

BIOLOGICAL FACTORS  
IN LATE LIFE DEPRESSION  
A population-based approach

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# **Biological factors in late life depression**

A population-based approach

## **Biologische factoren bij depressie op oudere leeftijd**

Een onderzoek in de algemene populatie

### Proefschrift

ter verkrijging van de graad van doctor aan de  
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für Gesa und Marleen



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# 1. Introduction



Community epidemiological surveys that examined the prevalence of psychiatric disorders have been conducted since the end of World War II.<sup>1,2</sup> We now know that up to half the general population meet criteria for one or more lifetime DSM disorder.<sup>3</sup> Depressive disorders are the most frequent psychiatric problem with a life-time incidence of around 25%. Although the published reports of high prevalence estimates initially were met with a good deal of scepticism, clinical reappraisal showed that they were accurate.<sup>4</sup> Public health experts now acknowledge that depression is one of the most important causes of disability world wide. Some expect it to become the number one cause of disability and premature death by 2020.<sup>5</sup>

More than 100 years ago psychiatrists began to systematically study biological factors as a cause of late life depression. In 1905 Gaupp, a college of Kraepelin in Munich, published the results of a case series of 300 patients aged 45 years and above consecutively admitted to the university psychiatric clinic in Heidelberg.<sup>6</sup> He delineated a subgroup of 23 patients that had "arteriosklerotische depressive Erkrankungen". Twenty years later the German psychiatrist Gilarowsky-Moskau took up the thread and proposed to systematically study atherosclerosis in the depressed elderly.<sup>7</sup> He concluded "es is schwierig, irgendwelche statistischen Schlüsse in bezug auf eine so geringe und schwer differenzierbare Gruppe zu machen". He also suggested to investigate "vasomotorische Störungen" because they reinforced "die mit der Arteriosklerose verbundenen psychischen Veränderungen". The efforts of German psychiatrists ended abrupt due to the impact of political despotism on individual careers and on the direction taken by scientific research. It was the British psychiatrist Post who resumed the study of vascular disease in the development of late life depression in the 1960s.<sup>8</sup> Another twenty years later, the advent of magnetic resonance imaging and other neuroimaging techniques made it possible to evaluate subtle vascular changes of the brain.<sup>9</sup> Vascular factors in late life depression were subsequently rediscovered by American psychiatrists.<sup>10</sup>

The contributions of immune mediators to the pathogenesis of psychiatric disorders have also been studied for over 100 years. In 1927 Wagner-Jauregg was the first of three psychiatrists ever to receive the Nobel prize. He had noted that the mental status of patients with fever improved and began treatment of dementia paralytica (syphilis) with the malaria virus.<sup>11</sup> Thousands of patients world-wide were treated with fever therapy until the introduction of penicillin. East German psychiatrists in the 1970s still induced local anaphy-

lactic reactions in attempts to treat depressive psychoses.<sup>12</sup> For decades mood disorders were related to a depressed immune system only.<sup>13</sup> The first report that considered a quantitative measure of the immune system in depressive disorders was published in 1978.<sup>14</sup> Since then, numerous investigators have compared immune markers of depressed persons with apparently healthy controls and more recently, showed an immune activation in depressed subjects.<sup>15,16</sup>

This thesis presents population-based studies of late-life depression. Biological correlates of depression were examined in community-dwelling persons. Our aim was not to detect a biological marker but to evaluate possible risk factors of depression. Many physiological measures have only been studied in clinical samples, others in population-based studies without careful assessment of depression and some have never been related to depression - such as arterial stiffness or vasomotor reactivity assessed by a Doppler technique. The basic idea of the thesis is, that it is possible to learn something about causes of depression if correlates are studied carefully. In chapter 2 we examined the physical health of depressed persons by constructing a frailty index. Chapter 3 is devoted to the associations of vascular pathology with depression in the elderly. In chapter 4 the role of immunological, nutritional and metabolic factors in late life depression are explored. Finally, in chapter 5 the reader will find a reappraisal of the formal and material aspects of the study. The chapter addresses the hypotheses our studies have generated and gives an outlook on future research.

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## **2. Frailty in late life depression**

**ABSTRACT**

Depression in the elderly is closely related to physical health. However, methodological problems arise when physical health is measured. A comprehensive assessment of somatic diseases is complex and possibly inefficient and self-reported measures may be biased in depressed persons. The aim of this study was to quantify the physical health of depressed persons and compare it to non-depressed persons. The authors used data from a large prospective population-based study of 4553 persons aged 55 and over (mean follow-up 9.3 years) to develop a study-specific index of physical health. A multivariate score function was derived using baseline information on all available variables independently related to mortality. Demographic factors, medical problems, functional disabilities and indicators of health service use were included in the index. However, most variables were based on physiologic measurements. Another score function was obtained by adding subjective items. Physical health of depressed (n=190) and non-depressed (n=2705) persons was assessed using data from a subsequent examination round. The association between the frailty score and late life depression was calculated with logistic regression. Odds ratio (OR) were 1.5 (95% confidence interval (CI): 1.3, 1.9) per standard deviation of score without subjective items and 1.7 (95% CI: 1.4, 2.0) for the score with subjective items. This was only moderately less than the association between the frailty score and stroke in older adults (OR: 1.7, 95% CI: 1.3, 2.2 and OR: 1.8, 95% CI: 1.4, 2.3, respectively). There was no indication that poorer health is related to more severe depressive disorders nor that a depressed subpopulation without somatic comorbidity can be distinguished in community-dwelling elderly. Nevertheless the score can be used to stratify populations into risk-homogenous subgroups by quantiles. The authors conclude that a frailty index is an appropriate tool to study the mortality risk and etiology of late life depression.

## INTRODUCTION

Depressive disorders in the elderly are intimately related to physical health.<sup>1-3</sup> Studies comparing the relative impact of risk factors found an overwhelming effect of physical illness on late life depression. Poor physical health largely explains the increase of depressive syndromes with age.<sup>4</sup> Compared to social support, life events, stress or genetic factors, physical health has the strongest correlation to depression in older community-dwelling adults.<sup>5,6</sup>

However, some methodological problems arise when physical health is assessed and quantified in depressed persons. Firstly, subjects who become depressed may report more negatively about their health.<sup>7</sup> This can bias self-report measures.<sup>8</sup> Secondly, depression and somatic disease may exhibit the same symptoms such as lack of concentration or appetite. An artificial correlation may result if rating scales are used to measure depression or physical health. Other problems encountered in the measurement of physical health are not specific for depression. Physical health can be conceptualized as specific pathology, impairment or functional limitations but combined measures are not available.<sup>9,10</sup> Furthermore, a comprehensive assessment of somatic diseases is very complex and possibly inefficient.

In most studies on late life depression, the investigators relied on self-reported measures of physical health. Besides assessing functional limitations, the number of comorbid conditions is simply summed and the resulting variable categorized.<sup>2,4</sup> This limits the understanding of the relation between physical health and depression. Weighing a select number of self-reported diseases equally makes comparisons of physical health disputable. It has remained unclear whether poorer physical health is associated with more severe depressive symptoms and whether distinct groups of elderly depressed subjects exist, those with 'pure depression' and those with somatic comorbidity.<sup>11</sup>

In the present study we constructed a study-specific score to assess physical health using all available correlates of mortality. Our assumption was that true comorbidity is related to worse health outcomes, of which mortality is the most straightforward and reliably measured. The primary aim of our study was to quantify the physical health of depressed subjects in order to examine whether subpopulations of depressed persons can be identified. Secondly, we wanted to compare the health of persons with different diseases. Finally, we give an example to show how a health score can be used in etiological

research.

