Vitamin D has a broader physiological role than simply as a contributor to bone health. Recent studies suggest that low 25-hydroxyvitamin D levels have clinical consequences on the cardiovascular system, and that vitamin D deficiency may be associated with an increased risk of hypertension, an elevated incidence of cardiovascular disease, and higher mortality in the general population.

During pregnancy, low maternal vitamin D levels have also been linked to adverse maternal outcomes such as preeclampsia, although some data are still controversial. Furthermore, studies on the relationship of serum vitamin D and maternal blood pressure are scarce and have several methodological flaws. Moreover, such studies have not yet been conducted on women at high risk of hypertensive disorders of pregnancy, such as patients with diabetes.

Therefore, the goal of this study was to evaluate the association between maternal blood pressure and serum 25-hydroxyvitamin D in pregnant women with gestational diabetes mellitus (GDM).

METHODS
Study population and research design
All women with a diagnosis of GDM who were referred to high-risk prenatal care at the Hospital de Clínicas de Porto Alegre, Brazil, between November 2009 and May 2012, were invited to participate in this study. Participants were included in the cohort during the third trimester of pregnancy and were prospectively followed until the postpartum period. All participants provided informed written consent, and the study was approved by the Research Ethics Committee of the hospital in which the study was conducted.

GDM was diagnosed based on the guidelines issued following the 2nd Meeting of the Diabetes and Pregnancy Task Force, which recommend the following criteria for diagnosis:

- Fasting plasma glucose (FPG) ≥ 126 mg/dL (7.0 mmol/L)
- 1-hour plasma glucose (1-h PG) ≥ 200 mg/dL (11.1 mmol/L)
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patients with positive screening results (fasting plasma glucose ≥85 mg/dl) underwent a 75-g oral glucose tolerance test, and GDM was diagnosed if fasting plasma glucose was ≥110 mg/dl or 2-hour plasma glucose was ≥140 mg/dl. Starting in 2010, the new diagnostic criteria issued by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) were also used to diagnose GDM: fasting plasma glucose ≥92 mg/dl, or 1-hour plasma glucose ≥180 mg/dl, or 2-hour plasma glucose ≥153 mg/dl after the 75-g load.

A standardized questionnaire about sociodemographic characteristics, medical history, and the pregnancy itself was answered by all women at enrollment, and physical examinations were performed in all prenatal visits. Body mass index (BMI) was calculated by dividing the reported prepregnancy body weight by the square of participant height. Blood pressure was measured according to World Health Organization recommendations. Women were calm and at rest for 10 minutes before taking blood pressure, and both arms were considered suitable for the measurement. The sitting position was acceptable for the first blood pressure measurement, but an abnormal reading was rechecked after 15 minutes with the patient lying on the left side. Mean blood pressure was calculated as (systolic blood pressure + diastolic blood pressure / 2). The first and the last blood pressure measurements were collected at the beginning and end of the third trimester of pregnancy, respectively. Gestational hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on at least 2 occasions after 20 weeks of gestation. Preeclampsia or preeclampsia superimposed on chronic hypertension was diagnosed in patients with proteinuria of at least 300 mg/L in 2 random urine specimens and who had been previously diagnosed with gestational hypertension or chronic hypertension, respectively. Lastly, eclampsia was defined as the occurrence of tonic-clonic seizures in hypertensive women.

Fasting maternal blood samples were collected for the assessment of 25-hydroxyvitamin D in the third trimester, and the serum was stored at −80 °C until the delivery of all pregnancies. Blood was sampled in all seasons. Serum 25-hydroxyvitamin D was measured by chemiluminescence immunoassay (DiaSorin LIAISON, Stillwater, MN), which can detect vitamin D levels between 4.0 and 150 ng/ml. The coefficients of variation of interassay analyses were <4% and <6% for 15 and 50 ng/ml values, respectively. Vitamin D deficiency was defined as serum 25-hydroxyvitamin D <20 ng/ml, and insufficiency was defined as levels <30 ng/ml, in agreement with current Endocrine Society guidelines.

