

ORIGINAL ARTICLE

# Epigenetic alteration of BDNF gene, social and health status as predictors of food and nutrition insecurity among familiar coffee farmers

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## Abstract

**Introduction:** state of Food and Nutritional Security (FNS) is one that should guarantee the right of permanent access to quality food and in sufficient quantity without prejudicing access to other basic rights. In Brazil, rural family farming establishments represent 84.4% of total agricultural establishments and contribute to more than 70% of all food consumed by Brazilians. In this context, the production of the coffee commodity stands out. However, despite being food producers, they do not earn a good income. Slow economic activity can lead to loss of wages and income, illness, as well as food insecurity (FNiS). In addition, the molecular effects of FNiS are poorly studied, especially epigenetic.

**Objective:** the objective of the present study is to analyze the association between Brain-Derived Neurotrophic Factor (BDNF) DNA methylation and socio demographic, lifestyle, and epigenetic factors, among coffee farmers in the Caparaó zone, in Espírito Santo, Southeast Brazil.

**Methods:** the study was carried out in 22 randomly selected coffee producing communities in Zona Caparaó, an area that produces coffee of recognized quality. A total of 570 coffee farming households, 18 to 60 years of age, were included in the study by answering a questionnaire about socioeconomic characteristics, land use and ownership, behavior, health, and working conditions. FNiS evaluation was carried out using the Brazilian Food Insecurity Scale. BDNF exon I methylation was examined by methylation-specific PCR. Body mass index and biochemical analyses were performed. Logistic regression models were used to verify factors associated with FNiS ( $p < 0.05$ ). Data were analyzed using the Stata® statistical software package version 14.

**Results:** the FNiS prevalence found was 23.68%. In multivariable logistic regression, the occurrence of FNiS was associated with hypermethylation of exon I of the BDNF promoter exon I [ORa = 5.03 (95% (1.98, 12.82))] when compared to the unmethylated gene. Moreover, FNiS was associated with excessive workload [ORa = 3.36 (1.23, 9.22)], possession of less land (hectares) [ORa = 0.77 (0.67, 0.90)] and greater number symptoms and / or illnesses in real life [ORa = 1.12 (1.04, 1.20)].

**Conclusion:** there is a high prevalence of Food Insecurity in the studied region. This phenomenon was associated with epigenetic factors (BDNF methylation), excessive workload, small land ownership and a greater number of diseases / symptoms. Food insecurity is a psychosocial stressor that can lead to epigenetic changes in the BDNF gene, responsible for regulating cognitive functions, neuronal survival and involved in the genesis of psychiatric diseases.

**Keywords:** coffee farmers, food and nutrition insecurity, BDNF, DNA methylation.

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## Authors summary

### Why was this study done?

There are few data on molecular changes resulting from food and nutrition insecurity, especially epigenetic effects.

### What did the researchers do and find?

Increased BDNF gene methylation was associated with food insecurity, in addition to smaller farm size, type of job, and health status

### What do these findings mean?

Food insecurity is a psychosocial stressor that can lead to epigenetic changes in the BDNF gene, responsible for regulating cognitive functions, neuronal survival and involved in the genesis of psychiatric diseases.

## INTRODUCTION

According to the most recent Food and Agricultural Organization (FAO-UN) estimates (2019), 26.4% of the world population, about 2 billion people, have food and nutrition insecurity (FNiS). Along with malnutrition, the prevalence of FNiS forms the main indicator for monitoring the eradication of hunger in the world. The Food insecurity provides a relevant global food outlook for all countries in the world: one that goes beyond hunger toward the goal of ensuring access to nutritious and sufficient food for all<sup>1</sup>.

Food insecure people are exposed to uncertainty about their ability to obtain food and have been forced to reduce, sometimes during the year, the quality and / or quantity of food they consume due to lack of money or other resources. Thus, it disrupts normal eating patterns and can have negative consequences for nutrition, health and well-being<sup>2</sup>.

There is increasing awareness and scientific recognition in the health community that health outcomes and disparities are most often driven by social determinants of health rather than health care<sup>3</sup>. Low income and food insecurity are social determinants of health and are associated with some of the country's most serious and costly health problems.

Social determinants of health include social, economic, physical, or other conditions in which people live, learn, work, and play that influence their health<sup>4</sup>.

In last years, geneticists recognized that the inheritance of traits was possible through mechanisms independent of genotype. Phenotypic changes where the sequence of DNA is not changed but expression of the gene is altered turned on or off so to speak are called epigenetic changes. The study of these phenomena is called epigenomics. Nutritional epigenetics is documenting how nutrition, one of the primary environmental factors that are in continuous interaction with individuals, may influence epigenetic changes with resultant consequences for health development<sup>5</sup>.

Since 2006, discussions on Food and Nutrition Insecurity (FNiS) in Brazil has involved efforts to increase access to food at appropriate quantities and quality, without compromising access to other essential needs, by means of food practices that will promote health while respecting cultural diversity and being environmentally, culturally, economically and socially sustainable<sup>6</sup>.

According to the National Household Sample Survey<sup>7</sup>, the prevalence of FNiS in Brazil fell from 39.8% in 2004 to 25.8% in 2013. In ES, FNiS was reduced from 32.2% in 2004 to 12.2% in 2013, a very significant reduction. In 2014, the Food and Agricultural

Organization (FAO)/United Nations (UN) excluded Brazil from the hunger map. The situation worsened by 74% after the first two years of the COVID-19 pandemic, in which approximately 33 million people were in a state of severe FNiS (hunger). This context highlights fragility in social public policies, which were dismantled before the beginning of the pandemic, such as the extinction of the National Council for Food and Nutrition Security (CONSEA)<sup>8</sup>.

