Choline in anxiety and depression: the Hordaland Health Study[1–3]

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ABSTRACT

Background: Despite its importance in the central nervous system as a precursor for acetylcholine and membrane phosphatidylcholine, the role of choline in mental illness has been little studied.

Objective: We examined the cross-sectional association between plasma choline concentrations and scores of anxiety and depression symptoms in a general population sample.

Design: We studied a subsample (n = 5918) of the Hordaland Health Study, including both sexes and 2 age groups of 46–49 and 70–74 y who had valid information on plasma choline concentrations and symptoms of anxiety and depression measured by the Hospital Anxiety and Depression Scale—the latter 2 as continuous measures and dichotomized at a score $\geq$8 for both subscales.

Results: The lowest choline quintile was significantly associated with high anxiety levels (odds ratio: 1.33; 95% CI: 1.06, 1.69) in the fully adjusted (age group, sex, time since last meal, educational level, and smoking habits) logistic regression model. Also, the trend test in the anxiety model was significant (P = 0.007). In the equivalent fully adjusted linear regression model, a significant inverse association was found between choline quintiles and anxiety levels (standardized regression coefficient = −0.027, P = 0.045). We found no significant associations in the corresponding analyses of the relation between plasma choline and depression symptoms.

Conclusion: In this large population–based study, choline concentrations were negatively associated with anxiety symptoms but not with depression symptoms. *Am J Clin Nutr* 2009;90:1056–60.

INTRODUCTION

Choline is an essential nutrient found in animal products such as liver, egg, and meat, but also in some vegetable products, including wheat germ and soy beans (1). Despite the presence of a pathway for endogenous synthesis of choline, a choline-deficient diet impairs growth, memory, and hepatic, renal, and pancreatic functions in mammals (2). Choline plays an important role in the central nervous system, both for the synthesis of membrane phosphatidylcholine and for acetylcholine (3).

Disturbed cholinergic transmission affects cognitive function (4); however, its influence on emotional regulation is less known (5). On the basis of animal studies and drug effects, Janowsky et al (6) postulated that there is relative or actual cholinergic hyperactivity in depression and the opposite in mania. However, no attempts have been made to test this hypothesis. In a case report of 2 patients with tardive dyskinesia, large doses of choline induced depressive symptoms in both patients (7). In an open-label clinical trial, patients (n = 13) with Alzheimer disease showed no significant change in the mean Geriatric Depression Scale score after treatment with tetrahydroaminoacridine (an acetylcholine-esterase inhibitor) and lecithin (phosphatidylcholine) for 10 wk (8). In a crossover, double-blind, placebo-controlled trial, patients (n = 11) with mania showed a significant improvement after 1 wk of treatment with lecithin, but not with placebo (9). In an open-label study, treatment-refractory rapid cycling bipolar patients (n = 6) taking mood-stabilizing medication had a reduced level of mania and other mood symptoms after treatment with choline bitartrate for 1 wk (10). During the past decade, there has been an interest in choline as a marker of neuronal activity in imaging studies with magnetic resonance spectroscopy. However, the number of studies has been few (11–14), and it is difficult to draw specific conclusions other than that choline concentrations in the central nervous system may be disturbed in mental illness.

In the present study we investigated the associations between plasma choline concentrations and anxiety and depression in a large sample of middle-aged and elderly men and women from the general population.

SUBJECTS AND METHODS

Subjects

The Hordaland Health Study was conducted from 1997 to 1999 as a collaboration between the National Health Screening Service, the University of Bergen, and local health services. In a subsample of the study, 9187 men and women aged 46–49 and 70–74 y who had participated in the Hordaland Homocysteine Study in 1992–1993 (15) were invited to participate; 7074 (77%) participated in the Hordaland Health Study. The subjects underwent a brief physical examination (height, weight, and blood pressure). Blood samples used for the preparation of plasma were drawn into evacuated tubes containing EDTA, placed in a refrigerator (4–5°C) within 15–30 min, and centrifuged, usually within 1 h (always within 3 h). EDTA plasma was stored at

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TABLE 1
Univariate associations of anxiety and depression with sex, age group, time since last meal, educational level, smoking habit, and plasma choline concentration: the Hordaland Health Study (n = 5918)

