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Anxiety disorders in childhood are associated with youth IL-6 levels: A mediation study including metabolic stress and childhood traumatic events

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ABSTRACT

Anxiety disorders (ADs) are chronic conditions that often have their onset in childhood and adolescence. Inflammation and oxidative stress markers have been associated with the vulnerability to ADs, however it is not known if ADs in childhood can influence these biomarkers levels longitudinally. This study aims to investigate a possible association between ADs and serum levels of IL-10, IL-6, IL-1β, TNF-α, BDNF, and protein carbonyl content, assessed after 5 years of follow-up. Moreover, we studied possible mediators for these associations, including physical activity, metabolic markers and childhood trauma. From 240 individuals evaluated at baseline, 73 were re-evaluated in the follow-up. Psychiatric diagnoses were assessed with the K-SADS or the MINI and child trauma questionnaire (CTQ) to evaluate presence of trauma. We searched serum levels of IL-10, IL-6, IL-1β and TNF-α (flow cytometry), BDNF (sandwich-ELISA) and carbonyl content in proteins (PCC method). We found a significant direct association between ADs at baseline and log IL-6 (B = 0.34, S.E. = 0.11, p = 0.002) and between AD and log BDNF (B = −0.10, S.E. = 0.05, p = 0.033) five years later. Searching for possible mediators of these association, we found that levels of HDL-cholesterol (ΔB = −0.148) partially mediated the association between ADs and IL-6. No significant mediators were found in the association between ADs and BDNF. Moreover, this association is no longer significant after controlling for the presence of depression. Our results demonstrated that previous AD diagnosis was associated with higher levels of IL-6 in the follow-up evaluation, suggesting that the presence of anxiety in childhood could influence altered inflammatory markers.

1. Introduction

Anxiety disorders (ADs) are the largest group of disabling mental disorders in most western societies (Craske et al., 2017). The onset of most ADs is during childhood, adolescence or early adulthood (Beesdo et al., 2011; Creswell et al., 2014; Salum et al., 2011) and if untreated, ADs tend to be chronic with waxing and waning course associated to the co-occurrence of depression and several other conditions (Beesdo et al., 2011, 2010). New studies have been designed to evaluate biological markers (biomarkers) with high predictive value to better understand the development of neuropsychiatric disorders, as well as to include its use into the standard diagnostic procedure and to evaluate preventive interventions (Cuthbert and Insel, 2013).

In line with this view, potential blood-based biomarkers, such as

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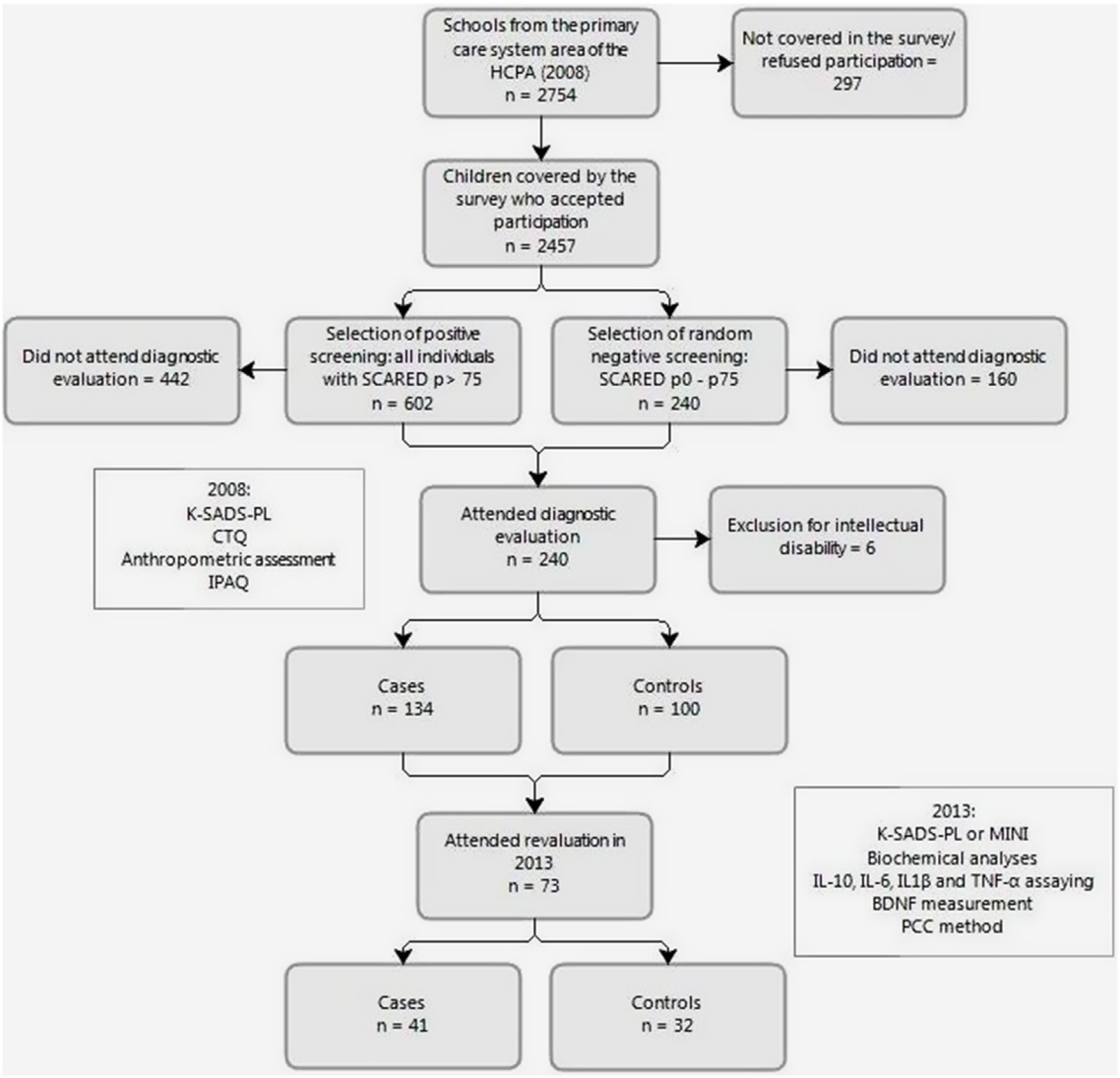