Statistical analysis

Descriptive statistics were used to analyze clinical and demographic variables. Continuous parametric variables were evaluated using Student t tests. Correlations between blood pressure and serum vitamin D levels were evaluated with Pearson correlation coefficients, and a multiple linear regression was used to study the predictors of blood pressure levels. A general mixed model regression (Poisson regression with robust standard errors) was used to study the predictors of hypertensive disorders of pregnancy (gestational hypertension, preeclampsia, or eclampsia). Models were adjusted for potential confounders such as age, blood pressure, ethnicity, previous hypertensive disorders of pregnancy, nulliparity, maternal glucose on an oral glucose tolerance test, HbA1c levels, BMI, use of diabetes medication, and season. Differences between ethnic subgroups were also investigated.

Statistical analyses were performed with the SPSS software, version 20.0 (SPSS, Chicago, IL). Statistical significance was considered when P < 0.05.

RESULTS

One hundred eighty-four women were included in the study. Eighteen women were diagnosed with GDM by IADPSG thresholds, whereas the remaining 166 met Brazilian and IADPSG criteria. Patients’ clinical and laboratory characteristics are shown in Table 1. Vitamin D deficiency was diagnosed in 33.2% of the sample, and insufficiency was found in 33.2% of the women. As expected, women with normal serum 25-hydroxyvitamin D had blood samples drawn more frequently in the summer (56% vs. 28%; P = 0.02). Mean gestational age of blood drawn was 31.5 ± 4 weeks. Gestational hypertension occurred in 26 case patients (14.1%), and preeclampsia or eclampsia occurred in 16 case patients (8.2%).

Serum 25-hydroxyvitamin D levels had a significant negative correlation with systolic blood pressure at the beginning of third trimester (r = −0.146; P = 0.049). However, this relationship was no longer significant (P = 0.08) after conducting a multiple linear regression adjusting for BMI and history of hypertensive disorders in a previous pregnancy. Other blood pressure measurements—diastolic blood pressure at the beginning of the third trimester and both systolic and diastolic blood pressure at the end of the third trimester—had no significant correlation with serum vitamin D.

When blood pressure was compared between women with and without vitamin D insufficiency, those with serum 25-hydroxyvitamin D <30 ng/ml were found to have higher blood pressure at the beginning and end of the monitoring period (Table 2). When the same analysis was performed with a vitamin D cutoff of 20 ng/ml, no differences were detected between the groups of women with and without vitamin D deficiency.

Blood pressure at the beginning (P < 0.001 for both systolic and diastolic) and end of the third trimester (P = 0.001 for both systolic and diastolic) was a significant predictor of hypertensive disorders of pregnancy (gestational hypertension, preeclampsia, or eclampsia) after adjusting for previous hypertensive disorders of pregnancies, season, ethnicity, and HbA1c levels.

Of the 48 non-white women in the sample, 24 were self-reported black and 24 self-identified as brown. Ethnic group analyses revealed that white women had a mean serum vitamin D concentration of 20.5 ± 8.8 ng/ml, whereas women with darker skin tones had concentrations of 17.5 ± 7.7 ng/ml (P = 0.04). We found no difference between white and dark-skinned women in the incidence of gestational hypertension (12.5% vs. 18.8%; P = 0.29) or of preeclampsia/eclampsia (8.1% vs. 10.4%; P = 0.63). Blood pressure was similar
between groups throughout the prenatal monitoring period. There were also no between-group differences in clinical or laboratory characteristics, except for urinary albumin excretion, which was higher in dark-skinned women (median 7.8 mg/24 hours, interquartile range 3.5–15.2 mg/24 hours vs. 3.9 mg/24 hours, 0–9.7 mg/24 hours; P = 0.005). In white women, serum 25-hydroxyvitamin D had a significant negative correlation with systolic blood pressure at the beginning and end of the third trimester (Figure 1). Diastolic blood pressure was not significantly correlated with vitamin D at the beginning (P = 0.12) or at the end (P = 0.06) of the third trimester. The association between serum 25-hydroxyvitamin D and blood pressure persisted even after adjusting for significant confounders (BMI and season) in a multiple linear regression analysis (β coefficient = −0.257, P = 0.009 for systolic blood pressure at the beginning of third trimester; β coefficient = −0.410, P = 0.006 for systolic blood pressure at the end of third trimester). In women with darker skin tones, vitamin D did not influence blood pressure after adjusting for confounders.

