## BRIEF COMMUNICATION

# TBARS and BDNF levels in newborns exposed to crack/cocaine during pregnancy: a comparative study

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**Objectives:** To compare levels of a marker of lipid peroxidation (thiobarbituric acid reactive substances, TBARS) and brain-derived neurotrophic factor (BDNF) in umbilical cord blood (UCB) between newborns exposed to crack/cocaine in utero (exposed newborns [EN], n=57) and non-exposed newborns (NEN, n=99), as well as in maternal peripheral blood at delivery.
**Methods:** This was a cross-sectional study. Potential confounders, including perinatal parameters, psychopathology, and use of other substances, were assessed.
**Results:** After adjusting for potential confounders, adjusted mean BDNF was significantly higher in EN (3.86 ng/mL, 95% confidence interval [95%CI] 2.29-5.43) than in NEN (0.85 ng/mL, 95%CI 0.47-1.23; $p < 0.001$; Cohen effect size: 1.12), and significantly lower in crack/cocaine mothers than in control mothers (4.03 ng/mL, 95%CI 2.87-5.18 vs. 6.67 ng/mL, 95%CI 5.60-7.74; p = 0.006). The adjusted mean TBARS level was significantly lower in EN (63.97 μM MDA, 95%CI 39.43-88.50) than NEN (177.04 μM MDA, 95%CI 140.93-213.14; $p < 0.001$; effect size = 0.84), with no difference between mother groups (p = 0.86).
**Conclusions:** The changes in TBARS levels observed in EN suggest that fetuses exposed to cocaine mobilize endogenous antioxidant routes since very early stages of development. The increase in BDNF levels in EN might indicate changes in fetal development, whereas the changes in BDNF levels in mothers provide evidence of the complex metabolic processes involved in drug use during pregnancy.

**Keywords:** TBARS; BDNF; pregnancy; crack/cocaine; umbilical cord blood; newborn

## Introduction

Cocaine and some of its metabolites are stored in both the myometrium and the placental membrane, and are continuously delivered into the amniotic fluid, exposing the fetus to a state of slow-release cocaine delivery.[1] Prenatal drug exposure affects neonatal brain functional connectivity, with cocaine having a specific effect on the amygdala-frontal network.[2] The pathways involved in such changes remain unclear. Brain-derived neurotrophic factor (BDNF) and other neurotrophic factors can play a crucial role in the development and maintenance of the central nervous system, and might influence the formation and elimination of neural connections.[3] It is reasonable to consider that intrauterine cocaine exposure (IUCE) may disrupt this process. Cocaine also contributes to an imbalance in the oxidative/antioxidant system by increasing the production of reactive oxygen species (ROS)[4] and disrupting the antioxidant system in the embryonic period.[5] Thus, IUCE could damage neurodevelopment since very early embryonic stages. Despite some studies on BDNF and oxidative stress (OE) in crack/cocaine-exposed adults and animal models, no data are available for newborns with a history of IUCE, nor for pregnant women. Thus, the purpose of this study was to compare OS (represented by lipid peroxidation, measured by thiobarbituric acid reactive substances, TBARS) and levels of BDNF in umbilical cord blood (UCB) from newborns with IUCE (exposed newborns [EN]) and non-exposed newborns (NEN), as well as in their respective mothers, in the immediate postpartum period.

## Methods

Data were collected at Hospital de Clínicas de Porto Alegre (HCPA) and Hospital Materno Infantil Presidente Vargas (HMIPV), in the city of Porto Alegre, state of

Rio Grande do Sul, Brazil. All mothers provided written informed consent, and the project was approved by the Ethics Committees of both hospitals.