There is some evidence that the main cause of FNS deficiency among family farmers in remote rural communities is the shortage of food rather than economic restrictions<sup>10</sup> and/or inequality of land ownership<sup>9</sup>. Brazil is a major agricultural power, with 15.6% of the population living in rural areas<sup>7</sup>. Although its area is just 0.5% of the country, the state of the Espírito Santo (ES) ranks second among the country's coffee producing states and ranks first in production of *Coffea canephora*<sup>10</sup>.

The phenomenon of epidemiological transition, initiated during the last century led to a higher incidence of chronic diseases, rather than infectious diseases, which has required a shift in public health efforts. The high levels of stress associated with nutritional deficits have had important effects on health status. In addition to the potential for sickness, there is an effect of food insecurity on mental health<sup>11</sup>. Food insecurity is linked to problems of drug use in adolescence<sup>10</sup> and suicidal ideation in adulthood<sup>12</sup>. FNiS is related to social vulnerability, as it results from a combination of factors that can lead to deterioration in the well-being of people, families and communities<sup>13</sup>.

Many health-related outcomes have been attributed to environmental influences, such as socioeconomic factors that cause epigenetic changes and can influence changes in gene expression and increased risk of disease<sup>14</sup>. Further, evidence shows that stress negatively affects health<sup>6</sup> and that environmental factors may have multi-generational impacts. Addressing social determinants of health is not only important for improving overall health, but also for reducing health disparities that are often rooted in social and economic disadvantages<sup>15</sup>.

Knowledge about mechanisms by which nutrition affects gene expression is recent<sup>16</sup>, although, in 1939, C.H. Waddington described the interactions between genes and their products in certain phenotypes. Later, epigenetic studies proved the existence of heritable patterns of gene expression that are not due to changes in DNA sequence<sup>17</sup>.

DNA methylation has received special attention as an epigenetic mediator and moderator of environmental

exposures on health-related outcomes<sup>14,18</sup>. It consists of adding a methyl group to cytosines preceded by guanines (CpG). Promoter CpG methylation is generally associated with transcriptional silencing or reduction of expression<sup>14</sup>.

In brain function, epigenetic mechanisms are involved with cognition, intelligence, eating disorders, autism, depression, schizophrenia and Alzheimer's disease<sup>17,19,20</sup>.

Brain-Derived Neurotrophic Factor (BDNF) is a protein that promotes neuronal survival, therefore paramount for the maintenance of brain functions. Decreases in its levels may be due to external (*e.g.* toxic agents, lack of nutrients) or internal (*e.g.* stress) insults, and could be involved with pathogenesis and treatment response in diverse neuropsychiatric conditions. Several studies have associated decreased BDNF brain and peripheral blood levels with increased BDNF promoter methylation<sup>21-23</sup>.

Considering BDNF's role in the central nervous system (CNS), especially in neurotransmission, and pathogenesis of neuropsychiatric conditions and considering the influence of stress on BDNF levels, it can be hypothesized that the stress caused by FNiS can lead to epigenetic changes in the BDNF gene. However, studies that evaluated the relationship between epigenetic changes and FNiS are scarce in the literature<sup>14</sup>, and there are no studies on the relationship between epigenetic changes in the BDNF gene and FNiS.

Thus, the aim of the study is analyze the association between BDNF promoter methylation and FNiS and the association of FNiS and socio demographic, lifestyle and epigenetic factors, among coffee farmers in Caparaó zone, in Espírito Santo, Southeast of Brazil.

This study can highlight the possible effects of food and nutritional insecurity and may guide public policies to reduce behavioral disorders and chronic diseases<sup>24</sup>, as well as encourage the development of more sustainable food production systems that will ensure nutrition security for rural populations<sup>8</sup>.

## METHODS

### Data source and procedures

The data were collected as part of the health evaluation of a project of the Brazilian Public Health System, studying small coffee growers from the "Espírito Santo" state, located in the Southeast region of Brazil, during the months of January to December 2015.

A cross-sectional survey was used to provide information on relationships among farmer's property, epigenetics, health and well-being. Information was collected by a personal interview with coffee growers aged 18 to 60 residing in the rural areas of 11 cities (Alegre, Divino São Lourenço, Dores do Rio Preto, Guaçuí, Ibatiba, Ibitirama, Irupí, Iúna, Jerônimo Monteiro, Muniz Freire, and São José do Calçado) located in of the Caparaó zone, Southwest of Brazil (between the latitudes of 20°12'23 "S to 20°46'50" S and longitudes of 41°07'44 "O to 42°05'14" O).

The small farmers of each municipality were randomly selected and the individuals Who volunteere designed the Free and Informed Consent Term (FICT), according to Resolution 466/12 of the National Health Council. Only one individual aged between 18 and 60 years

per household was interviewed. The criterion of inclusion was to be a family farmer. Individuals who presented incomplete data on Food and Nutritional Insecurity (FNiS) were excluded from the analysis.

Families who had a per capita income less than \$5 (five American dollars) per day were considered a low income<sup>25</sup>. Overweight status and obesity were classified according to body mass index (BMI)<sup>26</sup>.

### Sample size calculation

Sample size estimates used previous information about this particular region, which has 68,221 rural workers, according to data from the Brazilian Institute of Geography and Statistics<sup>7</sup>. For the calculation of the simple random sample, the municipalities involved were included with equal weight. In the absence of specific studies in a municipality, an FNiS prevalence of 50% was used. The estimated required sample size was calculated assuming a sampling error of 5% and a 95% confidence interval. Finally, 10% of losses were added and the sample size calculated was 423 family farmers<sup>27</sup>.

