Gestational diabetes mellitus (GDM) decreases butyrylcholinesterase (BChE) activity and changes its relationship with lipids

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Abstract

Many conditions interfere with butyrylcholinesterase (BChE) activity, e.g., pregnancy or presence of the BCHE gene variant -116A can decrease activity whereas obesity and types I and II diabetes mellitus can increase activity. In this study, we examined BChE activity, -116A and 1615A BCHE gene variants, and anthropometric and biochemical variables associated with diabetes in patients with gestational diabetes mellitus (GDM) and in healthy pregnant women. BChE activity was measured spectrophotometrically using propionylthiocholine as substrate and genotyping of the -116 and 1615 sites of the BCHE gene was done with a TaqMan SNP genotyping assay. Three groups were studied: 150 patients with GDM, 295 healthy pregnant women and 156 non-pregnant healthy women. Mean BChE activity was significantly lower in healthy pregnant women than in women from the general population and was further reduced in GDM patients. BChE activity was significantly reduced in carriers of -116A in GDM patients and healthy pregnant women. Although GDM patients had a significantly higher mean body mass index (BMI) and triglycerides than healthy pregnant women, they had lower mean BChE activity, suggesting that the lowering effect of GDM on BChE activity was stronger than the characteristic enhancing effect of increased BMI and triglycerides.

Key words: butyrylcholinesterase (BChE), gestational diabetes mellitus, -116A variant, 1615A variant.

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Introduction

Human butyrylcholinesterase (BChE; EC 3.1.1.8) is a serum esterase that hydrolyzes choline esters, as well as other esters, but its physiological function is not completely known (Chatonnet and Lockridge, 1989). BChE is found in plasma, liver, neuroglia, pancreas, digestive tube wall, lung, brain, muscle, kidney and heart (Siqueira et al., 1978). Obesity (Alcântara et al., 2003; Randell et al., 2005; Iwasaki et al., 2007), hypertension (Alcântara et al., 2002), and types I and II diabetes mellitus (DM1 and DM2) (Antopol et al., 1937; Abbott et al., 1993; Pavkovic et al., 1993) have been associated with an increase in BChE activity, whereas myocardial infarction (Whittaker, 1986) and some variants of the butyrylcholinesterase (BCHE) gene (Babaoğlu et al., 2004; Valle et al., 2006; Furtado-Alle et al., 2008) have been associated with a decrease in activity. A decrease in activity of ~25% has been observed after the tenth week of pregnancy, and a reduction of 33% occurs between ten days after delivery and six weeks of age (Whittaker, 1980). Evans and Wroe (1980) measured serum BChE activity in 941 pregnant women and observed a rapid fall during the first trimester to a level that did not change significantly during the remainder of pregnancy.

Gestational diabetes mellitus (GDM) affects 3-10% of pregnancies (Lobato, 2002) and is a risk factor for the mother and fetus (Schmidt et al., 2001). Mahmoud et al. (2006) and Omu et al. (2010) examined BChE activity in GDM and reported a significant increase in healthy pregnant women compared to GDM patients. However, the sample size was relatively small, the authors did not consider other variables that may influence BChE activity and they did not compare BChE activity between pregnant and non-pregnant women.
Variants of the \textit{BCHE} gene (3q26.1-q26.2) (Soreq \textit{et al.}, 1987) have been associated with DM1 and DM2 (Hashim \textit{et al.}, 2001; Alcântara \textit{et al.}, 2002, 2005; Lepienski \textit{et al.}, 2006; Iwasaki \textit{et al.}, 2007; Vaisi-Raygani \textit{et al.}, 2008). The K variant of the \textit{BCHE} gene (SNP: G/A; rs1126680; -116 nt) has a frequency of 18.4\% in Euro-Brazilians (Souza \textit{et al.}, 1998) and was originally associated with lower \textit{BCHE} activity. The -116A variant (SNP: G/A; rs1126680; -116 nt) is located in non-codifying exon 1 and has a frequency of 8\% in Euro-descendants, with -116A being found preferentially in \textit{cis} with the K variant (Bartels \textit{et al.}, 1990). Although the K variant was originally associated with lower BChE activity, this variant alone is not associated with a decrease in BChE activity since the -116A variant is required for this decrease (Furtado-Alle \textit{et al.}, 2008).

In view of the limited investigations into the physiopathology of GDM, in this study we examined \textit{BCHE} activity and behavior in GDM. Some of the variables that influence enzyme activity, \textit{e.g.}, -116A and 1615A variants of the \textit{BCHE} gene, age, body mass index (BMI), total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels, were also examined. To investigate variants in the regulatory region that may influence \textit{BCHE} activity, four tag SNPs were genotyped: two upstream (rs4440084 and rs2863381) and two downstream (rs7624915 and rs4387996) of the \textit{BCHE} gene.