		High anxiety levels[1]			High depression levels[2]		
	Total n	n[3]	Percentage	P[4]	n[3]	Percentage	P[4]
Sex				<0.001			0.223
Men	2609	305	11.7		256	9.8	
Women	3309	624	18.9		294	8.9	
Age group				<0.001			0.053
46–49 y	3084	551	17.9		265	8.6	
70–74 y	2834	378	13.3		285	10.1	
Time since last meal[5]				0.093			0.332
<2 h	2151	352	16.4		215	9.9	
2–3 h 59 min	2595	413	15.9		240	9.2	
4–5 h 59 min	912	130	14.3		83	9.0	
≥6 h	259	35	13.5		23	8.7	
Educational levels[5]				0.003			<0.001
<10 y	1718	314	18.3		208	12.1	
10–12 y	2329	338	14.5		209	9.0	
>12 y	1617	236	14.6		102	6.3	
Current smoker[5]				<0.001			<0.001
No	4391	605	13.8		365	8.3	
Yes	1172	246	21.0		146	12.5	
Plasma choline quintiles[6]				<0.001			0.622
1	1168	232	19.9		106	9.1	
2	1180	201	17.0		113	9.6	
3	1198	176	14.7		129	10.8	
4	1168	164	14.0		89	7.6	
5	1204	156	13.0		113	9.4	

[1] Defined as a Hospital Anxiety and Depression Scale anxiety subscale score ≥8.
[2] Defined as a Hospital Anxiety and Depression Scale depression subscale score ≥8.
[3] Number of cases with high anxiety or depression levels.
[4] Test for trends in proportions (chi-square test).
[5] The total number of participants and cases is somewhat lower because of missing data.
[6] Quintile 1: 4.09–7.96 μmol/L; quintile 2: 7.97–9.08 μmol/L; quintile 3: 9.09–10.10 μmol/L: quintile 4: 10.11–11.58 μmol/L; quintile 5: 11.60–24.70 μmol/L.

−80°C. The analyses of blood samples were completed in 2006; thus, plasma was stored for 7 to 9 y.

Information on health behaviors, subjective health, current or former diseases, use of medications, and demographic, socioeconomic, and psychosocial variables was obtained with a self-administered questionnaire. The study population included 5918 subjects with valid data on anxiety and depression symptoms, plasma choline, educational level, and smoking habits. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All participants signed a consent form.

Assessment of anxiety and depression

The Hospital Anxiety and Depression Scale (HADS) (16) is a self-administered questionnaire consisting of 14 items, 7 for anxiety (HADS-A subscale) and 7 for depression (HADS-D subscale). So that individuals do not feel as though they are being tested for mental disorders, symptoms of severe psychopathology are not included. HADS-A contains items mainly related to restlessness and worry and one item that reflects panic attacks. HADS-D focuses mainly on the reduced pleasure response aspect (anhedonia) of depression but also on psychomotor retardation and impaired mood. High anxiety levels were defined as a HADS-A ≥8 and high depression levels as a HADS-D ≥8 (17).

Biochemical measurements

Plasma free choline, betaine, and dimethylglycine were measured by using a method based on normal-phase chromatography–tandem mass spectrometry (18).

Statistical analyses

Logistic regression analyses were used to estimate odds ratios (OR) for being a case, comparing each quintile (quintile 1: 4.09–7.96 μmol/L; quintile 2: 7.97– 9.08 μmol/L; quintile 3: 9.09–10.10 μmol/L; quintile 4: 10.11–11.58 μmol/L) with the reference quintile (quintile 5: 11.60–24.70 μmol/L) of plasma choline in the total study population. The representation of the choline quintiles as indicator variables was used to allow for assessment of nonlinear dose-response relations, whereas a linear (1 df) representation was used to test for linear trends. Two logistic regression models were used, one with adjustment for age group, sex, and time since last meal (model 1) and one with additional adjustments for smoking status (smokers compared with nonsmokers and ex-smokers) and educational level (<10, 10–12, and >12 y) (model 2). To evaluate the possible effect modification of age, sex, and methylenetetrahydrofolate reductase 677 C→T polymorphism, product terms were added separately to the models. Two linear regression models were used, corresponding to the

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logistic regression models regarding explanatory variables. Here the continuous HADS-A and HADS-D scores were the outcome variables. The effect measure β is the standardized regression coefficient of choline concentration predicting either HADS-A or HADS-D scores.