Fig. 1. Flow diagram of subjects enrolled. HCPA: Hospital de Clínicas de Porto Alegre, Brazil; K-SADS-PL: Schedule for Affective Disorder and Schizophrenia for School-Age Children-Present and Lifetime Version; CTQ: Childhood Trauma Questionnaire; IPAQ: International Physical Activity Questionnaire; MINI: Mini International Neuropsychiatric Interview; IL-10: interleukin-10; IL-6: interleukin-6; IL-1 β: interleukin-1β; TNF-α: tumor necrosis factor-α; BDNF: brain-derived neurotrophic factor; PCC: protein carbonyl content.

inflammation and immune system components (Ogłodek et al., 2015a; Vogelzangs et al., 2013) and oxidative stress markers (Ercan et al., 2017) have been consistently associated with the vulnerability to AD. Pro- and anti-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), interferon-gamma (IFN- γ), C-reactive protein and monocyte chemoattractant protein-1 (MCP-1) have been implicated in the etiology and symptomatology of ADs (Belem da Silva et al., 2016; Duivis et al., 2013; Hou et al., 2017; Ogłodek et al., 2015b; Wohleb and Godbout, 2013). Moreover, chronic inflammation facilitates the release of potent reactive oxygen species (ROS) and an increased in oxidative stress status and these conditions have also been associated with ADs (Bouayed et al., 2009; Ercan et al., 2017).

However, it is still unclear whether an association among ADs, inflammation and oxidative stress could be direct or mediated through metabolic and environmental factors. For instance, a recent meta-analysis showed that anxiety had a significant positive association with metabolic syndrome in 18 cross-sectional studies (Tang et al., 2016). Moreover, our group has previously demonstrated that higher somatic symptoms of anxiety significantly predicted lower levels of physical activity (Belem da Silva et al., 2014), which could be potentially explained by variable degrees of avoidant behaviors. A sedentary lifestyle is a risk factor to metabolic syndrome development in susceptible individuals. This syndrome progressively breeds a mild inflammatory environment due to the activation of abnormal metabolic pathways, and this inflammatory state leads to redox equilibrium alterations with increased generation of oxidizing agents (Alves et al., 2016; Etchegoyen et al., 2018; Li et al., 2013). Adding to the complexity of potential mediating relationships, childhood life events and childhood trauma are both associated with ADs and immune system imbalances (Carr et al., 2013; Hovens et al., 2015; Menke et al., 2018; Nusslock and Miller, 2016). Altogether, these studies demonstrate that investigating potential mediators among ADs, inflammation and oxidative stress are issues of paramount interest.

Thus, the present study aims at investigating the possible association between childhood ADs and serum levels of interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), brain-derived neurotrophic factor (BDNF), as immune/inflammatory indicators, and protein carbonyl content, as an oxidative stress indicator, assessed after 5 years of follow-up. Moreover, we studied possible mediators for these associations, including metabolic markers and childhood trauma, in order to better understand our

findings.

2. Methods

2.1. Sample selection and psychiatric evaluation

This study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre, Brazil (protocol number 15–0349), and all parents and individuals over 18 years-old signed an informed consent form. The study was carried out in accordance with the latest version of the Declaration of Helsinki (World Medical Association, 2013).

Children and adolescents were selected from a larger community sample enrolled at schools within the area served by the Hospital de Clínicas de Porto Alegre (HCPA), Brazil, in 2008. From a total of 2.457 students that underwent a screening scale for anxiety disorders (Screen for Child and Anxiety Related Emotional Disorders - SCARED) (Birmaher et al., 1997; Isolan et al., 2011), 240 non-medicated individuals underwent an extensive psychiatric evaluation and diagnostic assessment (Schedule for Affective Disorder and Schizophrenia for School-Age Children-Present and Lifetime Version - K-SADS-PL) (Kaufman et al., 1997; Polanczyk et al., 2003). Exclusion criteria included: (1) a significant organic illness; (2) a history of pervasive developmental disorder, bipolar disorder, or any psychotic disorder; (3) a history of alcohol or drug abuse; or (4) an intellectual disability. Of the selected individuals, six were excluded due to intellectual disability, remaining a total of 234 adolescents: 134 with and 100 without anxiety disorders (classified as controls). The sampling procedures have been described in detail elsewhere (Salum et al., 2011). Besides the clinical interview, all participants were asked about their history of trauma, physical activity (PA) and underwent an anthropometric assessment.

From the initial cohort (n = 234) recruited in 2008, a sub-sample of 76 participants agreed to be re-evaluated 5 years later and 73 completed the whole evaluation, in 2013. In this follow-up evaluation, participants underwent a clinical and semi-structured interview with the K-SADS (under 18 years-old) or the Mini International Neuropsychiatric Interview - MINI (18 years-old or above) (Amorim, 2000) to confirm the diagnosis according to the DSM-IV-TR criteria. They also had their blood drawn for biochemical evaluations and biomarkers assessment. Fig. 1 shows a brief flow-chart of the subjects enrolled in the study. All the subjects were non-medicated and untreated throughout the study.

2.2. Childhood traumatic events

We investigated childhood trauma at baseline using the Brazilian version of Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003; Grassi-Oliveira et al., 2006). The CTQ is a self-report questionnaire constituted of 28-items. Each item is a 5-point likert type that evaluates one of five types of childhood trauma (emotional, physical and sexual abuse and emotional and physical neglect) in a retrospective basis.

2.3. Anthropometry

Anthropometric assessment was performed in the baseline evaluation, in the morning in fasting by trained researchers. We measured weight, height, and waist circumference using accurate and calibrated equipment (Toledo®, São Paulo, SP, BRA; Harpenden®, Holtain Limited, Crymych, UK). Body Mass Index (BMI) was calculated as weight (kg) divided by height squared (m^2).

2.4. Physical activity

We assessed physical activity (PA) at baseline evaluation, using International Physical Activity Questionnaire (IPAQ) short version, designed to access PA on a weekly basis (Craig et al., 2003). This instrument is composed by three items, each subdivided in two sub-items focusing on the number of days per week and on the mean daily time spent on each category of activity. An overall Metabolic Equivalent of Task (METS) score is computed multiplying both sub-items and a weighted score for each category, resulting in a total of MET-minutes/week.

2.5. Biochemical analysis

In the follow up evaluation, we collected blood samples for biochemical analyses from subjects after a 12-h fast in the early morning. Insulin and blood glucose levels were determined by chemiluminescence. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were determined by enzymatic colorimetric method. Low-density lipoprotein (LDL) cholesterol was estimated using the *Friedewald* equation (Friedewald et al., 1972).

2.6. Collection and processing of blood

Ten milliliters of blood were also collected in the follow-up evaluation, from all participants by venipuncture into a free-anticoagulant vacuum tube in the morning after 12-h of fasting. Immediately after withdrawal, blood was centrifuged at 2000g for 10 min and serum was aliquoted, labeled and stored at −80 °C until assaying.

2.7. Cytokine assaying

The concentration of serum cytokines at follow-up was determined by flow cytometry using the BD™ Cytometric Bead Array (CBA) Enhanced Sensitivity Flex Sets for Human IL-10, IL-6, IL1β and TNF-α (BD Biosciences, San Diego, CA). We processed the samples and analyzed the data according to the manufacturer's instructions. Data were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA) and results were generated using the BD CBA Analysis Software FCAP Array™ (BD Biosciences, San Diego, CA). Results are expressed in fg/mL.

2.8. Brain-Derived Neurotrophic Factor (BDNF) measurement

BDNF serum levels in the follow-up were determined by sandwich-ELISA using monoclonal antibodies specific for BDNF (R&D Systems, Minneapolis, Minnesota). Briefly, microliter plates (96-well flat-bottom) were coated overnight at room temperature with the monoclonal anti-BDNF antibody at 4 µg/mL in PBS. After, plates were washed three times with wash buffer (PBS, pH 7.4, with 0.05% Tween 20) and blocked for 1 h at room temperature with PBS containing 5% nonfat milk powder. After washing, plates were incubated for 3 h at room temperature with diluted samples 1:200 in sample diluent (PBS with 1% bovine serum albumin). A 2-fold range standard curve was run in duplicate and consisted in serial dilutions of BDNF ranged from 7.8 to 500 pg/mL. After samples incubation, plates were washed and biotinylated anti-BDNF antibody at 0.2 µg/mL was added and incubated for 2 h at room temperature. After washing, plates were incubated with streptavidin-peroxidase conjugate (diluted 1:200 in sample diluent) for 20 min at room temperature, washed and incubated with the substrate for 20 min at room temperature. Finally, stop solution (H_2SO_4 1M) was added and the amount of BDNF was determined by absorbance at 450 nm with correction at 540 nm. The concentration of BDNF was expressed in ng/mL.

2.9. Protein Carbonyl Content (PCC) method

Oxidative damage to proteins was analyzed by the determination of carbonyl content in proteins (PCC method), as previously described by Levine et al. (1990). This method is based on the reaction of carbonyl groups of proteins with dinitrophenylhydrazine reagent (DNPH) to

form Schiff bases that absorb at 380 nm wavelength. Analyses were performed in serum samples collected at follow-up and the values are expressed in nmol/mg of protein.

2.10. Statistical analysis

Sample characteristics were described as means and standard deviations, or percentages. We did multiple linear regression analysis to evaluate the associations between anxiety disorders (independent variable) and cytokines, BDNF or oxidative damage (dependent variables), controlling for the presence of depression. We used the logarithmic transformation of the potential biomarkers levels due to the asymmetric distribution of these variables.

We used mediation analysis after evaluating the significant associations to test: (I) the total effect of anxiety disorders on possible mediators (*a*); (II) the effect of mediators on (log) biomarker (*b*); (III) the direct effect of anxiety disorders on (log) biomarker, corrected for a × b (c'); (IV) the indirect effect of anxiety disorders on (log) biomarker, through the mediators (a × b). All mediator variables were standardized to create comparable effect sizes. Mediation analyses were performed using the Preacher and Hayes's SPSS macro, which estimates the indirect effects of the independent variable on the dependent variable through mediator variables, using bootstrap resampling procedures (5000 resamples). The indirect effect is deemed significant if the 95% bootstrap percentile confidence interval (CI) do not include zero (Hayes and Preacher, 2014; Révész et al., 2016).

The variables evaluated as mediators were: CTQ total trauma score, age, BMI, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, blood glucose, insulin and physical activity. Change in effect (ΔB) was calculated by subtracting c' from c, and dividing this residual by the c (e.g. ΔB = (c – c')/c) (Révész et al., 2016). All analyses were conducted using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

3. Results

The mean age of total sample (n = 73) was 12.6 (S.D. = 2.3) at baseline. Most individuals were female (60.3%) and caucasian (65%). Baseline characteristics of children are depicted in detail in Table 1. The 2013 subsample was representative of the whole sample cohort evaluated in 2008, as showed in Supplemental Table 1.

No associations were found among ADs at baseline and log transformed IL-10, IL1β, TNF-α and Carbonyl at follow-up (Table 2). We found significant associations between the diagnosis of ADs at baseline and log transformed IL-6 and BDNF evaluated in follow-up: ADs diagnosis in 2008 was associated with higher IL-6 levels (B = 0.34, S.E. = 0.11, p = 0.002) and lower BDNF levels (B = −0.10, S.E. = 0.05, p = 0.033) in 2013 (Table 3 and Table 4, respectively). Moreover, ADs at baseline were positively associated with HDL cholesterol and negatively associated with physical activity, whereas no associations were found among IL-6 and BDNF levels and the mediators evaluated (Tables 3 and 4).

Table 1
Baseline characteristics of children and adolescents (2008).

Variables	Cases (n = 41)	Controls (n = 32)	P
Gender, Female (%)	31 (75.6%)	13 (40.6%)	0.004[a]
Age, mean (SD), years	13 (2.3)	12 (2.3)	0.071[b]
Ethnicity			
Caucasians	26 (63%)	21 (68%)	0.376[a]
Brazilian Africans	7 (17%)	2 (6%)	
Other	8 (20%)	8 (26%)	
Psychiatric diagnosis			
Generalized Anxiety Disorder	30 (73.2%)	– – –	
Social Anxiety Disorder	16 (39%)	– – –	
Separation Anxiety Disorder	12 (29.3%)	– – –	
Agoraphobia	– – –	– – –	
Panic Disorder	4 (9.8%)	– – –	
Specific Phobia	17 (41.5%)	– – –	

[a] Chi-squared.
[b] Test t student. Statistical significance: P < 0.05. The ethnicity was determined by self-report.

Table 2
Linear regression analysis evaluating the association between anxiety disorders and cytokines, BDNF or oxidative damage (after logarithmic transformation).

	B[a]	S.E.	*p*
Log IL-1β	0.046	0.065	0.486
Log IL-6	0.342	0.109	0.002[b]
Log IL-10	−0.006	0.091	0.950
Log TNF-α	−0.065	0.060	0.286
Log PCC	0.000	0.043	0.991
Log BDNF	−0.100	0.046	0.033[b]

IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-10: Interleukin-10; TNF-α: Tumor Necrosis Factor-α; PCC: Protein Carbonyl Content; BDNF: Brain-Derived Neurotrophic Factor.
[a] Total effect of ADs (c) on each variable.
[b] Significant based on 95% CI (p < 0.05).

When analyzing mediating variables between ADs and IL-6 levels (column III, c' and column IV, a × b in Table 3), we found that higher levels of HDL-cholesterol (ΔB = −0.148 or 14.8%) were partially mediating this association (column IV, Table 3). The other studied variables were not significant mediators of this association. None of these variables significantly mediated the association between ADs and BDNF levels (column III, c' and column IV, a × b in Table 4). Mediation analyses are summarized in Fig. 2 (IL-6) and Fig. 3 (BDNF). When we performed the regression analysis controlling for depression, the association between ADs and log transformed IL-6 was maintained, but the association between ADs and log transformed BDNF was no longer significant (Supplemental Table 2).

The cross sectional analysis considering the presence of anxiety diagnosis and inflammatory markers in the follow-up evaluation showed no significant differences in IL6 and BDNF levels between anxious and non-anxious subjects (Supplemental Table 3).

4. Discussion

In the present study, we found that the diagnosis of ADs in childhood was significantly associated with higher levels of IL-6 after 5 years of follow-up. The association found herein was partially mediated by higher levels of HDL cholesterol. Furthermore, ADs was also associated with lower levels of BDNF 5 years later and no significant mediators were found for such association.

Our findings associating ADs and IL-6 in a longitudinal prospective are consistent with meta-analytical findings that reported a significant overall difference in IL-6 levels between healthy controls and individuals with anxiety disorders (P < 0.001, Hedge's g = −0.93) (Renna et al., 2018). IL-6 is a plurifunctional four-helix bundle cytokine that has been linked to numerous biological functions, particularly in the central nervous system (CNS)(Erta et al., 2012; Spooren et al., 2011). It is known that IL-6 produced peripherally can cross or send pro-inflammatory signals across the blood-brain barrier (Maier, 2003; Quagliato and Nardi, 2018), and change amygdaloid activity increasing anxiety-like behavior (Engler et al., 2011; Quagliato and Nardi, 2018). Moreover, psychiatric disorders are often associated with a Th1/Th2 cells unbalance, predominating humoral immunity (Th2) over the cellular immunity (Th1). Thereby, increases in IL-6 levels secreted by Th2 cells may result in anxiety symptoms (Martino et al., 2012). In agreement to this study, our group has previously demonstrated an association of current panic disorder with higher mean levels of IL-6 compared to remitted panic disorder, in a severity-dependent manner (Belem da Silva et al., 2016). These findings strengthen the relationship

Table 3
Mediation analyses with separate mediators between ADs and log IL-6 (n = 73).

Mediator	I. Effect of ADs on mediator (a)		II. Effect of mediator on log IL-6 (b)		Total effect of ADs on log IL-6 (c)			IV. Indirect effect of ADs on log IL-6 (a x b)
					B (S.E.)	p		
					0.342 (0.109)	0.002		
					III. Direct effect of ADs on log IL-6 (c')			
	B (S.E.)	P	B (S.E.)	P	B (S.E.)	P	ΔB[a]	B (95% CI)
Trauma	1.502 (3.576)	0.677	0.002 (0.007)	0.826	0.331 (0.157)	0.042	0.057	0.002 (−0.041 to 0.075)
BMI	1.165 (0.911)	0.205	0.006 (0.014)	0.680	0.335 (0.111)	0.004	0.046	0.007 (−0.014 to 0.071)
Total Cholesterol	12.681 (7.120)	0.079	−0.002 (0.002)	0.187	0.372 (0.111)	0.001	−0.060	−0.031 (−0.138 to 0.007)
LDL Cholesterol	8.117 (5.193)	0.123	−0.003 (0.003)	0.328	0.362 (0.111)	0.002	−0.031	0.030 (−0.119 to 0.011)
HDL Cholesterol	6.663 (2.688)	0.016	−0.009 (0.005)	0.058	0.403 (0.111)	0.001	−0.148	−0.061 (−0.157 to −0.010)[b]
Triglycerides	−10.497 (10.525)	0.322	0.001 (0.001)	0.716	0.346 (0.110)	0.003	0.014	−0.005 (−0.057 to 0.014)
Glycemia	0.544 (1.555)	0.728	−0.004 (0.008)	0.681	0.343 (0.110)	0.003	0.023	−0.002 (−0.056 to 0.017)
Insulin	2.880 (1.652)	0.086	0.001 (0.008)	0.951	0.340 (0.112)	0.003	0.031	0.001 (−0.040 to 0.047)
Physical Activity	−206.796 (66.468)	0.003	0.000 (0.0002)	0.907	0.346 (0.117)	0.004	0.014	−0.005 (−0.080 to 0.061)

IL-6: Interleukin-6; BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.
[a] Change in B was not part of mediation output, but calculated manually, as described in Statistical analyses.
[b] Significant based on 95% CI (p < 0.05).

Table 4
Mediation analyses with separate mediators between ADs and log BDNF (n = 73).

Mediator	I. Effect of ADs on mediator (a)		II. Effect of mediator on log BDNF (b)		Total effect of ADs on log BDNF (c)			IV. Indirect effect of ADs on log BDNF (a x b)
					B (S.E.)	P		
					−0.100 (0.046)	0.033		
					III. Direct effect of ADs on log BDNF (c')			
	B (S.E.)	P	B (S.E.)	P	B (S.E.)	P	ΔB[a]	B (95% CI)
Trauma	1.502 (3.576)	0.677	0.003 (0.003)	0.334	−0.106 (0.063)	0.102	0.577	0.004 (−0.013 to 0.060)
BMI	1.364 (0.913)	0.140	−0.0001 (0.006)	0.992	−0.100 (0.047)	0.037	0.601	−0.0001 (−0.021 to 0.017)
Total Cholesterol	12.828 (7.007)	0.071	−0.001 (0.001)	0.320	−0.090 (0.047)	0.059	0.641	−0.010 (−0.051 to 0.006)
LDL Cholesterol	7.959 (5.112)	0.124	−0.001 (0.001)	0.520	−0.094 (0.047)	0.048	0.625	−0.006 (−0.041 to 0.008)
HDL Cholesterol	6.806 (2.649)	0.012	0.000 (0.002)	0.995	−0.100 (0.048)	0.042	0.601	−0.0001 (−0.037 to 0.026)
Triglycerides	−9.686 (10.381)	0.354	−0.001 (0.001)	0.076	−0.109 (0.045)	0.019	0.565	0.045 (−0.039 to 0.188)
Glycemia	0.071 (1.586)	0.965	−0.005 (0.003)	0.115	−0.010 (0.045)	0.032	0.960	−0.0004 (−0.028 to 0.017)
Insulin	2.662 (1.637)	0.108	−0.001 (0.003)	0.690	−0.096 (0.047)	0.044	0.617	−0.004 (−0.031 to 0.011)
Physical Activity	−197.294 (65.943)	0.004	−0.0001 (0.0001)	0.414	−0.113 (0.049)	0.023	0.549	0.013 (−0.014 to 0.057)

BDNF: brain-derived neurotrophic factor; BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.
*Significant based on 95% CI (p < 0.05).
[a] Change in B was not part of mediation output, but calculated manually, as described in Statistical analyses.

between anxious symptoms and inflammation.

Moreover, we found that higher levels of HDL cholesterol partially mediated the association between ADs and IL-6, reducing the total relationship between ADs and IL-6 levels. HDL cholesterol is a strong antioxidant, and low levels are associated with increased oxidative stress and pro-inflammatory responses (Ganjali et al., 2018). A study with 5309 individuals in midlife demonstrated that the accumulation of stressful life events in childhood/adolescence or adulthood were associated with high levels of IL-6 and low levels of HDL in men (Dich et al., 2015), which is in agreement with the indirect relation reported by our present study.

Our findings also show an association between ADs and lower levels of BDNF in comparison to controls. It is important to note, however, that when we performed the regression analysis adjusting for depression, this association was no longer significant, indicating that this association may be due to all internalizing symptoms, including depressive symptoms, in our sample. The brain-derived neurotrophic factor (BDNF), a growth factor ligand of NTRK2 and TrkB receptor, is one of the most studied growth factors and plays an important role in neuroinflammation. Growth factors are cytokines that regulate development, cell migration, fate and survival in all body tissues, including the brain (Galvez-Contreras et al., 2016; Manju et al., 2017). BDNF modulates learning and memory in central nervous system, and may be considered a biomarker of hippocampal functions in humans. Low levels of BDNF have been proposed to be associated to schizophrenia, major depressive disorder, bipolar disorder and anxiety disorders (Galvez-Contreras et al., 2016; Lee and Kim, 2010; Polyakova et al., 2015). In recent years, there has been a growing interest in the role of BDNF signaling in fear and anxiety regulation. BDNF is involved in anxiety-like behaviors in preclinical models and diverse stressors reduced BDNF expression in different brain areas (Dalle Molle et al., 2012; Moreira et al., 2015).

Altogether, we demonstrated an important association between ADs and inflammation, represented here primarily by higher levels of IL-6. It is worth mentioning that inflammation is involved in a range of serious health problems such as coronary heart disease (CHD), cancers, chronic pain, and depression (Elinav et al., 2013; Louati and Berenbaum, 2015; Marsland et al., 2018; Miller and Raison, 2017). Thus inflammation may be the link between psychosocial adversity and health outcomes (Marsland et al., 2018; Steptoe et al., 2018). The study of such biomarkers in patients with ADs can improve our understanding on the impact of inflammatory processes on their general health, particularly

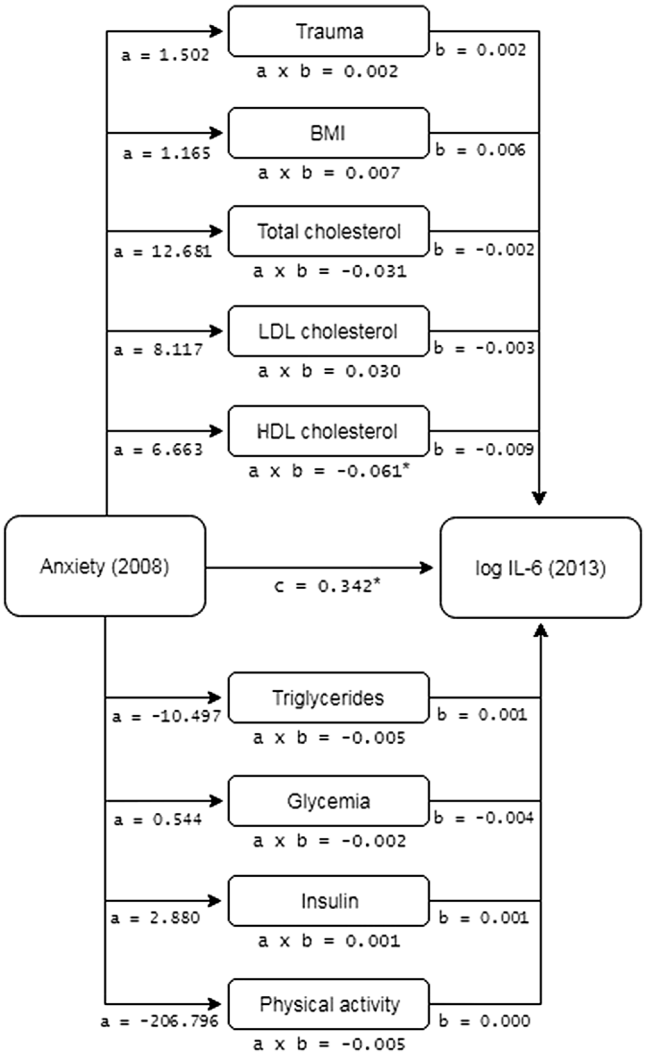

Fig. 2. Direct and indirect effects of anxiety disorders (Anxiety) at baseline (2008) on log-transformed interleukin-6 (log IL-6) in the follow-up (2013) in a mediation design. *p < 0.05; c, Original effect of ADs on log IL-6; a, effect of ADs on mediator; b, effect of mediator on log IL-6; a × b, indirect effect of ADs on log IL-6; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

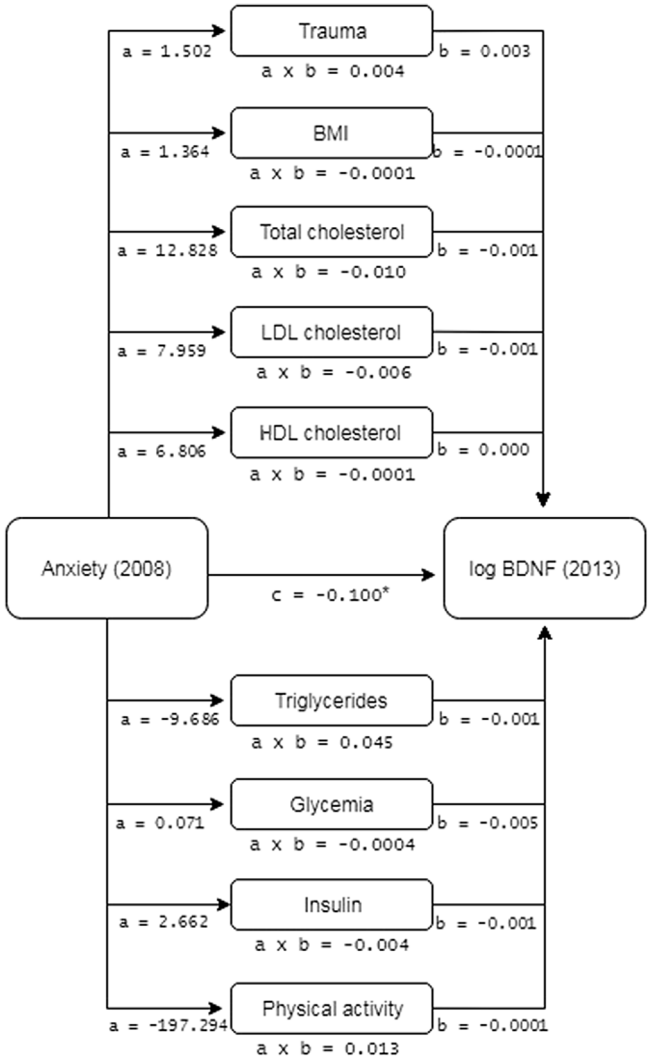

Fig. 3. Direct and indirect effects of anxiety disorders (Anxiety) at baseline (2008) on log-transformed BDNF (log BDNF) in the follow-up (2013) in a mediation design. *p < 0.05; c, Original effect of ADs on log BDNF; a, effect of ADs on mediator; b, effect of mediator on log BDNF; a × b, indirect effect of ADs on log BDNF; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

cardiovascular outcomes. Moreover, the ADs and BDNF association seems to be also influenced by depressive symptoms, so there is a need for more studies in order to clarify these findings.

In our study, we performed a longitudinal prospective design seeking to evaluate the relationship between a previous diagnosis of anxiety and inflammatory markers and oxidative stress in the future. The cross sectional analysis considering the presence of anxiety diagnosis and their association with inflammatory markers in the follow-up evaluation showed no differences in IL6 and BDNF levels between anxious and non-anxious subjects.

To our knowledge, this is the first study in non-medicated adolescents that longitudinally evaluated the influence of ADs on inflammation and oxidative stress. We have been able at demonstrating that the presence of ADs was associated with inflammation in adolescents. Some caveats of this study include the small sample size, which may have limited our power to detect associations of small to medium effect sizes. We also need to consider the potential bias in the follow up subsample, however, as evaluated, the subsample is representative for the whole sample evaluated in 2008. History of trauma, physical activity and anthropometry were evaluated only at baseline, not allowing a more complete evaluation of the role of these variables during follow up. Moreover, the biochemical markers were measured concurrently to the outcomes, limiting the mediation analysis. Finally, we did not assess serum biomarkers at baseline, which could somewhat limited our longitudinal inferences. Although our study does not allow determining if ADs are causes or effects of pro/anti-inflammatory changes, we pointed out potential biomarkers within ADs. Surely, further studies longitudinally evaluating these biomarkers in ADs are necessary to better clarify this relationship.

In summary, our results suggest that previous AD diagnosis was associated with higher IL-6 in comparison with non-anxious individuals, after 5-years follow-up. These findings also point to IL-6 and BDNF as potential biomarkers for ADs, highlighting that inflammatory phenomena might take place in the context of child and adolescent psychiatric disorders. Further studies should evaluate how these associations may affect anxiety morbidity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.05.011.

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