This was a cross-sectional study. The predictive variable was history of crack/cocaine use/exposure and the outcome measures were serum levels of BDNF and TBARS in UCB and in maternal peripheral blood at delivery. Participants were mother/newborn dyads exposed to crack/cocaine who underwent consecutive sampling at HMIPV. Control newborn/mother samples were obtained from a group of pregnant women who participated in the HCPA Umbilical Cord and Placental Blood Bank (Banco de Sangue de Cordão Umbilical e Placentário) project, according to the Foundation for the Accreditation of Cellular Therapy (FACT) criteria.[6] The maternal inclusion criteria for the EN group were crack/cocaine use and age 18-45 years, and the exclusion criteria were inability to understand and complete the psychiatric questionnaires. The inclusion and exclusion criteria for controls relied on FACT criteria. All mothers included in the control group were confirmed as non-drug users by history and urinary tests (Bioeasy® cocainetest, Alere™, Belo Horizonte, Brazil). Tobacco use was not an exclusion criterion for controls. Controls could not have abuse or dependence of alcohol or other drugs, according to the MINI interview. The potential confounders were: maternal intelligence quotient (IQ) (Wechsler Adult Intelligence Scale-III [WAIS-III])[7] and psychopathology (Mini International Neuropsychiatric Interview [MINI], Brazilian version 5.0.0/DSM-IV/Current[8]); use of other psychoactive substances (Alcohol, Smoking and Substance Involvement Screening Test [ASSIST][9]); sociodemographic data (age, self-reported ethnicity, marital status, educational attainment); and perinatal and obstetric data. The following neonatal variables were obtained by review of medical records: weight, Apgar score at 1 and 5 minutes, gestational age (GA) at neonatal examination, sex, and classification for GA. Data on the clinical status of the mother for relevant infections (syphilis, HIV, and/or hepatitis C), mode of delivery, and prenatal care were also collected from medical records. Blood collection and processing and BDNF and TBARS measurement were performed as usual in our laboratory.[10] The main outcome measure was assessed by generalized linear model (GLM) analyses (gamma distribution), while confounders were chosen on the basis of statistical criteria (association with both study factor and outcome measure at a significance level of $p \leqslant 0.2$). The accepted significance level was $p \leqslant 0.05$. Data were processed and analyzed in PASW Statistics 18.0.

## Results

The sample comprised 57 EN and 99 NEN. Selected characteristics of the newborns and their mothers are shown in Table 1. No mother reported use of intravenous drugs, club drugs, or hallucinogens. There was no difference between groups for gestational age (p = 0.37), infant gender (p = 0.72), maternal age (p = 0.39), or cesarean delivery (p = 1).

In the GLM analyses for BDNF in UCB, results were adjusted for intensity of alcohol, tobacco, and cannabis use in the last 3 months, presence of any maternal infection, infant weight, and marital status. Adjusted mean BDNF levels were significantly higher in newborns exposed to crack/cocaine in utero (3.86 ng/mL, 95%CI 2.29-5.43) than in NEN (0.85 ng/mL, 95%CI 0.47-1.23; p < 0.001; Wald = 20.75), as shown in Table 1. The Cohen effect size was 1.12 (very large). No variable other than crack/cocaine exposure was significant. For analysis of TBARS levels in UCB, results were adjusted for intensity of alcohol, tobacco, and cannabis use in the last 3 months, presence of any maternal psychopathology, maternal infectious disease, prenatal care, marital status, and estimated IQ. Variables that did not contribute to the model were withdrawn in descending order of p-value. Mean adjusted TBARS levels were significantly lower in newborns exposed to crack/cocaine in utero (63.97 µM MDA, 95%CI 39.43-88.50) than in NEN (177.04 µM MDA, 95%CI 140.93-213.14; p < 0.001; Wald = 19.71), with a large effect size (0.84). In addition to crack/cocaine exposure, presence of any maternal psychopathology (p = 0.04, Wald = 4.42) and estimated IQ (p < 0.001; Wald = 17.62) were also significant. According to GLM analysis, with a model including intensity of cannabis use in the last 3 months, presence of any maternal psychopathology, and 5-minute Apgar score, TBARS measures in the immediate postpartum period were not significantly different between crack/cocaine-using mothers (30.52 µM MDA, 95%CI 17.79-43.25) and control mothers (29.21 µM MDA, 95%CI 23.95-34.47; p = 0.86). However, there was a difference between groups for postpartum BDNF levels, which were significantly lower in crack/cocaine users than in controls (adjusted for intensity of use of alcohol and tobacco; 4.03 ng/mL, 95%CI 2.87-5.18 vs. 6.67 ng/mL, 95%CI 5.60-7.74, Wald = 7.52; p = 0.006).