The precision of the OR estimates was expressed with 95% CIs. Generalized additive logistic regression was used to provide a graphic representation of the dose-response relation between choline and high anxiety and depression levels. This technique is based on the generalized additive model (19) and allowed adjustment for age, sex, time since last meal, smoking status, and educational level. A 2-sided P value <0.05 was chosen to indicate statistical significance. The statistical analyses were conducted by using the software package SPSS 15.0 and S-Plus 6.1 (SPSS Inc, Chicago, IL).

RESULTS

Characteristics of the study population

The total numbers of subjects with high anxiety (HADS-A $\geq$ 8) or depression (HADS-D $\geq$ 8) levels were 929 (15.7%) and 550 (9.3%), respectively. High anxiety levels were most prevalent among middle-aged women (19.9%) and high depression levels were most prevalent among older men (10.1%). The educational level was higher among the middle-aged subjects, and this difference was most pronounced among women. Current smoking was more common among the middle-aged (29.5%) than among the older (12.2%) subjects, with only minor sex differences. The mean ($\pm$SD) plasma choline concentration was 9.87 $\pm$ 2.30 μmol/L, was lower in women than in men, and was lower in younger than in older subjects (20). Among subject with the shortest time (<2 h) since the last meal, mean plasma choline was highest (10.15 μmol/L) and gradually decreased in the next groups (2–3 h 59 min: 9.85 mol/L; 4–5 h 59 min: 9.42 μmol/L) to the lowest concentration (9.20 μmol/L) in subjects with the longest time ($\geq$6 h) since the last meal.

Univariate associations

The proportion of participants with high levels of anxiety or depression symptoms according to sex, age group, time since last meal, educational level, smoking habit, and choline quintiles is shown in **Table 1**. There was a negative association between choline concentrations and anxiety symptoms ($P < 0.001$) but not depression symptoms ($P = 0.622$). Higher educational levels (negatively) and current smoking (positively) were associated with both anxiety ($P = 0.003$ and $P < 0.001$, respectively) and depression ($P < 0.001$ and $P < 0.001$, respectively) symptom levels. A higher proportion of women than men reported anxiety symptoms ($P < 0.001$), whereas no sex differences were seen for depression. A higher proportion in the middle than in the oldest aged group reported anxiety symptoms ($P < 0.001$), and a higher proportion in the oldest than the middle aged group reported depression symptoms; however, it was of borderline significance ($P = 0.053$).

Multivariate associations

In the logistic regression analyses (**Table 2**), significant associations were found only between choline and high anxiety levels. Furthermore, only the lowest choline quintile was

TABLE 2
Associations between plasma choline concentrations and anxiety and depression symptoms by logistic regression analyses: the Hordaland Health Study (n = 5918)

	Model 1[1]				Model 2[2]			
Choline quintiles[3]	Odds ratio	95% CI	SE	P[4]	Odds ratio	95% CI	SE	P[4]
High anxiety symptom levels[5]								
1	1.36	1.08, 1.72	0.12	0.010	1.33	1.06, 1.69	0.12	0.016
2	1.18	0.94, 1.49	0.12	0.164	1.17	0.93, 1.48	0.12	0.190
3	1.03	0.81, 1.30	0.12	0.838	1.01	0.80, 1.29	0.12	0.908
4	1.03	0.82, 1.31	0.12	0.784	1.03	0.81, 1.31	0.12	0.809
5	1				1			
Test for trend (1 df)				0.004				0.007
Test for homogeneity (4 df)				0.038				0.056
High depression symptom levels[6]								
1	1.10	0.83, 1.48	0.15	0.504	1.09	0.82, 1.46	0.15	0.562
2	1.13	0.85, 1.49	0.14	0.399	1.12	0.85, 1.49	0.14	0.424
3	1.25	0.95, 1.63	0.14	0.107	1.24	0.95, 1.62	0.14	0.117
4	0.83	0.62, 1.10	0.15	0.193	0.83	0.62, 1.10	0.15	0.196
5	1							
Test for trend (1 df)				0.140				0.169
Test for homogeneity (4 df)				0.062				0.073