## **METHODS**

### **Study population**

This study is part of the Rotterdam Study, a population based cohort study of chronic and disabling diseases in the elderly.<sup>12</sup> All inhabitants of a district of Rotterdam aged 55 years or over were invited. People living in homes for elderly were also included. In total, 7983 subjects of those invited participated (78% response rate). The Medical Ethics Committee of Erasmus University Rotterdam approved the study. Written informed consent was obtained from all participants. Baseline measurements were performed from 1990 to 1993 and consisted of an interview and two visits to the research center for physical examination.

### **The frailty index**

We constructed a study-specific measure of physical health. Because of the absence of a gold standard we assumed that good health is associated with survival. All variables that correlate with mortality can thus be considered as indicators of poor physical health. To derive specific weights for these variables we developed a Cox-regression model with the binary variable alive/dead as the outcome. First, we selected all variates of the baseline data set that were related to mortality in an age- and gender-adjusted model. Secondly, we allowed these variates to compete for entry in a stepwise procedure. All variates that were not independently associated with mortality ( $p < 0.1$ ) were removed. The regression coefficients (betas) of retained variates constituted the score function. In the function all variates were entered as additive terms. Thus a particular value can be computed for each subject. The result is a unidimensional score for physical health. Because the score function included items not traditionally considered health indicators we termed it 'frailty index'.

The variates entered into the age-and gender-adjusted survival analyses included demographic and social factors, the presence of major medical problems, and risk indicators assessed by interview or physiologic measurement. We also tested indicators of functional limitations or disabilities as well as variates indicating health service use. The variates included in the score

**TABLE 1: Selected characteristics of the study subjects in the third examination round (Rotterdam Study 1997-1999)**

Variates included in the frailty index*	Non-depressed (n=2705)	Depressive symptoms (n=190)
Demographic characteristics		
Age, years, mean	73	75
Gender, % female	56	73
Diagnoses established with physiologic measurements		
Diabetes, %	9	11
Peripheral arterial disease, %	17	20
Atrium fibrillation, %	3	1
Diagnoses established by questioning		
Myocardial infarct, %	11	13
Cancer in last five years, %	10	17
Risk indicators established with physiologic measurements		
Diastolic blood pressure, mmHg †	75	73
Systolic blood pressure, mmHg	144	141
Cholesterol, mmol/l †	5.8	5.8
Carotid plaques, number	1.8	1.9
Intima media thickness, mm	0.87	0.88
Bone mineral density, T score	0.85	0.83
Cognitive score	28	27
Body mass index, kg/m <sup>2</sup>	26.8	26.9
Risk indicators established by questioning		
Smoking current, %	16	20
former, %	52	44
Dyspnoe, %	34	54
Health service use and medication		
Visits to specialist per year (more than one)	44	55
Number of medication taken daily (more than one)	42	56
Functional disabilities and limitations		
Problems with riding a bicycle, %	55	77
Activities of daily life score	0.5	0.9
Personal health assessment‡		
Health compared to members of age groups	good, %	54
	bad, %	10
Memory complaints, %	36	63

\* Note that C-reactive protein, creatinin, and unintentional weight loss in last three months were not included in the index because no data was available from the third examination round. History of fractures was not included because of a negative association of the baseline variable with mortality, whereas the association of a time dependent variable with mortality was positive.

† The category depressive symptoms includes all subjects with a score on the Center of Epidemiological Studies for Depression scale of 16 and above.

‡ Diastolic blood pressure and cholesterol were additionally entered into the index as squared terms. This accounted for the fact that both high and low levels were negatively associated with survival.

‡ The primary frailty index was calculated without these subjective variables.

function are listed in table 1.

For certain conditions or risk indicators the association with mortality was not independent of other variates (e.g. dementia, stroke, left ventricular hypertrophy diagnosed by electrocardiogram, albumin level, angina pectoris, instrumental activities of daily living score, number of chronic conditions, or use of antihypertensive medications). Consequently, these were not included in the frailty function. A second frailty score (with subjective items) was obtained by additionally including personal health assessments (e.g. subjective memory function), which were possibly related to depression. Again only variates were retained that independently predicted survival.

The frailty indexes were based on the data of 4553 subjects who participated in the baseline examination and had complete information on the selected variables. During 42,479 person years of follow-up (mean follow-up 9.33 years) 1188 deaths occurred before October 1st 2002, end of follow-up. However, assessment of depressive symptoms was added to the study protocol only in the third survey (1997-1999). Thus the actual frailty score in the present study is calculated with data from the third survey, whereas the function is derived from baseline data. Three variates, the inflammation marker C-reactive protein (CRP), urinary creatinin and unintentional weight loss, that were associated with mortality have not been assessed in this 3rd examination round. Thus the final model was adapted, rerun and the weights of the score function changed to account for this.

### **Assessment of baseline variables**

Measurements of all variables have been described in previous publications.<sup>12-14</sup> Information on smoking, history of cancer in the last five years, congestive heart failure, number of visits to specialist, medications, ability to ride a bike, memory complaints, and personal health judgment were obtained during the home interview. We used the Disability Index of the Stanford Health Assessment Questionnaire and a 9-item Instrumental Activities of Daily Living score to establish the functional status and disabilities.<sup>15,16</sup> Cognitive function was measured by the Mini Mental State Examination. History of myocardial infarction was primarily assessed by direct questioning but only self-reported events confirmed by additional information of general practitioner or specialist were taken into account. Diabetes was defined as random or postload serum glucose higher than 11.1 mmol/l or use of anti-diabetic medication. Serum cholesterol and CRP levels were assessed by an

automated procedure in non-fasting blood, creatinin levels were assessed in urine. A standard 12-lead electrocardiogram was recorded and analyzed using a computer program to detect atrium fibrillation. Bone mineral density measurement of the femoral neck was performed by X-ray absorptiometry. Ankle-brachial systolic blood pressure index was used as an indicator of peripheral arterial disease. Intima-media thickness and plaques in the carotid arteries were determined by an ultrasound technique and used as indicators of subclinical atherosclerosis.

### **Depression assessment**

In the third examination we added assessment of depressive symptoms to the study protocol. Depressive disorders were diagnosed using a two step procedure. First, participants were screened for depressive symptoms with the Dutch version of the original Center for Epidemiologic Studies Depression scale (CES-D) during the home interview. We used a score of 16 as a cut-off. Previous studies in the Netherlands have verified that this score represents clinically significant depressive symptoms and has a very high sensitivity for major depression in older subjects.<sup>17</sup> As a second step, screen positive subjects had a semi-structured psychiatric interview with the Schedules for Clinical Assessment in Neuropsychiatry.<sup>18</sup> Psychiatric disorders were classified according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria.<sup>19</sup> The diagnostic categories include major depression and dysthymia in addition to minor depression (as defined in the appendix of the DSM-IV).