### Food and nutrition Insecurity (FNiS) evaluation

Beginning in 2017, the prevalence of severe food insecurity based on the Food Insecurity Experience Scale (FIES) was also included in the report as another, complementary indicator of hunger using a different approach<sup>1</sup>. In the present study, FNiS was evaluated by the application of the Brazilian Food Insecurity Scale (BFIS)<sup>28</sup>, a validated instrument comprising 14 questions, aimed at families from the same household with and without member sunder 18 years of age; concerns evaluated were: lack of food at home, and having some member of the family spending a whole Day without eating in the last three months, among others. The degree of severity of FNiS (mild, moderate, and severe) was grouped in this study as FNiS.

### Biochemical evaluation

Blood tests were performed according to the manufacturer's recommendations (Bioclin® Kits), for: total protein, creatinine, urea, albumin, total cholesterol, high density lipoprotein, glycemia, oxalacetic transaminase, and pyruvic transaminase. Sample collections were performed from 6:00 a.m. to 7:00 a.m. after a 12-hour fasting interval. All analyzes were performed using the Bioclin® BS 120® automatic analyzer.

### Extraction and quantification of genomic DNA

Blood DNA was extracted using the salting-out protocol<sup>29</sup>. DNA quality and concentration was assessed by spectroscopy (NANODROP®, USA).

### BDNF gene methylation

To determine BDNF exon I promoter methylation, region (chr11: 27 743 605–27 744 379), specific primers<sup>30</sup> (listed in Table 1) were used for methylation-specific polymerase chain reaction (MS-PCR). CpGs where M primers are annealed were evaluated. After treatment with sodium bisulfite (Zymo®), samples were amplified and visualized by agarose gel electrophoresis (1.5%).

**Table 1:** BDNF primers

Methylated primers	PCR product size
Forward: 5'-GTA GTT TTC GTA GGA GGA TGA AGC-3 ' Reverse: 5'-ATA AAT AAT TAA CAA CCC CGA TAC G-3 ' Unmethylated primers	163 bp
Forward: 5'- GTA GTT TTT GTA GGA TGA GGA AGT G-3 ' Reverse: 5'-TAT AAA TTA ACA ACC CCA ATA CAC A-3 ' 	161 bp

**Response rate**

Response rate, defined as the proportion of households where the contact was made, and an interview completed, was 100%.

**Variables**

FNiS State was treated as the dependent variable. We assessed numerous independent variables for associations with FNiS: BDNF exon I methylation, self-reported health condition, symptoms and/or diseases, socioeconomic profile, property size in hectares, total household monthly income and per capita income, education, possession and use of land, number of produced cultivars, sex, age, life risk factors (smoking, excessive alcohol consumption), affiliation to cooperatives or associations, family composition (marital status, children and number of family members) and biochemical indicators (total protein, creatinine, urea, albumin, total cholesterol and high density lipoprotein, glycemia, oxalacetic transaminase, pyruvic transaminase, D vitamin and cortisol).

**Coding of variables**

Except for age, property size, number of symptoms and/or diseases and family size variables were coded. For independent variables, such as education, self-reported skin color, and monthly household income with per capita income, there were several response options. For FNiS a binary code was used. BDNF gene methylation was rated 0 (unmethylated) or 1 (methylated). Self-reported health conditions were 0 (good/very good) or 1 (fair/poor/very poor). Marital status was 0 (no stable partner) or 1 (stable partner). Schooling was 0 (uneducated) or 1 (incomplete elementary school, <5 years), 2 (complete elementary school, 8 years), 3 (high school, 11 years), and 4 (higher education, 15 or more years). Skin color was 0 (white) or 1 (not white). Poverty index was 0 (not low income) or 1 (low income). Social class was 1 (Class E, up to 2 minimum wages/month), 2 (Class D, 2 to 4 minimum wages/month) or 3 (no income). Presence of children, minor children, garden, access to leisure, extra work outside their property, formal contract, access to government assistance/benefits, belonging to a cooperative/union, garbage collection, were coded as 0 (yes) or 1 (no). Use of pesticides (glyphosate, tebuconazole, flutriafol, thiamethoxan, or copper oxychloride), smoking, consuming alcohol, continued use of medications, health risks due to work, and work impediment due to back pain were coded as 0 (no) or 1 (yes). BMI was rated 1 (normal), 2 (low weight), 3 (overweight) or 4 (obese). Sex was coded 0 (male) or 1 (female). Biochemical profile was 0 (normal) or 1 (altered).

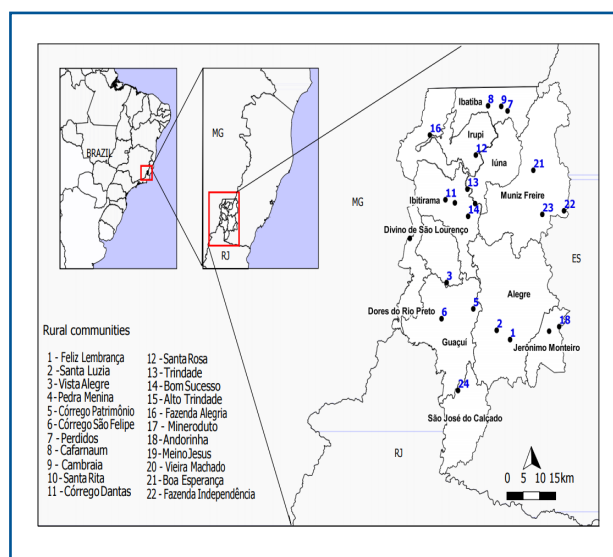
**Statistical analysis**

After sample coding, data were analyzed using the Stata® statistical software package version 14. Quantitative variables presented in medians and interquartile ranges, or means and standard deviations according to normality (Shapiro–Wilk test). Categorical variables presented in relative (%) and absolute (No) frequencies. In descriptive analyses, p-Value was presented after univariate logistic regression at 5% significance and FNiS as a dependent variable (p<0.05).

