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# Caffeine consumption during pregnancy and ADHD at the age of six years: A birth cohort study

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**Keywords:** Caffeine, pregnancy, ADHD and hyperactivity.

## ABSTRACT

**Objective:** Studies evaluating caffeine intake during pregnancy and long-term outcomes, such as the child's neurobehavior, are still scarce and their results are inconsistent. The objective of the present study was to evaluate the association between maternal consumption of caffeine during pregnancy and attention deficit hyperactivity disorder (ADHD) at the age of six years.

**Methodology:** All children born in the city of Pelotas, Brazil, during the year 2004, were selected for a cohort study. The mothers were interviewed at birth to obtain information on coffee and yerba mate consumption during pregnancy, among other matters. At the age of six years, presence of ADHD was evaluated using the Development and Well-Being Assessment (DAWBA) questionnaire, applied to the mothers. The prevalence of heavy caffeine consumption (≥300 mg/day) and ADHD were calculated, with 95% confidence intervals (95%CI). The association between caffeine consumption and ADHD was tested by means of logistic regression.

**Results:** 3507 children were included in the analyses. The prevalence of ADHD was 2.6% (2.1-3.2%): 3.4% (2.9-3.9%) among boys and 1.8% (1.4-2.2%) among girls. The prevalence of heavy caffeine consumption during the entire pregnancy and the first, second and third trimesters was 15.4% (11.0-19.8%), 19.2% (13.5-24.9%), 17.9% (12.6-23.2%) and 16.4% (11.6-21.2%), respectively. Heavy caffeine consumption in the entire pregnancy was not associated with ADHD in the crude (OR: 0.66; CI95%: 0.34 – 1.28) or adjusted analysis (OR: 0.59; CI95%: 0.30 – 1.16).

**Conclusion:** The present study did not show any association between maternal caffeine consumption during pregnancy and ADHD at the age of six years.

## ARTICLE SUMMARY

### Strengths

This was a longitudinal study ensuring that the temporal relationship between exposures and outcomes can be ascertained. Detailed information on caffeine consumption from coffee and yerba mate during the three trimesters of pregnancy was available. The outcome was evaluated by means of a validated instrument. A great number of potential confounding factors were adjusted for. There were a low percentage of losses and refusals during the follow-up of the study.

### Limitations

The outcome was ascertained by means of tests applied only to the mother. In addition, the amount of coffee and yerba mate consumed during pregnancy was obtained retrospectively, being subject to recall bias. Also, although caffeine consumption during pregnancy was assessed from the two main sources (coffee and mate) there are other caffeine sources (foods like chocolate, chocolate drink and cola-drinks as well as medicines) that were not measured.

## FINDINGS TO DATE

- Prevalence of heavy caffeine consumption (≥ 300 mg/day) during the entire pregnancy was high (15.4%; 95% Confidence interval: 11.0-19.8%).
- The prevalence of ADHD was 2.6% (95% CI: 2.1-3.2%); higher among boys (3.4%; 95%CI: 2.9-3.9%) than among girls (1.8%; 95%CI: 4-2.2%).
- The present cohort study, involving around 4000 children did not show any association between maternal caffeine consumption during pregnancy and ADHD.

## INTRODUÇÃO

Attention deficit hyperactivity disorder (ADHD) is a neurobiological disorder that affects around 6% of school-age children around the world.(1) It is the most prevalent mental disorder during childhood and the main reason why mental health services are sought for children and adolescents.(2) It is characterized by persistent symptoms of inattention, impulsivity and hyperactivity, which become present before the age of 12 years and are abnormal for the developmental stage.(3)

ADHD is a multi factorial disease with complex etiology and a large genetic component (heritability estimated as 76%).(4) Epidemiological studies have shown higher prevalence among boys and among children belonging to families with worse socioeconomic conditions.(5-13) Maternal caffeine consumption (17) as well as other nutritional factors during pregnancy, such as intake of folic acid,(14) iron(15) and omega-3(16) have been investigated as determinants of ADHD. In animals, intrauterine exposure to caffeine was associated with increased motor activity, thus suggesting a possible effect on attention deficit and hyperactivity on children born to mothers with high consumption of caffeine-rich foods and beverages during pregnancy.(17, 18) Moreover, exposure of rats to caffeine, during the prenatal period, resulted in gene expression alterations relating to formation of synapses, thereby showing some of the potential molecular effects of caffeine during fetal cerebral development.(19)

Caffeine is commonly consumed throughout the world, including by pregnant women, who present daily consumption prevalence ranging from 75% to 93%.(20) Studies evaluating caffeine intake during pregnancy and long-term outcomes, such as child neuro-behavior, are still scarce and their results are inconsistent. Among five articles identified in a systematic review of the literature (21) only one found that the higher maternal caffeine intake during pregnancy would increase the risk of ADHD.(22)

So, the objective of the present study was to evaluate the association between maternal caffeine consumption during pregnancy and ADHD at the age of six years, among children belonging to a birth cohort. The hypothesis is the ADHD is associated with heavy consumption (≥ 300 mg / day) of caffeine.

## METODOLOGIA

In 2004, a birth cohort study began in the city of Pelotas, Brazil. The original cohort population consisted of the 4231 newborns at the five hospitals in the city, who were the children of mothers living in the urban zone of Pelotas, corresponding to 99.2% of the births in that year. After delivery mothers were interviewed by trained interviewers, using standardized questionnaires, regarding their socioeconomic, demographic and reproductive characteristics, use of health services, prenatal attention and pregnancy complications (perinatal study). Further methodological details of the study can be found in other publications.(23-25)

So far, the cohort participants were followed-up at the ages of 3, 12, 24 and 48 months, and at 6 and 10 years. The mothers were interviewed regarding their children's growth, development, type of food and morbidity, and also answered questions about their own health.(24) Differently from the visits at 3, 12, 24, and 48 months that took place at the child's place, at the age of 6 and 10 years data-gathering was undertaken at a clinic that had been set up especially to attend to this research. Besides interviews, the children underwent a comprehensive health evaluation, which included psychological, psychiatric, anthropometric and body composition evaluations.(25)

The presence of ADHD was evaluated by means of the Development and Well-Being Assessment (DAWBA), an instrument employed for psychiatric diagnosis among children and teenagers aged from 5 to 17 years, and that uses diagnostic classifications

from the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) and the International Classification of Diseases (ICD-10).(26) DAWBA was applied to the mother during the six-year follow-up, by trained interviewers (psychologists). The DAWBA combines highly structured questions based on DSM-IV diagnostic criteria and ICD-10 with qualitative descriptions of all areas of difficulty. The generating program is a computer algorithm which provides a probability of a child to have any psychiatric disorder based on answers to structured questions. In the presence of positive symptoms in any area, additional questions (qualitative assessment) are made to assess the impact (loss) of these problems in the child's life. These questions concern specific areas covering distress and interference with family life, learning, friendship and leisure activities resulting in symptoms. Subsequently, a clinical evaluator combines the quantitative results with the qualitative date and then, based on the two information makes a judgment in regard to the presence or absence of the disorder. The clinic trial in this case was made by a child psychiatrist (rater), supervised by another child psychiatrist, who translated and validaded the DAWBA for the Brazilian population. To make the different psychiatric diagnosis from DAWBA evaluations, the rater needs to judge whether symptoms are present or not and the loss (impact) that they cause. DAWBA diagnoses are supplied dichotomously as “yes” or “no”, strictly respecting the diagnostic criteria defined by IDC-10 and DSM-IV diagnostic classifications. For this study the DSM-IV classification was employed. The DAWBA allows the identification of children currently under treatment for ADHD, such children were classified as positive for ADHD. The DAWBA questionnaire was translated and validated in Brazil by Bacy Fleitlich-Bilyk.(26)

The exposure of interest, i.e. caffeine consumption during pregnancy, was evaluated retrospectively, at the perinatal assessment by means of a series of questions

regarding consumption of the foods that are the main sources of caffeine at this region of the country: coffee and yerba mate (a typical hot beverage consumed in southern Brazil and neighboring countries, which is prepared from the leaves of the herb *Ilex paraguariensis)*. For each source of caffeine, the daily frequency of consumption was obtained, separately for each trimester of pregnancy. Information regarding the type of coffee (filtered or instant), preparation, concentration (strong, medium or weak) and quantity consumed per day was gathered, taking into consideration the size of the recipient (180 ml cup; 50 ml small cup; 200 ml glass and 190 ml mug). The estimated caffeine content from coffee and yerba mate was obtained from coffee samples collected from the homes of mothers who participated in a previous study conducted in the city of Pelotas,(27) and that were analyzed by chromatography. From these analyses, it was possible to infer the average caffeine content in mg per ml of coffee, according to the concentration at which it was consumed: strong coffee, 0.25 mg/ml; medium coffee, 0.20 mg/ml; and weak coffee, 0.11 mg/ml. For yerba mate, the analyses showed an average concentration of 17 mg of caffeine per 100 ml of the liquid. These results were used to estimate the caffeine intake of the entire sample. For instant coffee, the items investigated were the size of the spoon used to serve coffee (full coffee spoon, 2.6 g; level coffee spoon, 2.3 g; full small coffee spoon, 2.5 g; level small coffee spoon, 1.5 g; full dessert spoon, 7.5 g; and level dessert spoon, 7.0 g) and the number of spoons per portion. The spoon sizes were obtained from home measurements. Photographs of spoons were used during interviews to avoid classification errors. For instant coffee, the information used came from the manufacturer: an average of 3 mg of caffeine per gram of powdered coffee. For each mother, the average daily caffeine intake was calculated per trimester and during the entire pregnancy. Mothers who consumed ≥300 mg/day of caffeine were considered heavy consumers (the exposed group).

Potential confounding factors in the association between maternal caffeine consumption during pregnancy and ADHD were gathered at the perinatal study and considered in the adjusted analysis: National Economic Index (acronym IEN in Portuguese) presented in quintiles (in which mothers at Q1 were the poorest and at Q5 were the wealthiest); mother's and father's education levels, evaluated as years of study; maternal age, evaluated as complete years at the delivery; mother living with or without partner; mother smoking during pregnancy (at least one cigarette/day in at least one trimester of the pregnancy); father smoking during pregnancy (in at least one trimester of the pregnancy); alcohol consumption by the mother during pregnancy (yes or no); antenatal care (yes or no); number of antenatal care consultations; mood symptoms during pregnancy (through the question "During pregnancy, did you feel depressed or nervous?"); maternal nutritional state before pregnancy, evaluated according to the body mass index (BMI) and categorized as underweight (<18.5kg/m$^2$), normal weight (18.5-24.9kg/m$^2$), overweight (25-29.9kg/m$^2$) or obese (≥30 kg/m$^2$); the child gestational age at birth; type of delivery (normal or cesarean); and low birth weight (<2500 g) (yes or no).

Only children from single pregnancies were included in the analysis. The prevalence of ADHD and respective 95% confidence interval (95% CI) was calculated for the entire cohort and separately by sex (based on the current literature that consistently reports higher prevalence rates among boys).(5-13) Prevalence (95% CI) of heavy caffeine consumption in each trimester and during the entire pregnancy was also calculated. The association between maternal heavy caffeine consumption and ADHD was evaluated by means of the chi-square test. The strength of the association between caffeine consumption and ADHD was ascertained for the entire cohort and after stratification by sex, by means of logistic regression (crude and adjusted for

confounding factors). With the aim of evaluating the presence of dose-response effects, daily caffeine consumption was grouped in three categories: <100, 100-299 and ≥300 mg/day. In addition, analyses were performed with daily caffeine intake as a continuous variable.

A conceptual framework previously built by the authors describing the postulated hierarchical relationships between exposures (Figure 1) was used to drive the inclusion of potential confounders to the analytical model. Maternal mental health during pregnancy was the first variable included in the model, followed by father years of school and maternal socio-demographic characteristics (IEN, years of school, age and marital status). Subsequently the behavioral variables were added (maternal smoking and alcoholic beverage intake during pregnancy, paternal smoking during pregnancy, and number of antenatal care consultations). Only variables associated with the outcome at p-values ≥ 0.20 were kept at the final model.

The Pelotas 2004 Birth Cohort Study was approved by the Research Ethics Committee of the Medical School of the Federal University of Pelotas that is affiliate to the Brazilian National Commission for Research Ethics. Mothers signed an informed consent form at each folow-up, after being informed of the study objectives.

## RESULTADOS

The present study only used data from the perinatal evaluation (N=4231) and the follow-up at the age of six years (N=3721). A total of 3507 mother and children had full information on caffeine intake and mental health and were entered at the current analysis.

The prevalence of ADHD in the population studied was 2.6% (95% CI: 2.1-3.2%): 3.4% (95% CI: 2.9-3.9%) among boys and 1.8% (95% CI: 1.4-2.2%) among

girls. The prevalence of heavy caffeine consumption during the entire pregnancy and in the first, second and third trimesters was 15.4% (95% CI: 11.0-19.8%), 19.2% (95% CI: 13.5-24.9%), 17.9% (95% CI: 12.6-23.2%) and 16.4% (95% CI: 11.6-21.2%), respectively.

