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Keywords:	early psychosis, Brain-derived neurotrophic factor, stress, diet

ABSTRACT

OBJECTIVES: The brain-derived neurotrophic factor (BDNF) is a major participant in the regulation of food intake and may play a role in the regulation of the stress response. We aimed to investigate whether there is a gene-environment interaction in the relationship between stress and BDNF Val66Met polymorphism in relation to dietary patterns in a sample of subjects with early psychosis.

METHODS: We studied 124 early psychotic patients (PD), 36 At-Risk Mental States (ARMS) and 62 healthy subjects (HS). Dietary patterns were examined by a dietician. Physical activity, life stress and perceived stress were assessed by validated questionnaires. BDNF Val66Met polymorphism (rs6265) was genotyped. A gene-environment interaction was tested with multiple linear regression analysis while adjusting for covariates.

RESULTS: Perceived stress was not associated with calorie intake in HS. In ARMS subjects, Met-carriers who presented low-perceived stress were associated with increased caloric intake. Conversely, those who presented high-perceived stress were associated with reduced caloric intake. In PD, perceived stress was associated with increased calorie intake without an effect by BDNF genotype nor a gene-environment interaction. Perceived stress was associated with food craving in PD patients, independent of genotype, and in ARMS or HS who were Val homozygous.

CONCLUSIONS: Our study suggests that the common Val66Met polymorphism of the BDNF gene may modulate the relationship between life stress and calorie intake in subjects at risk for psychosis.

Keywords: Brain-derived neurotrophic factor (BDNF); BDNF Val66Met; early psychosis; stress; diet

1. Introduction

People with schizophrenia have a reduced life expectancy compared to the general population (1), which is mainly caused by cardiovascular disease (2). Although antipsychotic treatment is one of the main causes of weight gain and metabolic abnormalities in psychosis (3), life style factors including unhealthy diet and reduced physical activity also play a role (4,5). Life stress is associated with greater calorie intake and increased refined sugar consumption in individuals with early psychosis (6). This is in accordance with other studies in non-psychiatric populations, which have also found that chronic stress is associated with hyperphagia (7,8).

Psychosocial stress is implicated in the development of psychotic symptoms (9). However, individuals likely differ in their vulnerability to stress. A mechanism that could potentially explain between-subject differences is through a gene-environment interaction (10). Although life stress has been associated with dietary habits in subjects with early psychosis (6), there are no previous studies addressing whether there is a gene-environment interaction in the relationship between life stress and eating behaviour in early psychotic patients. Genes involved in regulating the adaptive behavioural response to stress represent plausible candidates to be explored in studies addressing why some individuals are more prone to develop dietary changes and metabolic abnormalities. In this sense, the brain-derived neurotrophic factor (BDNF) gene (OMIM 113505) is an excellent target because it has been implicated in several processes including food intake (11,12) or the regulation of stress responses (13,14). Moreover, it is thought to modulate the clinical expression of schizophrenia (15).

BDNF is the most profusely expressed neurotrophin in the central nervous system and is located predominantly within neurons. It is involved in growth,

differentiation, maturation and survival of neurons (16), and contributes to energy metabolism, food intake and body weight control by acting as an anorexigenic factor (11,12,17). Animal studies have shown reduced hypothalamic expression of BDNF, increased hyperphagia and risk of obesity in BDNF-deficient mice (18). Intra-cranial infusion of BDNF into the third ventricle can transiently reverse the eating behaviour and obesity. There is a functional single-nucleotide polymorphism (SNP) in the BDNF gene, a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met), that has an impact on BDNF protein. Met66 allele carriers have been linked with reduced BDNF activity-dependent secretion. Although there are no studies exploring the role of this polymorphism in the dietary patterns in subjects with psychoses, human studies in other clinical populations have reported an increased risk for food restriction (19,20), bulimia (20) and restrictive type anorexia nervosa (21) in Met-carriers.

Thus the aim of this study was to investigate whether there is a gene-environment interaction in the relationship between stress and BDNF Val66Met polymorphism in relation to dietary patterns in a sample of subjects with early psychosis.