Initially, univariate logistic regression was used to identify factors associated with FNiS. Then, through backward modeling, multivariate logistic regression was performed to investigate the relationship between FNiS and identified associated factors. At each step, the lowest F value variable was removed. Final model fit was made using the goodness-of-fit test model. Statistical significance was defined as p <0.05.

**RESULTS**

FNiS prevailed in 23.68% of 570 interviews carried out in 22 rural communities of 11 municipalities that compose the study region (Fig 1). Respondents were an average of 42.38 (SD ± 12.59) years old. BDNF exon I promoter methylation was present in 39.10% of the analyses.



**Figure 1:** Communities map.in Caparaó Zone, in Espírito Santo, Southeast of Brazil.

In addition, it was possible to associated socio-demographic and epigenetic alterations with farmers FNiS. For further details, the reader will be able to access the support material (Table S1 to S5) showing results of univariate logistic regression and the general

characterization of the data (S1- demographic, S2- socioeconomic, S3- health, S4- Activities S5- Biochemical tests of blood).

There were positive associations ( $p < 0.05$ ) with variables sex, education, number of living children, number of underage children, number of household residents, per capita income, social class, food prices, property size, property legal status, alcohol consumption, thiamethoxam

pesticide use, chronic medication use, number of reported symptoms and/or diseases, back pain, general self-reported health, body mass index, abdominal circumference and BDNF methylation. Other listed variables were chosen for multivariate analysis and were associated ( $p < 0.200$ ) with FNiS.

### Multivariate logistic regression analysis of FNiS variables

**Table 2:** Multivariate logistic regression using backward modeling of family farmers

Feature	Adjusted Odds Ratio (95% CI)	p value
BDNF methylation		
Negative	1	
Positive	5.03 (1.98–12.83)	0.001
Property size in hectare		
Continuous variable	0.77 (0.67–0.90)	0.001
Extra work outside one's property		
Negative	1	
Positive	3.36 (1.23 – 9.22)	0.018
Number of reported symptoms and/or diseases		
Continuous variable	1.12 (1.04– 1.20)	0.003

## DISCUSSION

The variation in the prices of coffee commodities considerably affects the income of family farmers. There are direct impacts emanating, since the change in commodity prices affects terms of trade, exchange rate adjustments and the balance of payments; and secondary indirect effects of these macroeconomic impacts on domestic prices, including food; unemployment, declining wages and loss of income; and social and health services<sup>1</sup>.

Similarly, the high prevalence (26.4%) of FNiS reported in the world<sup>1</sup>, found 23.68% of FNiS in regional coffee farmers surveyed. When considering only rural populations, this region showed a 1.28 times higher FNiS rate than the southeast Brazilian region (18.6%) and 3.17 fold higher than the ES state (7.5%)<sup>31</sup>.

This study contributes significantly to our current knowledge of epigenetic biomarkers and demonstrates the potential use of BDNF methylation to predict behavioral vulnerability induced by life adversities such as FNiS. This is the first such study to investigate FNiS on family farmers, associating epigenetic and socio-environmental mechanisms among their determinants.

The contribution of environmental factors to DNA methylation has drawn recent attention<sup>32</sup>. Our results reinforce these findings, showing an association of FNS with pathological methylation of BDNF exon I promoter, which has been linked to behavioral disorders such as depression and bulimia nervosa<sup>33</sup>.

Land size, low economic returns and higher reporting of symptoms/diseases, partially correlate with FNiS, which is associated with BDNF gene methylation.

### FNiS and number of symptoms and/or diseases reported

For high commodity-dependent countries

reductions in fiscal revenues in the wake of low or declining commodity prices can threaten the continuity of social programmes, safety nets, and other components of economic and social development plans. Cuts to health and social sector spending can have negative impacts on food security and nutrition<sup>1</sup>.

People reporting the largest number of symptoms and/or diseases had higher FNiS scores, Adjusted Odds Ratio (ORa) 1.12 (95% CI 1.04 to 1.20). Every symptom and/or reported illness increased by 12% the chance of co-occurrence of FNiS.

Health expenditure as a percentage of total government expenditure in countries with high dependence on goods during the period 2008-2015 contracted 1.3% for low-income countries<sup>1</sup>. This fact contributes to understanding the impact of poor health care for family farmers.

In the studied region, 17.11% of producers reported depression, and 47.99% self-rated health status as very bad, bad, or regular. The percentage of respondents who reported 11-33 diseases or symptoms was 50.53%, whereas 49.47% reported 10 or fewer symptoms. The most frequent complaints were muscle pain (64.73%), headache (62.08%), back pain (58.14%), muscle cramps (55.03%), eye irritation (52.38%), joint pain (50.44%), overweight/obesity (49.18%), dizziness and lightheadedness (48.22%), abdominal pain (44.27%), cough (42.68%), and turbid vision (38.62%). Unsustainable practices while working in agriculture contribute to FNiS<sup>34</sup>.

### FNiS and BDNF exon I promoter methylation

Slow economic activity because of falling commodity prices can lead to unemployment, loss of wages and, consequently, loss of income. All of this has a negative impact on food security and nutrition, with potentially lifelong and intergenerational implications for

health and development<sup>1</sup>.

The main health problems of people living in social vulnerability include increased exposure to environmental risk factors, poor nutritional status and difficulty in accessing food, health services and medicines, as well as other economic, psychosocial, cultural or of health. All these factors act as stressors, affecting well-being<sup>35</sup>.

Food and nutrition insecurity with or without hunger is an intense stressor related to concern about food scarcity and fear of hunger. It is related to lack of control, social stigma, family instability, fragility and insecurity. Therefore, FNiS can trigger the activation of the HPA axis involved with stress-response<sup>14,35</sup>.

Long-term stress can lead to HPA axis dysregulation leading to changes in stress responsiveness, and can affect brain health through epigenetic changes<sup>19</sup>. Psychosocial stress can increase vulnerability to depression and be related to increased central adiposity, contributing to increased morbidity<sup>14,35</sup>.

Our study showed that BDNF gene methylation was also associated with FNiS (ORa5.03, 95% CI 1.97 to 12.82).

Our study showed that BDNF gene methylation is also associated with FNiS (ORa5.03, 95% CI 1.97 to 12.82). In addition to the stressor effect of FNiS, which acts through epigenetic changes, there may be the effects of changes in nutrition. Nutrition effects on brain function are primarily mediated by gene expression changes. These changes have diverse characteristics, such as dynamic and short-termed, stable and long-termed, and some are now considered inheritable<sup>36,37</sup>.

Borçoi *et al.*, 2021 found in an adult population a positive association between FNiS and the hypermethylation of the NR3C1 gene, responsible for the expression of the glucocorticoid receptor and regulation of the HPA axis with modulating action on stress responsiveness. In addition, the authors reported a mediating action of NR3C1 gene methylation between FNiS and depression in these individuals. Thus, the authors suggested that the stressor action of the FNiS state could lead to methylation of the NR3C1 gene that regulates stress responsiveness and lead to the development of depression<sup>14</sup>.

In animal models exposed to Bisphenol A, blood BDNF methylation can be used as a predictor of brain BDNF methylation and gene expression, as well as behavioral vulnerability induced by early environmental exposure<sup>38</sup>, suggesting blood BDNF methylation as a potential new biomarker for early detection of psychiatric disease.

Rural worker lifestyle associated with low education may contribute to epigenetic changes in BDNF gene methylation. Strenuous exercises are related to increased reactive oxygen species, reduced BDNF expression and impaired synaptic plasticity and cognition<sup>39</sup>. Depression also increases BDNF exon I methylation, and it was found in 17% of farmers, being a possible cause of epigenetic dysfunction, reduced neurogenesis and neuronal plasticity damage<sup>32,40</sup>, these changes may persist into adulthood in hippocampal tissues<sup>41</sup>.

### **FNiS and extra work out of one's own property**

It was noted that workers, even with additional work out of their property, were more likely to report FNiS, ORa 3.36 (95% CI 1.23 to 9.21). Working in someone else's property is sporadic and does not create an employment bond. Data presented here show FNS deficiency even when governmental financial help was obtained by the farmer or when he was associated with Unions or Cooperatives.

Lack of FNiS is a marker of socioeconomic inequality and is associated with poverty and social imbalance. Therefore, estimates of poverty and social inequality may be used in a complementary way - for the prediction of FNiS<sup>42,43</sup>.

Despite being more frequent in poor countries, lack of FNiS also appears in 5 to 10% of households in developed nations. Between 2007 and 2008 the prevalence in France, United States of America, and Canada were 12.2, 14.5 and 7.7% respectively<sup>2</sup>. In 2013-14, the Trussell Trust, an European network of food banks, provided emergency food aid to more than 900,000 adults and children. Greek, Spanish and French organizations also reported an increase in the number of people seeking emergency food support<sup>44</sup>.

In this study, 74.14% of farmers reported an income per capita per day less than \$5 US dollars and were classified as low income<sup>25</sup>. Whereas our data was collected from the Southeast and richest region of the country, northeast is where most of the rural poverty in Brazil is located (53%)<sup>7</sup>.

Our results support data obtained from the Municipal Human Development Index Average (MHDIA), which has income as an FNiS indicator. The United Nations Development Program reported that the MHDIA for the studied region was 0.665, thus classified as "medium human development", compared to a value of 0.740 (high human development) found for the ES state as a whole<sup>45</sup>.

Although worrisome, Brazilian government data shows that, in recent years, the population's performance has improved. Overall, rural extreme poverty rate decreased from 21.8% in 2002 to 7.6% in 2014 (65.2% decrease); and poverty was reduced from 49% in 2002 to 20.2% in 2014 (58.7% decrease)<sup>8</sup>.

In addition to low income, produce market prices instability is also a major issue. Family farmers face relative disadvantages, including competition with imported goods and governmental subsidies to large producers, which retain privileged access to services and marketing channels<sup>46</sup>.

Farmers who did extra work out of their own properties had a 336% higher chance of experiencing FNiS (ORa = 3.36). Lower levels of education also contributed this<sup>47</sup>. Food security interventions in the setting should focus on gender, education and financial services rather<sup>48</sup>. These results corroborate other findings<sup>48,49</sup> associating the low socioeconomic status of rural households with higher intake of cheaper food with lower nutritional value.

### **FNiS and property size**

Most of the rural properties in the study were not big enough to provide a guarantee of subsistence and socioeconomic progress (Brazilian Law 4504/64)<sup>50</sup>. This represents a failure of Human Rights to Adequate Food (HRAF)<sup>51</sup>, mostly due to the concentration of land ownership in Brazil<sup>49,52</sup>.

Considering that the smallest land size that qualifies as a family farm is 21.6 hectares<sup>52,53</sup>, more than 90% of the farmers had a property area deficit. According to our data, an increase in property size of 1 hectare correlated with a decrease of 23% (ORa = 0.77) in FNiS.

Therefore, even after the social progress achieved in Brazil over the past 15 years, there are large discrepancies among social classes in the countryside, as found in this study. In general, to promote family farming and poverty/inequality reduction in rural areas, a greater diversification in food production, strengthening of food supply in remote locations and promotion of local economies is needed.

For the stated reasons, land reform could help FNiS in rural areas, generating employment and increasing access to nutritious foods<sup>13</sup>. Furthermore, understanding the underlying mechanisms linking nutrition, gene expression and brain function will allow for better nutrition and brain health.

This study has some limitations. A cross-sectional study design does not allow us to infer causality, only demonstrating a strong association of the variable with increased prevalence. A qualitative study of DNA methylation has quality, but determinations of DNA methylation with pyrosequencing after bisulfite conversion have been considered gold standard and are able to quantitatively detect the methylation profile of the sample.

This study significantly contributes to our current knowledge of epigenetic biomarkers and demonstrates the potential use of BDNF gene methylation to predict behavioral vulnerability induced by life adversities such as FNiS. To the best of our knowledge, this is the first study to address FNiS and link it to epigenetic changes in BDNF, gene of fundamental importance for cognitive health and mental health well-being.

In addition, this research can help guide public policies to reduce behavioral disorders and chronic diseases<sup>24</sup>, as well as stimulate the development of more sustainable food production systems that guarantee the nutritional security of rural populations<sup>8</sup>.

## ■ CONCLUSIONS

FNiS is a major problem for rural workers. A prevalence of 23.68% of FNiS among farmer families was observed in our study. The socioeconomic profile was marked by low levels of education and low per capita income. BDNF gene methylation, owning small properties,

## ■ REFERENCES

1. FAO I, UNICEF, WFP and WHO. The State of Food Security and Nutrition in the World 2019. Safeguarding against economic slowdowns and downturns. Rome 2019. 239 p.
2. Bocquier A, Vieux F, Lioret S, Dubuisson C, Caillavet F, Darmon N. Socio-economic characteristics, living conditions and diet quality are associated with food insecurity in France. *Public Health Nutr.* 2015;18(16):2952-61.
3. Hinton SAE. Beyond Health Care: The Role of Social Determinants in Promoting Health and Health Equity May 2018:13.
4. People H. Social Determinants of Health. 2020.
5. Josep C Jiménez-Chillarón 1 RD, Débora Martínez, Thais Pentinat, Marta Ramón-Krauel, Sílvia Ribó, Torsten Plösch. The Role of Nutrition on Epigenetic Modifications and Their Implications on Health. *Biochimie.* 2012 nov;94(11):2242-63.

large number of symptoms/diseases, and the need to work in someone else's property was associated with FNS deficiency. Governmental action to promote sustainable working practices is needed to improve food safety and health of small family coffee growers.

## Ethical approval and consent to participate

Ethical approval was obtained from the Ethics Committee on Health Human Research of the Federal University of Espírito Santo under number 662550. Participation in the study was voluntary. The consent form was attached to each questionnaire and interview before each study participant gave his consent.

## Availability of data and materials

The data supporting the conclusions of this article is included within the article (and its Additional file).

### Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

WMB, AMAS, IL, JBPS, LDC and CCJA: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Writing – Review & Editing, Supervision, Project Administration AND Writing – Review & Editing;. FVF, AAB, CCJA, JKA, JGS, ARB, JAP, ABA, SOM, LLM, JB and MMO: Formal Analysis, Investigation and Resources: All authors read and approved the final manuscript.

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6. Brasil. Law No. 11,346 Creates the National System of Food and Nutritional Security - SISAN with a view to ensuring the human right to adequate food and takes other measures. *Diário Oficial da União* 2006; 18 set. 2006.
7. IBGE. Instituto Brasileiro de Geografia e Estatística. Fornecido em meio eletrônico:[[www.ibge.gov.br/home/estatistica/populacao/censo2010/](http://www.ibge.gov.br/home/estatistica/populacao/censo2010/)] Acessado em. 2010;20(03):2012.
8. Mattei, L. Pobreza e fome no Brasil e em Santa Catarina no contexto da pandemia da Covid-19- Editorial. *Revista NECAT*. 2022; 11(21).
9. Bojanic AJ, França CG, Marques VPMA, Del Grossi ME. Superação da fome e da pobreza rural: iniciativas brasileiras. 2016.
10. Borges LRdOVAJ. Balanço Social 2014 Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural. 2014:38.
11. Melchior M, Caspi A, Howard LM, Ambler AP, Bolton H, Mountain N, et al. Mental health context of food insecurity: a representative cohort of families with young children. *Pediatrics*. 2009;124(4):e564-72.
12. Davison KM, Marshall-Fabien GL, Tecson A. Association of moderate and severe food insecurity with suicidal ideation in adults: national survey data from three Canadian provinces. *Soc Psychiatry Psychiatr Epidemiol*. 2015;50(6):963-72.
13. Valente FLS. Direito humano à alimentação: desafios e conquistas. *Direito humano à alimentação: desafios e conquistas*: Cortez; 2002.
14. Kim DH. The interactive effect of methyl-group diet and polymorphism of methylenetetrahydrofolate reductase on the risk of colorectal cancer. *Mutat Res*. 2007;622(1-2):14-8.
15. Raj Chetty NH, Patrick Kline, Emmanuel Saez. Where is the land of Opportunity? The Geography of Intergenerational Mobility in the United States. *The Quarterly Journal of Economics*. 2014;129(4):1553–623.
16. Dauncey M. Recent advances in nutrition, genes and brain health. *Proceedings of the Nutrition Society*. 2012;71(04):581-91.
17. Esteller M. DNA methylation and cancer therapy: new developments and expectations. *Curr Opin Oncol*. 2005;17(1):55-60.
18. Hou L, Zhang X, Wang D, Baccarelli A. Environmental chemical exposures and human epigenetics. *Int J Epidemiol*. 2012;41(1):79-105.
19. Borçoi AR, Mendes SO, Gasparini Dos Santos J, Mota de Oliveira M, Moreno IAA, Freitas FV, Pinheiro JA, Arpini JK, Cunha ER, Archanjo AB, Evangelista Monteiro de Assis AL, Sorroche BP, Rebolho Batista Arantes LM, Borloti E, Álvares-da-Silva AM. Risk factors for depression in adults: NR3C1 DNA methylation and lifestyle association. *J Psychiatr Res*. 2020 Feb;121:24-30. doi: 10.1016/j.jpsychires.2019.10.011. Epub 2019 Oct 16. PMID: 31731185..
20. Bockmühl Y, Patchev AV, Madejska A, Hoffmann A, Sousa JC, Sousa N, et al. Methylation at the CpG island shore region upregulates Nr3c1 promoter activity after early-life stress. *Epigenetics*. 2015;10(3):247-57.
21. Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol*. 2008;11(8):1169-80.
22. D'Addario C, Dell'Osso B, Galimberti D, Palazzo MC, Benatti B, Di Francesco A, et al. Epigenetic modulation of BDNF gene in patients with major depressive disorder. *Biol Psychiatry*. 2013;73(2):e6-7.
23. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. *Nat Neurosci*. 2007;10(9):1089-93.
24. Pryor L, Lioret S, van der Waerden J, Fombonne É, Falissard B, Melchior M. Food insecurity and mental health problems among a community sample of young adults. *Soc Psychiatry Psychiatr Epidemiol*. 2016;51(8):1073-81.
25. Neri MC. A nova classe média. Rio de Janeiro: FGV/Ibre, CPS. 2008:16.
26. Nuttall FQ. Body Mass Index: Obesity, BMI, and Health: A Critical Review. *Nutr Today*. 2015;50(3):117-28.
27. Triola MF. introdução á estatística: LTC; 2008.
28. Marin-Leon AMS-CL. A segurança alimentar no Brasil: proposição e usos da escala brasileira de medida da insegurança alimentar (EBIA) de 2003 a 2009 *Segurança Alimentar E Nutricional*. 2009;16(2):1-19.
29. Salazar LA, Hirata MH, Cavalli SA, Machado MO, Hirata RD. Optimized procedure for DNA isolation from fresh and cryopreserved clotted human blood useful in clinical molecular testing. *Clin Chem*. 1998;44(8 Pt 1):1748-50.



30. D'Addario C, Dell'Osso B, Palazzo MC, Benatti B, Lietti L, Cattaneo E, et al. Selective DNA methylation of BDNF promoter in bipolar disorder: differences among patients with BDI and BDII. *Neuropsychopharmacology*. 2012;37(7):1647-55.
31. Pereira ARVARFdRMdFPdOSM. Segurança Alimentar Espírito Santo, a partir da Pnad 2013. Instituto Jones dos Santos Neves - RESENHAS ESTRUTURAIAS. 2015:1-6.
32. Koenen KC, Uddin M, Chang SC, Aiello AE, Wildman DE, Goldmann E, et al. SLC6A4 methylation modifies the effect of the number of traumatic events on risk for posttraumatic stress disorder. *Depress Anxiety*. 2011;28(8):639-47.
33. Thaler L, Gauvin L, Joober R, Groleau P, de Guzman R, Ambalavanan A, et al. Methylation of BDNF in women with bulimic eating syndromes: associations with childhood abuse and borderline personality disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;54:43-9.
34. Wilde PE, Peterman JN. Individual weight change is associated with household food security status. *J Nutr*. 2006;136(5):1395-400.
35. Freitas FV, Barbosa WM, Silva LAA, Garozi MJO, Pinheiro JA, Borçoi AR, Conti CL, Arpini JK, de Paula H, de Oliveira MM, Archanjo AB, de Freitas ÉAS, de Oliveira DR, Borloti EB, Louro ID, Alvares-da-Silva AM. Psychosocial stress and central adiposity: A Brazilian study with a representative sample of the public health system users. *PLoS One*. 2018 Jul 31;13(7):e0197699. doi: 10.1371/journal.pone.0197699. PMID: 30063700; PMCID: PMC6067710.
36. Dauncey MJ. New insights into nutrition and cognitive neuroscience. *Proc Nutr Soc*. 2009;68(4):408-15.
37. Roth TL, Lubin FD, Funk AJ, Sweatt JD. Lasting Epigenetic Influence of Early-Life Adversity on the BDNF Gene. *Biological Psychiatry*. 2009;65(9):760-9.
38. Kundakovic M, Gudsnuk K, Herbstman JB, Tang D, Perera FP, Champagne FA. DNA methylation of BDNF as a biomarker of early-life adversity. *Proc Natl Acad Sci U S A*. 2015;112(22):6807-13.
39. Erickson KI, Miller DL, Roecklein KA. The aging hippocampus: interactions between exercise, depression, and BDNF. *Neuroscientist*. 2012;18(1):82-97.
40. Vaiserman A. Epidemiologic evidence for association between adverse environmental exposures in early life and epigenetic variation: a potential link to disease susceptibility? *Clin Epigenetics*. 2015;7:96.
41. Nemoda Z, Massart R, Suderman M, Hallett M, Li T, Coote M, et al. Maternal depression is associated with DNA methylation changes in cord blood T lymphocytes and adult hippocampi. *Transl Psychiatry*. 2015;5:e545.
42. SILVA JGd, DEL GROSSI ME, FRANÇA CGd. Fome Zero: a experiência brasileira. Brasília: Ministério do Desenvolvimento Agrário. 2010.
43. Morais DdC, Dutra LV, Franceschini SCC, Priore SE. Insegurança alimentar e indicadores antropométricos, dietéticos e sociais em estudos brasileiros: uma revisão sistemática. *Ciência & Saúde Coletiva*. 2014;19:1475-88.
44. Loopstra R, Reeves A, Stuckler D. Rising food insecurity in Europe. *The Lancet*. 2015;385(9982):2041.
45. Chaves JCAPMCNM. Brazilian Municipal Human Development Index. United Nations Development Program (UNDP). 2013:96.
46. Buainain AM, Romeiro AR, Guanziroli C. Agricultura familiar e o novo mundo rural. *Sociologias*. 2003;5(10).
47. McGregor J. Climate change and involuntary migration: implications for food security. *Food Policy*. 1994;19(2):120-32.
48. Hassen K, Zinab B, Belachew T. Gender and education as predictors of food insecurity among coffee farming households of the Jimma zone, Southwest of Ethiopia. *BMC Nutrition*. 2016;2(1):75.
49. Mondini L, Rosa TE, Gubert MB, Sato GS, Benício MHD. Insegurança alimentar e fatores sociodemográficos associados nas áreas urbana e rural do Brasil. 2011.
50. Brasil. Land Statute Law - Provides for the Land Statute, and makes other provisions. *Diário Oficial da República Federativa do Brasil*. 1964.
51. FAO UN-. The Right to Adequate Food. 2013;Fact Sheet No. 34.
52. Bergamim MC, CAMPOS JR CT, editors. Agricultura familiar no Espírito Santo: concentração fundiária e recomposição socioeconômica. *Anais XLIII Congresso da Sociedade Brasileira de Economia e Sociologia Rural*; 2005.
53. Brasil. NATIONAL RURAL REGISTRATION SYSTEM - BRAZILIAN BASIC INDICES OF 2013. 2013.

## Resumo

**Introdução:** o Estado de Segurança Alimentar e Nutricional (SAN) é aquele que deve garantir o direito de acesso permanente à alimentação de qualidade e em quantidade suficiente sem prejudicar o acesso a outros direitos básicos. No Brasil, os estabelecimentos de agricultura familiar rural representam 84,4% do total de estabelecimentos agropecuários e contribuem com mais de 70% de todos os alimentos consumidos pelos brasileiros. Nesse contexto, destaca-se a produção da commodity café. No entanto, apesar de serem produtores de alimentos, não auferem bons rendimentos. A lenta atividade econômica pode levar à perda de salários e renda, doenças e insegurança alimentar (INSAN). Além disso, os efeitos moleculares da INSAN são pouco estudados, sobretudo epigenéticos.

**Objetivos:** o objetivo do presente estudo é analisar a associação entre a metilação do promotor do BDNF e a INSAN e a associação da INSAN com fatores sociodemográficos, de estilo de vida e epigenéticos, em cafeicultores da zona do Caparaó, no Espírito Santo, Sudeste do Brasil.

**Método:** o estudo foi realizado em 22 comunidades cafezeiras selecionadas aleatoriamente na Zona do Caparaó, área que produz café de reconhecida qualidade. Um total de 570 famílias de cafeicultores, entre 18 a 60 anos, foram incluídos no estudo e responderam a um questionário sobre características socioeconômicas, uso e posse da terra, hábitos de vida, saúde e condições de trabalho. A avaliação da INSAN foi realizada por meio da Escala Brasileira de Insegurança Alimentar. A metilação do éxon I do BDNF foi examinada por PCR específica para metilação. Índice de massa corporal e análises bioquímicas foram realizadas. Modelos de regressão logística foram utilizados para verificar os fatores associados à INSAN ( $p < 0,05$ ). Os dados foram analisados usando o software estatístico Stata® versão 14.

**Resultados:** a prevalência de INSAN encontrada foi de 23,68%. Na regressão logística multivariada, a ocorrência de INSAN foi associada a hipermetilação do éxon I do promotor do gene BDNF [ORa = 5,03 (95% (1,98, 12,82))] quando comparado ao gene não metilado. Além disso, a INSAN foi associada a carga de trabalho excessiva [ORa = 3,36 (1,23, 9,22)], posse de menos hectares de terra [ORa = 0,77 (0,67, 0,90)] e maior número de sintomas e/ou doenças da vida real [ORa = 1,12 (1,04, 1,20)].

**Conclusão:** o estudo mostrou uma alta prevalência de Insegurança Alimentar na região analisada. Esse fenômeno foi associado a fatores epigenéticos (metilação do gene BDNF), carga horária excessiva, pequena propriedade de terra e maior número de doenças/sintomas. A INSAN pode ser um estressor capaz de promover alterações epigenéticas no gene BDNF, importante gene regulador da cognição, crescimento e sobrevivência neuronal e envolvido com doenças psiquiátricas.

**Palavras-chave:** cafeicultores, insegurança alimentar e nutricional, BDNF, metilação DNA.

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