Heavy-caffeine consumer mothers were more likely to present economic and behavioral exposures than the remaining mothers: they were poorer (28,5% versus 18,6% belonged to Q1 of the IEN; p= <0,001), had less years of formal education (28,0% versus 13,1% for mothers with education 0 to 4 years; p= <0,001), smoked (55,7% versus 22,4%; p= <0,001) and consumed alcoholic beverages (5,0 versus 2,9; p= <0,001) in pregnancy, attended a few number of antenatal care consultations (25,1% versus 16,1%; p= <0,001), and presented mood symptoms during pregnancy (24,7% versus 21,1; p= 0,035).

In Table 1, the prevalence of ADHD is presented according to family and child variables, for the total cohort and after stratification by sex. The IEN was inversely associated with ADHD: the higher the economic level of the family, the lower the prevalence of ADHD. The prevalence of ADHD was higher among the children whose mothers lived without a partner and presented mood symptoms during pregnancy. The prevalence of ADHD among the children of mothers who were heavy consumers of caffeine did not differ from what was observed among the children of mothers who consumed less than 300 mg/day during the entire pregnancy or in each trimester.

Figure 2 shows the prevalence of ADHD in relation to caffeine consumption in three categories (< 100, 100-299 and ≥ 300 mg/day) per trimester and at the entire pregnancy. There was no association between daily maternal consumption of caffeine and prevalence of ADHD.

The crude and adjusted analyses of the association between heavy caffeine consumption per trimester and during the entire pregnancy and ADHD are presented in Table 2. There was no association between caffeine consumption and ADHD, both in the crude and in the adjusted analysis, during the three pregnancy trimesters and at the entire pregnancy. All the 95% CI of the estimated odds ratios included the unit, thus showing that there was no association. The same result was shown in the analysis stratified according to sex.

The crude and adjusted analyses of caffeine divided into three categories (<100, 100-299 and ≥ 300 mg/day) (Table 3) and as a continuous variable (data not shown) also found no association.

## DISCUSSÃO

The present study did not show any association between caffeine consumption during pregnancy and ADHD. Contrary to the hypothesis of this study, the crude and adjusted analyses indicated that caffeine had no effect over the occurrence of ADHD. A recent review of the literature showed that there is a scarcity of studies evaluating the effect of caffeine consumption during pregnancy over the occurrence of ADHD, and concluded that the available evidence does not make it possible to confirm or deny the risk that this exposure might present with regard to development of this morbidity during childhood.(21) The five studies investigating the effect of maternal caffeine consumption over the occurrence of ADHD(21) differed in relation to the tools used to measure the outcome: only one evaluated ADHD by means of a diagnostic instrument and this did not find any association.(28) The remaining articles used screening tests: Conners' Continuous Performance Test II (CPT-II),(29) the Child Behavior Checklist (CBCL)(8, 22) and SDQ(30) and only one found an association(22) indicating that

caffeine consumption during pregnancy would increase the risk of ADHD. The difference between the instrument used for assessing the presence of ADHD generates issues that go beyond the lack of comparability. Screening instruments are more sensitive and less specific, and have a high capacity to recognize true positives, but they fail to discard false positives, thereby wrongly identifying healthy individuals as ill. For instance, in an analysis of data from another cohort conducted in Pelotas (the Pelotas 1993 Birth Cohort Study), to estimate the prevalence of psychiatric diseases among children aged 11 years, Anselmi et al(31) compared the results from DAWBA with those from the Strengths and Difficulties Questionnaire (SDQ), which is a screening instrument. They found that as a screening instrument for ADHD, SDQ presented weak performance, with a positive predictive value (PPV) of 48.2% and a negative predictive value (NPV) of 90.2%. Similar results have been found in other Brazilian studies.(26, 32)

There is a high inter-individual variability in the physiological response to caffeine consumption that may in part be due to genetic characteristics. The genes involved in caffeine metabolism, such as cytochrome P450 1A2 (CYP1A2), and in caffeine responses in the central nervous system, such as the adenosine 2A receptor (ADORA2A*)*, have been the main targets of genetic studies in this area.(33-38) Polymorphisms in genes in these pathways have been correlated with the habit of consuming coffee and have been shown to be important to modulate the response to caffeine consumption among adults, such as symptoms of anxiety, cognitive performance and insomnia.(33-38) On the other hand, little is known about the molecular response mechanisms to caffeine in the central nervous system while it is still developing; or about the way in which gene polymorphisms along these pathways might module the response to caffeine. Future studies adding genetic factors to caffeine

consumption during pregnancy could contribute towards better understanding the potential role that caffeine may play in the development of ADHD and other psychiatric disorders.

The present study presents some strengths and limitations. Among the strengths is the fact that this was a longitudinal study with data from a birth cohort of about 4,000 children, which facilitates the generalization of data. The longitudinal analysis is characterized by following up individuals over a period of time, which ensures that the temporal relationship between exposures and outcomes can be ascertained. Hence, among all the observational study designs this is the ideal for investigating the topic in question. Furthermore, detailed information on caffeine consumption from coffee and yerba mate during the three trimesters of pregnancy was available. The outcome was evaluated by means of an instrument that had been adapted and validated for the Brazilian population, which made it possible to confirm the diagnoses of ADHD.(26) Moreover, there was the possibility of controlling the analysis for a great number of potential confounding factors. Also noteworthy is the low percentage of losses and refusals during the follow-up of the study (90.2% from birth to 6 years of age).

Amongst the limitations, the diagnostic tests, including DAWBA, generally evaluate two contexts in the child's life: Home and school. The present study evaluated only the home context, because at the age of six years most of the children had not yet entered to school and the remainder were just joining school life, thus making it too early to expect teachers to be able to make an evaluation of their behavior. In addition, the amount of coffee and yerba mate consumed during pregnancy was obtained retrospectively, being subject to recall bias. Also, although caffeine consumption during pregnancy was assessed from the two main sources (coffee and mate) there are other caffeine sources (foods like chocolate, chocolate drink and cola-drinks as well as

medicines) that were not measured. However, daily consumption and caffeine intake from other food are low at this population (0,19 mg/ml in black tea; 0,10 mg/ml in soft drinks; and 0,67 mg/g in chocolate bars).(27)

## CONCLUSÃO

There is no evidence from the present study to support any deleterious effect of caffeine consumption during pregnancy over the occurrence of ADHD in the offspring.

## CONTRIBUTORS STATEMENT

Ms Silva BDP and Dr Santos IS participated in the design of the manuscript, carried out the initial analyses, drafted the initial manuscript, and approved the final manuscript as submitted.

Drs Matijasevich A and Santos IS designed the data collection instruments, and coordinated and supervised data collection, critically reviewed the manuscript, and approved the final manuscript as submitted.

Drs Tovo-Rodrigues L and Anselmi L participated in the drafting, critically reviewed the manuscript, and approved the final manuscript as submitted.

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## CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

## DATA SHARING STATEMENT

Extra data is available by emailing bianca.delponte@gmail.com

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Table 1. Prevalence of attention deficit hyperactivity disorder (ADHD) at the age of six years, according to the characteristics of the family and child.

| Variables | N (%) | Boys | | Girls | | Total | |
|---|---|---|---|---|---|---|---|
| | | ADHD % | P | ADHD % | p | ADHD % | P |
| IEN | 3472 | | 0.10* | | 0.07* | | 0.03* |
| Q1 | 709 (20.4) | 3.7 | | 2.2 | | 3.0 | |
| Q2 | 695 (20.0) | 4.5 | | 2.1 | | 3.3 | |
| Q3 | 689 (19.8) | 4.3 | | 1.8 | | 3.1 | |
| Q4 | 693 (20.0) | 3.1 | | 1.3 | | 2.3 | |
| Q5 | 686 (19.8) | 1.9 | | 0.6 | | 1.3 | |
| Maternal education level in years | 3506 | | 0.50* | | 0.14* | | 0.19* |
| 0-4 | 524 (15.0) | 2.6 | | 2.0 | | 2.3 | |
| 5-8 | 1461 (41.7) | 4.4 | | 1.9 | | 3.2 | |
| 9-11 | 1154 (32.9) | 3.3 | | 1.6 | | 2.5 | |
| 12 or over | 367 (10.5) | 2.0 | | 0.0 | | 1.1 | |
| Paternal education level in years | 2753 | | 0.30* | | 0.07* | | 0.07* |
| 0-4 | 485 (17.6) | 3.5 | | 3.2 | | 3.3 | |
| 5-8 | 976 (35.5) | 3.2 | | 1.7 | | 2.5 | |
| 9-11 | 989 (35.9) | 3.2 | | 1.9 | | 2.6 | |
| 12 or over | 303 (11.0) | 1.2 | | 0.0 | | 0.7 | |
| Maternal age | 3513 | | 0.29* | | 0.05* | | 0.05* |
| <20 | 675 (19,2) | 3.4 | | 2.5 | | 3.0 | |
| 20-35 | 2458 (70.0) | 3.8 | | 1.7 | | 2.9 | |
| >35 | 379 (10.8) | 1.1 | | 0 | | 0.5 | |
| Conjugal situation | 3513 | | 0.02 | | 0.38 | | 0.02 |
| With partner | 2957 (84.2) | 3.1 | | 1.5 | | 2.3 | |
| Without partner | 556 (15.8) | 5.7 | | 2.3 | | 4.1 | |
| Mother smoking during pregnancy | 3513 | | 0.59 | | 0.33 | | 0.34 |
| No | 2559 (72.8) | 3.4 | | 1.5 | | 2.5 | |
| Yes | 954 (27.2) | 3.9 | | 2.2 | | 3.0 | |

*Linear trend test

*Cont.* Table 1. Prevalence of attention deficit hyperactivity disorder (ADHD) at the age of six years, according to the characteristics of the family and child.

| Variables | N (%) | Boys | | Girls | | Total | |
|---|---|---|---|---|---|---|---|
| | | ADHD % | P | ADHD % | P | ADHD % | P |
| Father smoking during pregnancy | 3513 | | 0.29 | | 0.88 | | 0.35 |
| No | 2446 (69.6) | 3.2 | | 1.6 | | 2.5 | |
| Yes | 1067 (30.4) | 4.2 | | 1.7 | | 3.0 | |
| Alcohol consumption by the mother during pregnancy | 3513 | | 0.20 | | 0.24 | | 0.08 |
| No | 3396 (96.7) | 3.4 | | 1.6 | | 2.5 | |
| Yes | 117 (3.3) | 6.5 | | 3.6 | | 5.1 | |
| Prenatal care | 3513 | | 0.89 | | 0.58 | | 0.90 |
| No | 43 (1.2) | 4.0 | | 0.0 | | 2.3 | |
| Yes | 3470 (98.8) | 3.5 | | 1.7 | | 2.6 | |
| Number of prenatal consultations | 3327 | | 0.09 | | 0.06 | | 0.02 |
| ≥6 | 2776 (83.4) | 3.2 | | 1.4 | | 2.3 | |
| < 6 | 551 (16.6) | 5.2 | | 3.0 | | 4.2 | |
| Maternal mood symptoms during pregnancy | 3511 | | 0.20 | | 0.06 | | 0.01 |
| No | 2647 (75.4) | 3.1 | | 1.3 | | 2.2 | |
| Yes, not treated | 746 (21.3) | 4.9 | | 3.1 | | 4.0 | |
| Yes, treated | 118 (3.4) | 4.2 | | 2.1 | | 3.4 | |
| Pre-pregnancy BMI | 2080 | | 0.95* | | 0.07* | | 0.31* |
| Underweight | 69 (3.3) | 0.0 | | 0.0 | | 0.0 | |
| Normal weight | 1187 (57.1) | 4.4 | | 1.4 | | 3.0 | |
| Overweight | 567 (27.3) | 3.3 | | 1.9 | | 2.7 | |
| Obese | 257 (12.36) | 4.3 | | 3.6 | | 3.9 | |
| Gestational age | 3490 | | 0.92 | | 0.56 | | 0.88 |
| ≥ 37 weeks | 3124 (89.5) | 3.4 | | 1.6 | | 2.6 | |
| < 37 weeks | 366 (10.5) | 3.5 | | 2.1 | | 2.7 | |

*Linear trend test

*Continuation*

| Variables | N (%) | Boys ADHD % | P | Girls ADHD % | p | Total ADHD % | P |
|---|---|---|---|---|---|---|---|
| Type of delivery | 3513 | | 0.50 | | 0.23 | | 0.26 |
| Normal | 1577 (44.9) | 3.2 | | 1.2 | | 2.3 | |
| Cesarean | 1936 (55.1) | 3.8 | | 2.0 | | 2.9 | |
| Low birth weight | 3512 | | 0.78 | | 0.33 | | 0.64 |
| No | 3237 (92.2) | 3.5 | | 1.8 | | 2.7 | |
| Yes | 275 (7.8) | 3.9 | | 0.7 | | 2.2 | |
| Heavy caffeine consumption during the first trimester of pregnancy | 3507 | | 0.62 | | 0.52 | | 0.45 |
| No | 2829 (80.7) | 3.6 | | 1.7 | | 2.7 | |
| Yes | 678 (19.3) | 3.1 | | 1.3 | | 2.2 | |
| Heavy caffeine consumption during the second trimester of pregnancy | 3505 | | 0.40 | | 2.95 | | 0.20 |
| No | 2869 (81.9) | 3.7 | | 1.82 | | 2.8 | |
| Yes | 636 (18.1) | 2.4 | | 0.98 | | 1.9 | |
| Heavy caffeine consumption during the third trimester of pregnancy | 3506 | | 0.37 | | 0.76 | | 0.36 |
| No | 2926 (83.5) | 3.7 | | 1.7 | | 2.7 | |
| Yes | 580 (16.5) | 2.6 | | 1.5 | | 2.1 | |
| Heavy caffeine consumption during the entire pregnancy | 3503 | | 0.32 | | 0.48 | | 0.22 |
| No | 2961 (84.5) | 3.7 | | 1.8 | | 2.8 | |
| Yes | 542 (15.5) | 2.5 | | 1.2 | | 1.9 | |