[1] Adjusted for sex, age group (46–49 and 70–74 y), and time since last meal.
[2] Adjusted for sex, age group (46–49 and 70–74 y), time since last meal, educational level, and smoking habit.
[3] Quintile 1: 4.09–7.96 μmol/L; quintile 2: 7.97–9.08 μmol/L; quintile 3: 9.09–10.10 μmol/L: quintile 4: 10.11–11.58 μmol/L; quintile 5: 11.60–24.70 μmol/L.
[4] Wald statistic.
[5] Defined as a Hospital Anxiety and Depression Scale anxiety subscale score $\geq$8.
[6] Defined as a Hospital Anxiety and Depression Scale depression subscale score $\geq$8.

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TABLE 3
Associations between plasma choline concentrations and anxiety and depression symptoms by linear regression: the Hordaland Health Study (n = 5918)

	Model 1[1]			Model 2[2]		
	SE	β[3]	P[4]	SE	β[3]	P[4]
Anxiety[5]	0.03	−0.030	0.028	0.03	−0.027	0.045
Depression[6]	0.03	−0.025	0.065	0.03	−0.023	0.086

[1] Adjusted for sex, age group (46–49 and 70–74 y), and time since last meal.
[2] Adjusted for sex, age group (46–49 and 70–74 y), time since last meal, educational level, and smoking habit.
[3] Standardized regression coefficient.
[4] t test.
[5] Defined as a Hospital Anxiety and Depression Scale anxiety subscale score ≥8.
[6] Defined as a Hospital Anxiety and Depression Scale depression subscale score ≥8.

significantly associated with high anxiety levels. Subjects with plasma choline concentrations in the lowest (first) quintile [compared with the highest (fifth)] had an OR of 1.36 (95% CI: 1.08, 1.72) in the model that adjusted for sex, age group, and time since the last meal (model 1), which was similar to the results in the fully adjusted model 2 (OR: 1.33; 95% CI: 1.06, 1.69). The trend tests for these models were significant (P = 0.004 and P = 0.007 for models 1 and 2, respectively). Adjustments for coffee consumption, physical exercise, body mass index, cardiovascular disease, and serum creatinine were performed as well and had essentially no effect on the associations between plasma choline and anxiety. There was no significant effect modification of age (P = 0.525) or of the methylenetetrahydrofolate reductase 677 C→T polymorphism (P = 0.944) on the association. The choline-sex interaction was significant (P = 0.010); however, when stratified on sex, no significant associations between choline and anxiety symptoms were found (data not shown). In the logistic regression models predicting high depression levels, no significant associations were found for either separate choline quintiles or trend through the choline quintiles.

A significant inverse association was also found by linear regression analyses, only between choline and anxiety levels (**Table 3**), after adjustment for sex, age group, and time since last meal [standardized regression coefficient (β) = −0.030, P = 0.028]. Further adjustment for smoking and educational level somewhat weakened the association (β = −0.027, P = 0.045). In the linear regression models predicting depression symptoms, no significant associations were found.

An inverse dose-response association between plasma choline concentrations and high anxiety symptom levels and a possible inverse dose-response association between plasma choline concentrations and high depression symptom levels were indicated by the generalized additive model analyses (**Figure 1**). Because of the increasing widening of the 95% pointwise CI toward the tails of the distribution [plasma choline <7.0 μmol/L (8th percentile) and >12.0 μmol/L (84th percentile)], these values are not shown.

Having access to the concentration of plasma betaine and dimethylglycine from the assessment process of plasma choline (chromatography–tandem mass spectrometry), the associations between these metabolites and symptoms of anxiety and depression were analyzed as well, however, without any significant findings (data not shown).

DISCUSSION

We investigated the association between plasma choline concentrations and anxiety and depression symptoms in a cohort of 5918 community-dwelling subjects and found that choline was inversely related to anxiety, but not to depression symptoms.

The present study has a cross-sectional design. Although this design allows the assessment of associations between variables, causality could not be determined. Because high levels of anxiety and depression may influence dietary habits, it is conceivable that these mental conditions may alter choline status through dietary changes. Unfortunately, we did not have information on dietary choline intake.

Although the primary participation rate of the present study was relatively high (77%), the recruitment might have been affected by mental disorders themselves, because persons with high levels of anxiety and depression symptoms may have been less likely to participate. Hence, selection bias cannot be ruled out. However, in this study, such possible bias would probably attenuate the true associations because of reduced variance in the outcome measure.

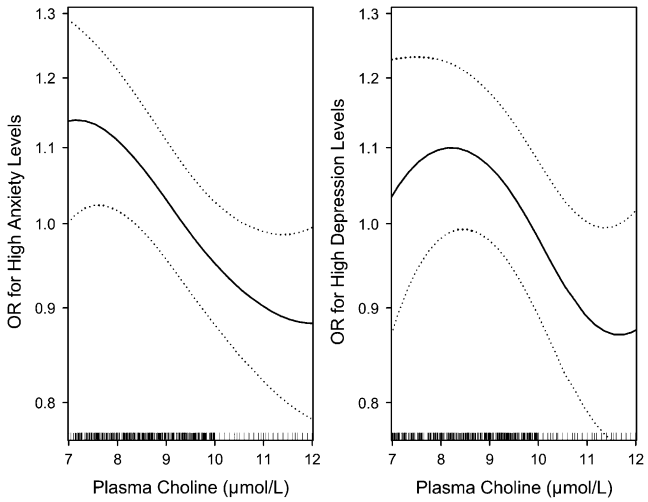

FIGURE 1. Dose-response relations between plama choline and high levels of anxiety and depression symptoms from generalized additive regression analyses (19) adjusted for sex, age group, time since last meal, smoking habit, and educational level in the Hordaland Health Study (n = 5918). The dotted lines indicate 95% pointwise CIs. Every 10th observation is marked in the rug-plot on the x axis, which indicates the distribution of plasma choline concentrations. Because of increasing widening of the 95% pointwise CIs, the results from the tails of the distribution are not shown. OR, odds ratio.

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There has probably been no or only minor change in free choline during storage of the EDTA samples before analysis. Stability of free choline in the presence of EDTA and at a low temperature was previously documented (18) and is corroborated by the mean choline concentration (9.87 μmol/L) of the study population not exceeding the concentration previously measured in fresh EDTA samples from nonfasting healthy subjects (18). Furthermore, instability of plasma choline would have attenuated rather than strengthened the observed associations.

We studied free circulating choline, which is a precursor of brain acetylcholine. Acetylcholine is of great importance in cognitive function and diseases, including Alzheimer disease (21). Impaired cognitive function might cause problems with the management of daily life tasks, which in turn might increase the level of anxiety symptoms. However, in a substudy of the oldest age group (n = 1930) in whom cognitive function was assessed, we found no association between cognitive function and anxiety symptoms (22).

Data on the role of acetylcholine in other mental disorders and in emotional regulation in humans in particular are sparse (5). In animal models (rats), dorsal hippocampal cholinergic transmission mediates an anxiolytic effect (23). Nicotine, an acetylcholine receptor agonist, affects anxiety in both animal and human studies (24). Stress, an anxiety component, induces an increase in adrenal catecholamines, which increases the demand for methylation reactions required in norepinephrine and adrenaline synthesis (25). Synthesis of biogenic amines may increase the demand for one-carbon units for methionine synthesis, derived from choline, as well as folate-dependent pathways. However, we found no associations between components of one-carbon metabolism and anxiety symptoms in a previous study of the same population (26). In summary, results from this large population-based study suggest a role of choline in anxiety disorders. However, our results are preliminary, and prospective studies need to be conducted.

The authors' responsibilities were as follows—IB: analyzed the data and primarily responsible for writing the manuscript; GST: Principal Investigator of the Hordaland Health Study, responsible for the design of the study and data collection; and provided significant advice and consultation concerning the writing of the manuscript; SEV: involved in the design of the Hordaland Health Study, provided significant advice and consultation concerning the analysis of the data, and critically read and commented on the manuscript; SK: performed the plasma choline assessments in the blood samples and critically read and commented on the manuscript; and PMU: involved in the design of the Hordaland Health Study, in charge of the plasma choline assessments, and provided significant advice and consultation concerning the writing process of the manuscript. None of the authors had any conflicts of interest.

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