\textbf{Materials and Methods}

\textbf{Subjects}

Three groups were studied: (1) 150 pregnant women with GDM, (2) 295 healthy pregnant women and (3) 156 female blood donors. The GDM group was recruited from the Diabetes Unit of the Endocrinology and Metabolism Service (SEMPR) of the Clinical Hospital at the Federal University of Paraná (UFPR) and the healthy pregnant women were recruited from the Municipal Laboratory in Curitiba. The group of female blood donors (BMI = 24.24 ± 3.08; \(n = 156\)) was collected at HEMEPAR in Curitiba. GDM was defined based on carbohydrate intolerance and resulted in hyperglycemia that began in or was first detected during pregnancy (World Health Organization, 1999). GDM was diagnosed using the criteria of the Brazilian Diabetes Society (Sociedade Brasileira de Diabetes, 2009), based on a Brazilian study of Gestational Diabetes (Schmidt \textit{et al.}, 2001) and on World Health Organization criteria (World Health Organization, 2006). An initial screening of fasting glucose was done during the first appointment: fasting glucose between 85 and 109 mg/dL indicated the need for an oral glucose tolerance test (OGTT). OGTT values > 110 and/or 140 mg/dL in fasting and postprandial conditions, respectively, confirmed GDM. Before initiating the diet, fasting glucose ≥ 110 mg/dL was again examined and another value in this range characterized GDM. The healthy pregnant women group had fasting glucose < 85 mg/dL or OGTT values < 110 mg/dL during fasting and < 140 mg/dL in the postprandial period. Patients who reported renal failure and cardiovascular disease were excluded. Blood samples and clinical and anthropometric variables were collected from GDM patients following diagnosis and before the beginning of treatment. Pregnant women with GDM and healthy controls were paired in relation to gestational age.

This study was approved by the Research Ethics Committee of the Health Science Sector of UFPR and by the Research Ethics Committee of the Municipal Health Department of the City Hall of Curitiba.

\textbf{Laboratory methods}

Peripheral blood samples were collected without anticoagulant in dry tubes containing a separator gel and then centrifuged; the resulting serum was stored at -20 °C. The biochemical parameters were quantified using an Architect automated system (Abbott) with Trulab calibrators and controls.

BChE activity was measured spectrophotometrically ([A\textsubscript{410 nm}] at 25 °C using propyonilthiocoline as substrate, as described by Dietz \textit{et al.} (1972) and modified by Evans and Wroe (1978).

Genomic DNA was extracted by the salting-out method (Lahiri and Nurnberger Jr, 1991) with modifications, or with a Blood Genomic Prep Mini Spin (GE Healthcare) and stored at -20 °C. All DNA samples were adjusted to a concentration of 100 ng/μL.

Genotyping of the -116 and 1615 sites of the \textit{BCHE} gene (rs1126680 and rs1803274, respectively) and the four SNPs near this gene (rs4440084, rs2863381, rs7624915 and rs4387996) was done using a TaqMan SNP genotyping kit (Applied Biosystems, reference code 27847456), performed on a Mastercycler Realplex 2 equipment (Eppendorf). To 8.5 μL of reaction mix (4.0 μL of TaqMan Universal PCR Master Mix, 0.3 μL of specified TaqMan SNP genotyping kit and 4.2 μL of ultrapure water) was added 2 μL (20 ng/μL) of DNA. The amplification involved 45 cycles of 2 min at 50 °C, 10 min at 95 °C and 15 s at 95 °C intercalated with 60 s at 60 °C.

\textbf{Statistical analysis}

Frequency distributions, means ± SE, variances and the t-test were calculated using Statistica for Windows software (StatSoft, Inc.) and a 5\% significance level was adopted. Data normality was tested by the Shapiro-Wilk normality test. Arlequin 2000 (Schneider \textit{et al.}, 2000) was used to estimate the haplotypes and Bonferroni’s correction was applied for comparisons between groups. Allelic and genotypic frequencies were obtained by direct counting and compared between groups via the chi-square test using Clump (Sham and Curtis, 1995). Pearson’s correlation anal-
ysis between BChE activity and variables related to GDM was done using Statistica for Windows software. Regression analysis was done using SPSS for Windows (SPSS Inc.) to verify whether BChE activity was dependent on variables related to GDM and BCHE gene variants.

Results

Clinical and anthropometric features

Table 1 shows the mean ± SD and variances of age, BMI and clinical data for pregnant women (GDM and controls), along with their t and F test values. The variables age, BMI, total cholesterol, HDL cholesterol and triglycerides, differed significantly between the groups.

BChE activity

Table 2 summarizes the results for BChE activity in women with GDM and controls. BChE activity was significantly higher in healthy pregnant women than in women with GDM (2.83 ± 0.89 KU/L vs. 2.26 ± 0.59 KU/L; \( t = -7.06, p = 6.66 \times 10^{-12} \)), but was significantly lower in both groups than in the general population sample (4.68 ± 1.51 KU/L; \( t = 14.06 \) and \( p = 0.00 \) for healthy pregnant women; \( t = 18.54 \) and \( p = 0.00 \) for GDM).