*Linear trend test

Table 2. Association between heavy caffeine consumption (≥300 mg/day) during the entire pregnancy and per trimester and presence of attention deficit hyperactivity disorder (ADHD) at the age of six years.

| | Total | | | | Boys | | | | Girls | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Crude analysis (N= 3507) | | Adjusted analysis* (N= 3282) | | Crude analysis (N= 1827) | | Adjusted analysis* (N= 1707) | | Crude analysis (N= 1680) | | Adjusted analysis* (N= 1575) | |
| | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | p | OR (95% CI) | p | OR (95% CI) | P | OR (95% CI) | p |
| Caffeine consumption 1st trimester | | | | | | | | | | | | |
| No | 1 | 0.46 | 1 | 0.18 | 1 | 0.62 | 1 | 0.49 | 1 | 0.52 | 1 | 0.35 |
| Yes | 0.80 (0.46-1.41) | | 0.74 (0.42-1.30) | | 0.84 (0.44-1.64) | | 0.79 (0.41-1.54) | | 0.70(0.24-2.05) | | 0.59(0.20-1,76) | |
| 2nd trimester | | | | | | | | | | | | |
| No | 1 | 0.20 | 1 | | 1 | 0.41 | 1 | 0.32 | 1 | 0.30 | 1 | 0.20 |
| Yes | 0.67 (0.36-1.24) | | 0.61 (0.33-1.14) | 0.12 | 0.74 (0.36-1.51) | | 0.69 (0.34-1.43) | | 0.53 (0.16-1.77) | | 0.45 (0.13-1.52) | |
| 3rd trimester | | | | | | | | | | | | |
| No | 1 | 0.36 | 1 | 0.19 | 1 | 0.37 | 1 | 0.30 | 1 | 0.76 | 1 | 0.52 |
| Yes | 0.75 (0.41-1.39) | | 0.69 (0.37-1.27) | | 0.71 (0.33-1.50) | | 0.67 (0.31-1,43) | | 0.76 (0.29-2.45) | | 0.69 (0.24-2.07) | |
| Entire pregnancy | | | | | | | | | | | | |
| No | 1 | 0.22 | 1 | 0.13 | 1 | 0.32 | 1 | 0.25 | 1 | 0.48 | 1 | 0.31 |
| Yes | 0.66 (0.34-1.28) | | 0.59 (0.30-1.16) | | 0.67 (0.30-1.48) | | 0.63 (0.28-1,39) | | 0.65 (0.19-2.16) | | 0.53 (0.15-1,79) | |

OR, odds ratio; IC, confidence interval

* Adjusted for IEN and maternal marital status, smoking and mood symptoms during pregnancy

Table 3. Association between caffeine consumption during the entire pregnancy and per trimester and the presence of attention deficit hyperactivity disorder (ADHD) at the age of six years.

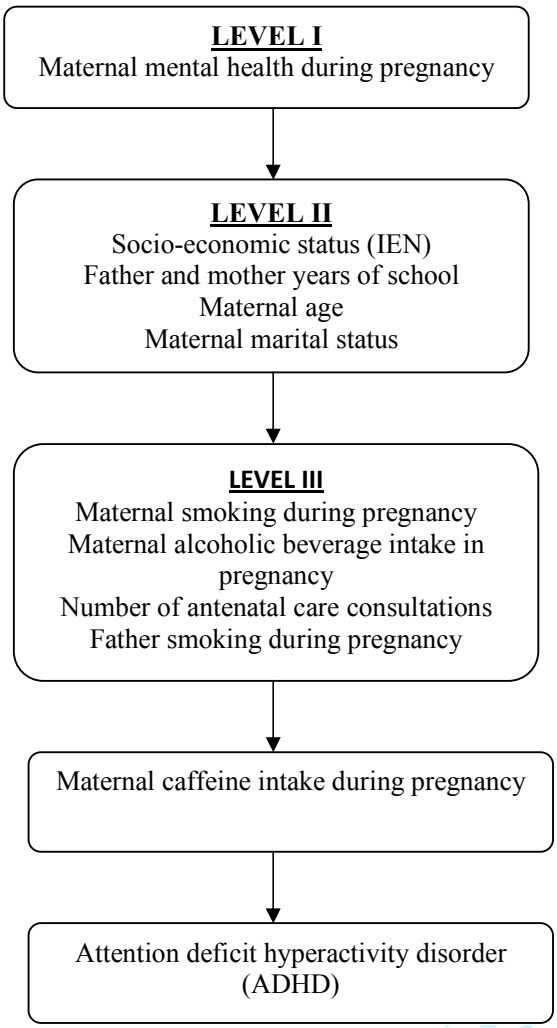
| | Total | | | | Boys | | | | Girls | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Crude analysis N= 3507 | | Adjusted analysis* N= 3282 | | Crude analysis N= 1827 | | Adjusted analysis* N= 1707 | | Crude analysis N= 1680 | | Adjusted analysis* N= 1575 | |
| | OR (95% CI) | P | OR (95% CI) | p | OR (95% CI) | p | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | P |
| Caffeine consumption | | | | | | | | | | | | |
| 1st trimester | | 0,71 | | 0,69 | | 0,89 | | 0,30 | | 0,75 | | 0,37 |
| <100mg/day | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | |
| 100-299 mg/day | 0.91 (0.54-1.54) | | 0.90 (0.53-1.53) | | 0.99 (0.52-1.88) | | 0.99 (0.52-1.88) | | 0.84 (0.33-2.14) | | 0.80 (0.31-2.09) | |
| ≥300 mg day | 0.79 (0.45-1.41) | | 0.71 (0.40-1.28) | | 0.85 (0.43-1.68) | | 0.79 (0.40-1.58) | | 0.67 (0.23-2.00) | | 0.56 (0.18-1.70) | |
| 2nd trimester | | 0,30 | | 0,09 | | 0,67 | | 0,76 | | 0,39 | | 0,18 |
| <100mg/day | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | |
| 100-299 mg/day | 0.81 (0.47-1.39) | | 0.78 (0.46-1.37) | | 0.92 (0.48-1.76) | | 0.90 (0.47-1.75) | | 0.68 (0.25-1.82) | | 0.63 (0.23-1.73) | |
| ≥300 mg day | 0.64 (0.34-1.19) | | 0.58 (0.31-1.09) | | 0.72 (0.35-1.50) | | 0.67 (0.32-1.41) | | 0.49 (0.14-1.64) | | 0.39 (0.11-1.37) | |
| 3rd trimester | | 0,52 | | 0,18 | | 0,62 | | 0,48 | | 0,89 | | 0,55 |
| <100mg/day | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | |
| 100-299 mg/day | 0.83 (0.47-1.47) | | 0.80 (0.45-1.43) | | 0.89 (0.45-1.79) | | 0.87 (0.43-1.75) | | 0.83 (0.31-2.25) | | 0.78 (0.28-2.29) | |
| ≥300 mg day | 0.73 (0.39-1.35) | | 0.65 (0.35-1.23) | | 0.69 (0.33-1.49) | | 0.65 (0.30-1.41) | | 0.81 (0.27-2.40) | | 0.66 (0.21-2.00) | |
| Entire pregnancy | | 0,21 | | 0,37 | | 0,31 | | 0,48 | | 0,61 | | 0,58 |
| <100mg/day | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | |
| 100-299 mg/day | 1.13 (0.70-1.84) | | 1.11 (0.68-1.82) | | 1.26 (0.71-2.26) | | 1.24 (0.69-2.23) | | 0.98 (0.41-2.36) | | 0.93 (0.38-2.29) | |
| ≥300 mg/day | 0.03 (0.31-1.20) | | 0.54 (0.27-1.08) | | 0.65 (0.29-1.46) | | 0.60 (0.26-1.37) | | 0.56 (0.16-1.92) | | 0.45 (0.13-1.57) | |

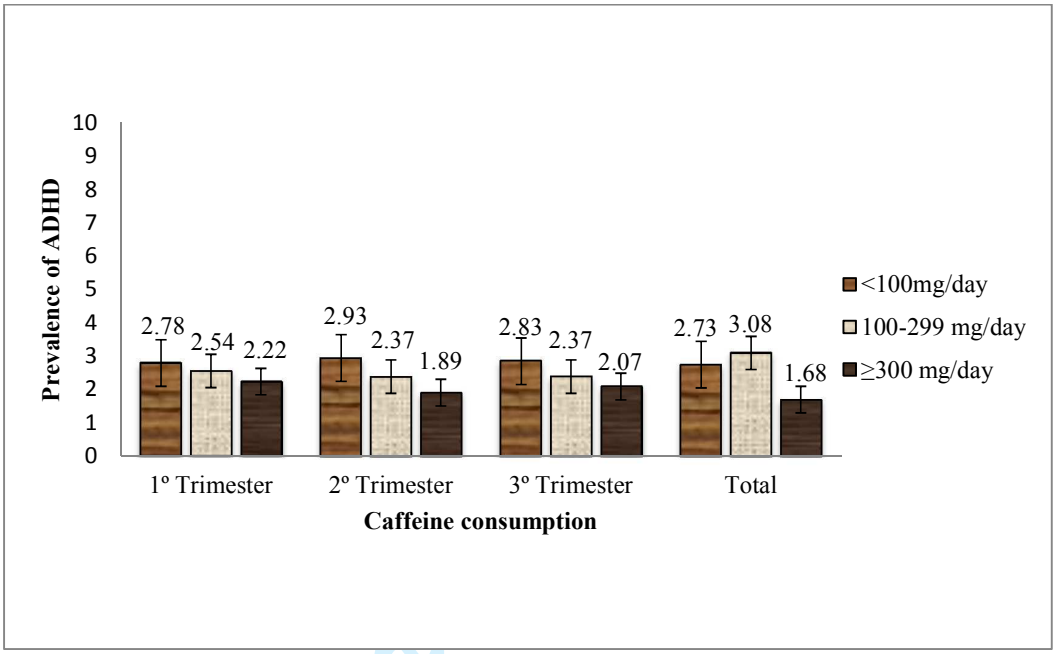
OR, odds ratio; IC, confidence interval

* Adjusted for IEN and maternal marital status, smoking and mood symptoms during pregnancy

Figure 1. Conceptual framework for the association between maternal caffeine consumption during pregnancy and offspring ADHD at the age of six years.

Figure 2. Prevalence of attention deficit hyperactivity disorder (ADHD) at the age of six years, according to maternal caffeine consumption (mg/day) in each trimester and during the entire pregnancy.





STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation | Page in article |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | 5 |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6, 7 and 8 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | |
| Bias | 9 | Describe any efforts to address potential sources of bias | 8 |
| Study size | 10 | Explain how the study size was arrived at | 6 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 9 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 9 |
| | | (*c*) Explain how missing data were addressed | |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | |
| | | (*e*) Describe any sensitivity analyses | |

Continued on next page

| Results | | | Page in article |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 9 |
| | | (b) Give reasons for non-participation at each stage | 9 |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | 9 |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 11 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 11 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 11 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 11 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 13 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11 and 12 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 13 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 15 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

# Caffeine consumption during pregnancy and ADHD at the age of eleven years: A birth cohort study



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# Caffeine consumption during pregnancy and ADHD at the age of eleven years: A birth cohort study

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**Keywords:** Caffeine, pregnancy, ADHD and hyperactivity.

## ABSTRACT

**Objective:** Studies evaluating caffeine intake during pregnancy and long-term outcomes, such as the child's neurobehavior, are still scarce and their results are inconsistent. The objective of the present study was to evaluate the association between maternal consumption of caffeine during pregnancy and attention deficit hyperactivity disorder (ADHD) at the age of eleven years.

**Methodology:** All children born in the city of Pelotas, Brazil, during the year 2004, were selected for a cohort study. The mothers were interviewed at birth to obtain information on coffee and yerba mate consumption during pregnancy, among other matters. At the age of eleven years, presence of ADHD was evaluated using the Development and Well-Being Assessment (DAWBA) questionnaire, applied to the mothers. The prevalence of ADHD were calculated, with 95% confidence intervals (95%CI). The association between caffeine consumption and ADHD was tested by means of logistic regression.

**Results:** 3485 children were included in the analyses. The prevalence of ADHD was 4,1% (95% CI: 3,4-4,7%): 5,8% (95% CI: 4,7-6,9) among boys and 2,3% (95% CI: 1,5-3,0%) among girls. The prevalence of caffeine consumption during the entire pregnancy and in the first, second and third trimesters was 7,7% (6,9-8,5%), 11,3% (10,3-12,2%), 13,5% (12,5-14,6%) and 17,0% (15,8-18,1%), respectively. The caffeine consumption during the entire pregnancy and the first, second and third trimesters not associated with ADHD in the crude or adjusted analysis.