## Discussion

In our study, we were able to demonstrate differences in a general state of oxidative stress, as measured by TBARS, in neonates exposed to crack/cocaine in utero. Among possible explanations, cocaine and amphetamine regulated transcript (CART), an endogenous antioxidant stimulated by dopamine presence, should be considered. According to animal models, CART is expressed very early during embryonic neurodevelopment,[11] being more present in the cortex, nucleus accumbens, and ventral tegmental area and decreasing progressively in expression as the fetus develops.[12] Dopaminergic stimulation, as seen in crack/cocaine dependence, is a regulator of CART expression. In our study, high levels of dopamine due to maternal crack/cocaine intake might have affected fetal homeostasis. The maturational decrease in CART levels, among other factors, could help explain the absence of difference in TBARS levels between exposed and control pregnant women.

BDNF plays a major role in cellular plasticity during development and maturation of the brain. In agreement with our results, young rats treated with stimulants exhibited increased BDNF protein levels in the prefrontal cortex compared to a saline-treated group.[13] In our study, higher levels of BDNF in exposed newborns might indicate an acute stress reaction. Increased BDNF in this

**Table 1** Sociodemographic, clinical characteristics, TBARS and BDNF levels of crack/cocaine-exposed mother-infant pairs and controls

|  | Cases (n=57) | Controls (n=99) | p-value |
|---|---|---|---|
| Infant variables, mean (SD) |  |  |  |
| Weight (g) | 2,882.15 (473.32) | 3,144.80 (439.39) | 0.001 |
| Apgar, 1-minute | 7.8 (2.12) | 8.41 (1.31) | 0.06 |
| Apgar, 5-minute | 8.94 (1.24) | 9.38 (0.74) | 0.007 |
|  |  |  |  |
| Maternal variables |  |  |  |
| Ethnicity, n (%) |  |  |  |
| White | 17 (32.1) | 74 (76.3) | < 0.001 |
| Nonwhite | 36 (67.9) | 23 (23.7) |  |
| IQ, mean (SD) | 77.39 (8.75) | 84.17 (9.59) | < 0.001 |
| Educational attainment, n (%) |  |  |  |
| Some primary, completed primary, or some secondary | 39 (81.3) | 51 (53.1) |  |
| Completed secondary, some higher, or completed higher | 9 (18.8) | 45 (53.1) | 0.002 |
| Prenatal care, n (%)* | 36 (73.5) | 99 (100) | < 0.001 |
| Presence of infectious diseases (syphilis, HIV, and/or hepatitis C), n (%)* | 26 (46.4) | 0 | < 0.001 |
| Marital status (married/cohabiting), n (%)* | 29 (50.9) | 94 (94.9) | < 0.001 |
|  |  |  |  |
| Drug use by mothers during pregnancy – ASSIST total score, median (range)[†] |  |  |  |
| Total score for nicotine | 17.50 (0-31) | 0.00 (0-39) | < 0.001 |
| Total score for alcohol | 4.00 (0-33) | 0.00 (0-11) | < 0.001 |
| Total score for cannabis | 0.00 (0-25) | - | - |
|  |  |  |  |
| Presence of current psychopathology in mothers, n (%) |  |  |  |
| MINI positive for any diagnosis | 25 (61.0) | 32 (33.3) | 0.005 |
|  |  |  |  |
| Biomarkers (general linear models), adjusted mean (95%CI) |  |  |  |
| BDBF, umbilical cord blood[‡] | 3.86 (2.29-5.43) | 0.85 (0.47-1.23) | < 0.001 |
| TBARS, umbilical cord blood ($\mu$M MDA)[§] | 63.97 (39.43-88.50) | 177.04 (140.93-213.14) | < 0.001 |
| TBARS, maternal peripheral blood ($\mu$M MDA)[‖] | 30.52 (17.79-43.25) | 29.21 (23.95-34.47) | 0.86 |
| BDNF, maternal peripheral blood (ng/mL)[¶] | 4.03 (2.87-5.18) | 6.67 (5.60-7.74) | 0.006 |