In the GDM group, the correlation coefficients (r) between BChE activity and BMI and BChE activity and LDL cholesterol were 0.18 (p < 0.05) and -0.19 (p < 0.05), respectively. In pregnant women of the control group, total cholesterol, HDL cholesterol and triglyceride levels were negatively correlated with BChE activity (r = -0.12; r = -0.49 and r = -0.19, respectively, p < 0.05). Since BChE activity differed between patients and controls, a forward step-wise multiple regression analysis was done in which BChE activity was the dependent variable and GDM/pregnant women without the disease, age, BMI, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides,

| Groups | Mean ± SD (KU/L) | SD² | RV |
|--------|-----------------|-----|----|
| GDM (N = 150) | 2.26 ± 0.59 | 0.36 | 0.67-4.39 |
| Healthy pregnant women (N = 250) | 2.83 ± 0.89 | 0.79 | 0.76-5.56 |
| Female blood donors (N = 156) | 4.68 ± 1.51 | 2.28 | 0.16-11.3 |

Comparison between the groups

| Comparison | t (p) | \( F (p) \) |
|------------|------|-------|
| GDM vs. healthy pregnant women | -7.06 (6.66 \times 10^{-12})* | 2.22 (1.22 \times 10^{5})* |
| GDM vs. female blood donors | 18.54 (0.00)* | 6.41 (1.25 \times 10^{28})* |
| Healthy pregnant women x female blood donors | 14.06 (0.00)* | 2.89 (5.51 \times 10^{15})* |

BMI - body mass index. OGTT - oral glucose tolerance test 75 g. SD² - variance. The total cholesterol, LDL cholesterol and triglyceride levels were available for 122 patients with GDM whereas the HDL cholesterol values were available for 139 patients with GDM. BMI values were available for 142 patients with GDM. OGTT with normal values < 110 and 140 mg/dL in fasting and post-prandial (2 h after ingestion) conditions, respectively. The OGTT was done in 142 patients. The t values for Student’s t-test (comparison of means) and the f values for the F-test (equality of two variances) are shown. *p < 0.05.
-116A and 1615A variants of the \textit{BCHE} gene and the four tag SNPs were the independent variables. This analysis showed that \textit{BCHE} activity was significantly dependent on the sample group - GDM/pregnant women without the disease ($\beta = 0.198, p = 0.00152$) and presence of the \textit{BCHE} gene variant -116A ($\beta = -0.166, p = 0.000812$). In the patients and control group, \textit{BCHE} activity was significantly higher in pregnant women homozygous for the usual allele (-116G) than in heterozygous individuals (-116AG) (2.31 ± 0.61 KU/L vs. 1.93 ± 0.42 KU/L; $t = 2.53$, $p = 0.01$, for GDM; and 2.88 ± 0.88 KU/L vs. 2.39 ± 0.81 KU/L; $t = 2.96$, $p = 0.003$, for healthy pregnant women, respectively).

**Frequencies of -116 and 1615 site variants**

The genotypic frequencies for both sites were in Hardy-Weinberg equilibrium and did not differ between the GDM patients and controls ($\chi^2 = 1.92$, $p = 0.38$ for the -116 site and $\chi^2 = 0.56$; $p = 0.75$ for the 1615 site). The frequency of the -116A allele did not differ between the GDM patients and controls (7.69 ± 0.09% vs. 5.76 ± 0.04%, respectively, $\chi^2 = 1.20$, $p = 0.27$). The 1615A allele frequency did not differ between the GDM patients and controls (17.13 ± 0.13% vs. 17.12 ± 0.06%, respectively, $\chi^2 = 0.00$, $p = 0.99$).

**Haplotype frequencies**

For haplotype inferences, in addition to the -116A and 1615A variants, the four SNPs close to the \textit{BCHE} gene were also included (rs4440084, rs2863381, rs7624915 and rs4387996). The genotype frequencies of these four SNPs were in Hardy-Weinberg equilibrium and the allele and genotype frequencies did not differ between GDM patients and healthy pregnant women. After the application of Bonferroni’s correction there was no significant difference in the haplotype frequencies between healthy pregnant women and pregnant women with GDM.

**Discussion**

Pregnancy is associated with physiological changes that include an increase in caloric intake, greater accumulation of fat mass and a progressive increase in insulin resistance (Tham \textit{et al.}, 2009). Towards the end of pregnancy, the insulin resistance approaches that of DM2 patients. Insulin resistance during pregnancy most likely reflects a combination of maternal body fat excess and placental hormone effects that reduce the sensitivity to insulin. Resistance to insulin is normally compensated for by an increase in insulin secretion by pancreatic \textit{b}-cells. Hyperglycemia in GDM results from inadequate insulin supplement to the tissues (Buchanan and Xiang, 2005).