**Conclusion:** The present study did not show any association between maternal caffeine consumption during pregnancy and ADHD at the age of eleven years.

## ARTICLE SUMMARY

### Strengths

This was a longitudinal study ensuring that the temporal relationship between exposure and outcome can be ascertained. Detailed information on caffeine consumption from coffee and yerba mate during the three trimesters of pregnancy was available. The outcome was evaluated by means of a validated instrument. Information on a number of potential confounding factors was gathered and their role over the association between maternal caffeine intake and ADHD was formally tested. There were a low percentage of losses and refusals during the follow-up of the study.

### Limitations

The outcome was ascertained by means of a test applied only to the mother. In addition, the reported amount of coffee and yerba mate consumed during pregnancy may be subject to recall bias. Also, although caffeine consumption during pregnancy was assessed from the two main sources (coffee and mate) there are other caffeine sources (foods like chocolate, chocolate drink and cola-drinks as well as medicines) that were not measured.

## FINDINGS TO DATE

- The prevalence caffeine consumption during the entire pregnancy was high (7,7%; 6,9-8,5%).
- The prevalence of ADHD was 4,1% (3,4-4,7%); higher among boys (5,8%; 4,7-6,9%) than among girls (2,3%; 1,5-3,0%).
- The present cohort study, involving around 3500 children did not show any association between maternal caffeine consumption during pregnancy and ADHD.

## INTRODUÇÃO

Attention deficit hyperactivity disorder (ADHD) is a neurobiological disorder that affects around 6% of school-age children around the world.(1) It is the most prevalent mental disorder during childhood and the main reason why mental health services are sought for children and adolescents.(2) It is characterized by persistent symptoms of inattention, impulsivity and hyperactivity, which become present before the age of 12 years and are abnormal for the developmental stage.(3)

ADHD is a multi factorial disease with complex etiology and a large genetic component (heritability estimated as 76%).(4) Epidemiological studies have shown higher prevalence among boys and among children belonging to families with worse socioeconomic conditions.(5-13) Maternal caffeine consumption (14) as well as other nutritional factors during pregnancy, such as intake of folic acid,(15) iron(16) and omega-3(17) have been investigated as determinants of ADHD. In animals, intrauterine exposure to caffeine was associated with increased motor activity, thus suggesting a possible effect on attention deficit and hyperactivity on children born to mothers with high consumption of caffeine-rich foods and beverages during pregnancy.(18, 19) Moreover, exposure of rats to caffeine, during the prenatal period, resulted in gene expression alterations relating to formation of synapses, thereby showing some of the potential molecular effects of caffeine during fetal cerebral development.(20)

Caffeine is commonly consumed throughout the world, including by pregnant women, who present daily consumption prevalence ranging from 75% to 93%.(21) Studies evaluating caffeine intake during pregnancy and long-term outcomes, such as child neuro-behavior, are still scarce and their results are inconsistent. Among five articles identified in a systematic review of the literature (22) only one found that the higher maternal caffeine intake during pregnancy would increase the risk of ADHD.(14)

The objective of the present study was to evaluate the association between maternal caffeine consumption during pregnancy and ADHD at the age of eleven years, among children belonging to a birth cohort. The hypothesis of the study was that maternal caffeine consumption during pregnancy was associated with ADHD at the age of eleven years.

## METODOLOGIA

In 2004, a birth cohort study was begun in the city of Pelotas, Brazil. The original cohort population consisted of the 4231 newborns at the five hospitals in the city, who were the children of mothers living in the urban zone of Pelotas, corresponding to 99.2% of the births in that year. After delivery (perinatal study) mothers were interviewed by trained interviewers, using standardized questionnaires, regarding their socioeconomic, demographic and reproductive characteristics, use of health services, prenatal attention and pregnancy complications. Further methodological details of the study can be found in other publications.(23-25)

So far, the cohort participants were followed-up at the ages of 3, 12, 24 and 48 months, and at 6 and 11 years. The mothers were interviewed regarding their children's growth, development, type of food, and morbidity, and also answered questions about their own health.(24) Differently from the visits at 3, 12, 24, and 48 months that took place at the child's place, at the age of 6 and 11 years data-gathering was undertaken at a clinic that had been set up especially to attend to this research. Besides interviews, the children underwent a comprehensive health evaluation, which included psychological, psychiatric, anthropometric and body composition evaluations.(25)

The presence of ADHD was evaluated by means of the Development and Well-Being Assessment (DAWBA), an instrument employed for psychiatric diagnosis among

children and teenagers aged from 5 to 17 years, and that uses diagnostic classifications from the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V) and the International Classification of Diseases (ICD-10).(26) DAWBA was reported by mothers during the eleven -year follow-up, by trained interviewers (psychologists). The DAWBA combines highly structured questions based on DSM-V diagnostic criteria and ICD-10 with qualitative descriptions of all areas of difficulty. The generating program is a computer algorithm which provides a probability of a child to have any psychiatric disorder based on answers to structured questions. In the presence of positive symptoms in any area, additional questions (qualitative assessment) are made to assess the impact (loss) of these problems in the child's life. These questions concern specific areas covering distress and interference with family life, learning, friendship and leisure activities resulting in symptoms. Subsequently, a clinical evaluator, based on the collected information, combines the quantitative results with the qualitative date and makes a judgment in regard to the presence or absence of the disorder. The clinic trial in this case was made by a child psychiatrist *(rater)*, supervised by another child psychiatrist, who translated and validaded the DAWBA for the Brazilian population. To make the different psychiatric diagnosis from DAWBA evaluations, the *rater* needs to judge whether symptoms are present or not and the loss (impact) that they cause. DAWBA diagnoses are supplied dichotomously as “yes” or “no”, strictly respecting the diagnostic criteria defined by IDC-10 and DSM-V diagnostic classifications. For this study the DSM-V classification was employed. The DAWBA allows the identification of children currently under treatment for ADHD, such children were classified as positive for ADHD. The DAWBA questionnaire was translated and validated in Brazil by Bacy Fleitlich-Bilyk.(26)

The exposure of interest, daily caffeine consumption during pregnancy was evaluated at the perinatal study by means of a series of questions regarding consumption of the foods that are the main sources of caffeine at this region of the country: coffee and yerba mate (a typical hot beverage consumed in southern Brazil and neighboring countries, which is prepared from the leaves of the herb *Ilex paraguariensis)*. For each source of caffeine, the daily frequency of consumption was obtained, separately for each trimester of pregnancy. Information regarding the type of coffee (filtered or instant), preparation, concentration (strong, medium or weak) and quantity consumed per day was gathered, taking into consideration the size of the recipient (180 ml cup; 50 ml small cup; 200 ml glass and 190 ml mug). The estimated caffeine content from coffee and yerba mate was obtained from coffee samples collected from the homes of mothers who participated in a previous study conducted in the city of Pelotas,(27) and that were analyzed by chromatography. From these analyses, it was possible to infer the average caffeine content in mg per ml of coffee, according to the concentration at which it was consumed: strong coffee, 0.25 mg/ml; medium coffee, 0.20 mg/ml; and weak coffee, 0.11 mg/ml. For yerba mate, the analyses showed an average concentration of 17 mg of caffeine per 100 ml of the liquid. These results were used to estimate the caffeine intake of the entire sample. For instant coffee, the items investigated were the size of the spoon used to serve coffee (full coffee spoon, 2.6 g; level coffee spoon, 2.3 g; full small coffee spoon, 2.5 g; level small coffee spoon, 1.5 g; full dessert spoon, 7.5 g; and level dessert spoon, 7.0 g) and the number of spoons per portion. The spoon sizes were obtained from home measurements. Photographs of spoons were used during interviews to avoid classification errors. For instant coffee, the information used came from the manufacturer: an average of 3 mg of caffeine per gram of powdered coffee. For each

mother, the average daily caffeine intake was calculated per trimester and during the entire pregnancy.

Potential confounding factors in the association between maternal caffeine consumption during pregnancy and ADHD were gathered at the perinatal study and considered in the adjusted analysis: National Economic Index (acronym IEN in Portuguese) presented in quintiles (in which mothers at Q1 were the poorest and at Q5 were the wealthiest); mother's and father's education levels, evaluated as years of study; maternal age, evaluated as complete years at the delivery; mother living with or without partner; number of cigarettes/day smoked by the mother during pregnancy; number of cigarettes/day smoked by the father in the mother's presence during pregnancy; alcohol consumption by the mother during pregnancy (yes or no); number of antenatal care consultations; mood symptoms during pregnancy (through the question "During pregnancy, did you feel depressed or nervous?"); maternal nutritional state before pregnancy, evaluated according to the body mass index (BMI) and categorized as underweight (<18.5kg/m$^2$), normal weight (18.5-24.9kg/m$^2$), overweight (25-29.9kg/m$^2$) or obese (≥30 kg/m$^2$); the child gestational age at birth; birth weight; and sex of the child.

The twins were not included in the analyses (N=84). The prevalence of ADHD and respective 95% confidence interval (95% CI) was calculated for the entire cohort and separately by sex (based on the current literature that consistently reports higher prevalence rates among boys).(5-13) The association between maternal caffeine consumption grouped in three categories <100, 100-299 and ≥300 mg/day and ADHD was evaluated by means of the chi-square test. The strength of the association between caffeine consumption grouped in three categories and ADHD was ascertained for the entire cohort and after stratification by sex, by means of logistic regression (crude and

adjusted for confounding factors). In addition, analyses were performed with daily caffeine intake as a continuous variable.

A conceptual framework previously built by the authors describing the postulated hierarchical relationships between exposures (Figure 1) was used to drive the inclusion of potential confounders to the analytical model. Maternal mental health during pregnancy was the first variable included in the model, followed by father years of school and maternal socio-demographic characteristics (IEN, years of school, age and marital status). Subsequently the behavioral variables were added (maternal smoking and alcoholic beverage intake during pregnancy, paternal smoking during pregnancy, and number of antenatal care consultations). Only variables associated with the outcome at p-values $\leq 0.20$ were kept at the final model.

Loss to follow-up rates according to some of the child parents characteristics were not homogeneously distributed, the effect of missing outcome data was analysed as a sensitivity analysis, estimated by multiple imputation (mi Stata command) by the Bayesian paradigm from the frequentist (randomization-based) perspective. Least squares regression and 20 multiple datasets for the missing values were used.

The Pelotas 2004 Birth Cohort Study was approved by the Research Ethics Committee of the Medical School of the Federal University of Pelotas that is affiliate to the Brazilian National Commission for Research Ethics. Mothers signed an informed consent form at each folow-up, after being informed of the study objectives.

**RESULTADOS**

The present study used data from the perinatal study that included 4231 newborns and the follow-up at the age of eleven years (mean age of 10.9; standard deviation 0.3 years) that included 3566 children (follow-up rate of 86.6%). A total of

3485 mothers and children (82.4% of the original cohort) had full information on caffeine intake and mental health and were entered at the current analyses.

Table 1 shows the loss to follow-up rate at 11 years according to maternal caffeine intake in the entire pregnancy, IEN, paternal education level, mother living with or without a partner, and maternal mood symptoms during pregnancy. There were no differences in losses to follow-up by level of caffeine intake throughout pregnancy. Losses were higher for children from families in the extremes of IEN (18.9% among the poorest and 15.8% among the richest), with highly educated fathers (20.8%). Greater proportion of losses was also seen among children of mothers that lived with a partner and presented mood symptoms during pregnancy (Table 1).

The prevalence of ADHD was 4,1% (3,4-4,7%): 5,8% (4,7-6,9) among boys and 2,3% (1,5-3,0%) among girls. Table 2 shows the sample distribution and the prevalence of ADHD according to family and child variables. The ADHD was inversely associated with IEN: the higher the economic level of the family, the lower the prevalence of ADHD; maternal education level in years; paternal education level in years; number of prenatal consultations. The ADHD was directly associated with number of cigarettes smoked per day by the mother during pregnancy. The prevalence of ADHD was higher among children whose mothers lived without a partner, consumed alcohol during pregnancy and in boys.

Table 3 shows the prevalence and intensity of caffeine intake during pregnancy and the prevalence of ADHD among children of mothers who consumed between 100-299 mg/day or 300 or more mg/day of caffeine, compared to those from mothers who consumed less than 100 mg/day, taken as the reference group. The prevalence of caffeine consumption during the entire pregnancy and in the first, second and third trimesters was 7,7% (6,9-8,5%), 11,3% (10,3-12,2%), 13,5% (12,5-14,6%) and 17,0%

(15,8-18,1%), respectively. Most of the mothers consumed <100 mg/day of caffeine in the entire and in each trimester of pregnancy, whereas nearly one in every five mothers consumed ≥300 mg/day in every trimester and throughout pregnancy. Caffeine consumers were more likely to present economic and behavioral exposures than the remaining mothers, smoked and consumed alcoholic beverages in pregnancy, attended a few number of antenatal care consultations, and presented mood symptoms during pregnancy (data not shown). There was no difference in ADHD prevalence according to the mean amount of maternal daily caffeine consumption (Table 2).