Of the 4703 subjects who participated in the home interview of the third examination, 2973 had information on all variables included in the frailty index. Of these, 197 (6.6%) were screen-positive on the CES-D and in 184 (93%) psychiatric interviews had been conducted. The psychiatric interviews revealed that 78 cases had a depressive disorder according to DSM IV criteria. The remaining 106 subjects were diagnosed as having either anxiety or another psychiatric disorder (n=18) or did not meet criteria for an axis I psychiatric disorder (n=88, subthreshold depressive symptoms).

### **Assessment of stroke and age-related maculopathy**

The frailty index was used to compare the physical health of persons with depression to those with a history of stroke and those with age-related maculopathy. Neither stroke nor indicators of eye disease were included in the frailty index, because they did not predict mortality independent of other

variates. Stroke is a leading cause of death and disability in the Netherlands. Subjects participating in the Rotterdam Study were continuously monitored for stroke through automated linkage of the study database with the files from general practitioners. Additional information is obtained by scrutinizing hospital discharge records.<sup>20</sup> Age-related maculopathy is the most important cause of incurable blindness in the elderly. A positive association of eye diseases with mortality has been reported.<sup>21</sup> Like depression, the presence of age-related maculopathy was established in the third examination round. The screening and diagnostic procedure have been described in detail elsewhere.<sup>22</sup> Color transparencies were taken of the macula area and graded in a detailed manner to identify all features of age-related maculopathy. Subjects with early stages of the disease were also included.

### **Data analysis**

The frailty score was logistically transformed after adding 4.7 to avoid negative values. Through this computation a normal distribution in the non-depressed persons was achieved. Thereafter we divided the score by its standard deviation, which yields more meaningful values. Secondly, we assessed the association of frailty with depressive symptoms, depressive disorders and subthreshold depressive symptoms using age- and gender-adjusted logistic regression. Thirdly, the distribution of the frailty score in subjects with depressive symptoms was examined in order to study whether latent subpopulations with depression exist. For the graphical presentation the frailty score was categorized in 25 categories with equal ranges. Next, the association of the frailty score with stroke and age-related maculopathy was quantified with logistic regression. Finally, we illustrate the use of the frailty score in etiologic research. For this aim, we stratified the population at the median of the frailty score in two groups more homogenous for physical health. The relation between cytokine interleukin-6 and depression was examined in the resulting strata. Interleukin-6 is an inflammatory protein that indicates immune activation and can be measured in plasma. Numerous small hospital and two population-based studies have reported an association between depression and interleukin-6.<sup>23,24</sup>

## RESULTS

The characteristics selected for the frailty score in persons with and without depressive symptoms are presented in table 1. All indicators of frailty were found to be more frequent or had higher levels in the depressed persons except atrium fibrillation and male gender.

Table 2 shows the relation of the frailty scores with depressive symptoms. The odds ratio of depressive symptoms increased by 50% per standard deviation of frailty score without and by 70% of the score with subjective items. In a further analysis, screen positive subjects were classified according to the severity of the depressive symptoms. Persons with subthreshold depressive symptoms and subjects with DSM-IV depressive disorders were analyzed as distinct groups. Estimates for the association of the two frailty scores with depressive disorders and subthreshold depressive symptoms symptoms (data not shown) were exactly the same as for depressive symptoms (table 2). Thus we found no indication that more severe depressive disorders were associated with poorer health.

Figure 1 gives a graphical representation of the distribution of the frailty score in subjects with depressive symptoms. The distribution is normal (median: 5.5, interquartile range 4.9 to 6.1). No bimodal distribution was found and we could not identify latent classes in the group with depressive symptoms.

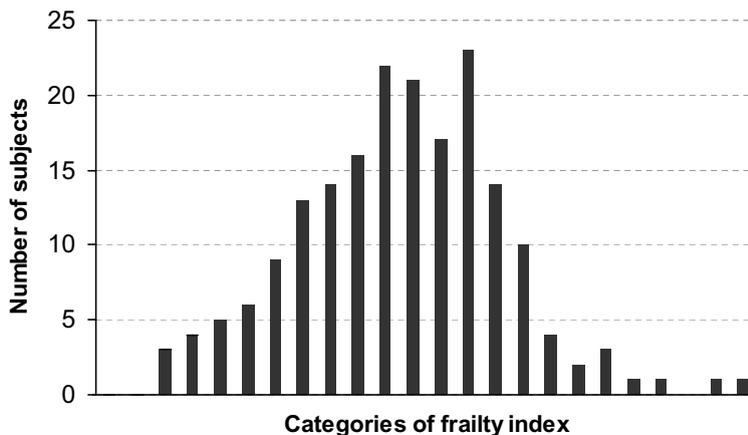
**TABLE 2: The association of physical health with depression, stroke and age-related maculopathy assessed with a study-specific frailty index**

Frailty score (per SD)	Depressive symptoms* (n=190)	History of stroke (n=90)	Age-related maculopathy* (n=341)
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Without subjective items	1.5 (1.3, 1.9)	1.7 (1.3, 2.2)	1.1 (0.9, 1.3)
With subjective items <sup>†</sup>	1.7 (1.4, 2.0)	1.8 (1.4, 2.3)	1.0 (0.9, 1.3)

Results were obtained with logistic regression adjusted for age-and gender. See table 1 for list of variates included in the frailty index.

\*The category depressive symptoms includes all subjects with a score on the Center of Epidemiological Studies for Depression scale of 16 and above. The category age-related maculopathy includes subjects in early stages of the disease.

<sup>†</sup> This frailty index additionally includes memory complaints and judgement of health compared to members of same age group.



**Figure 1** Distribution of physical health in persons with depressive symptoms

For this graphical representation the frailty score was divided in 25 categories with equal ranges. No subjects with depressive symptoms were found in the first three categories.

The association of the frailty score with stroke was slightly stronger than with depressive symptoms (table 2). The unadjusted odds ratios (OR) of the frailty scores also suggested a relation between physical health and age-related maculopathy (both OR were 1.5, 95% confidence interval (CI): 1.4, 1.7). However, the association disappeared after adjustment for age and gender.

Finally, we illustrate the use of the frailty score with an example of etiological research. In a previous paper we reported on associations between depressive disorders and plasma concentrations of inflammatory proteins in the present study population.<sup>25</sup> There was an association between cytokine interleukin-6 and depressive disorders after adjustment for age, gender, history of stroke, smoking and functional disability (OR per standard deviation: 1.5, 95% CI: 1.2, 1.9). This odds ratio remained unchanged after exclusion of subjects in whom no frailty score could be calculated. We used the median of the frailty score to stratify the population according to subjects with relatively good (n=256, of which 36 subjects with depressive disorders) or relatively poor physical health (n=248, of which 38 with depressive disorders). Subsequently, we reran the analyses. There was no relation between interleukin-6 and depressive disorders in subjects with good health (OR: 1.2, 95% CI: 0.7, 1.9), only in those with relatively poor health (OR: 1.9, 95% CI: 1.1, 3.3). In accordance with this, we found that the interaction term between the frailty score

and interleukin-6 concentrations was associated with depressive disorders ( $p=0.04$ ).