In the present study, the significant increase in BMI in GDM probably favored insulin resistance, which meant that pregnant women belonged to a GDM risk group. The significantly higher age, cholesterol and triglyceride levels in patients compared to the control group, were additional factors that increased the risk for patients. Age, BMI, cholesterol and high triglycerides levels are consistent with criteria established by Carpenter and Coustan (1982) for GDM risk factors and used as parameters in the American Diabetes Association recommendations (American Diabetes Association, 2004).

There was a positive correlation between \textit{BCHE} activity and BMI in the GDM group, in agreement with previous studies (Alcântara 	extit{et al.}, 2005; Randell 	extit{et al.}, 2005; Valle \textit{et al.}, 2006; Das, 2007). Despite the decreased enzyme activity, the patients had significantly higher BMI and triglycerides compared to healthy pregnant women, which could explain the positive correlation between BMI and \textit{BCHE} activity and the absence of this correlation in pregnant women group without the disease. In addition, the negative correlation between \textit{BCHE} activity, total cholesterol and triglyceride levels observed in healthy pregnant women was usually positive. Only for HDL cholesterol, that generally correlates negatively with \textit{BCHE} activity (Randell \textit{et al.}, 2005; Alcântara \textit{et al.}, 2005; Valle \textit{et al.}, 2006), were the results in agreement with those for pregnant women group without GDM. In the GDM group, most of the correlations between \textit{BCHE} activity and lipoproteins reported in the literature were not observed and there was an unexpected negative correlation with LDL cholesterol (Randell \textit{et al.}, 2005; Alcântara \textit{et al.}, 2005; Valle \textit{et al.}, 2006; Iwasaki \textit{et al.}, 2007). Considering that pregnancy is an environmental stressor that changes the organisms homeostasis and that GDM promotes further changes, the unexpected correlation coefficients found between \textit{BCHE} activity and lipoproteins in pregnant women with or without GDM can be explained by the ability of pregnancy to change the relationship between \textit{BCHE} and lipid metabolism.

Even though GDM has a similar etiology to DM2, \textit{BCHE} activity behaves differently in these diseases. The determinant factor for this change is pregnancy. During healthy pregnancy, there is generally a decrease in \textit{BCHE} activity (Blitt \textit{et al.}, 1977; Howard \textit{et al.}, 1978; Whittaker, 1986), a finding corroborated here when the enzyme activity of pregnant women in the control group was compared with that of women from the general population. Wyse \textit{et al.} (2004) suggest that there is a decrease in \textit{BCHE} activity in situations of oxidative stress, and pregnancy is considered an important environmental stressor (Robitaille and Grant, 2008).

Pregnancy results in much higher oxidative stress levels than under normal circumstances, and hyperglycemia intensifies this stress (Ramkumar \textit{et al.}, 2005). Orhan \textit{et al.} (2003) suggested that oxidative stress accompanies complications associated with diseases such as diabetes mellitus during pregnancy. Thus, the decrease in \textit{BCHE} activity in GDM was not only due to pregnancy, but to the overall effect of pregnancy and disease.
BChE activity is lower in the presence of the -116A variant (Furtado-Alle et al., 2008). As shown here, there was a significant decrease in BChE activity in both groups of pregnant women when -116GG was compared with -116AG. It should be noted that the -116A variant and the physiology of GDM and pregnancy contribute independently to BChE activity. Pregnancy leads to a decrease in BChE activity and the -116A mutation, when present in this state, also decreases BChE activity. Although the variants -116A and -116A are in linkage disequilibrium (Souza et al., 1998), regression analysis identified only the variant -116A as an independent factor for decreasing BChE activity, in agreement with a study showing that the 1615A enzyme is similar to the normal enzyme with regard to substrate affinity and rate of catalysis, rate of secretion and formation of tetramers, i.e., these parameters would have no effect on the structure or activity of BChE (Altamirano et al., 2000).

In conclusion, BChE is associated with lipid metabolism in GDM, a condition in which the activity of this enzyme is very low. Although women with GDM had significantly higher values of BMI and triglycerides, they had lower enzyme activity than healthy pregnant women, indicating that the GDM-induced decrease in BChE activity was stronger than the enhancer effect characteristic of obesity and overweight. Interestingly, the -116A variant leads to a decrease in BChE activity that is independent of pregnancy and GDM. Hence, each of these three factors (-116A variant, pregnancy and GDM) alters enzyme activity independently: healthy pregnant women have significantly lower enzyme activity than healthy pregnant women, and in both groups, as well as in the general population (Furtado-Alle et al., 2008), the -116A variant leads to a decrease in BChE activity.

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