The results of crude and adjusted analyses of the association between caffeine intake in three categories (<100, 100-299 and ≥ 300 mg/day) per trimester and during the entire pregnancy and ADHD are presented in Table 4. There was no association between caffeine consumption and ADHD, both in the crude and in the adjusted analysis, during the three pregnancy trimesters and at the entire pregnancy. All the 95% CI of the estimated odds ratios included the unit, thus showing that there was no association. The same result was shown in the analysis stratified by sex. Analyses with caffeine as a continuous variable also found no association (data not shown).

The multiple imputation data for the primary outcome produced imputed estimates that were similar to the available data. This similarity showed that all analyses were not affected by missing data or differential rates of follow-up.

## DISCUSSÃO

The present study found a prevalence of TDAH of 4,1% (95% CI: 3,4-4,7%): 5,8% (95% CI: 4,7-6,9) among boys and 2,3% (95% CI: 1,5-3,0%) among girls. This finding is consistent with results from other studies that employed DAWBA as the evaluation tool and the DSM-IV as the diagnostic criterion in Brazil.(5, 28, 29) The

prevalence of ADHD in school children in Brazil ranges from 0.9% (30) to 26.8% (31). The former was a population-based study carried out with 5-10 year-old children using clinical criteria (DSM-IV) obtained from two environments (home and school) and taking into account the impact of the symptoms at the child familiar and social relationships.(30) The later investigated a sample of 6-15 year-old school children employing clinical assessment (DSM-IV), but considering only the report by the teachers and without considering the impact of the symptoms. The variability between the estimates may be due to different factors, from type of sample, evaluation tools, diagnostic criteria, and mainly the source of information (parents, children, adolescents or teachers).(32) Prevalence of ADHD is generally higher in school samples than in population-based samples.(31).

This study did not show any association between caffeine consumption during pregnancy and ADHD. Contrary to the hypothesis of this study, the crude and adjusted analyses indicated that caffeine had no effect over the occurrence of ADHD. A recent review of the literature showed that there is a scarcity of studies evaluating the effect of caffeine consumption during pregnancy over the occurrence of ADHD, and concluded that the available evidence does not make it possible to confirm or deny the risk that this exposure might present with regard to development of this morbidity during childhood.(22) The five studies investigating the effect of maternal caffeine consumption over the occurrence of ADHD(22) differed in relation to the tools used to measure the outcome: The only one that evaluated the presence of ADHD by means of a diagnostic instrument did not find any association.(33) The remaining articles used screening tests: Conners' Continuous Performance Test II (CPT-II),(34) the Child Behavior Checklist (CBCL)(8, 14) and SDQ(35) and only one found an association(14) indicating that caffeine consumption during pregnancy would increase the risk of

ADHD. The difference between the instrument used for assessing the presence of ADHD generates issues that go beyond the lack of comparability. Screening instruments are more sensitive and less specific, and have a high capacity to recognize true positives, but they fail to discard false positives, thereby wrongly identifying healthy individuals as ill. For instance, in an analysis of data from another cohort conducted in Pelotas (the Pelotas 1993 Birth Cohort Study), to estimate the prevalence of psychiatric diseases among children aged 11 years, Anselmi et al(29) compared the results from DAWBA with those from the Strengths and Difficulties Questionnaire (SDQ), which is a screening instrument. They found that as a screening instrument for ADHD, SDQ presented weak performance, with a positive predictive value (PPV) of 48.2% and a negative predictive value (NPV) of 90.2%. Similar results have been found in other Brazilian studies.(26, 30)

There is a high inter-individual variability in the physiological response to caffeine consumption that may in part be due to genetic characteristics. The genes involved in caffeine metabolism, such as cytochrome P450 1A2 (CYP1A2), and in caffeine responses in the central nervous system, such as the adenosine 2A receptor (ADORA2A*)*, have been the main targets of genetic studies in this area.(36-41) Polymorphisms in genes in these pathways have been correlated with the habit of consuming coffee and have been shown to be important to modulate the response to caffeine consumption among adults, such as symptoms of anxiety, cognitive performance and insomnia.(36-41) On the other hand, little is known about the molecular response mechanisms to caffeine in the central nervous system while it is still developing; or about the way in which gene polymorphisms along these pathways might module the response to caffeine. Future studies adding genetic factors to caffeine consumption during pregnancy could contribute towards better understanding the

potential role that caffeine may play in the development of ADHD and other psychiatric disorders.

The present study presents some strengths and limitations. Among the strengths is the fact that this was a longitudinal study with data from a birth cohort of about 4,000 children, which facilitates the generalization of data. The longitudinal analysis is characterized by following up individuals over a period of time, which ensures that the temporal relationship between exposures and outcomes can be ascertained. Hence, among all the observational study designs this is the ideal for investigating the topic in question. Furthermore, detailed information on caffeine consumption from coffee and yerba mate during the three trimesters of pregnancy was available. The outcome was evaluated by means of an instrument that had been adapted and validated for the Brazilian population, which made it possible to confirm the diagnoses of ADHD.(26) Moreover, there was the possibility of controlling the analysis for a number of potential confounding factors. Also noteworthy is the low percentage of losses and refusals during the follow-up of the study (13.4% from birth to eleven years of age). Post-hoc analyses indicated that the study had a power of 82% to detect as statistically significant odds ratios $\geq 1.5$, setting alfa at 0.05 two-tailed.

Some limitations of the study need to be taken in consideration. The lack of information on the presence of ADHD in the mothers is among the limitations. Perhaps mothers with some degree of ADHD may not consider excessive activity in her child as unusual. In addition, the amount of coffee and yerba mate consumed during pregnancy may have been subject to recall bias. Also, although caffeine consumption during pregnancy was assessed from the two main sources (coffee and mate) there are other caffeine sources (foods like chocolate, chocolate drink and cola-drinks as well as medicines) that were not measured. However, daily consumption from other sources is

low at this population representing less than 10% of all caffeine consumed by pregnant women.(27)

## CONCLUSION

There is no evidence from the present study to support any deleterious effect of caffeine consumption during pregnancy over the occurrence of ADHD in the offspring.

## CONTRIBUTORS STATEMENT

Ms Silva BDP and Dr Santos IS participated in the design of the manuscript, carried out the initial analyses, drafted the initial manuscript, and approved the final manuscript as submitted.

Drs Matijasevich A and Santos IS designed the data collection instruments, and coordinated and supervised data collection, critically reviewed the manuscript, and approved the final manuscript as submitted.

Drs Tovo-Rodrigues L and Anselmi L Munhoz TN participated in the drafting, critically reviewed the manuscript, and approved the final manuscript as submitted.

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## CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

## DATA SHARING STATEMENT

Extra data is available by emailing bianca.delponte@gmail.com

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Table 1. Socio-demographic characteristics of mothers and children enrolled in the 2004 Pelotas Birth Cohort, and loss to follow-up rate at 11 year. Pelotas 2004 Birth Cohort Study.

| Variables | Perinatal study | Loss to follow-up rate | |
|---|---|---|---|
| | N (%) | | p* |
| Caffeine intake in the entire pregnancy | | | 0.338 |
| <100 mg/day | 1534 | 17,1 | |
| 100-299 mg/day | 902 | 14.4 | |
| ≥300 mg day | 698 | 16.6 | |
| IEN | | | <0.001 |
| Q1 | 641 | 18.9 | |
| Q 2 | 659 | 13.4 | |
| Q 3 | 623 | 10.0 | |
| Q 4 | 640 | 8.8 | |
| Q 5 | 639 | 15.8 | |
| Paternal education level | | | 0.021 |
| 1-4 | 568 | 17.1 | |
| 5-8 | 1133 | 16.2 | |
| 9-11 | 1159 | 14.1 | |
| 12 or more | 375 | 20.8 | |
| Maternal conjugal situation | | | 0.001 |
| With partner | 3468 | 20.5 | |
| Without partner | 679 | 15.3 | |
| Maternal mood symptoms in pregnancy | | | <0.001 |
| No | 3107 | 14.9 | |
| Yes, treated | 898 | 20.5 | |
| Yes, not treated | 140 | 15.7 | |

Table 2. Prevalence of attention deficit hyperactivity disorder (ADHD) at the age of eleven years, according to characteristics of the family and the child.

| Variables | 11-year follow-up | | |
|---|---|---|---|
| | N (%) | ADHD % | P |
| IEN | 2780 | | 0.003** |
| Q1 | 521 (18.7) | 5.0 | |
| Q2 | 574 (20.7) | 5.6 | |
| Q3 | 562 (20.2) | 3.0 | |
| Q4 | 584 (21.0) | 4.1 | |
| Q5 | 539 (19.4) | 1.9 | |
| Maternal education level in years | 3452 | | 0.003** |
| 0-4 | 512 (14.8) | 6.8 | |
| 5-8 | 1429 (41.4) | 4.1 | |
| 9-11 | 1175 (34.1) | 3.2 | |
| 12 or over | 336 (9,7) | 3.3 | |
| Paternal education level in years | 2717 | | 0.004** |
| 0-4 | 471 (17.3) | 5.5 | |
| 5-8 | 952 (35.0) | 4.5 | |
| 9-11 | 996 (36.7) | 3.2 | |
| 12 or over | 298 (11.0) | 2.0 | |
| Maternal age | 3483 | | 0.291** |
| <20 | 660 (18.9) | 4.9 | |
| 20-35 | 2441 (70,1) | 3.9 | |
| >35 | 382 (11.0) | 3.7 | |
| Maternal marital status | | | 0.001* |
| With partner | 2943 (84.5) | 3.6 | |
| Without partner | 542 (15.6) | 7.0 | |
| Nº cigarette smoked /day by the mother | 3485 | | 0.006** |
| 0 | 2618 (75.1) | 3.7 | |
| 1-9 | 520 (14.9) | 4.4 | |
| 10 or more | 347 (10.0) | 6.9 | |
| Maternal passive smoke (nº cigarette/day smoked by the father | 2917 | | 0.381** |
| 0 | 2458 (84.3) | 3.7 | |
| 1-9 | 262 (9.0) | 3.1 | |
| 10 or more | 197 (6.7) | 5.6 | |
| Alcohol consumption by the mother during pregnancy | 3485 | | 0.025* |
| No | 3372 (96.8) | 3.9 | |
| Yes | 113 (3.2) | 8.8 | |
| Number of antenatal care consultations | 3340 | | 0.006** |
| <3 | 120 (3.6) | 5.8 | |
| 3-5 | 452 (13.5) | 6.4 | |
| ≥ 6 | 2768 (82.9) | 3.5 | |
| Maternal mood symptoms during pregnancy | 3483 | | 0.090* |
| No | 2647 (76.0) | 3.7 | |
| Yes, not treated | 718 (20.6) | 5.4 | |
| Yes, treated | 118 (3.4) | 5.1 | |
| Maternal pre-pregnancy BMI | 2054 | | 0.315** |
| Underweight | 68 (3.3) | 5.9 | |
| Normal weight | 1165 (56.7) | 3.6 | |
| Overweight | 566 (27.6) | 4.2 | |
| Obese | 255 (12.4) | 5.5 | |

BMI: body mass index
* Fischer Exact Test
** Test for linear trend

*Cont.* Table 2. Prevalence of attention deficit hyperactivity disorder (ADHD) at the age of eleven years, according to characteristics of the family and the child.

| Variables | Follow-up 11 years | | |
|---|---|---|---|
| | N (%) | ADHD % | P |
| IG (weeks) | 3464 | | 0.264** |
| < 33 | 39 (1.1) | 7.7 | |
| 34-36 | 325 (9.4) | 4.6 | |
| ≥ 37 | 3100 (89.5) | 4.0 | |
| Birth weight (g) | 3484 | | 0.956** |
| <2500 | 283 (8.1) | 4.2 | |
| 2500-2999 | 883 (25.4) | 4.2 | |
| 3000-3499 | 1395 (40.0) | 3.9 | |
| ≥ 3500 | 923 (26.5) | 4.3 | |
| Sex | 3485 | | <0.001* |
| Male | 1803 (51.7) | 5.8 | |
| Female | 1644 (48.2) | 2.3 | |

* Fischer Exact Test

** Test for linear trend

Table 3. Prevalence of ADHD at eleven years of age according to maternal caffeine intake in each trimester and at the entire pregnancy stratified by sex.