## DISCUSSION

In this large population-based study we quantified physical health by constructing a study-specific frailty index. A multivariate score function was derived using baseline information on all available variables related to mortality. The score function was then used to assess the physical health of depressed and non-depressed persons with data from a following examination round. We found that persons with poor physical health were more likely to have depressive symptoms even when subjective measures of health were excluded. The association of the frailty score with depression was only moderately weaker than the association with stroke. There was no indication that poorer health was related to more severe depressive disorders nor that a depressed subpopulation without somatic comorbidity can be distinguished in community-dwelling elderly.

Previous population-based studies of depression mostly relied on self-reported measures of physical health only. Besides functional limitations, the number of chronic conditions was assessed. Participants were explicitly asked whether they have certain diseases.<sup>4,26</sup> The result may be biased and 'number of co-occurring conditions' is not considered an overall measure of physical health.<sup>8</sup> This may explain why some investigators found an association between severity of depressive symptoms and poor physical health.<sup>27</sup> On the other hand, our observations are in line with the strong evidence that depressive symptoms in the elderly are frequently accompanied by very poor health.<sup>5,6,28</sup>

It is a challenge to formalize the identification and measurement of physical disease for research purposes and different approaches exist.<sup>29</sup> Comorbidity scores are increasingly used in population-based research.<sup>30-32</sup> The popular instruments use a grading system which weigh either specific diseases or the severity of comorbidity within each organ system. For some diseases agreement between questionnaire and general practitioner data is poor (e.g. peripheral atherosclerosis).<sup>33</sup> A recent review questioned the use of standardized comorbidity scores in epidemiological research.<sup>34</sup> The indexes performed reasonably well in the setting they were developed for but added

little to adjustment or stratification for age in other populations.

More recently epidemiologists have also developed frailty indexes to summarize individual differences in health status.<sup>35-37</sup> There is no common concept of frailty but the aim of the indexes is to predict mortality.<sup>38</sup> Most frailty indexes use items traditionally included in measures of functional limitations such as grip strength, gait abnormality or visual problems. These measures are frequently superior to 'number of co-occurring conditions' in the ability to control for morbidity risks in community-dwelling populations.<sup>35,39</sup> However, functional limitations and disabilities capture only one aspect of disease and specific pathology is important for future health.

Another approach is to derive study specific weights to aggregate comorbidity information instead of using a standardized instrument. The outcome is modeled as a function of all comorbid conditions and regression scores are used to weigh individual diseases.<sup>34</sup> In the present study we decided to aggregate all variables available related to mortality. The main advantages are that an overall measure of health is derived and different dimensions of health can be incorporated. Furthermore all available information is used and specific weights account for the quality of diagnostic data. We are not aware of a risk function or a index that has taken this approach to quantify physical health.

However, the items used in our score to measure frailty are known to be related to mortality in older adults. In a seminal paper on the 5-year mortality in older adults the Cardiovascular Health Study Collaborative Research Group (CHS) identified 20 independent risk factors.<sup>40</sup> The overlap is striking, 14 of these characteristics were the same as in our study population. Minor differences originate from the availability of data, e.g. lung function was not assessed quantitatively in the Rotterdam Study. Other differences may be due to the population studied, the measurement technique, allowance for quadratic terms and the health care system. This probably explains why albumin, cholesterol, not being able to ride a bicycle or income are independent risk factors of mortality in our or in the CHS study.

Few clinical diseases that were assessed by history independently predicted mortality. Most of the conditions that characterize comorbidity scores compared badly to the variates incorporated in our index. We retained only cancer and myocardial infarction and included mostly objective, non-invasive measures in our score. This is a strong argument to use a study-specific score if physiological measurements are available. Using only diagnoses

as established by interview leads to more misclassification. Further, many conditions are correlated, because of shared risk factors, common pathological processes or their relation to health care use. Moreover, quantitative measures indicate both subclinical and clinical disease and do not dichotomize diseased and non-diseased. They do not rely on arbitrary cut-offs and self-report, which is especially appealing if the physical health of depressed subjects is examined.

There are some limitations to our study. The frailty score lacks a clear biological interpretation. Persons with the same score are at a similar mortality risk but with possibly very different patterns of risk factors.<sup>41</sup> When persons are grouped on biological or functional characteristics they may seem relatively risk-homogenous. We have seen, however, that there are a large number of independent risk factors for mortality. Meaningful groups cannot be defined without data aggregation. Thus a score function is useful if exposure and outcome are related to a large number of variates. However, it should be derived from an outcome relevant to the subject matter. In case a cardiovascular risk factor is studied one may want to use cardiovascular mortality as an outcome, provided that there is no substantial misclassification of this outcome.

Furthermore, the weights and the combination of items in our frailty index were study specific. The score function cannot be used across studies. However, only age and gender, the most simple and widely used "comorbidity scores", are ubiquitously and accurately recorded in all studies.

It should also be pointed out that our approach is less feasible if the psychosocial impact of somatic diseases is the issue. There is good evidence that lack of controllability and functional limitations have a strong impact on mood in chronically diseased persons.<sup>42,43</sup> This is a major conceptual difference between frailty and comorbidity. Comorbidity refers to co-occurring disease,<sup>29</sup> frailty to mortality risk.<sup>38</sup> Measures of subclinical disease fit better into the frailty concept. On the other hand, this makes it questionable to use mortality as the gold standard of a comorbidity index.<sup>44</sup>

Our approach is related to Miettinen's multivariate confounder score and Rubin's propensity score.<sup>45,46</sup> All variates associated with an outcome or an exposure are summarized in these score methods. Diseases, functional and personal characteristics are aggregated with study specific weights. The multivariate confounder score appears to have fallen into disuse. It was shown to be flawed as a test of confounding.<sup>47</sup> However, it was developed to enable stratification in fairly risk-homogenous subgroups. Indeed, with our frailty

index we could show that inflammatory proteins are only associated with depression in persons of relatively poor health. This suggests that immune activation may have a stronger effect in the presence of other pathological processes.

In summary, our frailty index allowed useful descriptive comparisons. Depressed persons have a much poorer health than persons with age-related maculopathy. They have a mortality risk which is comparable to persons who survived a stroke. This is consistent with the results of longitudinal studies that documented an increased mortality in depressed persons.<sup>48-50</sup> However, quantitative assessments of disease were not performed in the population-based cohort studies. Control for disease severity was only possible in clinical studies that showed a high risk of depressed symptoms for further events.<sup>51,52</sup> Thus two questions arise, (a) does confounding by subclinical disease explain the increased mortality in depressed community dwelling subjects, and (b) does depression increase the mortality risk predominantly in persons with poor health. This study showed that both possibilities must be investigated and that a frailty score may be an appropriate tool to study the latter question.

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### **3. Vascular factors in late life depression**



3.1

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Atherosclerosis

## **ABSTRACT**

*Context:* Depression in late life has been associated with vascular pathology. Several studies have demonstrated that persons with brain infarcts or cerebral white matter lesions are more likely to have depressive disorders. Furthermore, depression is related to the subsequent development of ischemic heart disease. However, whether atherosclerosis is related to depression in the general population has not yet been investigated.

*Objective:* To investigate the relation between atherosclerosis at different locations and depression.

*Design:* Cross-sectional population-based study.

*Setting:* The Rotterdam Study, a population-based cohort study.