Data presented as mean (standard deviation), unless otherwise specified.
95% confidence interval = 95%CI; ASSIST = Alcohol, Smoking and Substance Involvement Screening Test; BDNF = brain-derived neurotrophic factor; IQ = intelligence quotient; MINI = Mini International Neuropsychiatric Interview; SD = standard deviation; TBARS = thiobarbituric acid reactive substances.
* Fisher's exact test. [†] Mann-Whitney *U* test.
[‡] Adjusted for intensity of alcohol, tobacco, and cannabis use in the last 3 months, presence of any maternal infection, infant weight, and marital status.
[§] Adjusted for intensity of alcohol, tobacco, and cannabis use in the last 3 months, presence of any maternal psychopathology, maternal infectious disease (HIV, syphilis, and/or hepatitis C), prenatal care, marital status, and estimated IQ.
[‖] Adjusted for intensity of cannabis use in the last 3 months, presence of any maternal psychopathology, and 5-minute Apgar.
[¶] Adjusted for intensity of use of alcohol and tobacco.

scenario might represent as a compensatory search for neuroprotection.[10] However, this does not necessarily imply a better outcome for these children. In an animal model, the long-term effect of high BDNF levels has been suggested to affect neuronal migration and maturation through different developmental periods.[14] These changes could, in fact, cause impairment in brain function in adult life, which would help understand the complex pathway to cognitive and behavioral problems seen in exposed offspring.[14] Pregnancy, however, is characterized by several metabolic changes that interact with circulating BDNF levels. In usual circumstances, BDNF levels are expected to be lowest in the first trimester of pregnancy and increase thereafter.[15] In puerperal women, decreased BDNF levels might indicate chronicity of stressor exposure, as BDNF decreases in chronic stress.[16]

The present study has some limitations. While our chosen measure for oxidative imbalance (TBARS) is widely used, other markers are available. Our sample was composed of pregnant polydrug users; this increases the external validity of the study and, on multivariate analysis, the data were adjusted for use of other substances. We did not measure maternal body mass index because, although it is a good measure for the pre-gestational stage, it is not useful when data collection takes place at the time of delivery, as in our study. Additionally, it is important to note that our sample of cases comprised a difficult-to-reach population of women who received, for instance, incomplete prenatal care. Finally, as this was a cross-sectional study, we cannot establish causal associations. Specific strengths of our study included measuring TBARS and BDNF in UCB, before newborns had been exposed to other stressors. To the best of our knowledge, ours is the first report of these biomarkers in newborns with a history of crack/cocaine exposure.

Taken together, our findings in EN and their mothers could be understood in the same direction, suggesting age-dependent neuroprotective changes in the oxidation-reduction status (redox) (lower TBARS in EN; no significant difference among women in the postpartum period) and a neuroplastic response (higher BDNF in EN; lower BDNF among women in the postpartum period) promoted by crack/cocaine. Our data suggest a need for further investigations into the fetal endogenous antioxidant

system, including CART, and into the impact of drug use on pregnancy.

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## Disclosure

LAR has received honoraria, has been on the speakers' bureau/advisory board, and/or has acted as a consultant for Eli-Lilly, Janssen-Cilag, Novartis, and Shire in the last 3 years; receives authorship royalties from Oxford Press and ArtMed; and has received travel awards from Shire for taking part in 2014 APA and 2015 WFADHD meetings. The ADHD and Juvenile Bipolar Disorder Outpatient Programs chaired by him received unrestricted educational and research support from the following pharmaceutical companies in the last 3 years: Eli-Lilly, Janssen-Cilag, Novartis, and Shire. CMS has been on the speakers' bureau for Novartis. The other authors report no conflicts of interest.