2. Materials and methods

2.1. Participants

The study sample included 160 individuals who were attending an Early Intervention Service for Psychosis (Hospital Universitari Institut Pere Mata, Reus, Tarragona, Spain): 1) 124 patients with a psychotic disorder (PD, 82 [66.1%] were first

episodes of psychosis) with less than 5 years from the onset of the illness; 2) 36 individuals with prodromal psychotic symptoms fulfilling the set criteria for At-Risk Mental States (ARMS) (22). Recruitment of PD and ARMS individuals was conducted by consecutive sampling. We included a control group of healthy subjects (HS, n=62) who were recruited by advertisements. All HS were screened to rule out past or current histories of psychiatric disorders by direct interviewing by an experienced psychiatrist. Exclusion criteria for all participants (HS, ARMS, PD) were: pregnancy, mental retardation, severe head injury or neurological disease, active glucocorticoid treatment, active substance dependence (other than tobacco or cannabis) and type 1 diabetes mellitus. In order to include relatively stable PD patients, clinical assessment was performed when subjects had been treated at the program for at least three months. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the local ethics committee. After complete description of the study to the subjects, written informed consent was obtained.

2.2. Clinical Assessment

2.2.1. Clinical diagnosis

All patients were assessed with the Schedules for Clinical Assessment in Neuropsychiatry (23). The Operational Criteria Checklist for Psychotic and Affective Illness (OPCRIT 4 Windows) was used to generate DSM-IV diagnosis for psychotic disorders (schizophreniform disorder [n=22], schizophrenia [n=20], schizoaffective disorder [n=12]), and psychotic disorder not otherwise specified [n=70]). ARMS subjects were also assessed with the Comprehensive Assessment of At-Risk Mental States (CAARMS), to ensure that subjects met criteria for any of the three ultra high

risk groups defined by the CAARMS (22): 1) attenuated psychosis (n=28), 2) brief limited intermittent psychotic symptoms (n=5), and 3) vulnerability (n=7), that includes subjects with a family history of psychosis in first degree relative or schizotypal personality disorder in identified patient with a 30% drop in Global Assessment of Functioning (GAF) score from premorbid level, sustained for 1 month.

2.2.2. Stress measures

Stressful life events in the previous 6 months were assessed with the Holmes-Rahe Social Readjustment Scale (24). This scale was initially developed to explore the relationship between social readjustment, stress and susceptibility to illness. It explores 43 life events and gives a "stress score" for each item, obtaining a final score by adding the scores of all present life events. This scale has been validated and used in Spanish populations (25). Previous studies include the use of this scale to explore the relationship between life events and subclinical psychotic symptoms in the general population (26) or metabolic abnormalities in healthy individuals (27). The 14-item Perceived Stress Scale (28) was used to explore the psychological repercussion of stress. This instrument is a self-report scale that assesses the perception of stressful experiences over the previous month.

2.2.3. Dietary assessment and obesity measures

Dietary patterns were assessed by means of clinical interview conducted by a dietician. Food intake was registered by 24 h recall. Dietary recall was applied to all participants considering one of the first four working days of the week (from monday to thursday) in order to avoid potential changes in dietary habits over the weekend. Specialized software (Centre d'Ensenyament Superior de Nutrició i Dietètica,

University of Barcelona, Santa Coloma de Gramenet, Barcelona, Spain) was used to calculate the daily calorie and nutrient intake. The Food Craving Questionnaire-State (FCQ-S) was used to assess craving for foods (29). Cravings have been defined as strong desires that, arising from either physiological or psychological underlying states, promote drug and food consumption. The FCQ-S covers five domains: 1) an intense desire to eat, 2) anticipation of positive reinforcement, 3) anticipation of relief from negative states and feelings, 4) preoccupation with food and lack of control over eating, 5) feelings of hunger. We calculated the full-scale total by adding all item scores. The International Physical Activity Questionnaire-short form (IPAQ-SF) (30), was used to calculate the level of physical activity in metabolic equivalents (MET-min/week). Weight, height, waist circumference and blood pressure were assessed by physical examination. Body Mass Index (BMI) was calculated with the formula weight (kg)/height $(m)^2$.

2.2.4. Treatments and other clinical information

Antipsychotic treatment and other socio-demographic and clinical variables were requested by semi-structured interview. All patients received second-generation antipsychotics. Of all 36 ARMS individuals, 27 (75%) were not receiving antipsychotic drugs, 7 (19.4%) were on antipsychotic monotherapy (risperidone [n=1], olanzapine [n=3], aripiprazole [n=3]) and 3 were receiving two antipsychotics in combination. Of all 124 PD patients, 72 (58.1%) were on antipsychotic monotherapy (risperidone [n=31], paliperidone [n=13], olanzapine [n=17], quetiapine [n=1], aripiprazole [n=10]), 33 (26.6%) were receiving two antipsychotics in combination and 19 (15.3%) were not receiving antipsychotic drugs.

2.3. DNA extraction and BDNF genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells using the Gentra Puregene Blood Kit (QIAGEN Iberia S.L., L'Hospitalet de Llobregat, Barcelona, Spain) according to the manufacturer's instructions. The extraction was carried out at the Biobank of the Institut d'Investigació Sanitària Pere Virgili (IISPV) (Reus, Tarragona, Spain). DNA was genotyped using a TaqMan SNP genotyping assay for the rs6265 SNP (assay ID C_11592758_10; Life Technologies, Alcobendas, Madrid, Spain). Each 5 µL of PCR reaction mix contained 40 ng of DNA, 2.5 µL of TaqMan Universal PCR Master Mix, 0.25 µL of 20X TaqMan SNP Genotyping Assay and 2.25 µL of DNase-free water. PCR conditions were 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The reactions were carried out on an ABI 7900HT Fast Real-Time PCR System (Life Technologies, Alcobendas, Madrid, Spain). Five percent of samples were run in duplicate for quality control with 100% concordance.

2.4. Statistical analyses

The Statistical Package for the Social Sciences (SPSS) version 19.0 for Windows (IBM Corporation Software Group, Somers, New York, USA) was used for statistical analysis.

2.4.1. Univariate analyses

T-Student Test or ANOVA was used to compare continuous data between groups. Bonferroni adjustment was used for post-hoc comparisons. Chi-square tests were used to compare categorical data between groups. Pearson correlations were used to explore

the association between continuous variables. Significance was set at $p<0.05$ (two-tailed).

2.4.2. Multivariate analyses

We conducted a multiple lineal regression analysis to investigate the relationship between stress variables and dietary variables (e.g. calorie intake) while controlling for potential confounders with a confidence interval of 95%. In this analysis, stress measures (e.g. PSS score) and BDNF genes SNP (Met-carriers vs Val/Val homozygotes) were used as independent variables. Potential confounders (sex, BMI, substance use) were also used as independent variables. Dietary measures were used as dependent variables. In order to explore whether there was a gene-environment interaction, we tested the interaction between BDNF gene Val66Met SNP and stress measures (perceived stress and stressful life events). Those significant interaction terms were kept in the final equation. We conducted a multivariate analysis stratified by diagnosis (HS vs ARMS vs PD), so three multiple linear regression analyses were conducted.

3. Results

3.1. Clinical differences between groups

Socio-demographic and genotype characteristics of samples are described in Table 1. Smoking and cannabis use were more prevalent in PD patients when compared to ARMS and HS. Conversely, alcohol consumption among patients was low compared to HS. We found significant differences in perceived stress (but not in stressful life events) between diagnostic groups. Among all groups, the ARMS subjects had greater scores in the PSS scale.

Lifestyle variables (dietary habits and physical activity) and obesity measures are described in Table 2. ARMS subjects and PD patients reported an increased energy intake and reduced protein consumption, when compared to HS. Both ARMS subjects and PD patients reported reduced physical activity when compared to HS. Finally, individuals with PD had a greater BMI than other groups.

3.2. Diet and stress measures by diagnostic group and genotype

Diet and stress measures by diagnostic group and genotype are presented in Table 3. We did not find significant differences in each of these variables between Met-carriers or Val homozygotes. However, when we explored the relationship between stress and lifestyle variables or obesity measures by genotype, we found significant differences (Table 4). Perceived stress was associated with a different pattern in calorie intake, depending on genotype and diagnostic group: perceived stress was associated with a reduced caloric intake in ARMS subjects who were Met-carriers, whereas a positive relationship was found in PD patients who were Val homozygous. Perceived stress was associated with food craving in PD patients, independent of genotype, and in ARMS or HS who were Val homozygous. In HS, perceived stress was also associated with increased lipid and fatty acid consumption, reduced protein intake and lower BMI.

3.3. Multivariate analysis

We also conducted multiple linear regression analysis that was stratified by diagnosis, in order to explore the relationship between perceived stress and calorie intake while adjusting for confounders (cannabis and tobacco use, sex and BMI) and exploring the gene-environment interaction. In HS, perceived stress was not associated with calorie intake (Figure 1a). In ARMS subjects, BDNF genotype (Met-carriers) was associated with an increased calorie intake (Standardized β= 1.36, p=0.040). However, a

significant negative interaction between perceived stress and Val66Met SNP (Met-carriers) was found (Standardized β= -1.37, p=0.047). Thus, in ARMS subjects, BDNF genotype (Met-carrier in Val66Met SNP) was associated with an increased calorie intake, but that in situations of increased perceived stress, Met-carriers reported reduced calorie consumption (Figure 1b). On the other hand, in PD patients, perceived stress was associated with an increased calorie intake (Standardized β= 0.217, p= 0.021), without an effect by genotype or a gene-environment interaction (Figure 1c). We did not find an interaction between stressful life events and genotype.

4. Discussion

In this cross-sectional study exploring the relationship between stress and dietary habits in three diagnostic groups (HS, ARMS subjects and PD patients) in relation to a SNP of the BDNF gene (Val66Met), we found a gene-environment interaction in ARMS subjects only. While in both HS and PD patients this genetic polymorphism did not seem to affect the relationship between stress and energy intake, in ARMS subjects carriers of the Met-allele, a negative relationship between stress and diet was found (a lower calorie intake was reported by those subjects with increased perceived stress).

These findings are in accordance with previous genetic studies that have linked eating disorders, particularly restrictive type anorexia nervosa, with Val66Met Met-allele carriers (21). Other studies in healthy populations have also found a restricted energy intake in Met-carriers adolescents with maladaptive or problematic eating attitudes and behaviours (19), or adolescent girls with food restrictive behaviours (20). Although BDNF plays an essential role in neuronal survival and differentiation, as well

as neuronal plasticity, it is also an anorexigenic factor involved in the regulation of food intake (12,17). Interestingly, in animal models using BDNF knock-out heterozygous mice with only one functional BDNF allele, a reduction of BDNF expression in the hypothalamus has been demonstrated with associated hyperphagia and obesity (18,31).

Of all diagnostic groups, we only found a gene-environment effect in the ARMS group. ARMS subjects showed increased perceived stress, which fits well with other studies in the literature reporting similar findings (32). The existing clinical differences between ARMS and PD groups, or a different stage of the illness (prodromal vs established psychosis), may explain a distinct pattern in the relationship between BDNF gene and perceived stress, in relation to dietary habits. In a previous study by our group (6) we also found a different pattern by diagnosis in the relationship between intake of 'comfort foods' and stressful life events: In PD subjects, life stress was associated with increased intake of refined sugar, whereas in ARMS and HS subjects it was related to a decreased intake of refined sugar. In our current study we have also included information on craving for foods. Food craving refers to an intense desire or urge to eat specific foods of which chocolate is the most often craved one among other highly palatable foods (33). In line with our previous study (6), ARMS individuals that are Met-allele carriers of the BDNF Val66Met polymorphism also report lower craving for foods (with similar FCQ-S scores as HS), when compared to PD patients that are Met-allele carriers. The mechanistic pathways leading to a distinct relationship between perceived stress, calorie intake and BDNF genotype in ARMS and PD groups are uncertain. We wonder whether distinct patterns may reflect different levels of cumulative stress and adaptation. The brain controls and coordinates behavioural and physiological adjustments to meet the demands imposed by stressors (34). The active process of responding to a challenge to the body by triggering chemical mediators of

adaptation (hypothalamic-pituitary-adrenal, autonomic, metabolic, immune) can be adaptive in the short term (allostasis) and maladaptive in the long term (allostatic load). Lifestyle behaviour is another important aspect of individual response to stress in relation to allostasis and allostatic load. Although speculative, ARMS individuals may have a lower allostatic load than patients with a PD, because it may be hypothesized that disease has emerged in this latter group as a failure to adapt to stress. In line with this, acute and chronic stress have different effects on appetite, as acute stress is associated with anorexia and chronic stress with hyperphagia (35). In acute stress, CRH stimulates POMC neurons of the arcuate nucleus which elicit anorexic signals, via a-MSH release, and suppress neuropeptide Y, a potent orexigen. In turn, chronic stress and the increase of circulating glucocorticoid concentration eventually promote the intake of carbohydrates and fat and decrease energy expenditure by suppressing CRH and stimulating NPY hypothalamic secretion. The different pattern in ARMS and PD in those individuals with a genetic vulnerability to stress (e.g. Met-allele carriers of the BDNF Val66Met polymorphism), could be explained by a lower allostatic load and maintenance of adaptative responses in ARMS individuals (that would mimic acute effect responses on appetite with reduced calorie intake by stress) and an increased allostatic load and lack of adaptation to chronic stress in PD subjects (leading to increased calorie intake by stress in this group).

Another potential explanation of the differences between ARMS and PD groups in the relationship between stress and energy intake is that PD patients were receiving more antipsychotic treatment. It is plausible that the effect of perceived stress on dietary habits may be modified by antipsychotic treatment, which interacts with the dopaminergic and serotoninergic systems (33), that are also involved in the control of eating behaviour. Thus, a potential gene-environment interaction in PD subjects may be

obscured by treatment with antipsychotic drugs in this subgroup. Cannabis use is another factor that may partially explain some of the differences in the results between ARMS and PD groups, because PD patients reported more daily cannabis consumption. Cannabinoids promote energy intake by their action at specific brain regions that are important in the control of eating motivation (37).

Several limitations must be acknowledged. The cross-sectional design of the study does not allow inferring causality in the relationship between life stress and dietary habits. Some variables were retrospectively assessed with questionnaires (24), which may induce a recall bias. BDNF-serotonin transporter gene-gene interactions were not controlled. BDNF and serotonin systems interact with each other to regulate the development and plasticity of neural circuits (35). It is plausible that environmental exposures could trigger the expression of a gene that in turn modifies other genes. Future studies may address whether the effects of BDNF Val66Met polymorphism interact with other genes such as the serotonin transporter gene. Finally the sample size was relatively small, in particular for the ARMS group, thus some negative findings could be influenced by a lack of statistical power. Small samples also increase the rates of false-positive results due to a type I error. For this reason, it is important to replicate our findings with larger samples.

On the other hand, our study has several strengths among which it should be emphasized that is the first study of the gene-environment interaction in ARMS subjects and PD patients exploring how BDNF polymorphism (Val66Met) affects the diet in correlation with stress life events, adding important information to our previous study that assessed dietary habits and stress measures in ARMS subjects and PD patients groups without considering genetic implications (6). Besides, a detailed and thorough dietary assessment was conducted by a dietician, who administrated a semi structured

interview and registered calorie intake with a special software and a control group of healthy volunteers was included.

Further longitudinal studies are required to describe temporal changes in eating behaviour, before or after the diagnosis of schizophrenia or other PD, in order to elucidate whether these changes are linked to the illness of may be considered a consequence of psychopharmacological treatment. Future studies addressing this topic need to consider controlling for multi-genic interactions and measuring BDNF levels. This is important in the design of future preventive interventions that may target improvement of dietary habits and strategies to cope with stress in subjects with early psychosis, particularly in subjects at high risk for psychosis.

A deeper study on this topic and future advances in the understanding of the complex interaction between gene, environment and nutrition may help to find new ways to prevent this illness and lower the costs, raising the quality of life. Our study suggests that the BDNF is a candidate gene that may help to identify vulnerable people with stress-related dietary habits in the field of early psychosis, and highlights the need to continue exploring the potential role of neurotrophins in the interplay between stress and diet in individuals who are at risk for psychosis.

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Figure 1. Scatter plot of the relationship between perceived stress and calorie intake in healthy subjects (1a), at-risk mental states (1b) and patients with a psychotic disorder (1c). Regression lines for each Val66Met genotype group (Val/Val vs Met-carriers) are presented. A gene-environment effect was observed in ARMS individuals (1b).

Table 1. Clinical, stress and genetic variables of the sample.

	Healthy Subjects N=62	ARMS N=36	Psychotic Disorder N=124	P value
Age	23.5 (3.5)	22.2 (4.6)	24.7 (4.9)	0.098
Sex				
Male	32 (51.6)	26 (72.2)	81 (65.3)	0.082
Female	30 (48.4)	10 (27.8)	43 (34.7)	
Ethnic Group				
Caucasian	59 (95.2)	32 (88.9)	95 (76.6)	0.204
Black	0 (0)	0 (0)	1 (0.8)	
Gipsy	0 (0)	0 (0)	5 (4.0)	
Asian	0 (0)	0 (0)	1 (0.8)	
Arabian	1 (1.6)	1 (2.8)	10 (8.1)	
Latinoamerican	2 (3.2)	3 (8.3)	12 (9.7)	
Civil Status				
Single	43 (69.4)	29 (80.6)	99 (79.8)	0.207
Lives with couple/ Married	19 (30.6)	6 (16.7)	22 (17.7)	
Divorced	0	1 (2.8)	3 (2.4)	
Work Status				
Employed/ Student	55 (88.7)	24 (66.7)	44 (35,5)	<0.001
Unemployed	7 (11.3)	12 (33.3)	80 (64.5)	
Drug Use				
Tobacco				
No	41 (66.1)	20 (55.6)	33 (26.6)	<0.001
Occasionally	5 (8.1)	1 (2.8)	4 (3.2)	
Daily	16 (25.8)	15 (41.7)	87 (70.2)	
Cannabis				
No	49 (79.0)	26 (72.2)	70 (56.5)	<0.001
Occasionally	11 (17.7)	5 (13.9)	12 (9.7)	
Daily	2 (3.2)	5 (13.9)	42 (33.9)	
Alcohol				
No	5 (8.1)	12 (33.3)	41 (33.1)	<0.001
Occasionally	56 (90.3)	23 (63.9)	66 (53.2)	
Daily	1 (1.6)	1 (2.8)	17 (13.7)	
Stress measures				
PSS	18.7 (7.4)	32.5 (11.0)	24.8 (9.0)	<0.001[a,b,c]
SLE (Holmes-Rahe score)	107.1 (96.7)	150.5 (83.9)	158.1 (118.0)	0.052
BDNF (rs6262) genotype				
Val/Val	34 (54.8)	20 (55.5)	72 (58.0)	0.908
Val/Met	25 (40.3)	14 (38.9)	43 (34.7)	
Met/Met	3 (4.8)	2 (5.5)	9 (7.3)	

Data are mean (SD) or N (%).

Significant ANOVA post-hoc comparisons (after a Bonferroni adjustment) are highlighted: [a] HS vs ARMS; [b] HS vs PD; [c] ARMS vs PD

Abbreviation: ARMS= At-Risk Mental State; PSS= Perceived Stress Scale; SLE= Stressful life events; Val= Valine; Met= Methionine.

2. Lifestyle variables and obesity measures among diagnostic groups.

	Healthy Subjects N=62	ARMS N=36	Psychotic Disorder N=124	P value
Dietary Intake (24h recall):				
Total energy (Kcal)	1741.6 (424.3)	2424.1 (863.0)	2509.4 (653.3)	<0.001[a,b]
Lipids (%)	35.9 (6.0)	36.6 (10.3)	37.6 (6.8)	0.318
Proteins (%)	20.3 (4.9)	15.6 (4.5)	16.11 (3.5)	<0.001[a,b]
Carbohydrates (%)	43.4 (6.3)	46.4 (11.4)	45.7 (8.0)	0.121
Saturated fatty acids (%)	10.9 (2.9)	13.1 (5.1)	12.5 (3.3)	0.003
Refined sugar (%)	18.4 (6.4)	21.1 (11.9)	20.1 (6.6)	0.197
Food craving (FCQ-S)	28.3 (10.5)	32.0 (12.7)	33.6 (14.3)	0.038
Physical activity (IPAQ)				
MET-min/week	2954.7 (2227.3)	1289.5 (1144.1)	1672.5 (1370.3)	<0.001[a,b]
Obesity measures				
BMI (kg/m^2)	22.2 (3.8)	22.2 (3.5)	24.3 (4.6)	0.002[b,c]
Weight classification by BMI				
Underweight (<18.5 kg/m^2)	5 (8.2)	4 (11.1)	5 (4.1)	0.012
Normal (18.5-24.9 kg/m^2)	46 (75.4)	22 (61.1)	77 (62.6)	
Overweight (25-29.9 kg/m^2)	7 (11.5)	10 (27.8)	28 (22.8)	
Obesity(≥30 kg/m^2)	3 (4.9)	0	13 (10.6)	

Data are mean (SD) or N (%).

Significant ANOVA post-hoc comparisons (after a Bonferroni adjustment) are highlighted: [a] HS vs ARMS; [b]HS vs PD; [c]ARMS vs PD

Abbreviation: ARMS= At-Risk Mental State; IPAQ= International Physical Activity Questionnaire; FCQ-S= Food Craving Questionnaire-State; IPAQ= International Physical Activity Questionnaire; MET: Metabolic Equivalent of Task; BMI= Body mass index.

Table 3. Diet and stress measures. Stratified analysis by diagnosis and BDNF Val66Met polymorphism.

	Healthy Subjects N=62		ARMS N=36		Psychotic Disorder N=124	
	Val/Val N=34	Met Carriers N=28	Val/Val N=20	Met Carriers N=16	Val/Val N=72	Met Carriers N=52
Dietary intake (24h recall)						
Total energy (Kcal)	1762.5 (468.4)	1716.3 (370.7)	2329.5 (904.1)	2542.4 (821.9)	2476.7 (626.5)	2554.8 (692.3)
Lipids (%)	36.2 (6.1)	35.5 (5.9)	36.7 (10.9)	36.4 (10.0)	36.8 (6.3)	38.7 (7.3)
Proteins (%)	19.2 (5.2)	21.6 (4.2)	15.5 (4.6)	15.8 (4.5)	15.9 (3.7)	16.4 (3.2)
Carbohydrates (%)	43.9 (7.1)	42.7 (5.1)	46.3 (12.1)	46.6 (10.9)	46.8 (8.1)	44.2 (7.7)
Saturated fatty acids (%)	11.1 (2.8)	10.6 (2.9)	12.8 (4.3)	13.4 (6.1)	12.8 (3.5)	11.9 (2.8)
Refined sugar (%)	19.1 (6.3)	17.6 (6.3)	20.0 (13.2)	22.5 (10.3)	20.8 (6.8)	19.0 (6.3)
Food craving (FCQ-S)	27.7 (9.6)	29.1 (11.6)	34.5 (13.6)	28.9 (11.2)	34.3 (14.7)	32.6 (13.9)
Stress measures						
PSS	19.3 (8.0)	18.0 (6.6)	32.4 (13.0)	32.7 (8.6)	25.2 (8.7)	24.2 (9.3)
SLE (Holmes-Rahe score)	112.7 (106.9)	100.3 (84.5)	172.0 (90.2)	124.9 (70.0)	176.5 (124.6)	133.3 (104.8)

Abbreviation: Val/Val= homozygous for valine at codon 66; ARMS= At-Risk Mental State; PSS= Perceived Stress Scale; SLE= Stressful life events; FCQ-S= Food Craving Questionnaire-State.

Data are mean (SD)= Standard Deviation

Table 4. Correlations between lifestyle variables and stress measures. Stratified analysis by diagnosis and BDNF Val66Met polymorphism.

	Healthy Subjects N=62				ARMS N=36				Psychotic Disorder N=124			
	Val Homozygous N=34		Met Carriers N=28		Val Homozygous N=20		Met Carriers N=16		Val Homozygous N=72		Met Carriers N=52	
	SLE (HR)	PSS	SLE (HR)	PSS	SLE (HR)	PSS	SLE (HR)	PSS	SLE (HR)	PSS	SLE (HR)	PSS
Dietary intake (24h recall)												
Total energy (Kcal)	-0.257	0.018	0.050	0.081	0.059	0.119	-0.303	-0.594* (0.015)	0.137	0.262* (0.033)	0.255	0.242
Lipids (%)	0.126	0.048	-0.029	0.558* (0.004)	0.151	-0.294	-0.362	-0.404	-0.142	-0.019	-0.056	-0.083
Proteins (%)	-0.118	-0.254	0.083	-0.444* (0.026)	-0.287	-0.408	0.203	0.168	-0.046	-0.228	-0.394* (0.007)	-0.255
Carbohydrates (%)	-0.079	0.072	0.060	-0.260	0.128	0.332	0.206	0.193	0.140	0.176	0.245	0.198
Saturated fatty acids (%)	-0.100	0.119	-0.247	0.444* (0.026)	0.182	-0.235	-0.156	-0.187	-0.084	0.059	0.012	0.016
Refined sugar (%)	-0.216	-0.186	-0.252	-0.308	-0.144	0.151	0.132	0.127	0.189	0.107	0.230	0.281
Food craving (FCQ-S)	0.366* (0.047)	0.489* (0.005)	0.114	0.196	0.252	0.546* (0.016)	0.192	0.380	0.112	0.327* (0.008)	0.464* (0.001)	0.430* (0.003)
Physical Activity (IPAQ)	0.192	0.148	0.174	0.206	0.225	-0.343	0.413	-0.005	0.135	-0.365* (0.003)	-0.215	-0.115
BMI	0.175	0.237	-0.011	-0.457* (0.032)	-0.006	0.340	0.094	-0.341	-0.104	-0.079	-0.021	-0.086

*Significant P values are shown.

Abbreviation: ARMS= At-Risk Mental State; SLE (HR)= Stressful life events (Homes-Rahe score); PSS= Perceived Stress Scale; IPAQ= International Physical Activity Questionnaire; FCQ-S= Food Craving Questionnaire-State; BMI= Body mass index.

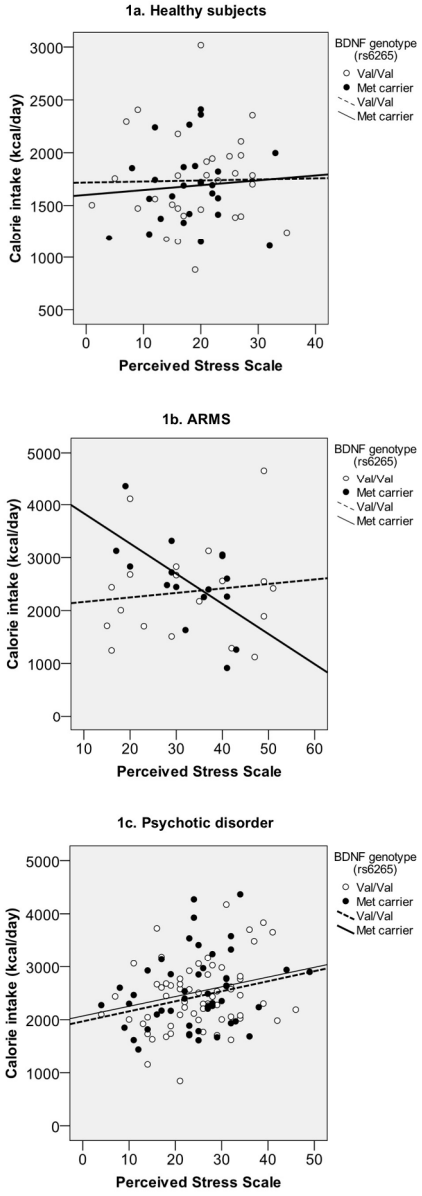

Figure 1. Scatter plot of the relationship between perceived stress and calorie intake in healthy subjects (1a), at-risk mental states (1b) and patients with a psychotic disorder (1c). Regression lines for each Val66Met genotype group (Val/Val vs Met-carriers) are presented. A gene-environment effect was observed in ARMS individuals (1b).
106x287mm (240 x 240 DPI)