*Participants:* 4019 men and women aged 60 and above in whom we assessed atherosclerosis at different locations, including common carotid intima-media thickness, plaques in the carotid arteries, the ankle-brachial blood pressure index, and aortic atherosclerosis. An overall measure of extracoronary atherosclerosis was obtained in 3747 persons by computing the principal component of these extracoronary atherosclerosis measures. In a subgroup of 1874 persons we additionally measured coronary calcifications.

*Main Outcome Measure:* All subjects were screened for depressive symptoms. Screen positive subjects had a psychiatric interview to diagnose depressive disorder. Logistic regression controlled for age, gender, cognitive score and cardiovascular risk factors was used to examine the association of the atherosclerosis measures with depression.

*Results:* More severe extracoronary atherosclerosis was associated with a higher prevalence of depressive disorders. For every standard deviation increase the prevalence increased with 30%. Further, we found a strong relation of severe coronary and aortic calcifications with depressive disorders (odds ratio: 3.8, 95% confidence interval (CI): 1.5-9.3 and 2.0, 95% CI: 1.0-3.9, respectively).

*Conclusions:* Atherosclerosis and depression are associated in the elderly. Our population-based study supports the existence of vascular depression. It suggests that in some elderly depressed persons a treatment more akin to the management of cardiac disease needs to be investigated.

## INTRODUCTION

Several lines of evidence suggest that there is a relation between vascular factors and late life depression. Both symptomatic and silent brain infarcts are associated with subsequent depression.<sup>1</sup> Further, subtle white matter and deep grey matter abnormalities were found more frequently in depressed than in non-depressed persons.<sup>2</sup> A vascular depression hypothesis has been proposed. It postulates that structural changes in the brain due to atherosclerosis are of primary importance in late life depression.<sup>3,4</sup>

On the other hand, depressive symptoms are also related to subsequent cardiovascular disease. Several prospective population-based studies of depressed persons showed an increased risk of myocardial infarct.<sup>5-8</sup> However, none of these studies included measurements of vascular pathology that might underlie the observed association. Most clinical studies on the cardiovascular risk associated with depressive symptoms did not assess atherosclerosis either but focussed on other measures like platelet function or heart rate variability.<sup>9</sup> Epidemiological evidence for a link between atherosclerosis and depression in the general population is lacking. Moreover, the vascular depression hypothesis is mainly supported by neuroimaging studies.

We examined the association between atherosclerosis at different locations in the body and depression in the Rotterdam Study, a large, community-based population of the elderly subjects.

## METHODS

### Subjects

The study was conducted as part of the Rotterdam Study, a population based cohort study ongoing since 1990 for which all inhabitants aged 55 and over of a suburb of Rotterdam were invited. The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants. In the third survey (1997 to 1999) we added assessment of depressive symptoms to the study protocol. Of the 5901 subjects that were invited, 4,730 persons (response rate 80%) participated in the home interview. Of these, 4019 came to the research center for non-invasive assessments of atherosclerosis. The 711 subjects with no assessment of atherosclerosis were on average older (78 versus 72 years), more likely to be female (69%

versus 58%) and had more depressive symptoms (11.8% versus 6.9%, overall prevalence 7.8%).

Additionally, non-institutionalized participants younger than 85 years who had completed the third survey were invited for a coronary atherosclerosis scan at a second research site. Of the 3371 eligible subjects, 2263 agreed to undergo electron-beam computer tomography (CT). Because of archiving problems the scores of only 1986 participants were available for analysis.

### **Assessment of depression**

Depressive disorders were assessed using a two step procedure. First, participant completed the Dutch version of the original Center for Epidemiology Studies Depression scale (CES-D) during the home interview. The CES-D is a 20-item self-reported measure of symptoms scored on a scale of 0 to 3 points.<sup>10</sup> We used a score of 16 as a cut-off to indicate depressive symptoms. This cut-off had a very high sensitivity for major depression in a random sample of older subjects in the Netherlands.<sup>11</sup> Previous studies have verified that a score of 16 and above on the CES-D represents clinically significant depressive symptoms.<sup>12</sup> In a second step, screen positive subjects had a psychiatric work-up. They were evaluated by the Dutch version of the Present State Examination (PSE-10), a semi-structured psychiatric interview included in the Schedules for Clinical Assessment in Neuropsychiatry.<sup>13</sup> All interviews were conducted by one or other of two experienced clinicians. Psychiatric disorders were classified according to the DSM-IV criteria with an algorithm based on the PSE-10 scores. The diagnostic criteria include minor depression as defined in the appendix of the DSM-IV.

Of the 4019 subjects included in the analyses, 285 (7.1%) were screen-positive for depressive symptoms as measured by the CES-D. A psychiatric work-up was performed in 259 (93 %) of these participants. Twelve subjects refused to participate in this evaluation; four screen positive subjects could not be reached. A depressive disorder as defined by the DSM-IV criteria was established in 119 cases. The remaining subjects were either classified as anxiety disorders or other psychiatric diseases (n=30) or did not meet the criteria for an Axis I psychiatric disorder (n=110, subthreshold depressive symptoms).

### **Assessment of atherosclerosis**

We measured atherosclerosis non-invasively with four established methods, i.e. the ankle-brachial blood pressure index, intima-media thickness in the

common carotid arteries, the presence of plaques in the carotid arteries and aortic atherosclerosis. These four measures assess extracoronary atherosclerosis at different locations in the body. Furthermore, in a subgroup of participants we measured coronary atherosclerosis.

Ankle-brachial blood pressure index is an indicator of peripheral arterial disease.<sup>14</sup> Systolic blood pressure at the right brachial artery was calculated as the mean of two consecutive measurements with a random-zero sphygmomanometer. A single systolic blood pressure reading was taken both at the left and the right posterior tibial artery with a Doppler ultrasound 8 MHz transducer (Huntleigh 500 D, Huntleigh Technology, Bedfordshire, UK) while the subject was in supine position. In agreement with the approach followed by Fowkes et al., peripheral arterial disease was considered present when the ankle-brachial blood pressure index was lower than 0.90 in at least one leg.<sup>14</sup>

Intima-media thickness was measured by recording of ultrasonographic images of both the left and right carotid artery, using a 7.5-MHz linear-array transducer (ATL Ultra-Mark IV, Advanced Technology Laboratories, Bethel, Washington, USA).<sup>15</sup> The lumen-intima interface and the media-adventitia interface of the distal common carotid artery were measured off-line. The common carotid intima-media thickness was determined as the average of near and far wall measurements of both left and right side.

The presence of plaques in the carotid artery was assessed by evaluating the ultrasonographic images of the common, internal, and bifurcation sites of the carotid artery for the presence of atherosclerotic lesions.<sup>16</sup> Plaques were defined as a focal widening relative to adjacent segments and composed of calcified or non-calcified components. The total carotid plaque score of each subject was defined by summation of the presence of plaques at far and near walls of left and right sides at 3 locations. For the analyses we used four categories of plaque scores (no, 1, 2-3, and 4 or more plaques).

Atherosclerosis of the abdominal aorta was determined with a lateral x-ray of the lumbar spine. Calcified plaques were considered present when linear densities were clearly visible in an area parallel and anterior to the lumbar spine.<sup>17</sup> Values for the extent of calcification were scored according to the length of the involved area (<1 cm, 1-2.5 cm, 2.5-5cm, and >5 cm)

We used coronary calcifications as a measure of coronary atherosclerosis. Imaging of the epicardial coronary arteries was performed with an electron beam CT-scan (C-150, Imatron, San Francisco, USA). From the root of the

aorta through the heart 38 images were obtained with 3 mm slice thickness. Using Agatston's method a calcium score was obtained by multiplying each area of interest with a factor indicating peak density within the area. We summated the scores for the calcifications to obtain a score for the entire epicardial system.

### **Other measurements**

Age, gender, cognitive function, education, cigarette smoking, total cholesterol, blood pressure, history of myocardial infarction and stroke were considered as possible confounders. Cognitive function was assessed by the Mini Mental State examination. Education was measured on an ordinal scale and later dichotomized into low (primary education only) and high education. Cigarette smoking was analyzed in categories of non-smoker, current and former smoker. Fasting blood samples were obtained from all subjects in this analysis and serum total cholesterol was determined using an automatic enzymatic procedure. A history of myocardial infarction and stroke were obtained by direct questioning and computerized linkage with general practitioner medical records. It was considered positive when verified by a physician.

### **Statistical analysis**

The association of atherosclerosis with depression was analyzed in two ways. First, we assessed the relation of individual atherosclerosis measures with depressive disorders using logistic regression. Subjects who were screen positive for depressive symptoms but did not fulfill the DSM-criteria for depressive disorders were excluded. Intima-media thickness was entered into the model as a continuous variable divided by one standard deviation. Ankle-brachial blood pressure index, carotid plaques and aortic calcifications were analyzed in the categories defined above. For the coronary calcifications three calcium score categories were defined as described previously: 0 to 100, 101 to 500, and above 500.<sup>18</sup>

Second, we used the different extracoronary atherosclerosis measures to obtain an overall atherosclerosis score. A principal component analysis was performed. This is a standard procedure to combine related measures.<sup>19</sup> Original variables were replaced with a linear combinations of these variables. For this aim we used the continuous measure ankle-brachial blood pressure index, intima-media thickness, plaques in the carotid arteries and aortic ath-

erosclerosis. We were able to reduce the four measures in our original data set to one principal component. The association with extracoronary atherosclerosis was studied by regressing depressive disorders on this continuous measure divided by its standard deviation. A principal component analysis increases the power to reveal relationships. Unlike other methods – such as the construction of a composite score – it does not rely on arbitrary cut-off points (categorizations).

To check whether confounding existed we added the potential confounders into the basic model, which already contained age (continuous) and gender. The variables were included in further analysis if the result differed meaningfully.<sup>20</sup> The analyses were run with and without additional adjustment for a history of stroke and myocardial infarction because they can be seen as an intermediate in the relation between atherosclerosis and depressive disorders.

Of all 3747 subjects who had at least three measures of atherosclerosis performed, 742 had a missing value on the fourth measure. To decrease possible bias these missing atherosclerosis values can be filled in (imputed) using the multiple imputation method.<sup>21</sup> Imputation is based on the correlation between the missing variable and other variables including the atherosclerosis measures. This correlation can be estimated using the subjects in whom the measurement was performed. We followed the method described by Van Buuren et al.,<sup>22</sup> which accounts for the uncertainty around estimated values. Imputations were performed with the Multivariate Imputations by Chained Equations (MICE<sup>®</sup>) statistical package. For the present analyses, imputed values were used for the principal component analyses only.

## RESULTS

Table 1 presents characteristics of the non-depressed reference subjects and the cases with depressive disorders.

In table 2 the associations of the extracoronary atherosclerosis measures with depressive disorders are shown. There was a consistent pattern across the four locations at which atherosclerosis was measured. The persons with a more severe disease process were more likely to be depressed. For intima media thickness and severe aortic calcifications the relation with depressive disorders reached statistical significance.

**TABLE 1: Characteristics of the study subjects**

	Non-depressed (n=3734)	Depressive disorders <sup>†</sup> (n=119)
Age, years <sup>‡</sup>	72.2 (61-97)	74.3** (61-87)
Gender, % female	56.7	72.3***
Primary education only, %	47.5	57.1
MMSE-score <sup>‡</sup>	27.7 (2.0)	26.5*** (3.4)
History of stroke, %	2.8	8.4**
History of MI, %	11.3	17.4
Smoking: current smoker, %	15.9	19.3
ex-smoker, %	50.4	39.5
Diastolic blood pressure (mmHg) <sup>‡</sup>	75 (11)	73 (12)
Systolic blood pressure (mmHg) <sup>‡</sup>	144 (21)	139* (24)
Total cholesterol <sup>‡</sup> (mmol/l)	5.8 (1.0)	5.8 (1.0)
Common carotid intima-media thickness <sup>‡</sup> (mm)	0.87 (0.15)	0.90 (0.16)
Peripheral arterial disease, %	16.8	21.8
Carotid plaques, %	70.2	73.8
Aortic calcifications, %	78.1	82.8
Coronary calcifications, <sup>¶</sup> %	54.3	75.0

\*p < 0.05; \*\*p<0.01, \*\*\*p<0.001 for comparison with reference group; Continuous variables analyzed by Ancova and categorical variables by logistic regression, adjusted for age and gender were appropriate.

<sup>†</sup> The category “depressive disorders” includes persons with major or minor depression and dysthymia.

<sup>‡</sup> Values are means and ranges for age, and means and standard deviations for MMSE-score and blood pressure, cholesterol, and intima-media thickness.

<sup>¶</sup> Numbers for coronary calcifications were 1871 and 36 in non-depressed and depressive disorder group, respectively

Table 3 shows that subjects with higher levels of coronary calcifications have substantially more depressive disorders than subjects without extracoronary atherosclerosis do.

In table 4 we present the results based on the overall measure of atherosclerosis. Extracoronary atherosclerosis is related to depressive disorders if different measures are combined. The associations were very similar if imputed data of subjects with a missing atherosclerosis measurement were included. The odds ratio for this all case analysis was 1.27 (95% confidence interval: 1.02, 1.59; p=0.03 with 102 cases with depressive symptoms and 3491 non-

**TABLE 2: Relation between different extracoronary measures of atherosclerosis and depression expressed as odds ratios\***

	Depressive disorders <sup>†</sup>	
	Cases/ Controls	Odds ratio (95% CI)
Intima-media thickness (per SD <sup>‡</sup> increase)	102/3588	1.22 (1.01, 1.48)
Peripheral arterial disease <sup>§</sup>	110/3580	1.15 (0.70, 1.88)
Carotid plaques: none	25/1037	1.0 Referent
mild	19/ 646	1.16 (0.64, 2.13)
moderate	29/1137	1.19 (0.70, 2.03)
severe	21/ 661	1.52 (0.83, 2.82)
Aortic calcifications: none	15/ 686	1.0 Referent
mild	29/1112	1.20 (0.63, 2.29)
moderate	13/ 699	0.86 (0.40, 1.87)
severe	29/635	1.99 (1.01, 3.94)

\* Odds ratios were calculated with logistic regression adjusted for age, gender, total cholesterol, blood pressure, cognitive score, smoking and history of stroke and myocardial infarction. To test statistical significance of the categorical variables we calculated overall p values for depressive disorders with a test for trend. For carotid plaques we obtained p=0.20, for aortic calcifications p=0.06.