### DISCUSSION

Serum vitamin D insufficiency was associated with higher blood pressure levels during the third trimester of pregnancy in a cohort of women with GDM. To our knowledge, this is the first study to evaluate blood pressure in hyperglycemic pregnant women who are at high risk of gestational hypertension and preeclampsia.

In general, studies on the association between preeclampsia and vitamin D do not display blood pressure data. One exception to this observation is the nested case–control study conducted by Powe et al., which did not find any association between total 25-hydroxyvitamin D and blood pressure in the first trimester of pregnancy. Similar results were reported when only white women were analyzed. A study of Pakistani parturients found maternal 25-hydroxyvitamin D levels to be inversely correlated with mean blood pressure at the time of admission to the labor unit. Another nested case–control study conducted in pregnant Chinese women showed an independent association between serum 25-hydroxyvitamin D levels and systolic blood pressure 26–28 weeks into pregnancy in women who had normal glucose tolerance. Therefore, although studies of the association between blood pressure and vitamin D levels in pregnancy are scarce, the available data, including our results, suggest that vitamin D levels may influence blood pressure at the end of pregnancy but not during the first trimester. This correlation does not necessarily indicate causality, and further studies are imperative to determine the role of 25-hydroxyvitamin D in blood pressure levels during pregnancy.

### Table 1. Clinical and laboratory characteristics in women according to vitamin D status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>25-hydroxyvitamin D ≥30 ng/ml (n = 25)</th>
<th>25-hydroxyvitamin D &lt;30 ng/ml (n = 159)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-hydroxyvitamin D, ng/ml</td>
<td>33.2 ± 3.8</td>
<td>17.6 ± 7.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>32.3 ± 6.4</td>
<td>32.0 ± 6.0</td>
<td>0.32</td>
</tr>
<tr>
<td>White race</td>
<td>21 (84.0)</td>
<td>115 (72.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>Parity, number of pregnancies</td>
<td>2 (2–4)</td>
<td>3 (2–4)</td>
<td>0.54</td>
</tr>
<tr>
<td>Previous HDP</td>
<td>4 (16.0)</td>
<td>36 (22.6)</td>
<td>0.61</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>1 (4.0)</td>
<td>20 (12.6)</td>
<td>0.32</td>
</tr>
<tr>
<td>Smoking</td>
<td>2 (8.0)</td>
<td>19 (11.9)</td>
<td>0.74</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.7 ± 5.1</td>
<td>30.2 ± 6.9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

| 75-g OGTT, ng/dl                |                                     |                                       |         |
| Fasting glucose                 | 97 ± 33                              | 101 ± 26                              | 0.46    |
| 1-h glucose                     | 166 ± 49                             | 179 ± 26                              | 0.46    |
| 2-h glucose                     | 163 ± 19                             | 163 ± 28                              | 0.96    |
| Urinary albumin excretion, mg/24 h | 3.0 (0–4.2)                         | 5.4 (0–11.8)                          | 0.04    |

Data are expressed as No. (%), mean± SD, or median (interquartile range).

Abbreviations: BMI, body mass index; HDP, hypertensive disorder of pregnancy; OGTT, oral glucose tolerance test.

### Table 2. Blood pressure measurements in women with high vs. low vitamin D levels

<table>
<thead>
<tr>
<th>Blood pressure measurement, mm Hg</th>
<th>25-hydroxyvitamin D ≥30 ng/ml (n = 25)</th>
<th>25-hydroxyvitamin D &lt;30 ng/ml (n = 159)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First blood pressure measurement, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>109 ± 12</td>
<td>116 ± 11</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>68 ± 11</td>
<td>73 ± 9</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean</td>
<td>82 ± 11</td>
<td>88 ± 9</td>
<td>0.006</td>
</tr>
<tr>
<td>Last blood pressure measurement, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>113 ± 10</td>
<td>120 ± 17</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic</td>
<td>73 ± 9</td>
<td>78 ± 11</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean</td>
<td>86 ± 8</td>
<td>92 ± 11</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD.
Hypovitaminosis D and Maternal Blood Pressure