## References

1  De Giovanni N, Marchetti D. Cocaine and its metabolites in the placenta: a systematic review of the literature. Reprod Toxicol. 2012;33:1-14.

2  Salzwedel AP, Grewen KM, Vachet C, Gerig G, Lin W, Gao W. Prenatal drug exposure affects neonatal brain functional connectivity. J Neurosci. 2015;35:5860-9.

3  Barde YA. The nerve growth factor family. Prog Growth Factor Res. 1990;2:237-48.

4  Kovacic P. Role of oxidative metabolites of cocaine in toxicity and addiction: oxidative stress and electron transfer. Med Hypotheses. 2005;64:350-6.

5  Peng Y, Kwok KH, Yang PH, Ng SS, Liu J, Wong OG, et al. Ascorbic acid inhibits ROS production, NF-kappa B activation and prevents ethanol-induced growth retardation and microencephaly. Neuropharmacology. 2005;48:426-34.

6  Foundation for the Accreditation of Cellular Therapy (FACT). Net Cord-FACT international standards for cord blood collection, banking and release for administration [Internet]. 5th ed. 2013 [cited 2016 Oct 10]. factweb.org/forms/store/ProductFormPublic/fifth-edition-net-cord-fact-international-standards-for-cord-blood-collection-banking-and-release-for-administration-free-download.

7  Wechsler D. WAIS-III: Escala de Inteligência Wechsler para adultos. 3ª ed. São Paulo: Casa do Psicólogo; 2004.

8  Amorim P. Mini International Neuropsychiatric Interview (MINI): validação de entrevista breve para diagnóstico de transtornos mentais. Rev Bras Psiquiatr. 2000;22:106-15.

9  Henrique IF, De Micheli D, Lacerda RB, Lacerda LA, Formigoni ML. [Validation of the Brazilian version of Alcohol, Smoking and Substance Involvement Screening Test (ASSIST)]. Rev Assoc Med Bras (1992). 2004;50:199-206.

10  Narvaez JC, Magalhaes PV, Fries GR, Colpo GD, Czepielewski LS, Vianna P, et al. Peripheral toxicity in crack cocaine use disorders. Neurosci Lett. 2013;544:80-4.

11  Risold PY, Bernard-Franchi G, Collard C, Jacquemard C, La Roche A, Griffond B. Ontogenetic expression of CART-peptides in the central nervous system and the periphery: a possible neurotrophic role? Peptides. 2006;27:1938-41.

12  Rodrigues BC, Cavalcante JC, Elias CF. Expression of cocaine- and amphetamine-regulated transcript in the rat forebrain during postnatal development. Neuroscience. 2011;195:201-14.

13  Simchon Tenenbaum Y, Weizman A, Rehavi M. The impact of chronic early administration of psychostimulants on brain expression of BDNF and other neuroplasticity-relevant proteins. J Mol Neurosci. 2015;57:231-42.

14  McCarthy DM, Zhang X, Darnell SB, Sangrey GR, Yanagawa Y, Sadri-Vakili G, et al. Cocaine alters BDNF expression and neuronal migration in the embryonic mouse forebrain. J Neurosci. 2011;31: 13400-11.

15  Garcés MF, Sanchez E, Torres-Sierra AL, Ruíz-Parra AI, Angel-Müller E, Alzate JP, et al. Brain-derived neurotrophic factor is expressed in rat and human placenta and its serum levels are similarly regulated throughout pregnancy in both species. Clin Endocrinol (Oxf). 2014;81:141-51.

16  Hauck S, Kapczinski F, Roesler R, de Moura Silveira E Jr, Magalhães PV, Kruel LR, Schestatsky SS, Ceitlin LH. Serum brain-derived neurotrophic factor in patients with trauma psychopathology. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34:459-62.

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