For flow cytometric analysis, equal numbers of leukocytes (1x10^6) were incubated for 30 minutes at 4°C with antibodies against rat CD4 conjugate to fluorescein isothiocyanate (BD Biosciences Pharmingen, San Diego, CA). As a negative control for each individual rat, cells were treated exactly as described above except that they were incubated with anti-fluorescein isothiocyanate secondary antibodies alone. Subsequently, cells were washed and suspended in 500 µL of Roswell Park Memorial Institute medium and analyzed for single staining on a Gallios flow cytometer (Beckman Coulter, Brea, CA). The percentage of positive staining cells above the negative control was collected for each individual rat, and mean value for each experimental group were calculated.

**Western blotting analysis of endothelial nitric oxide synthase (eNOS)**

Vascular eNOS expression was evaluated in the aortas (n=5-7/group). Briefly, aortic extracts were homogenized in cold RIPA-buffer. Fifty-five micrograms of protein extracts were separated by SDS-PAGE using a polyacrylamide gel (4-20%) and the proteins were transferred onto nitrocellulose membranes (BioRad, USA).
Different membranes were blocked with Blocking Buffer (LI-COR, Biosciences, Lincoln, NE) for 1 h at room temperature and incubated overnight at 4°C with primary antibody directed against eNOS (1:250; BD Transduction Laboratories). The membranes were then incubated with secondary antibody (IRDeye700-conjugated affinity-purified anti-mouse IgG (1:5000; Rockland, Gilbertsville, PA) and scanned using Odyssey Infrared Imaging System (LI-COR, Biosciences, Lincoln, NE). The intensity of specific bands was quantified by densitometry using Image J (National Institutes of Health, USA) and aortic eNOS expression was normalized with respect to β-actin expression (1:5000, Cambridge, USA).

**Determination of TNF-α levels**

Circulating TNF-α was measured using rat parameter TNF-α ELISA kit from R&D Systems (Quantikine) according the manufacturer’s instructions. This assay displayed a sensitivity level of 5 pg/mL, with inter- and intra-assay variability of 10% and 5.1%, respectively.

**Circulating nitrate/nitrite bioavailability**

Forty microliters maternal plasma in duplicate (n=6-8/group) were used to measure circulating total nitrate/nitrite evaluated by Nitrate/Nitrite Colorimetric Assay Kit from Cayman Chemical following instructions outlined by the manufacturer. The inter-assay coefficient of variation is 3.4% while intra-assay coefficient of variation is 2.7%.

**Determination of plasma 8-isoprostane concentrations**

To evaluate oxidative stress, plasma 8-isoprostane (8-isoPGF₂α) concentrations (n=3-6/group) were measured in duplicate with commercially available enzyme linked immunosorbent (ELISA) assays kits (Cayman Chemical Company, Ann Arbor, MI, USA), according to manufacturer’s instructions.
References:
