

Phobic Anxiety Is Associated With Higher Serum Concentrations of Adipokines and Cytokines in Women With Diabetes

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OBJECTIVE — Phobic anxiety has been associated with increased risk of cardiovascular disease (CVD), but the underlying mechanisms are poorly understood. We aimed to determine whether associations of phobic anxiety with several known markers of CVD might be contributors.

RESEARCH DESIGN AND METHODS — We used a 16-point validated index (Crown-Crisp) measured in 1988 to categorize 984 women with type 2 diabetes from the Nurses' Health Study as having low, moderate, or high phobic anxiety. Groups were then compared for differences in adipokines (adiponectin and leptin), inflammatory markers (C-reactive protein and tumor necrosis factor [TNF]- α receptor II), and markers of endothelial function (sE-selectin, soluble intercellular adhesion molecule [sICAM]-1) measured on blood samples provided between 1989 and 1990.

RESULTS — Higher levels of phobic anxiety were associated with higher BMI and lower education. Higher levels of phobic anxiety were also associated with higher leptin and soluble TNF- α receptor II in both crude analyses and after adjustment for potential confounders. sICAM and sE-selectin were higher in the highest tertile compared with the middle tertile, but there was no significant trend across tertiles. We found no association between phobic anxiety and adiponectin.

CONCLUSIONS — High levels of phobic anxiety are associated with increased levels of leptin and inflammatory markers, which may in part explain the previously observed relationship between anxiety and other psychosocial disorders with CVD.

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Cardiovascular disease (CVD) is the leading cause of death in women in the U.S. and most of the developed world. Despite advances in our understanding and treatment of several cardiovascular risk factors, a large proportion of the CVD burden remains unexplained by traditional risk factors. Moreover, among women with diabetes, CVD mortality has not improved in the last three decades, despite improvements among diabetic

men (1). Psychosocial factors including anxiety have been proposed as risk factors for the development of CVD (2), and, unlike most traditional risk factors, anxiety is more common in women. Phobic anxiety in particular has been associated with increased risk of death from CVD in several prospective cohort studies in men (3). In a large prospective cohort study in women (4), increased phobic anxiety was associated with increased risk of fatal cor-

onary heart disease and sudden cardiac death.

The association between phobic anxiety and increased risk from coronary heart disease is not completely explained by established CVD risk factors (4), and pathways involving behavioral factors, increased sympathetic tone (4), endothelial dysfunction (5), dyslipidemia (6), and increased inflammatory biomarkers (7) have been proposed. Increased anxiety may also have an impact on adipose tissue, and several studies have linked psychological factors with changes in body fat distribution and the metabolic syndrome, possibly mediated through changes in the sympathetic nervous system (8). Despite the high prevalence of anxiety in the general population, few studies have focused on the relationship between inflammatory biomarkers and anxiety, and the relation of anxiety to circulating leptin and adiponectin levels has never been examined in a study large enough to allow adjustment for possible confounders. Therefore, to examine the association between phobic anxiety, as measured by the Crown-Crisp index (CCI), adipokine levels, and inflammatory biomarkers, we conducted a cohort study in 984 women with diabetes. Our objective was to determine whether high phobic anxiety is associated with increased levels of inflammatory biomarkers, markers of endothelial function and leptin levels, and/or with lower adiponectin levels in women with diabetes.

RESEARCH DESIGN AND METHODS

The Nurses' Health Study began in 1976 with the enrollment of 121,700 female nurses aged 30–55 years who received biennially mailed questionnaires on lifestyle factors and health outcomes. Blood samples were obtained from 32,826 study participants from 1989 to 1990. Among participants who returned blood samples, 1,188 women had a confirmed diagnosis of type 2 diabetes, which was validated as previously reported (9). The current analysis includes 984 women with diabetes, for whom measures of hormones and inflammatory markers, as well as data to calcu-

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late level of phobic anxiety, were available and who were free of coronary heart disease (myocardial infarction, coronary artery bypass grafting, or percutaneous transluminal coronary angioplasty) and stroke at blood draw in 1990.

Assessment of phobic anxiety

The Crown-Crisp phobic anxiety subscale was completed by participants in the Nurses' Health Study in 1988. The CCI (also called the Middlesex Hospital Questionnaire) is a standardized, self-rating questionnaire, and the phobic anxiety subscale measures common symptoms of phobic anxiety. It includes eight questions with two to three levels of possible responses to each question, allowing scores ranging from 0 to 16, with higher scores given to higher levels of phobic anxiety (10). The CCI has been validated in psychiatric outpatient clinic settings and found to discriminate patients with anxiety disorders and agoraphobia from healthy control subjects (10,11). The mean score on the phobic anxiety subscale in subjects with psychiatrist diagnosed anxiety disorder is 5.6 (11). The internal validity of the phobic anxiety subscale of the CCI in the Nurses' Health Study population has been tested previously where it was associated with use of tranquilizer medications in 2000 (4). The individual questions and response options have been reported previously (4). For those with missing data on one or two questions ($n = 59$), the total score was divided by the fraction of questions answered and then rounded to the nearest whole number consistent with previous studies that have used the CCI (4). We categorized the total score into the categories of 0 or 1, 2–3, and 4, or higher (highest levels of phobic anxiety) based on frequency distribution similar to previous reports (4). These categories also separated the population into approximate tertiles.

Assessment of hormones and inflammatory markers

Blood samples were drawn in 1989 or 1990. Participants were sent a blood-set kit that included supplies and instructions. Participants arranged for the blood to be drawn into tubes containing liquid EDTA and sent the samples back by prepaid overnight courier. Most samples arrived within 24 h of the blood draw. After arrival in the laboratory, samples were centrifuged and aliquotted into cryotubes as plasma, buffy coat, and erythrocytes.

Cryotubes were stored in liquid-nitrogen freezers at -130°C or lower.

Quantification of biomarker levels in the Nurses' Health Study has been described in detail previously (9). Adiponectin and leptin were assayed using a radioimmunoassay from Linco Research (St. Charles, MO), which has a sensitivity of 0.78 ng/ml and an intra-assay coefficient of variation (CV) of 1.8–6.2% for adiponectin and a sensitivity of 0.5 ng/ml and an intra-assay CV of 8.3% for leptin. Plasma C-reactive protein (CRP) was measured using the U.S. CRP ELISA Kit (Diagnostic Systems Laboratories, Webster, TX), with a CV range of 2.8–5.1%. Soluble intercellular adhesion molecule (sICAM)-1, soluble E-selectin (sE-selectin), and soluble tumor necrosis factor (sTNF)- α RII were assayed in plasma using ELISA kits (R&D Systems, Minneapolis, MN) with CVs $<8.8\%$.

Assessment of covariates

Data on demographic and lifestyle factors were obtained from Nurses' Health Study questionnaires. BMI was calculated from self-reported weight (in kg) in 1990 divided by the square of height (in m^2) reported in 1976. Physical activity in metabolic equivalent tasks (METs) per week was computed from 1986 data on duration and intensity of exercise performed. Medication use and family history of diabetes were both assessed by the 1988 questionnaire. Smoking status and status of self-reported hypertension and hypercholesterolemia were obtained from questionnaires administered in 1990, and race, marital status, education, and employment were addressed on the 1992 form. Alcohol and caffeine intake were determined from the 1990 questionnaire using a previously validated (12) semi-quantitative food frequency questionnaire. Sleep duration was assessed by questionnaire in 1986.

Statistical analyses

Comparisons of descriptive measures were conducted using linear regression for continuous variables and appropriate χ^2 tests for categorical variables across groups of phobic anxiety (CCI 0–1, 2–3, or ≥ 4). Associations of the CCI group with biomarker concentrations were evaluated using simple linear regression models for crude analysis and multiple linear regression analysis to calculate adjusted means, with logarithmic transformation of outcome values to more closely approximate the normal distribution. We

adjusted for potential confounders in multivariate analyses, including age, BMI, employment status, education, marital status, physical activity, smoking, hypertensive status, hypercholesterolemia, A1C concentration, family history of diabetes, sleep duration, and medication usage, with continuous variables categorized in quintiles and modeled as four dichotomous indicator variables in the multivariate model. In a secondary analysis, we also ran CCI as a continuous variable in a model adjusting for BMI as a continuous variable. Post hoc tests for differences between CCI groups were conducted using Bonferroni adjustment for multiple comparisons within each variable (P value of <0.0125 was considered significant on post hoc test). Tests for interaction by age, BMI, smoking status, alcohol consumption, education, work status, marital status, and duration of diabetes and the relationship between phobic anxiety and biochemical variables were conducted using linear regression with multiplicative interaction terms. These tests were conducted for each biomarker separately. All analyses were conducted using the SAS statistical package (SAS version 8.2 for UNIX; SAS Institute, Cary, NC). Reported P values are two sided, and values <0.05 were used to determine statistical significance.

RESULTS — Descriptive characteristics of 984 women with type 2 diabetes by level of phobic anxiety are presented in Table 1. Women with phobic anxiety scores ≥ 4 had significantly higher BMI and were less highly educated than women with scores of ≤ 3 . These women were also more likely to have a history of hypertension and to smoke. With respect to medical history, women with the highest phobic anxiety were more likely to have been diagnosed with diabetes for <2 years and were marginally less likely to be taking oral diabetes medications. Phobic anxiety was also associated with use of thiazide diuretics, calcium channel blockers, and other antihypertensive medications. We found no significant differences among varying levels of phobic anxiety in age, energy intake and physical activity, alcohol consumption, marital status, or employment, nor did we detect significant differences in other medical history or other medication use (Table 1). Women with high scores had significantly elevated concentrations of leptin, sICAM-1, sTNF- α RII, and a trend toward elevated CRP concentrations (Ta-

Table 1—Descriptive characteristics of women (n = 984) with diabetes by category of CCI measuring phobic anxiety

	CCI (0–16 points)			<i>P</i> _{trend}
	0 or 1	2 or 3	≥4	
<i>n</i>	288	318	378	
Demographic				
Age (years)	59.3 ± 6.5	58.8 ± 6.7	58.5 ± 6.7	0.13
BMI (kg/m ²)	29.9 ± 6.4	29.2 ± 6.1	30.6 ± 6.4*	0.10
Physical activity (METs/week)	12.6 ± 15.2	12.4 ± 16.9	10.6 ± 14.2	0.10
Total energy intake (kcal)	1,742 ± 503	1,784 ± 514	1,796 ± 537	0.21
Alcohol (g/day)	3.0 ± 7.4	2.7 ± 6.6	2.8 ± 8.6	0.68
Current smoker	29 (10.2)	47 (14.9)	60 (15.9)	0.04
Caucasian race	285 (98.9)	307 (96.5)	364 (96.3)	0.10
Married	228 (83.2)	249 (82.2)	294 (80.6)	0.38
Education (Bachelor's degree)	88 (32.2)	70 (23.2)	71 (19.4)	0.0002
Full-time employment	82 (30.0)	76 (25.1)	98 (26.9)	0.43
Medical history				
Hypertension	106 (36.8)	128 (40.3)	172 (45.5)	0.02
Hypercholesterolemia	123 (42.7)	119 (37.4)	153 (40.5)	0.63
Duration of diabetes ≥2 years	126 (43.8)	143 (45.0)	131 (34.7)	0.01
Family history of diabetes	149 (51.7)	164 (51.6)	198 (52.4)	0.86
Insulin	60 (20.8)	59 (18.6)	69 (18.3)	0.42
Oral diabetes medication	67 (23.3)	81 (25.5)	68 (18.0)	0.08
A1C (%)	7.0 ± 1.8	6.9 ± 1.7	6.8 ± 1.7	0.12
Medications				
Aspirin	133 (46.2)	146 (45.9)	158 (41.8)	0.24
Postmenopausal hormones	101 (35.1)	106 (33.3)	120 (31.8)	0.37
Thiazide diuretics	78 (27.1)	110 (35.6)	134 (35.5)	0.03
Other hypertension medication	21 (7.3)	29 (9.1)	48 (12.7)	0.02
β-Blocker	40 (13.9)	51 (16.0)	70 (18.5)	0.11
ACE inhibitor	25 (8.7)	23 (7.2)	44 (11.6)	0.16
Calcium channel blocker	6 (2.1)	21 (6.6)	24 (6.4)	0.02
Cholesterol-lowering medication	17 (5.9)	15 (4.7)	13 (3.4)	0.13
Biomarkers				
Adiponectin (μg/ml)	5.3 (1.0–31.7)	6.1 (1.3–38.4)	5.4 (1.2–41.8)	0.35
Leptin (ng/ml)	44.0 (1.6–144)	40.1 (1.5–229)	47.7 (2.0–162)*†	0.006
CRP (mg/l)	3.5 (0.6–34)	3.4 (0.7–35)	3.9 (0.7–35)	0.06
sE-selectin (ng/ml)	59.0 (16.2–229)	55.9 (18.8–327)	61.0 (14.2–525)*	0.20
sICAM-1 (ng/dl)	290 (130–799)	285 (100–849)	305 (9.0–1,074)*	0.02
sTNF-αRII (pg/ml)	2,387 (939–6,946)	2,310 (1,005–6,861)	2,496 (1,046–9,355)*	0.03

Data are means ± SD, *n* (%), or median (range). *Significantly different from group 2 (Bonferroni-adjusted *P* value <0.05). †Significantly different from group 1 (Bonferroni-adjusted *P* value <0.05).

ble 1). The CCI was not associated with adiponectin.

Since there was no evidence of interaction by age (*P* = 0.45–0.81), BMI (*P* = 0.09–0.91), smoking status (*P* = 0.36–0.92), alcohol consumption (*P* = 0.06–0.97), education (*P* = 0.42–0.94), or duration of diabetes (*P* = 0.13–0.96) for any of the biomarkers, crude and adjusted estimates of biomarkers by CCI group from all subjects are presented (Table 2). Adiponectin levels were not associated with phobic anxiety in either crude or adjusted models or after mutual adjustment for leptin. After controlling for age, BMI,

and race, the marginal association between CRP and phobic anxiety was attenuated to null and remained unassociated with subsequent adjustments (Table 2). sICAM-1 levels were also associated with phobic anxiety in the crude analysis, but these lost significance after adjustment for age, BMI, and lifestyle factors (*P* = 0.08). Compared with the group with moderate anxiety, sICAM-1 levels remained higher in the group with higher anxiety in the fully adjusted model. Similarly, sE-selectin levels were significantly higher in the high-anxiety group compared with the group with moderate anxiety before

and after adjustment for potential confounders, but the linear trend across tertiles was nonsignificant (Table 2). For each model, post hoc tests were conducted using Bonferroni adjustments for multiple comparisons, and comparisons with a *P* value of <0.012 were considered significant (Table 2). In the final, fully adjusted model (model 3), mean leptin concentrations were significantly higher for women who scored ≥4 on the CCI compared with both the first (16% higher) and second (13% higher) tertile of CCI score. For sICAM-1, sE-selectin, and sTNF-αRII, significant differences were

Table 2—Estimated crude (95% CI) and adjusted least-squares mean biomarker concentrations by CCI category for women (n = 984) with diabetes

	CCI (0–16 points)			P _{trend}
	0 or 1	2–3	≥4	
n	288	318	378	
Adiponectin (μg/ml)	5.7 (5.3–6.2)	6.2 (5.7–6.7)	5.5 (5.1–5.9)	0.35
Model 1	5.4	5.7	5.3	0.68
Model 2	5.0	5.4	5.1	0.78
Model 3	6.0	6.6	6.4	0.34
Leptin (ng/ml)	34.7 (31.6–38.2)	33.1 (30.2–36.2)	40.9 (37.7–44.4)*†	0.006
Model 1	41.6	41.7	46.6	0.01
Model 2	37.0	38.1	43.3*†	0.002
Model 3	37.3	38.3	43.4*†	0.004
CRP (mg/l)	3.7 (3.3–4.1)	3.6 (3.2–3.9)	4.2 (3.8–4.6)	0.06
Model 1	4.1	4.1	4.5	0.11
Model 2	4.4	4.5	4.7	0.25
Model 3	4.4	4.5	4.7	0.38
sE-selectin (ng/ml)	59.0 (55.7–62.4)	55.1 (57.8–57.7)	61.5 (58.5–64.6)†	0.20
Model 1	61.2	57.6	63.4†	0.27
Model 2	61.4	57.6	63.1	0.40
Model 3	54.7	50.5	55.9†	0.43
sICAM-1 (ng/dl)	295 (284–306)	291 (281–302)	312 (302–322) †	0.02
Model 1	290	288	307*†	0.01
Model 2	325	318	340†	0.07
Model 3	311	303	325†	0.08
sTNF-αRII (pg/ml)	2,397 (2,318–2,480)	2,382 (2,307–2,461)	2,516 (2,443–2,591)†	0.03
Model 1	2,481	2,490	2,611	0.02
Model 2	2,416	2,424	2,529	0.04
Model 3	2,487	2,495	2,629†	0.01

Model 1: adjusted for age, BMI, and race; model 2: adjusted additionally for physical activity, total energy intake, alcohol intake, smoking status, full-time work status, education level, and marital status; model 3: adjusted additionally for hypertension, hypercholesterolemia, A1C, family history of diabetes, aspirin, postmenopausal hormone, insulin, oral diabetes medication use, sleep duration, and duration of diabetes. *Significantly different from group 1 (Bonferroni-adjusted *P* value <0.05). †Significantly different from group 2 (Bonferroni-adjusted *P* value <0.05).

found between women who scored ≥4 and women who scored 2 or 3 on the CCI after full adjustment. sE-selectin was 11% higher, sICAM-1 was 7% higher, and sTNF-αRII was 5% higher in the third tertile compared with the second tertile of CCI score. The associations of CCI ≥4 with increased leptin and sTNF-αRII remained significant after adjusting for age, BMI, and race.

To address the possibility of residual confounding by BMI, we repeated the analysis treating BMI as a continuous variable. The results were not materially different from those presented (data not shown). A total of 180 individuals in our cohort had a score of ≥6 on the CCI; these individuals are more likely to have a psychiatrist-diagnosed anxiety disorder (11). When we separated these individuals out from the highest tertile and examined for a trend across groups, the association between higher phobic anxiety and higher leptin and sTNF-αRII persisted (leptin *P* for trend = 0.0006, sTNF-

αRII = 0.004 in fully adjusted model). Treating the CCI as a continuous variable in the models yielded similar results to those presented herein. Mutual adjustment for leptin attenuated the associations between phobic anxiety and sTNF-αRII (*P* for trend = 0.06), indicating that variation in leptin levels may partly explain the association with sTNF-αRII. In contrast, leptin remained significantly associated with the CCI, even after adjustment for all other biomarkers.

CONCLUSIONS— We found that higher levels of phobic anxiety, as measured by the CCI in 984 women with type 2 diabetes, were associated with increased leptin and sTNF-αRII measured within 2 years. Also, compared with women with low or moderate phobic anxiety, women with high levels of phobic anxiety had higher sE-selectin and sICAM-1. In contrast, there was no association between phobic anxiety and adiponectin levels. The association between higher phobic

anxiety and higher leptin and sTNF-αRII remained significant after adjustment for BMI, lifestyle, and CVD risk factors in the final, fully adjusted model.

Elevations of inflammatory biomarkers (TNF-α and CRP) have been associated with increased risk of CVD in multiple prospective studies (13). In the current study, we measured sTNF-RII, the cleaved extracellular part of the cell surface receptor for TNF-α that correlates highly with TNF-α and is less susceptible to short-term variation. Inflammatory biomarkers are increased in states of acute psychological stress, and these results have been extended to states of chronic psychological distress, such as posttraumatic stress disorder (14). We demonstrate that phobic anxiety is associated with increased inflammatory biomarkers and markers of endothelial dysfunction, which may explain, in part, the association between phobic anxiety and increased CVD risk. Previous studies in other groups, using other measures of

chronic anxiety to investigate the association with inflammatory biomarkers, have reported results consistent with those presented herein. A study of healthy adult men and women from Greece (7) found that higher anxiety, as assessed by the Spielberger State Anxiety Inventory, was associated with higher CRP, interleukin-6, homocystine, and fibrinogen levels in women, but this study did not adjust for all potential confounding variables, and only a small proportion of these women had diabetes. We found only a trend toward a significant association between CRP and phobic anxiety in women with diabetes ($P = 0.06$ in crude analysis), but this became nonsignificant after adjustment for BMI, age, and race, indicating that these variables may mediate any association between phobic anxiety and CRP. We did document significantly increased levels of sTNF-RII in subjects with higher anxiety indicating immune activation. This association became nonsignificant after adjustment for leptin, which may indicate an underlying role for leptin, or overall fat mass as expressed by leptin, to mediate this association.

We have also documented increased markers of endothelial activation (i.e., sE-selectin and sICAM-1) in subjects with higher anxiety. While the overall trend across tertiles in a linear model was not significant, levels of sE-selectin and sICAM were higher in women with scores of ≥ 4 compared with women with scores of 2 or 3. These cellular adhesion markers have been associated with vascular damage and are elevated in hypertension and diabetes (15). Previous studies have demonstrated increased expression of cell adhesion molecules on leukocytes in response to stress, both acute and chronic (16). To our knowledge, this is the first study to document increased circulating vascular adhesion markers in a chronic anxiety state independently of other confounding factors, and these data need to be confirmed by future studies.

We demonstrate herein that higher phobic anxiety is associated with higher circulating leptin levels that remained significant after adjustment for possible confounders and other biomarkers. In mice, leptin is an important regulator of the hypothalamic-pituitary-adrenal axis in response to stress (17), but the role of leptin in the response to chronic psychological stress in humans is incompletely understood. Although acute physical stress, such as exposure to cold, results in a re-

duction in circulating leptin levels, acute psychological stress has been associated with increased leptin levels (18). In states of chronic psychological stress, increased leptin levels have also been described in several studies, including a large cross-sectional study in Japanese men that found that higher perceived psychological stress was associated with higher circulating leptin levels (19) and a further study documenting higher leptin levels in subjects with posttraumatic stress disorder (20). Furthermore, in one study (21), the increase in leptin levels in response to acute stress was significantly correlated with waist circumference, but it remains unclear whether increased waist circumference is the cause or the effect. The results presented herein confirm these earlier studies by reporting significantly higher leptin levels in women with higher phobic anxiety scores and may represent an adaptive response to chronic stress exposure, potentially resulting in chronically increased sympathetic nervous system activation in these subjects. While we adjusted for BMI in our analyses, we were unable to adjust for overall fat mass, which is a stronger predictor of leptin levels and may underlie the association observed herein. Future studies are required to investigate whether higher fat mass confounds this association and to determine whether leptin has any role in the hypothalamic-pituitary-adrenal axis response to stress in humans.

In contrast to leptin, we found no association between phobic anxiety and adiponectin, an adipocyte-secreted hormone with an important role in insulin resistance. Reduced adiponectin levels have been associated with increased risk of CVD in prospective cohort studies (22), and increased sympathetic nervous stimulation has been shown to reduce adiponectin expression in cultured adipocytes, and sympatholytic therapy in humans is associated with increased serum adiponectin (23,24). We know of no other study that has examined the association of phobic anxiety with adiponectin. Although we hypothesized that adiponectin might potentially mediate the association between phobic anxiety, a condition associated with increased activity of the sympathetic nervous system, and CVD risk, we did not observe any significant association either before or after adjustment for demographic and lifestyle variables, indicating that this adipokine is unlikely to be mechanistically involved in

any association between phobic anxiety and CVD.

Our study has several strengths, including its large sample size and prospective design, as well as the blinded assessment of outcomes using state-of-the-art methodology. Potential limitations also need to be considered. Behavioral characteristics such as decreased physical activity and smoking may be on the causal pathway of the association between phobic anxiety and CVD risk markers. If this was the case, then adjustment for these covariates would have attenuated any association between phobic anxiety and biomarkers. Our study was limited to women with diabetes, and whether these findings apply to men or other groups of patients remains unknown. Similar to any observational study, a further limitation of the study is the possibility for uncontrolled and/or residual confounding. While we adjust for multiple possible confounders such as sleep duration and CVD risk factors in our analyses, other unmeasured variables, such as fat mass, may confound the observed relationship. Random error in the measurement of outcome variables may have resulted in misclassification, which could have depressed effect estimates toward the null but could not have accounted for the statistically significant results presented herein. Despite these potential limitations, this is the first study to assess the association between many of the biomarkers and adipokines reported herein and phobic anxiety. These data need to be confirmed and extended in future studies.

In summary, our findings suggest that the increased CVD risk in women with phobic anxiety may be mediated in part by an association with elevated inflammatory biomarkers. The finding of elevated adipokine levels in women with higher levels of phobic anxiety may signify a role for adipokines in the adaptation to a chronic anxiety state and deserves further study.

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