Chronic hyperleptinemia results in the development of hypertension in pregnant rats

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Palei AC, Spradley FT, Granger JP. Chronic hyperleptinemia results in the development of hypertension in pregnant rats. Am J Physiol Regul Integr Comp Physiol 308: R855–R861, 2015. First published March 11, 2015; doi:10.1152/ajpregu.00286.2014.—Despite the fact that obesity is a major risk factor for preeclampsia (PE), the pathophysiological mechanisms whereby obesity and metabolic factors such as leptin increase this risk are unclear. While human data have shown that hyperleptinemia is associated with PE, the long-term effect of hyperleptinemia on blood pressure during pregnancy is unknown. Thus we tested the hypothesis whether chronic circulating leptin elevations in pregnant rats increase blood pressure and placental factors known to play a role in PE. On gestational day (GD)14, rats were assigned to the normal pregnant group with food intake ad libitum (control), leptin-treated (0.5 μg·kg−1·min−1 ip) pregnant group with food intake ad libitum (pregnant+LEP), and normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (pregnant-FR). On GD19, mean arterial pressure (MAP) was assessed and tissues were collected. Serum leptin concentration was elevated in pregnant+LEP compared with control and pregnant-FR (18.0 ± 2.8 vs. 0.8 ± 0.1 vs. 0.3 ± 0.1 ng/ml; P < 0.05), which was associated with increased MAP (121.3 ± 8.1 vs. 102.4 ± 2.4 vs. 101.3 ± 1.8 mmHg; P < 0.05). Food intake and body weight were reduced in pregnant+LEP and pregnant-FR by the end of gestation. Additionally, placentas and fetuses of these groups were lighter than those of control. However, placental expression of tumor necrosis factor-α was significantly greater in pregnant+LEP compared with controls (1.6 ± 0.1 vs. 1.1 ± 0.1 pg/mg; P < 0.05). In conclusion, leptin increases blood pressure and placental tumor necrosis factor-α during pregnancy despite its effect of reducing food intake and body weight, and represents a mechanism whereby obesity can promote the development of hypertension in PE.

hyperleptinemia; blood pressure; pregnancy; hypertension; preeclampsia

PREECLAMPSIA is a serious pregnancy disorder characterized by hypertension and proteinuria, which affects between 3% and 5% of pregnancies worldwide (21). Besides being a major cause of maternal and perinatal mortality and morbidity (16, 24), preeclamptic women and their offspring are at increased risk for developing cardiovascular diseases later in life (13, 35). In addition, there are several recognized risk factors for preeclampsia, including preexisting chronic diseases such as hypertension, diabetes, and obesity (14). Indeed, epidemiological studies indicate that obesity (defined as a body mass index ≥30 kg/m2) is associated with an up to fivefold increase in the rate of preeclampsia (34).

Many metabolic adaptations occur during pregnancy to provide proper nutrient supply to the growing fetus. At first and second trimesters, maternal metabolism is predominantly anabolic targeting the storage of a large amount of nutrients, as evidenced by an accumulation of depots of body fat. At the third trimester, however, maternal metabolism is catabolic directing the transfer of nutrients to the fetus through the placenta, as verified by increases in circulating levels of cholesterol, triglycerides, and free fatty acids during this last stage of pregnancy (56). The adipose tissue also exerts endocrine and paracrine functions in gestation as it produces and secretes hormones such as leptin. (55). Leptin is the peptide product of the obese (ob) gene, and it has been identified as a modulator of numerous physiological processes including food intake, adipose storage, and reproduction (19, 58). Indeed, circulating leptin levels are greater in pregnant women than their nonpregnant counterparts. However, hyperleptinemia over the levels seen in normal pregnancy has been associated with preeclampsia (19, 47). This is important because leptin has been shown to have direct effects on blood pressure regulation in male rats (51). Clinical studies have also shown that increased circulating leptin levels are associated with increased risk for the development of preeclampsia (43, 50), and the degree of hyperleptinemia in preeclamptic women is correlated with disease severity (1). Therefore, because hyperleptinemia is consistently observed in obese humans and animal models (58), this is a potential mechanism that links obesity with the development of hypertension in preeclampsia.

Growing evidence supports that incomplete remodeling of uterine spiral arteries results in reduced placental perfusion. The hypoxic/ischemic placenta, in turn, releases multiple mediators into the maternal circulation, such as the anti-angiogenic factor soluble fms-like tyrosine kinase (sFlt)-1 and the inflammatory cytokine tumor necrosis factor (TNF)-α, which leads to widespread maternal endothelial dysfunction and then the clinical symptoms of preeclampsia (45). Recent clinical studies have reported a positive association of circulating leptin with sFlt-1 (15) or TNF-α (7) in preeclampsia. However, the direct effect of hyperleptinemia on these placental factors and blood pressure regulation during pregnancy is unknown. Therefore, the purpose of the present study was to test the hypothesis that chronic exposure to hyperleptinemia increases blood pressure and placental sFlt-1 and TNF-α levels in pregnant rats.

METHODS

Animals. All protocols were approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Age-matched, timed-pregnant Sprague-Dawley rats (Harlan, Indianapolis, IN) were received on gestational day (GD) 11. They were maintained on a 12–12-h light:dark cycle at
23°C. On GD 14, rats were randomly assigned to the normal pregnant group with food intake ad libitum (pregnant, n = 8–12), leptin-treated pregnant group with food intake ad libitum (pregnant+LEP, n = 8–12), or normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (pregnant-FR, n = 11). Water was provided ad libitum to all groups. From GD 13 to GD 19, body weight (BW), food intake (FI), and water intake (WI) were recorded daily. We did not consider FI and WI on GD 15 and 19 because animals were still recovering from the surgeries performed on GD 14 (mini-pump placement, see Chronic leptin treatment) and GD 18 (carotid catheter placement, see Blood pressure measurement in conscious rats), respectively.

Chronic leptin treatment. On GD 14, under isoflurane anesthesia (Butler Schein Animal Health, Dublin, OH), which was delivered by an anesthetic vaporizer (Ohmeda, BOC Health Care, Steeton, UK), an osmotic minipump (model 2ML1, Alzet, Cupertino, CA) was placed intraperitoneally in the pregnant+LEP group to deliver leptin (National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA) at a dose of 0.5 μg·kg⁻¹·min⁻¹ (25) for 5 days.

Blood pressure measurement in conscious rats. On GD 18, rats were anesthetized with isoflurane as described above and implanted with indwelling carotid catheters consisting of V-1 tubing attached to V-3 tubing (Scientific Commodities, Lake Havasu City, AZ). Catheters were tunneled under the skin and externalized at the scapular region. On GD 19, rats were placed in individual restraining cages and catheters connected to pressure transducers (MLT0699, ADInstruments, Colorado Springs, CO) coupled to a computerized data acquisition system (PowerLab and Lab Chart Pro V7 software, ADInstruments). Animals were allowed to acclimate to restraint for approximately 1 h. Once hemodynamic readings stabilized, mean arterial blood pressure was recorded for at least another 1 h.

Tissue harvest. On GD 19, rats were anesthetized with isoflurane as mentioned before and a ventral midline incision was made to externalize the uterus. Blood was collected into Corvac separator tubes (for collection of serum; Tyco Healthcare Kendall, Mansfield, MA) and Vacutainer K₂EDTA tubes (for collection of plasma; BD, Franklin Lakes, NJ) by puncturing the abdominal aorta. The number of viable and reabsorbed fetuses in each animal was recorded, and individual fetuses and placentas were weighed. Representative placentas from each horn were flash frozen in liquid nitrogen and stored at −80°C until processed. Serum and plasma samples were obtained by centrifugation of whole blood at 2,000 g for 12 min at 4°C and stored at −20°C until assayed.

Serum and plasma measurements. Serum leptin and insulin concentrations were quantified by ELISA (both from R&D Systems, Minneapolis, MN) following the manufacturer’s instructions. Plasma total cholesterol (Cayman Chemical, Ann Arbor, MI) was quantified by fluorimetric assay, whereas glucose (Cayman Chemical), triglycerides (Cayman Chemical), and free fatty acids (Zen-bio, Durham, NC) were quantified by colorimetric assays.

Placental measurements. Frozen placentas were crunched with mortar and pestle in liquid nitrogen, and tissue fragments were suspended in radioimmunoprecipitation (RIPA) lysis buffer with a protease inhibitor cocktail containing phenylmethyl-sulfonyl fluoride and sodium orthovanadate (all from Santa Cruz Biotechnology, Santa Cruz, CA). After homogenization with a glass dounce tissue grinder, homogenates were centrifuged at 14,000 g for 25 min at 4°C, and supernatants were used to quantify sFlt-1 and TNF-α by ELISA (both from R&D Systems). Total protein concentration in samples was measured using the bicinchoninic acid (BCA) method (Thermo Scientific, Rockford, IL).

Statistical analysis. Graphs and statistical analysis were prepared using GraphPad Prism 5.0 software (San Diego, CA). Comparisons among groups were performed using ANOVA followed by Tukey’s multiple comparison test. Values are shown as means ± SE. A value of P < 0.05 was considered statistically significant.

**RESULTS**

Maternal circulating leptin and blood pressure. At GD 19, chronic leptin treatment produced a significant increase in serum leptin concentration compared with those observed in pregnant and pregnant-FR groups (Fig. 1A; P = 0.0001). Mean arterial pressure (MAP) was also significantly increased by ~20 mmHg in pregnant+LEP rats compared with pregnant and pregnant-FR rats as measured at GD 19 (Fig. 1B; P < 0.05).

Placental anti-angiogenic and inflammatory factors. At GD 19, placental TNF-α concentration was increased in pregnant+LEP rats compared with pregnant and pregnant-FR rats (Fig. 2A; pregnant+LEP vs. pregnant P < 0.01), but pregnant-FR rats presented statistically similar TNF-α levels to pregnant and pregnant+LEP rats (Fig. 2A; both P > 0.05). There was no difference in placental sFlt-1 concentration among groups (Fig. 2B; P > 0.05).

Fetal and placental weights. As shown in Fig. 3, placental and fetal weights from pregnant-FR and pregnant+LEP groups were significantly reduced compared with those from the pregnant group as measured at GD 19 (Fig. 3, B and C; P < 0.001 and P < 0.0001, respectively). However, there was no
difference in the ratio of fetal weight to maternal BW among pregnant fed ad libitum, pregnant with reduced food intake, and leptin-treated pregnant rats (0.0070 ± 0.0003 vs. 0.0073 ± 0.0001 vs. 0.0069 ± 0.0003, respectively; P > 0.05). In addition, litter size was not statistically different among groups (Fig. 3A; P > 0.05).

Maternal body weight and food intake. Initial BW at GD 14 was similar among rats designated for the pregnant group (257.0 ± 4.7 g), the pregnant-FR group (264.4 ± 2.1 g), and the pregnant + LEP group (263.4 ± 2.9 g) (Fig. 4A; P > 0.05). Baseline FI and WI were assessed from GD 13 to 14, and no differences in FI (15.8 ± 1.5 vs. 17.3 ± 0.5 vs. 17.0 ± 1.0 g/day; Fig. 1B; P > 0.05) or WI (38.0 ± 4.6 vs. 37.3 ± 4.7 vs. 36.1 ± 3.0 ml/day; P > 0.05) were found among pregnant, pregnant-FR, and pregnant + LEP groups, respectively.

By the end of the study, at GD 18, BWs in pregnant-FR and pregnant + LEP rats (280.1 ± 2.6 and 284.1 ± 3.9 g, respectively) were decreased compared with pregnant rats (310.3 ± 6.6 g) (Fig. 4A; P < 0.01). From GD 16 to 18, pregnant + LEP rats had lower FI than pregnant rats (GD 16: 6.8 ± 0.8 vs. 21.5 ± 0.4 g/day; GD 17: 9.3 ± 1.1 vs. 23.8 ± 0.7 g/day; and GD 18: 11.3 ± 1.2 vs. 25.2 ± 1.1 g/day; Fig. 4B; all P < 0.0001). Since the mean GD 16–18 FI of pregnant + LEP rat was 8.6 ± 0.8 g/day, we fed pregnant-FR rat 9.0 g/day of chow from GD 14 to 19. WI was lower in pregnant-FR and pregnant + LEP dams than pregnant dams on GD 16–18 (GD 16: 37.8 ± 2.0 vs. 23.5 ± 0.9 vs. 49.9 ± 1.7 ml/day; GD 17: 37.5 ± 2.9 vs. 29.5 ± 1.9 vs. 58.9 ± 2.2 ml/day, and GD 18: 33.7 ± 2.3 vs. 38.3 ± 2.6 vs. 60.0 ± 2.1 ml/day; all P < 0.0001), respectively.

Maternal circulating metabolic factors. As shown in Table 1, plasma glucose and serum insulin were not statistically different between pregnant and pregnant + LEP groups as measured at GD 19 (both P > 0.05). However, the pregnant-FR group presented lower circulating glucose levels compared with both pregnant and pregnant + LEP groups (P < 0.0001).
DISCUSSION

The main finding reported here is that chronic hyperleptinemia increases MAP in pregnant rats. This alteration during chronic leptin excess was associated with increased placental TNF-α. In parallel to decreases in FI and BW, circulating cholesterol, triglyceride, and free fatty acid levels were reduced in leptin-treated pregnant rats. These data suggest that the hyperleptinemia encountered in obesity is itself, in the absence of additional metabolic disturbances, an important link between obesity and the development of hypertension during pregnancy.

In support of the involvement of leptin in the pathophysiology of preeclampsia are several reports showing that preeclamptic women have increased circulating leptin concentrations compared with normal pregnant women (1, 6, 18, 20, 36, 38, 40, 41, 47). Importantly, some reports have suggested that serum leptin can be used as a predictive marker for this syndrome. Hyperleptinemia appears to develop in preeclamptic women during first and second trimester (5, 11, 36, 43, 46, 50), i.e., before the manifestation of the clinical symptoms. Moreover, positive correlations between circulating levels of leptin and sFlt-1 (15) and between leptin and TNF-α (7) have been noted in preeclamptic women. Although these association studies did not show a cause or effect relationship between hyperleptinemia and preeclampsia, collectively these findings prompted us to examine whether hyperleptinemia alters blood pressure and placental anti-angiogenic and inflammatory factors during pregnancy.

Our study clearly demonstrated that chronic leptin treatment increases MAP in pregnant rats. One pathway by which hyperleptinemia may elicit hypertension during pregnancy is through its role in regulating angiogenic and inflammatory processes (8, 44), which are known to be implicated in preeclampsia. Interestingly, the increased blood pressure in our leptin-treated pregnant rats was accompanied by an elevation in placental TNF-α; however, sustained hyperleptinemia did not alter placental sFlt-1. As demonstrated previously, leptin can stimulate the release of TNF-α from placental tissue explants (29). In addition, leptin in doses comparable to those achieved during first and third trimester of gestation can act as a proinflammatory cytokine upregulating the production of TNF-α by mononuclear leukocytes (44). Moreover, serum leptin (39) and serum and placental TNF-α (26, 27) are elevated in the reduced uterine perfusion pressure (RUPP) model of preeclampsia in rats. Furthermore, chronic TNF-α infusion also increases MAP in normal pregnant rats, and inhibition of TNF-α with entanercept attenuates the blood pressure (42).

and higher insulin levels compared with the pregnant+LEP group (P < 0.05). In addition, plasma cholesterol and triglyceride concentrations were decreased in pregnant-FR and pregnant+LEP rats compared with their normal pregnant counterparts (P < 0.01 and P < 0.001, respectively). Moreover, plasma free fatty acid was reduced in pregnant+LEP rats compared with pregnant rats (P < 0.01), but these levels were similar to the free fatty acid level observed in the pregnant-FR group.

Table 1. Effects of chronic leptin infusion and food restriction in pregnant rats on circulating metabolic measurements at gestational day 19

<table>
<thead>
<tr>
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<th>Pregnant</th>
<th>Pregnant-FR</th>
<th>Pregnant + LEP</th>
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</thead>
<tbody>
<tr>
<td>Plasma glucose concentration, mg/dl</td>
<td>161.8 ± 14.2</td>
<td>104.6 ± 3.1*</td>
<td>160.5 ± 5.8#</td>
</tr>
<tr>
<td>Serum insulin concentration, μU/ml</td>
<td>9.6 ± 0.8</td>
<td>14.4 ± 2.4</td>
<td>7.7 ± 2.3#</td>
</tr>
<tr>
<td>Plasma cholesterol concentration, mg/dl</td>
<td>250.5 ± 21.9</td>
<td>105.4 ± 6.9*</td>
<td>111.8 ± 27.7*</td>
</tr>
<tr>
<td>Plasma triglyceride concentration, mg/dl</td>
<td>494.5 ± 92.1</td>
<td>160.1 ± 23.1*</td>
<td>91.1 ± 33.7*</td>
</tr>
<tr>
<td>Plasma FFA concentration, mg/dl</td>
<td>105.1 ± 27.9</td>
<td>53.0 ± 7.7</td>
<td>33.7 ± 11.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Pregnant, normal pregnant group with food intake ad libitum (n = 8); pregnant+LEP, leptin (0.5 μg·kg⁻¹·min⁻¹)-ip-treated pregnant group with food intake ad libitum (n = 8); pregnant-FR, normal pregnant group with food intake adjusted to the food intake of pregnant+LEP rats (n = 11); FFA, free fatty acids. *P < 0.05 vs. pregnant. #P < 0.05 vs. pregnant-FR.
pressure response in RUPP rats (3, 26). TNF-α-induced hyper-
tension in pregnant rats is mainly due to activation of the 
endothelin (ET) system (28). Nonetheless, leptin is also able to 
increase ET-1 production and ET-1 receptors in endothelial 
cells (23). Thus another mechanism whereby hyperleptinemia 
may increase blood pressure is through exacerbation of ET-1 
synthesis directly or indirectly via TNF-α. Since we observed 
an increase in circulating cholesterol, triglyceride, and free fatty 
acid levels in leptin-treated pregnant rats, a role for hyperlip-
idemia in mediating the blood pressure response to chronic 
hyperleptinemia can be disregarded.

An alternative mechanism that may play a role in how 
hyperleptinemia elicits increases in blood pressure during preg-
nancy is by stimulation of the sympathetic nervous system. 
Earlier studies in male rats by Hall and colleagues have found 
that chronic leptin infusion increases MAP and heart rate (10, 
25, 51). They showed that pharmacological antagonism of α1-
and β-adrenergic receptors prevented the blood pressure and 
cardiac responses to hyperleptinemia (10). Additional evidence 
for the hypertensive effect of leptin derives from studies with 
transgenic mice overexpressing leptin in the liver, which pre-
sewed higher plasma leptin levels and MAP than nontrans-
genic control mice. The increased blood pressure in these 
animals was also normalized after adrenergic or ganglionic 
blockade (2). The proposed pathway for leptin-induced hyper-
tension involves activation of neurons in the brain which 
stimulate the renal sympathetic nerves, leading ultimately to an 
increase in blood pressure (12). However, blood pressure 
regulation is different in male and female, especially during 
gestation (32, 57). Indeed, the blood pressure response to leptin 
observed in our pregnant rats (ΔMAP ~20 mmHg) is higher 
than that documented in male rats (ΔMAP ~6–8 mmHg) (10, 
25, 51). Interestingly, a recent report found that acute intra-
rebroventricular infusion of leptin enhances renal sympathetic 
activity, but not MAP, in nonpregnant female rats (52). This 
effect of leptin on stimulating renal nerves was observed only 
during the pro-estrous phase, suggesting that high estrogen 
levels are necessary to increase MAP in response to chronic 
leptin infusions. Therefore, while increased renal sympathetic 
activity has been reported to play a role in leptin-induced 
hypertension in males, the involvement of the sympathetic 
nervous system in modulating the blood pressure response to 
leptin during pregnancy remains to be determined.

Although human obesity is associated with hyperleptinemia, 
prior studies indicate that obese subjects are resistant to the 
anorexics effects but remain sensitive to the hypertensive ac-
tions of leptin. Most rat and mouse models of obesity also 
develop hyperleptinemia and increased caloric intake with 
preserved blood pressure responsiveness to leptin. Distinct 
intracellular signaling pathways of the leptin receptors allow 
leptin to control separately these physiological processes (17, 
58). This pattern of selective leptin resistance seems also to 
occur in preeclampsia. Obesity markedly increases the risk for 
developing preeclampsia (49) and preeclamptic women have 
exacerbated hyperleptinemia and hypertension. Therefore, it 
was not surprising that using lean Sprague-Dawley normal 
pregnant rats, we noted both metabolic and cardiovascular 
effects of leptin. In addition, caloric intake was 
similar between normal pregnant rats with food restriction 
and leptin-treated pregnant rats, leptin interestingly abol-
ished the effects of food restriction on decreasing plasma 
glucose and increasing serum insulin. All these results (i.e., 
reduced food intake and body weight, no difference in 
circulating glucose but reduced insulin levels, and elevated 
blood pressure) are in line with previous findings in lean 
Sprague-Dawley male rats (25).

Intriguingly, both obese (20, 36, 43) and nonobese (5, 18, 
50) preeclamptic women present with exacerbated hyperlep-
tinemia, suggesting that another source of leptin besides the 
adipose tissue contributes to the increased circulating leptin 
levels seen in preeclampsia. Both human (9, 33) and rat (4, 53) 
placentas have been shown to produce leptin as well express 
leptin receptors. Indeed, placental leptin gene and protein 
expressions are elevated in preeclampsia (18, 22, 31, 48). 
However, it is unknown to what extent leptin derived from 
adipose and/or placental tissues is involved in pregnancy-
duced hypertension.

Regarding fetal outcome, we observed a decrease in fetal 
and placental weights in leptin-treated pregnant rats. An in-
verse correlation between maternal circulating leptin and birth 
weight has been noted in humans (6, 37, 38, 46), suggesting 
that hyperleptinemia can lead to decreased fetal growth. 
Indeed, pregnant women with intrauterine growth restriction 
(IUGR) exhibit exacerbated hyperleptinemia (30, 37). Like-
wise, preeclamptic women with IUGR have higher circulating 
leptin levels than preeclamptic women with normal fetal 
growth (40). However, since fetal and placental weights were 
comparable in normal pregnant rats with food restriction and 
leptin-treated pregnant rats, we conclude that the decrease in 
fetal and placental weights following leptin infusion resulted 
from the reduced food intake of dams instead of a direct effect 
of leptin. Previous reports have already addressed whether 
maternal hyperleptinemia over the levels found in normal 
pregnancy affects fetal programming, and they found that in 
uterine exposure to high leptin levels modifies the development 
of energy balance regulatory systems (54) and leads to lean 
offspring with reduced skeletal growth (42).

Perspectives and Significance

In this study, we tested the hypothesis that chronic hyper-
leptinemia increases blood pressure and placental factors in 
pregnant rats. Our results show that sustained hyperleptinemia 
does raise blood pressure and increase placental TNF-α. 
Understanding the molecular pathways that regulate the actions 
of leptin in the cardiovascular system during pregnancy may offer 
novel pharmacological agents for the treatment of preeclampsia.

Obesity is a major risk factor for the onset of preeclampsia. 
Recent epidemiological data suggest that the rate of preeclampsia 
has increased largely due to a significant increase in the 
incidence of metabolic diseases such as obesity, but the mecha-

isms explaining this relationship are still unclear. Preeclamps-
tic pregnancies are associated with exacerbated hyperleptine-
mia. Importantly, we have direct evidence that chronic hyper-
leptinemia in pregnant rats increases blood pressure despite 
decreasing body weight and numerous metabolic factors com-
monly associated with the obese milieu. Therefore, our data 
implicate hyperleptinemia in itself as an important link be-
 tween obesity and the development of hypertension during 
preeclampsia.
HYPERLEPTINEMIA INCREASES BLOOD PRESSURE IN PREGNANT RATS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.C.P. and J.P.G. conception and design of research; A.C.P. performed experiments; A.C.P., F.T.S., and J.P.G. interpreted results of experiments; A.C.P., F.T.S., and J.P.G. drafted manuscript; A.C.P., F.T.S., and J.P.G. edited and revised manuscript; A.C.P., F.T.S., and J.P.G. approved final version of manuscript.

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