| | Total | | | Boys | | | Girls | | |
|---|---|---|---|---|---|---|---|---|---|
| Caffeine consumption | N (%) | ADHD | P* | N (%) | ADHD | P* | N (%) | ADHD | P* |
| 1st trimester | 3485 | | 0.249 | 1803 | | 0.267 | 1682 | | 0.563 |
| <100 mg/day | 2072 (59.5) | 3.8 | | 1097 (60.8) | 5.4 | | 975 (58.0) | 2.1 | |
| 100-299 mg/day | 746 (21.4) | 4.3 | | 360 (20.0) | 6.1 | | 386 (23.0) | 3.0 | |
| ≥300 mg day | 667 (19.1) | 4.8 | | 346 (19.2) | 6.9 | | 321 (19.0) | 2.5 | |
| 2nd trimester | 3483 | | 0.393 | 1803 | | 0.500 | 1680 | | 0.463 |
| <100 mg/day | 2151 (61.8) | 3.9 | | 1141 (63.3) | 5.6 | | 1010 (60.1) | 2.0 | |
| 100-299 mg/day | 710 (20.4) | 4.2 | | 345 (19.1) | 5.8 | | 365 (21.7) | 2.7 | |
| ≥300 mg day | 622 (17.8) | 4.7 | | 317 (18.6) | 6.6 | | 305 (18.2) | 2.6 | |
| 3rd trimester | 3484 | | 0.151 | 1803 | | 0.368 | 1681 | | 0.141 |
| <100 mg/day | 2289 (65.7) | 3.9 | | 1216 (67.4) | 5.7 | | 1073 (63.8) | 1.9 | |
| 100-299 mg/day | 628 (18.0) | 3.8 | | 295 (16.4) | 5.1 | | 333 (19.8) | 2.7 | |
| ≥300 mg day | 567 (16.3) | 5.3 | | 292 (16.2) | 7.2 | | 275 (16.4) | 3.3 | |
| Entire pregnancy | 3481 | | 0.40 | 1803 | | 0.475 | 1678 | | 0.350 |
| <100 mg/day | 2124 (61.0) | 3.8 | | 1131 (62.7) | 5.5 | | 993 (59.2) | 1.9 | |
| 100-299 mg/day | 773 (22.2) | 4.5 | | 379 (21.0) | 6.3 | | 394 (23.5) | 2.8 | |
| ≥300 mg day | 584 (16.8) | 4.6 | | 293 (16.3) | 6.5 | | 291 (17.3) | 2.8 | |

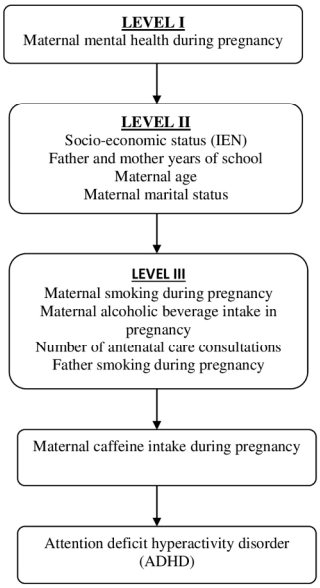
*Test for linear trend

Table 4. Association of maternal caffeine consumption in each trimester and during the entire pregnancy with the presence of attention deficit hyperactivity disorder (ADHD) at the age of eleven years.

| Caffeine consumption | Total | | Boys | | Gilrs | |
|---|---|---|---|---|---|---|
| | Crude analysis N= 3481 OR (95% CI) | Adjusted analysis* N= 2491 OR (95% CI) | Crude analysis N= 1803 OR (95% CI) | Adjusted analysis* N= 1274 OR (95% CI) | Crude analysis N= 1682 OR (95% CI) | Adjusted analysis* N= 1217 OR (95% CI) |
| 1st trimester (N=3485) | | | | | | |
| <100 mg/day | 1 | 1 | 1 | 1 | 1 | 1 |
| 100-299 mg/day | 1.13 (0.74-1.72) | 1.04 (0.62-1.73) | 1.15 (0.69-1.89) | 1.06 (0.57-1.98) | 1.27 (0.59-2.74) | 1.13 (0.44-2.90) |
| ≥300 mg day | 1.27 (0.84-1.94) | 0.93 (0.55-1.60) | 1.31 (0.80-2.14) | 1.06 (0.57-1.96) | 1.22 (0.53-2.80) | 0.68 (0.21-2.17) |
| 2nd trimester (N= 3483) | | | | | | |
| <100 mg/day | 1 | 1 | 1 | 1 | 1 | 1 |
| 100-299 mg/day | 1.9 (0.71-1.66) | 1.03 (0.61-1.74) | 1.04 (0.62-1.74) | 1.03 (0.55-1.94) | 1.39 (0.65-3.01) | 1.24 (0.48-3.22) |
| ≥300 mg day | 1.20 (0.78-1.85) | 0.95 (0.55-1.63) | 1.19 (0.72-1.99) | 1.09 (0.58-2.03) | 1.33 (0.58-3.06) | 0.75 (0.24-2.38) |
| 3rd trimester (N=3484) | | | | | | |
| <100 mg/day | 1 | 1 | 1 | 1 | 1 | 1 |
| 100-299 mg/day | 0.98 (0.62-1.56) | 0.96 (0.55-1.68) | 0.89 (0.50-1.58) | 0.82 (0.40-1.68) | 1.46 (0.66-3.24) | 1.68(0.64-4.40) |
| ≥300 mg day | 1.38 (0.90-2.11) | 1,05 (0.61-1.81) | 1.28 (0.78-2.14) | 1.07 (0.57-2.02) | 1.78 (0.80-3.95) | 1.22 (0.41-3.60) |
| Entire pregnancy (n=3481) | | | | | | |
| <100 mg/day | 1 | 1 | 1 | 1 | 1 | 1 |
| 100-299 mg/day | 1.19 (0.79-1.79) | 1.12 (0.68-1.84) | 1.16 (0.72-1.90) | 1.05 (0.57-1.92) | 1.47 (0.69-3.12) | 1.46 (0.58-3.68) |
| ≥300 mg day | 1.22 (0.78-1.91) | 0.90 (0.51-1.59) | 1.20 (0.70-2.03) | 1.01 (0.52-1.95) | 1.45 (0.69-3.35) | 0.82 (0.25-2.65) |

*Analysis adjusted for maternal mental health during pregnancy, IEN, paternal education level and maternal conjugal situation.

Figure 1. Conceptual framework for the association between maternal caffeine consumption during pregnancy and offspring ADHD at the age of eleven years.

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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation | Page in article |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | 5 |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6, 7 and 8 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | |
| Bias | 9 | Describe any efforts to address potential sources of bias | 8 |
| Study size | 10 | Explain how the study size was arrived at | 6 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 9 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 9 |
| | | (*c*) Explain how missing data were addressed | |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | |
| | | (*e*) Describe any sensitivity analyses | |

Continued on next page

| **Results** | | | **Page in article** |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 9 |
| | | (b) Give reasons for non-participation at each stage | 9 |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | 9 |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 11 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 11 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 11 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 11 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 13 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11 and 12 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 13 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 15 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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# Caffeine consumption during pregnancy and ADHD at the age of eleven years: A birth cohort study



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# Caffeine consumption during pregnancy and ADHD at the age of eleven years: A birth cohort study

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**Keywords:** Caffeine, pregnancy, ADHD and hyperactivity.

## ABSTRACT

**Objective:** Studies evaluating caffeine intake during pregnancy and long-term outcomes, such as the child's neuro-behaviour, are still scarce and their results are inconsistent. The objective of the present study was to evaluate the association between maternal consumption of caffeine during pregnancy and attention deficit hyperactivity disorder (ADHD) at the age of eleven years.

**Methodology:** All children born in the city of Pelotas, Brazil, during the year 2004, were selected for a cohort study. The mothers were interviewed at birth to obtain information on coffee and yerba mate consumption during pregnancy, among other matters. At the age of eleven years, presence of ADHD was evaluated using the Development and Well-Being Assessment (DAWBA) questionnaire, applied to the mothers. The prevalence of ADHD was calculated, with 95% confidence intervals (95%CI). The association between caffeine consumption and ADHD was tested by means of logistic regression.

**Results:** 3485 children were included in the analyses. The prevalence of ADHD was 4,1% (95% CI: 3,4-4,7%): 5,8% (95% CI: 4,7-6,9) among boys and 2,3% (95% CI: 1,5-3,0%) among girls. The prevalence of caffeine consumption during the entire pregnancy and in the first, second and third trimesters was 88,7% (87,7-89,7%), 86,5% (85,4-87,5%), 83,0% (81,8-84,2%) and 92,3% (91,4-93,1%), respectively. The caffeine consumption during the entire pregnancy and the first, second and third trimesters not associated with ADHD in the crude or adjusted analysis.

**Conclusion:** The present study did not show any association between maternal caffeine consumption during pregnancy and ADHD at the age of eleven years.

## ARTICLE SUMMARY

### Strengths

- This was a longitudinal study.
- Detailed information on caffeine consumption from coffee and yerba mate during the three trimesters of pregnancy was available.
- The outcome was evaluated by means of a validated instrument.
- Information on a number of potential confounding factors was gathered and formally tested.
- There were a low percentage of losses and refusals during the follow-up of the study.

### Limitations

- The outcome was ascertained by means of a test applied only to the mother.
- The reported amount of coffee and yerba mate consumed during pregnancy may be subject to recall bias.
- Only two sources of caffeine (coffee and yerba mate) were assessed.

## INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurobiological disorder that affects around 6% of school-age children around the world.(1) It is the most prevalent mental disorder during childhood and the main reason why mental health services are sought for children and adolescents.(2) It is characterized by persistent symptoms of inattention, impulsivity and hyperactivity, which become present before the age of 12 years and are abnormal for the developmental stage.(3)

ADHD is a multi factorial disease with complex aetiology and a large genetic component (heritability estimated as 76%).(4) Epidemiological studies have shown higher prevalence among boys and among children belonging to families with worse socioeconomic conditions.(5-13) Maternal caffeine consumption (14) as well as other nutritional factors during pregnancy, such as intake of folic acid,(15) iron(16) and omega-3(17) have been investigated as determinants of ADHD. In animals, intrauterine exposure to caffeine was associated with increased motor activity, thus suggesting a possible effect on attention deficit and hyperactivity on children born to mothers with high consumption of caffeine-rich foods and beverages during pregnancy.(18, 19) Moreover, exposure of rats to caffeine, during the prenatal period, resulted in gene expression alterations relating to formation of synapses, thereby showing some of the potential molecular effects of caffeine during foetal cerebral development.(20)

Caffeine is commonly consumed throughout the world, including by pregnant women, who present daily consumption prevalence ranging from 75% to 93%.(21) Studies evaluating caffeine intake during pregnancy and long-term outcomes, such as child neuro-behaviour, are still scarce and their results are inconsistent. Among five articles identified in a systematic review of the literature (22) only one found that the higher maternal caffeine intake during pregnancy would increase the risk of ADHD.(14)

The objective of the present study was to evaluate the association between maternal caffeine consumption during pregnancy and ADHD at the age of eleven years, among children belonging to a birth cohort. The hypothesis of the study was that maternal caffeine consumption during pregnancy was associated with ADHD at the age of eleven years.

## METHODOLOGY

In 2004, a birth cohort study was begun in the city of Pelotas, Brazil. The original cohort population consisted of the 4231 newborns at the five hospitals in the city, who were the children of mothers living in the urban zone of Pelotas, corresponding to 99.2% of the births in that year. The mothers were interviewed after delivery (perinatal study) by trained interviewers, using standardized questionnaires, regarding their socioeconomic, demographic and reproductive characteristics, use of health services, prenatal attention and pregnancy complications. Further methodological details of the study can be found in other publications.(23-25)

So far, the cohort participants were followed-up at the ages of 3, 12, 24 and 48 months, and at 6 and 11 years. The mothers were interviewed regarding their children's growth, development, type of food, and morbidity, and also answered questions about their own health.(24) Differently from the visits at 3, 12, 24, and 48 months that took place at the child's place, at the age of 6 and 11 years data-gathering was undertaken at a clinic that had been set up especially to attend to this research. Besides interviews, the children underwent a comprehensive health evaluation, which included psychological, psychiatric, anthropometric and body composition evaluations.(25)

The presence of ADHD was evaluated by means of the Development and Well-Being Assessment (DAWBA), an instrument employed for psychiatric diagnosis among

children and teenagers aged from 5 to 17 years, and that uses diagnostic classifications from the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V) and the International Classification of Diseases (ICD-10).(26) DAWBA was reported by mothers during the eleven-year follow-up by trained interviewers (psychologists). The DAWBA combines highly structured questions based on DSM-V diagnostic criteria and ICD-10 with qualitative descriptions of all areas of difficulty. The generating program is a computer algorithm that provides a probability of a child to have any psychiatric disorder based on answers to structured questions. In the presence of positive symptoms in any area, additional questions (qualitative assessment) are made to assess the impact (loss) of these problems in the child's life. These questions concern specific areas covering distress and interference with family life, learning, friendship and leisure activities resulting in symptoms. Subsequently, a clinical evaluator, based on the collected information, combines the quantitative results with the qualitative date and makes a judgment in regard to the presence or absence of the disorder. The clinic trial in this case was made by a child psychiatrist *(rater)*, supervised by another child psychiatrist, who translated and validated the DAWBA for the Brazilian population. To make the different psychiatric diagnosis from DAWBA evaluations, the *rater* needs to judge whether symptoms are present or not and the loss (impact) that they cause. DAWBA diagnoses are supplied dichotomously as “yes” or “no”, strictly respecting the diagnostic criteria defined by IDC-10 and DSM-V diagnostic classifications. For this study the DSM-V classification was employed. The DAWBA allows the identification of children currently under treatment for ADHD, such children were classified as positive for ADHD. The DAWBA questionnaire was translated and validated in Brazil by Bacy Fleitlich-Bilyk.(26)

The exposure of interest, daily caffeine consumption during pregnancy was evaluated at the perinatal study by means of a series of questions regarding consumption of the foods that are the main sources of caffeine at this region of the country: coffee and yerba mate (a typical hot beverage consumed in southern Brazil and neighbouring countries, which is prepared from the leaves of the herb *Ilex paraguariensis)*. For each source of caffeine, the daily frequency of consumption was obtained, separately for each trimester of pregnancy. Information regarding the type of coffee (filtered or instant), preparation, concentration (strong, medium or weak) and quantity consumed per day was gathered, taking into consideration the size of the recipient (180 ml cup; 50 ml small cup; 200 ml glass and 190 ml mug). The estimated caffeine content from coffee and yerba mate was obtained from coffee samples collected from the homes of mothers who participated in a previous study conducted in the city of Pelotas,(27) and that were analysed by chromatography. From these analyses, it was possible to infer the average caffeine content in mg per ml of coffee, according to the concentration at which it was consumed: strong coffee, 0.25 mg/ml; medium coffee, 0.20 mg/ml; and weak coffee, 0.11 mg/ml. For yerba mate, the analyses showed an average concentration of 17 mg of caffeine per 100 ml of the liquid. These results were used to estimate the caffeine intake of the entire sample. For instant coffee, the items investigated were the size of the spoon used to serve coffee (full coffee spoon, 2.6 g; level coffee spoon, 2.3 g; full small coffee spoon, 2.5 g; level small coffee spoon, 1.5 g; full dessert spoon, 7.5 g; and level dessert spoon, 7.0 g) and the number of spoons per portion. The spoon sizes were obtained from home measurements. Photographs of spoons were used during interviews to avoid classification errors. For instant coffee, the information used came from the manufacturer: an average of 3 mg of caffeine per gram of powdered coffee. For each

mother, the average daily caffeine intake was calculated per trimester and during the entire pregnancy.