<sup>†</sup> The category "depressive disorders" includes cases with major or minor depression and dysthymia. Screen positive subjects with subclinical depressive symptoms, other psychiatric disorders or without a psychiatric work-up were excluded.

<sup>‡</sup> SD = Standard deviation.

<sup>§</sup> Peripheral arterial disease was defined as an ankle-brachial index below 0.90.

**TABLE 3: Relation between coronary calcifications and depression expressed as odds ratios\***

	Depressive disorders <sup>†</sup>	
	Cases/ Controls	Odds ratio (95% CI)
Coronary calcification: (0-100)	9/865	1.0 Referent
(101-500)	11/463	2.42 (0.97, 6.02)
(>500)	16/511	3.74 (1.51, 9.25)

\* Odds ratios were calculated with logistic regression adjusted for age, gender, total cholesterol, cognitive score, blood pressure, smoking and history of stroke. To test statistical significance of the association between coronary calcifications and depressive disorders we calculated the overall p value with a test for trend: p=0.004.

<sup>†</sup> The category "depressive disorders" includes cases with major or minor depression and dysthymia. Screen positive subjects with subclinical depressive symptoms, other psychiatric disorders or without a psychiatric work-up were excluded.

depressed persons). Additional adjustment for stroke and myocardial infarction moderately changed estimates.

**TABLE 4: The association between the measure of generalized extracoronary atherosclerosis and depression**

Principal component (per SD) of extra-coronary atherosclerosis measures	Depressive disorders*		
	Cases/ Controls	OR (95% CI)	p-value
Adjusted†	77/2798	1.30 (1.01, 1.66)	0.04
Additionally adjusted for stroke and myocardial infarction	77/2798	1.28 (1.01, 1.66)	0.05

CI = confidence interval, OR = Odds ratio, SD = Standard deviation.  
 In this complete case analysis only subjects with all four measurements of atherosclerosis were included.  
 \* The category "depressive disorders" includes cases with major or minor depression and dysthymia. Screen positive subjects with subclinical depressive symptoms, other psychiatric disorders or no psychiatric work-up were excluded.  
 † Odds ratio and 95% CI were calculated with logistic regression adjusted for age, gender, total cholesterol, cognitive score, systolic blood pressure, smoking.

## DISCUSSION

In this population-based study we found that subjects with atherosclerosis were more likely to be depressed. A combined measure of extracoronary atherosclerosis was related to depressive disorders although at some of the different locations the association was only moderate and non-significant. Further, we found a substantial relation of severe coronary and aortic calcifications with depressive disorders.

The strengths of this study are the large number of elderly people participating and its population-based design. Furthermore, the psychiatric work-up in subjects who were screen positive on the CES-D enabled us to determine in which group depressive symptoms were due to depressive disorders. A previous study in an elderly Dutch population reported a high sensitivity using the same cut off point and misclassification of disease is therefore unlikely to have influenced our results.<sup>11</sup>

Some methodological issues of this study must be discussed. First, this is a cross-sectional study and cannot demonstrate the chronology of the observed relationship. Second, the prevalence of subjects with depressive symptoms in this study (7.8%) was relatively low. However, it is comparable to the community prevalence observed by Blazer et al (1991) in the US (9.0%) who also used the CES-D.<sup>12</sup> Furthermore, the rate falls within the variable range recently

reported in a review of depressive symptoms in the elderly (2.8% to 35%).<sup>23</sup>

To our knowledge the present study is the first to examine the relation between measures of atherosclerosis and depressive disorders in community dwelling subjects. Many clinical studies have been performed in patients with pre-existing vascular disease.<sup>24-26</sup> These studies generally show a high risk of comorbid depression on survival after a cardiovascular event. So far few studies were conducted in community dwelling subjects.<sup>27,28</sup> A recent study in US Army personnel using electron beam tomography observed no correlation between psychological factors and coronary atherosclerosis.<sup>28</sup> We studied an elderly population which encompassed a very broad spectrum of the degree of atherosclerosis. This may help to detect an association not found in a homogenous population of middle aged men. Further, the previous population-based research, which investigated potential pathophysiological mechanisms, concentrated on personality traits rather than specific mood states.<sup>27-29</sup> It is not clear in how far a single trait (e.g. negative affectivity) can account for the associations of depressive disorders with increased cardiovascular morbidity. This makes it even more difficult to relate previous findings to the present study. Moreover, personality traits are much more stable than specific mood states such as depression. Studying distinct diseases has been advocated in order to identify potential treatments.<sup>9,30</sup>

Data from mostly cross-sectional studies suggest that cerebrovascular disease contributes to the development or persistence of depression.<sup>31</sup> These studies, few of which were population based, have utilized different neuro-imaging techniques.<sup>2,4,32-35</sup> It is assumed that the observed neuroradiological findings in depressed patients are due to vascular disease.<sup>3</sup> Our findings confirm the postulated link between vascular factors and depression. The present study cannot establish a causal role of atherosclerosis but provides evidence that a generalized atherosclerotic process accompanies late life depression. Although there are gaps in knowledge, the vascular depression hypothesis potentially has wide clinical implications.<sup>31</sup> Assessment of atherosclerosis in persons with late life depression and a treatment more akin to the management of cardiac disease and vascular dementia need to be investigated.<sup>36</sup>

Another explanation for our results needs to be discussed. Previously, longitudinal population-based studies established depression as a risk factor for cardiovascular disease. Depressive status at baseline was an independent risk factor for ischemic heart disease and death.<sup>5-7,37</sup> Numerous biological processes that have been associated with depression may underlie this increased

cardiovascular risk: decreased myocardial perfusion, abnormalities in platelet reactivity, cardiac arrhythmia, inflammatory processes and noradrenergic hyperactivity.<sup>38-40,41</sup> These pathophysiological changes are generally accompanied by increased atherosclerosis. Even if behavioral factors such as difficulties in smoking cessation or poor adherence to medical treatment account for the relation of depression with vascular disease one would expect to find increased levels of atherosclerosis in depressed persons. Our results are compatible with this notion. Increased cardiovascular mortality in depressed persons can be envisaged because depression was associated with generalized atherosclerosis in the present study population. The observed association was somewhat more marked for coronary calcifications. But this should be interpreted carefully because electron beam CT was not available in all subjects. Moreover, it may reflect the good assessment of atherosclerosis rather than indicating a specific cardiac process.

In summary, in this large study of community-dwelling elderly subjects we found that depression and atherosclerosis were related. Like other cardiovascular risk factors such as cholesterol or blood pressure, depressive disorders may be accompanied by a generalized atherosclerotic process. This suggests that intensive treatment of atherosclerosis or cardiovascular risk factors may prevent depressive disorders in late life. However, to further elucidate the pathophysiology underlying the association between depressive disorders and vascular disease prospective studies are needed that include different biological measures and psychiatric interviews.

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Arterial stiffness

## **ABSTRACT**

*Objective:* Neuroimaging studies suggest that vascular pathology may be a causal factor in late life depression. This study was performed to determine whether arterial stiffness is associated with depression in the elderly.