One of the possible mechanisms for the relationship between serum vitamin D and blood pressure is the regulation of the renin-angiotensin system (RAS). Experimental studies suggest that 1,25-hydroxyvitamin D is a negative endocrine regulator of the RAS. Vitamin D receptor–null mice had an elevation of renin expression and, consequently, an increase in plasma angiotensin II production, leading to hypertension. Moreover, 1,25-hydroxyvitamin D treatment resulted in renin suppression. These findings were independent of calcium metabolism. In humans, recent data from a large cohort showed that vitamin D decreasing values were independently related to RAS upregulation. This relationship between vitamin D, hypertension, and RAS may also be relevant in pregnancy, and RAS dysregulation has recently been linked with preeclampsia. Overexpression of many components of the RAS occurs in normal pregnancy, such as an increase in renin, angiotensinogen liver, and angiotensin II. In patients with preeclampsia, the levels of RAS components are lower than in uncomplicated pregnancies, but there is an increased sensitivity to angiotensin II, with an exaggerated pressor response in these patients.

Our study also found that serum 25-hydroxyvitamin D is an independent predictor of blood pressure in white, but not in dark-skinned, women. Many disparities exist between the adverse outcomes rates calculated for white and dark-skinned women, and vitamin D has been considered one of the potential causative factors for such differences. Moreover, there are few data on pregnancy outcomes in black women, in spite of the fact that vitamin D deficiency is especially common in this ethnic group. Our data could contribute to the understanding of the differences between ethnic groups because vitamin D was found to be a predictor of blood pressure only in white women. Differences between the RAS activation in black and white women may explain the lack of significant correlation between vitamin D and blood pressure in this subgroup. Lastly, the darker-skinned women in our cohort had higher urinary albumin excretion values than the white women, a finding that is in agreement with reports of elevated rates of target-organ injury in black individuals as well as studies of RAS activation.

The importance of assessing blood pressure levels during pregnancy stems from the fact that higher blood pressure levels are a predictor of preeclampsia. Our results are in agreement with this finding. Preeclampsia is the leading cause of maternal morbidity and mortality, and its pathogenesis has not yet been fully elucidated. Therefore, it is important to obtain an improved knowledge of modifiable risk factors for higher blood pressure and preeclampsia.

The benefits of vitamin D supplementation in pregnancy and its effects on blood pressure also need further evaluation. One trial of combined vitamin D and calcium supplementation reported significantly lower blood pressure in the supplemented group at 32 and 36 weeks into pregnancy. Another more recent and smaller study randomly assigned 48 pregnant women to receive 400 IU/d of cholecalciferol supplements or placebo during the third trimester of pregnancy. Lower blood pressure was among the benefits reported in the metabolic profile of treated women. This finding is in agreement with our results.

Our study has some methodological strengths, such as its longitudinal design, the homogeneity of the population of pregnant women with GDM entered into the study, and the fact that this population has not been previously evaluated. On the other hand, some limitations were also present. First, vitamin D binding globulin was not measured. Second, the relatively small sample size of dark-skinned women may explain the lack of significant correlation between vitamin D and blood pressure in this subgroup. Third, GDM was diagnosed based on 2 different criteria. However, both the Brazilian and the IADPSG criteria for GDM are acceptable because there has been no consensus among international associations as to the best diagnostic criteria for the condition and the cutpoints for diagnosis have not yet been defined. Analysis of data after exclusion of women whose diagnosis was made solely by the IADPSG criteria did not change the results.

Figure 1. Correlation between 25-hydroxyvitamin D levels and blood pressure in white women. (a) Systolic blood pressure at the beginning of the third trimester. (b) Systolic blood pressure at the end of the third trimester.
In conclusion, serum vitamin D concentrations <30 ng/ml were associated with higher systolic and diastolic blood pressure levels during the third trimester of pregnancy in this cohort of women with gestational diabetes. Vitamin D levels were not independently correlated with blood pressure when the group was analyzed as a whole, but ethnic subgroup analyses revealed it to be an independent predictor of systolic blood pressure levels in white women. More studies are necessary to clarify the association between vitamin D levels and blood pressure in pregnancy.

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DISCLOSURE

The authors declared no conflict of interest.

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