Potential confounding factors in the association between maternal caffeine consumption during pregnancy and ADHD were gathered at the perinatal study and considered in the adjusted analysis: National Economic Index (acronym IEN in Portuguese) presented in quintiles (in which mothers at Q1 were the poorest and at Q5 were the wealthiest); mother's and father's education levels, evaluated as years of study; maternal age, evaluated as complete years at the delivery; mother living with or without partner; number of cigarettes/day smoked by the mother during pregnancy; number of cigarettes/day smoked by the father in the mother's presence during pregnancy; alcohol consumption by the mother during pregnancy (yes or no); number of antenatal care consultations; mood symptoms during pregnancy (through the question "During pregnancy, did you feel depressed or nervous?"); maternal nutritional state before pregnancy, evaluated according to the body mass index (BMI) and categorized as underweight (<18.5kg/m$^2$), normal weight (18.5-24.9kg/m$^2$), overweight (25-29.9kg/m$^2$) or obese (≥30 kg/m$^2$); the child gestational age at birth; birth weight; and sex of the child.

The twins were not included in the analyses (N=84). The prevalence of ADHD and respective 95% confidence interval (95% CI) was calculated for the entire cohort and separately by sex (based on the current literature that consistently reports higher prevalence rates among boys).(5-13) The association between maternal caffeine consumption grouped in three categories <100, 100-299 and ≥300 mg/day and ADHD was evaluated by means of the chi-square test. The strength of the association between caffeine consumption grouped in three categories and ADHD was ascertained for the entire cohort and after stratification by sex, by means of logistic regression (crude and

adjusted for confounding factors). In addition, analyses were performed with daily caffeine intake as a continuous variable.

A conceptual framework previously built by the authors describing the postulated hierarchical relationships between exposures (Figure 1) was used to drive the inclusion of potential confounders to the analytical model. Maternal mental health during pregnancy was the first variable included in the model, followed by father years of school and maternal socio-demographic characteristics (IEN, years of school, age and marital status). Subsequently the behavioural variables were added (maternal smoking and alcoholic beverage intake during pregnancy, paternal smoking during pregnancy, and number of antenatal care consultations). Only variables associated with the outcome at p-values $\leq 0.20$ were kept at the final model.

Loss to follow-up rates according to some of the child parents characteristics were not homogeneously distributed, the effect of missing outcome data was analysed as a sensitivity analysis, estimated by multiple imputation (mi Stata command) by the Bayesian paradigm from the frequentist (randomization-based) perspective. Least squares regression and 20 multiple datasets for the missing values were used.

The Pelotas 2004 Birth Cohort Study was approved by the Research Ethics Committee of the Medical School of the Federal University of Pelotas that is affiliate to the Brazilian National Commission for Research Ethics. Mothers signed an informed consent form at each follow-up, after being informed of the study objectives.

## RESULTS

The present study used data from the perinatal study that included 4231 newborns and the follow-up at the age of eleven years (mean age of 10.9; standard deviation 0.3 years) that included 3566 children (follow-up rate of 86.6%). A total of

3485 mothers and children (82.4% of the original cohort) had full information on caffeine intake and on ADHD and were entered at the current analyses.

Table 1 shows the loss to follow-up rate at 11 years according to maternal caffeine intake in the entire pregnancy, IEN, paternal education level, mother living with or without a partner, and maternal mood symptoms during pregnancy. There were no differences in losses to follow-up by level of caffeine intake throughout pregnancy. Losses were higher for children from families in the extremes of IEN (18.9% among the poorest and 15.8% among the richest), with highly educated fathers (20.8%). Greater proportion of losses was also seen among children of mothers that lived with a partner and presented mood symptoms during pregnancy (Table 1).

The prevalence of ADHD was 4,1% (3,4-4,7%): 5,8% (4,7-6,9) among boys and 2,3% (1,5-3,0%) among girls. Table 2 shows the sample distribution and the prevalence of ADHD according to family and child variables. The ADHD was more frequent among children from families of lower socio-economic status (first quintile of IEN), from less educated mothers (0-4 years of formal education), living without a partner, who had attended to less than six antenatal care consultations, who smoked more than 10 cigarettes a day and consumed alcoholic beverages during pregnancy. ADHD was also more frequent in children from less educated fathers (0-4 years of schooling) and among boys.

Table 3 shows the prevalence and intensity of caffeine intake during pregnancy and the prevalence of ADHD among children of mothers who consumed between 100-299 mg/day or 300 or more mg/day of caffeine, compared to those from mothers who consumed less than 100 mg/day, taken as the reference group. The prevalence of caffeine consumption during the entire pregnancy and in the first, second and third trimesters was 88,7% (87,7-89,7%), 86,5% (85,4-87,5%), 83,0% (81,8-84,2%) and

92,3% (91,4-93,1%), respectively. Most of the mothers consumed <100 mg/day of caffeine in the entire and in each trimester of pregnancy, whereas nearly one in every five mothers consumed ≥300 mg/day in every trimester and throughout pregnancy. Heavy caffeine consumers were more likely to belong to families from low socio-economic stratus and to present behavioural exposures (smoking and consumption of alcoholic beverages in pregnancy) than the remaining mothers. Caffeine consumer mothers attended a few number of antenatal care consultations and presented mood symptoms during pregnancy more frequently than non-consumers (data not shown). There was no difference in ADHD prevalence according to the mean amount of maternal daily caffeine consumption (Table 2).

The results of crude and adjusted analyses of the association between caffeine intake in three categories (<100, 100-299 and ≥ 300 mg/day) per trimester and during the entire pregnancy and ADHD are presented in Table 4. There was no association between caffeine consumption and ADHD, both in the crude and in the adjusted analysis, during the three pregnancy trimesters and at the entire pregnancy. All the 95% CI of the estimated odds ratios included the unit, thus showing that there was no association. The same result was shown in the analysis stratified by sex. Analyses with caffeine as a continuous variable also found no association (data not shown).

The multiple imputation data for the primary outcome produced imputed estimates that were similar to the available data. This similarity showed that all analyses were not affected by missing data or differential rates of follow-up.

## DISCUSSION

The present study found a prevalence of ADHD of 4,1% (95% CI: 3,4-4,7%): 5,8% (95% CI: 4,7-6,9) among boys and 2,3% (95% CI: 1,5-3,0%) among girls. This finding is consistent with results from other studies that employed DAWBA as the

evaluation tool and the DSM-IV as the diagnostic criterion in Brazil.(5, 28, 29) The prevalence of ADHD in school children in Brazil ranges from 0.9% (30) to 26.8% (31). The former was a population-based study carried out with 5-10 year-old children using clinical criteria (DSM-IV) obtained from two environments (home and school) and taking into account the impact of the symptoms at the child familiar and social relationships.(30) The later investigated a sample of 6-15 year-old school children employing clinical assessment (DSM-IV), but considering only the report by the teachers and without considering the impact of the symptoms. The variability between the estimates may be due to different factors, from type of sample, evaluation tools, diagnostic criteria, and mainly the source of information (parents, children, adolescents or teachers).(32) Prevalence of ADHD is generally higher in school samples than in population-based samples.(31).

This study did not show any association between caffeine consumption during pregnancy and ADHD. Contrary to the hypothesis of this study, the crude and adjusted analyses indicated that caffeine had no effect over the occurrence of ADHD. A recent review of the literature showed that there is a scarcity of studies evaluating the effect of caffeine consumption during pregnancy over the occurrence of ADHD, and concluded that the available evidence does not make it possible to confirm or deny the risk that this exposure might present with regard to development of this morbidity during childhood.(22) The five studies investigating the effect of maternal caffeine consumption over the occurrence of ADHD(22) differed in relation to the tools used to measure the outcome: The only one that evaluated the presence of ADHD by means of a diagnostic instrument did not find any association.(33) The remaining articles used screening tests: Conners' Continuous Performance Test II (CPT-II),(34) the Child Behavior Checklist (CBCL)(8, 14) and SDQ(35) and only one found an association(14)

indicating that caffeine consumption during pregnancy would increase the risk of ADHD. The difference between the instruments used for assessing the presence of ADHD generates issues that go beyond the lack of comparability. Screening instruments are more sensitive and less specific, and have a high capacity to recognize true positives, but they fail to discard false positives, thereby wrongly identifying healthy individuals as ill. For instance, in an analysis of data from another cohort conducted in Pelotas (the Pelotas 1993 Birth Cohort Study), to estimate the prevalence of psychiatric diseases among children aged 11 years, Anselmi et al(29) compared the results from DAWBA with those from the Strengths and Difficulties Questionnaire (SDQ), which is a screening instrument. They found that as a screening instrument for ADHD, SDQ presented weak performance, with a positive predictive value (PPV) of 48.2% and a negative predictive value (NPV) of 90.2%. Similar results have been found in other Brazilian studies.(26, 30)

There is a high inter-individual variability in the physiological response to caffeine consumption that may in part be due to genetic characteristics. The genes involved in caffeine metabolism, such as cytochrome P450 1A2 (CYP1A2), and in caffeine responses in the central nervous system, such as the adenosine 2A receptor (ADORA2A*)*, have been the main targets of genetic studies in this area.(36-41) Polymorphisms in genes in these pathways have been correlated with the habit of consuming coffee and have been shown to be important to modulate the response to caffeine consumption among adults, such as symptoms of anxiety, cognitive performance and insomnia.(36-41) On the other hand, little is known about the molecular response mechanisms to caffeine in the central nervous system while it is still developing; or about the way in which gene polymorphisms along these pathways might module the response to caffeine. Future studies adding genetic factors to caffeine

consumption during pregnancy could contribute towards better understanding the potential role that caffeine may play in the development of ADHD and other psychiatric disorders.

The present study presents some strengths and limitations. Among the strengths is the fact that this was a longitudinal study with data from a birth cohort of about 4,000 children, which facilitates the generalization of data. The longitudinal analysis is characterized by following up individuals over a period of time, which ensures that the temporal relationship between exposures and outcomes can be ascertained. Hence, among all the observational study designs this is the ideal for investigating the topic in question. Furthermore, detailed information on caffeine consumption from coffee and yerba mate during the three trimesters of pregnancy was available. The outcome was evaluated by means of an instrument that had been adapted and validated for the Brazilian population, which made it possible to confirm the diagnoses of ADHD.(26) Moreover, there was the possibility of controlling the analysis for a number of potential confounding factors. Also noteworthy is the low percentage of losses and refusals during the follow-up of the study (13.4% from birth to eleven years of age). Post-hoc analyses indicated that the study had a power of 82% to detect as statistically significant odds ratios $\geq 1.5$, setting alpha at 0.05 two-tailed.

Some limitations of the study need to be taken in consideration. The lack of information on the presence of ADHD in the mothers is among the limitations. Perhaps mothers with some degree of ADHD may not consider excessive activity in her child as unusual. In addition, the amount of coffee and yerba mate consumed during pregnancy may have been subject to recall bias. Also, although caffeine consumption during pregnancy was assessed from the two main sources (coffee and mate) there are other caffeine sources (foods like chocolate, chocolate drink and cola-drinks as well as

medicines) that were not measured. However, daily consumption from other sources is low at this population representing less than 10% of all caffeine consumed by pregnant women.(27) The findings of this study can be generalized to other settings with socio-economic characteristics similar to that of Pelotas and where women largely consume caffeine during pregnancy.

## CONCLUSION

There is no evidence from the present study to support any deleterious effect of caffeine consumption during pregnancy over the occurrence of ADHD in the offspring.

## CONTRIBUTORS STATEMENT

Ms Silva BDP and Dr Santos IS participated in the design of the manuscript, carried out the initial analyses, drafted the initial manuscript, and approved the final manuscript as submitted.

Drs Matijasevich A and Santos IS designed the data collection instruments, and coordinated and supervised data collection, critically reviewed the manuscript, and approved the final manuscript as submitted.

Drs Tovo-Rodrigues L and Anselmi L Munhoz TN participated in the drafting, critically reviewed the manuscript, and approved the final manuscript as submitted.

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## ROLE OF THE FUNDING SOURCE

The funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; in preparation of the article; nor in the decision to submit the article for publication.

## CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

## DATA SHARING STATEMENT

Extra data is available by emailing bianca.delponte@gmail.com

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Table 1. Socio-demographic characteristics of mothers and children enrolled in the 2004 Pelotas Birth Cohort, and loss to follow-up rate at 11 year. Pelotas 2004 Birth Cohort Study.

| Variables | Perinatal study | Loss to follow-up rate | |
|---|---|---|---|
| | N (%) | | p* |
| Caffeine intake in the entire pregnancy | | | 0.338 |
| <100 mg/day | 1534 | 17,1 | |
| 100-299 mg/day | 902 | 14.4 | |
| ≥300 mg day | 698 | 16.6 | |
| IEN | | | <0.001 |
| Q1 | 641 | 18.9 | |
| Q 2 | 659 | 13.4 | |
| Q 3 | 623 | 10.0 | |
| Q 4 | 640 | 8.8 | |
| Q 5 | 639 | 15.8 | |
| Paternal education level | | | 0.021 |
| 1-4 | 568 | 17.1 | |
| 5-8 | 1133 | 16.2 | |
| 9-11 | 1159 | 14.1 | |
| 12 or more | 375 | 20.8 | |
| Maternal conjugal situation | | | 0.001 |
| With partner | 3468 | 20.5 | |
| Without partner | 679 | 15.3 | |
| Maternal mood symptoms in pregnancy | | | <0.001 |
| No | 3107 | 14.9 | |
| Yes, treated | 898 | 20.5 | |
| Yes, not treated | 140 | 15.7 | |

Table 2. Prevalence of attention deficit hyperactivity disorder (ADHD) at the age of eleven years, according to characteristics of the family and the child.

| Variables | 11-year follow-up | | |
|---|---|---|---|
| | N (%) | ADHD % | P |
| IEN | 2780 | | 0.003** |
| Q1 | 521 (18.7) | 5.0 | |
| Q2 | 574 (20.7) | 5.6 | |
| Q3 | 562 (20.2) | 3.0 | |
| Q4 | 584 (21.0) | 4.1 | |
| Q5 | 539 (19.4) | 1.9 | |
| Maternal education level in years | 3452 | | 0.003** |
| 0-4 | 512 (14.8) | 6.8 | |
| 5-8 | 1429 (41.4) | 4.1 | |
| 9-11 | 1175 (34.1) | 3.2 | |
| 12 or over | 336 (9,7) | 3.3 | |
| Paternal education level in years | 2717 | | 0.004** |
| 0-4 | 471 (17.3) | 5.5 | |
| 5-8 | 952 (35.0) | 4.5 | |
| 9-11 | 996 (36.7) | 3.2 | |
| 12 or over | 298 (11.0) | 2.0 | |
| Maternal age | 3483 | | 0.291** |
| <20 | 660 (18.9) | 4.9 | |
| 20-35 | 2441 (70,1) | 3.9 | |
| >35 | 382 (11.0) | 3.7 | |
| Maternal marital status | | | 0.001* |
| With partner | 2943 (84.5) | 3.6 | |
| Without partner | 542 (15.6) | 7.0 | |
| Nº cigarette smoked /day by the mother | 3485 | | 0.006** |
| 0 | 2618 (75.1) | 3.7 | |
| 1-9 | 520 (14.9) | 4.4 | |
| 10 or more | 347 (10.0) | 6.9 | |
| Maternal passive smoke (nº cigarette/day smoked by the father | 2917 | | 0.381** |
| 0 | 2458 (84.3) | 3.7 | |
| 1-9 | 262 (9.0) | 3.1 | |
| 10 or more | 197 (6.7) | 5.6 | |
| Alcohol consumption by the mother during pregnancy | 3485 | | 0.025* |
| No | 3372 (96.8) | 3.9 | |
| Yes | 113 (3.2) | 8.8 | |
| Number of antenatal care consultations | 3340 | | 0.006** |
| <3 | 120 (3.6) | 5.8 | |
| 3-5 | 452 (13.5) | 6.4 | |
| ≥ 6 | 2768 (82.9) | 3.5 | |
| Maternal mood symptoms during pregnancy | 3483 | | 0.090* |
| No | 2647 (76.0) | 3.7 | |
| Yes, not treated | 718 (20.6) | 5.4 | |
| Yes, treated | 118 (3.4) | 5.1 | |

BMI: body mass index
* Fischer Exact Test
** Test for linear trend

*Cont.* Table 2. Prevalence of attention deficit hyperactivity disorder (ADHD) at the age of eleven years, according to characteristics of the family and the child.

| Variables | Follow-up 11 years | | |
|---|---|---|---|
| | N (%) | ADHD % | P |
| Maternal pre-pregnancy BMI | 2054 | | 0.315** |
| Underweight | 68 (3.3) | 5.9 | |
| Normal weight | 1165 (56.7) | 3.6 | |
| Overweight | 566 (27.6) | 4.2 | |
| Obese | 255 (12.4) | 5.5 | |
| IG (weeks) | 3464 | | 0.264** |
| < 33 | 39 (1.1) | 7.7 | |
| 34-36 | 325 (9.4) | 4.6 | |
| ≥ 37 | 3100 (89.5) | 4.0 | |
| Birth weight (g) | 3484 | | 0.956** |
| <2500 | 283 (8.1) | 4.2 | |
| 2500-2999 | 883 (25.4) | 4.2 | |
| 3000-3499 | 1395 (40.0) | 3.9 | |
| ≥ 3500 | 923 (26.5) | 4.3 | |
| Sex | 3485 | | <0.001* |
| Male | 1803 (51.7) | 5.8 | |
| Female | 1644 (48.2) | 2.3 | |

* Fischer Exact Test

** Test for linear trend

Table 3. Prevalence of ADHD at eleven years of age according to maternal caffeine intake in each trimester and at the entire pregnancy, stratified by sex.

| | Total | | | Boys | | | Girls | | |
|---|---|---|---|---|---|---|---|---|---|
| Caffeine consumption | N (%) | ADHD | P* | N (%) | ADHD | P* | N (%) | ADHD | P* |
| 1st trimester | 3485 | | 0.249 | 1803 | | 0.267 | 1682 | | 0.563 |
| <100 mg/day | 2072 (59.5) | 3.8 | | 1097 (60.8) | 5.4 | | 975 (58.0) | 2.1 | |
| 100-299 mg/day | 746 (21.4) | 4.3 | | 360 (20.0) | 6.1 | | 386 (23.0) | 3.0 | |
| ≥300 mg day | 667 (19.1) | 4.8 | | 346 (19.2) | 6.9 | | 321 (19.0) | 2.5 | |
| 2nd trimester | 3483 | | 0.393 | 1803 | | 0.500 | 1680 | | 0.463 |
| <100 mg/day | 2151 (61.8) | 3.9 | | 1141 (63.3) | 5.6 | | 1010 (60.1) | 2.0 | |
| 100-299 mg/day | 710 (20.4) | 4.2 | | 345 (19.1) | 5.8 | | 365 (21.7) | 2.7 | |
| ≥300 mg day | 622 (17.8) | 4.7 | | 317 (18.6) | 6.6 | | 305 (18.2) | 2.6 | |
| 3rd trimester | 3484 | | 0.151 | 1803 | | 0.368 | 1681 | | 0.141 |
| <100 mg/day | 2289 (65.7) | 3.9 | | 1216 (67.4) | 5.7 | | 1073 (63.8) | 1.9 | |
| 100-299 mg/day | 628 (18.0) | 3.8 | | 295 (16.4) | 5.1 | | 333 (19.8) | 2.7 | |
| ≥300 mg day | 567 (16.3) | 5.3 | | 292 (16.2) | 7.2 | | 275 (16.4) | 3.3 | |
| Entire pregnancy | 3481 | | 0.40 | 1803 | | 0.475 | 1678 | | 0.350 |
| <100 mg/day | 2124 (61.0) | 3.8 | | 1131 (62.7) | 5.5 | | 993 (59.2) | 1.9 | |
| 100-299 mg/day | 773 (22.2) | 4.5 | | 379 (21.0) | 6.3 | | 394 (23.5) | 2.8 | |
| ≥300 mg day | 584 (16.8) | 4.6 | | 293 (16.3) | 6.5 | | 291 (17.3) | 2.8 | |

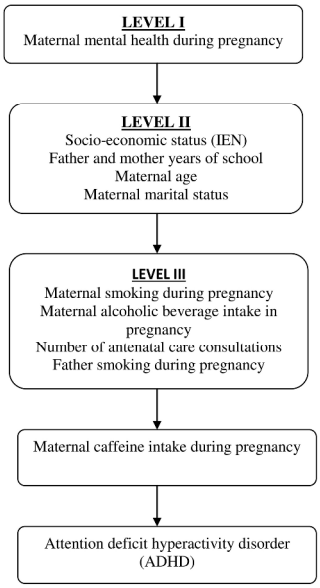
*Test for linear trend

Table 4. Association of maternal caffeine consumption in each trimester and during the entire pregnancy with the presence of attention deficit hyperactivity disorder (ADHD) at the age of eleven years.

| Caffeine consumption | Total | | Boys | | Gilrs | |
|---|---|---|---|---|---|---|
| | Crude analysis N= 3481 OR (95% CI) | Adjusted analysis* N= 2491 OR (95% CI) | Crude analysis N= 1803 OR (95% CI) | Adjusted analysis* N= 1274 OR (95% CI) | Crude analysis N= 1682 OR (95% CI) | Adjusted analysis* N= 1217 OR (95% CI) |
| 1st trimester (N=3485) | | | | | | |
| <100 mg/day | 1 | 1 | 1 | 1 | 1 | 1 |
| 100-299 mg/day | 1.13 (0.74-1.72) | 1.04 (0.62-1.73) | 1.15 (0.69-1.89) | 1.06 (0.57-1.98) | 1.27 (0.59-2.74) | 1.13 (0.44-2.90) |
| ≥300 mg day | 1.27 (0.84-1.94) | 0.93 (0.55-1.60) | 1.31 (0.80-2.14) | 1.06 (0.57-1.96) | 1.22 (0.53-2.80) | 0.68 (0.21-2.17) |
| 2nd trimester (N= 3483) | | | | | | |
| <100 mg/day | 1 | 1 | 1 | 1 | 1 | 1 |
| 100-299 mg/day | 1.9 (0.71-1.66) | 1.03 (0.61-1.74) | 1.04 (0.62-1.74) | 1.03 (0.55-1.94) | 1.39 (0.65-3.01) | 1.24 (0.48-3.22) |
| ≥300 mg day | 1.20 (0.78-1.85) | 0.95 (0.55-1.63) | 1.19 (0.72-1.99) | 1.09 (0.58-2.03) | 1.33 (0.58-3.06) | 0.75 (0.24-2.38) |
| 3rd trimester (N=3484) | | | | | | |
| <100 mg/day | 1 | 1 | 1 | 1 | 1 | 1 |
| 100-299 mg/day | 0.98 (0.62-1.56) | 0.96 (0.55-1.68) | 0.89 (0.50-1.58) | 0.82 (0.40-1.68) | 1.46 (0.66-3.24) | 1.68(0.64-4.40) |
| ≥300 mg day | 1.38 (0.90-2.11) | 1,05 (0.61-1.81) | 1.28 (0.78-2.14) | 1.07 (0.57-2.02) | 1.78 (0.80-3.95) | 1.22 (0.41-3.60) |
| Entire pregnancy (n=3481) | | | | | | |
| <100 mg/day | 1 | 1 | 1 | 1 | 1 | 1 |
| 100-299 mg/day | 1.19 (0.79-1.79) | 1.12 (0.68-1.84) | 1.16 (0.72-1.90) | 1.05 (0.57-1.92) | 1.47 (0.69-3.12) | 1.46 (0.58-3.68) |
| ≥300 mg day | 1.22 (0.78-1.91) | 0.90 (0.51-1.59) | 1.20 (0.70-2.03) | 1.01 (0.52-1.95) | 1.45 (0.69-3.35) | 0.82 (0.25-2.65) |

*Analysis adjusted for maternal mood symptoms during pregnancy, IEN, paternal education level and maternal conjugal situation.

Figure 1. Conceptual framework for the association between maternal caffeine consumption during pregnancy and offspring ADHD at the age of eleven years.

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STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation | Page in article |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5 |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | 5 |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5-8 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5-8 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 9 |
| Study size | 10 | Explain how the study size was arrived at | |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 8 and 9 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 8 |
| | | (*c*) Explain how missing data were addressed | 9 and 11 |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | 9 |
| | | (*e*) Describe any sensitivity analyses | |

Continued on next page

| Results | | | Page in article |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 9 and 10 |
| | | (b) Give reasons for non-participation at each stage | 9 and 10 |
| | | (c) Consider use of a flow diagram | - |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 10 and Table2 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 10 and Table2 |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | 10 |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 11 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 10 and 11 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | - |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 11 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 11 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 14 and 15 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 12 and 13 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 15 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 16 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.