*Design:* Population-based cross-sectional study.

*Setting:* Ommoord, a suburb of Rotterdam, Netherlands.

*Participants:* 3704 subjects of the Rotterdam Study aged 60 years and over.

*Measurements:* We assessed arterial stiffness by the distensibility of the carotid artery and the carotid-femoral pulse wave velocity. All participants were screened for depressive symptoms with the Center of Epidemiological Studies Depression Scale. Those with depressive symptoms had a psychiatric work-up to establish a diagnosis of depressive disorders according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria.

*Results:* Participants with increased arterial stiffness were more likely to have depressive symptoms. Odds ratios for depressive symptoms were 1.24 (95% confidence interval (CI): 1.01, 1.52) per standard deviation decrease in carotid distensibility, and 1.17 (95%CI: 1.00, 1.38) per standard deviation increase in aortic pulse wave velocity. The association was stronger for depressive disorders meeting DSM-IV criteria (odds ratios 1.44 (95%CI: 1.03, 2.03) and 1.48 (95%CI: 1.16, 1.90), respectively). Control for atherosclerosis, as measured by the ankle-to-brachial index or presence of plaques in the carotid artery, did not change the associations.

*Conclusions:* This study shows an association between arterial stiffness and depression in the elderly. The findings are compatible with the vascular depression hypothesis. Our data suggest that the proposed relation between vascular factors and depression may partly be caused by arterial stiffness.

## INTRODUCTION

Depression is an important health problem in older people. The causes of depression in the elderly are poorly understood but current research puts particular emphasis on biological risk factors. It has been suggested that vascular pathology may cause depression in the elderly.<sup>1</sup> A "vascular depression hypothesis" was introduced by Alexopoulos.<sup>2</sup> He postulated that late life depression encompasses a high percentage of patients with cerebrovascular disease. While few clinical differences exist between early and late-onset depression the hypothesis has been supported by neuroimaging studies showing that persons with late-onset depression have more vascular abnormalities than non-depressed.<sup>3</sup> Research on measures of atherosclerosis and cerebrovascular risk factors has yielded mixed results.<sup>4</sup> Factors such as smoking, hypertension and atrial fibrillation were not found more frequently in late onset depression.

The level of arterial stiffness is an important characteristic of the vascular system and increased arterial stiffness is related to cerebrovascular disease independent of atherosclerosis.<sup>5,6</sup> Furthermore, increased arterial stiffness is a determinant of blood pressure. Low blood pressure has repeatedly been associated with depressive symptoms in the elderly, but the underlying mechanism remains unclear.<sup>7,8</sup> We hypothesized that arterial stiffness could mediate the associations between vascular pathology and depression. Therefore, we investigated the relation between arterial stiffness and depression in a large population-based study among elderly subjects.

## SUBJECTS AND METHODS

### Study population

This study was conducted as part of the Rotterdam Study, a population based study that is ongoing since 1990. All inhabitants aged 55 years and over of a district of Rotterdam were invited at baseline; 7983 persons participated. In the third survey (1997-1999) we added assessments of depressive symptoms and arterial stiffness to the study protocol. Measurements included a home interview and a visit to the research center. Of the 4703 persons (80% response) who participated in this examination round, 3704 were screened for depressive symptoms and had at least one measurement of arterial

stiffness performed. Common carotid artery distensibility was measured in 3014 and carotid-femoral pulse wave velocity in 3374 participants. The 999 subjects in whom arterial stiffness was not assessed were on average older (77.2 vs. 72.1 years), more likely to be female (67% vs. 58%), and had more depressive symptoms (11.0% vs. 6.7%, overall prevalence 7.8%). The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants.

### **Depression assessment**

Depressive disorders were assessed using a two step procedure. First participants completed the Dutch version of the original Center for Epidemiology Studies Depression scale. This is a 20-item self-reported measure of symptoms scored on a scale of 0 to 3 points. We used a score of 16 as a cut-off to indicate clinically relevant depressive symptoms. Beekman et al. (1997) reported a very high sensitivity of this cut-off for major depression in older subjects in the Netherlands.<sup>9</sup> Second, screen positive subjects had a psychiatric work-up using the Dutch version of the Present State Examination (PSE-10), a semi-structured psychiatric interview included in the Schedules for Clinical Assessment in Neuropsychiatry.<sup>10</sup> All interviews were conducted by two experienced clinicians. Psychiatric disorders were classified according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria. Of the 3704 subjects included in this study 250 (6.7%) were screen-positive. Psychiatric disorders were subsequently assessed in 234 (93.6%) participants. The diagnostic categories of depression included dysthymia, major and minor depression as defined in the appendix of the DSM-IV.

### **Measures of arterial stiffness**

Both common carotid arterial and aortic stiffness were measured at the research center. The distensibility coefficient of the right common carotid artery was used as a measure of common carotid arterial stiffness. The vessel motion was measured by means of Doppler scanner (Ultramark IV, ATL, Bothell, Washington, USA). The arterial wall distensibility coefficient was calculated by dividing the relative stroke change in the diameter of the arterial wall through the pulse pressure.<sup>11</sup> Pulse pressure was defined as systolic blood pressure minus diastolic blood pressure.

Carotid-femoral pulse wave velocity was assessed using an automatic device (Complior, Colson, Garge-lès-Gonesse Cx, France). The time delay

between the rapid upstroke of simultaneously recorded pulse waves in the carotid artery and the femoral artery was measured. Pulse wave velocity was calculated as the ratio between the distance traveled by the pulse wave and the time delay.<sup>12</sup> It was expressed in meters per second. The average of at least 10 successive measurements was used in the analyses.

### **Other measurements**

The following variables were considered as possible confounding variables: age, gender, education, smoking, cognitive function, antihypertensive, antidepressant medication, body mass index, total and HDL-cholesterol, diabetes mellitus and a history of stroke. Educational level was assessed on an ordinal scale but dichotomized for analysis according to whether only primary education was attained. Cigarette smoking was analyzed in categories of current and former smoker. Cognitive function was measured by the Mini Mental State Examination. Information on current antidepressant or antihypertensive medication was obtained during the home interview and included a cabinet check, but treatment indication was not ascertained. Body mass index was computed as weight in kg divided by height in m<sup>2</sup>. Overnight fasting blood samples were taken and serum total and HDL-cholesterol was measured using an automated procedure. Diabetes mellitus was defined as the use of insulin or oral blood glucose lowering drugs, or serum glucose concentrations of more than 11.0 mmol/l. A history of stroke was obtained from all subjects through direct questioning and computerized linkage with general practitioner medical records, and verified by a neurologist.

Because the effects of arterial stiffness might depend on those of atherosclerosis and blood pressure as risk factors for depression we included measures of atherosclerosis and blood pressure in the present study. Atherosclerosis was assessed by three different measures: ankle-to-brachial index, the presence of plaques in the carotid arteries assessed by ultrasonography,<sup>13</sup> and presence of aortic atherosclerosis, which was determined on a lateral abdominal x-ray.<sup>14</sup> Ankle-to-brachial index was dichotomized using a threshold value of 0.90 that indicates peripheral arterial disease.<sup>15</sup>

### **Statistical analysis**

The association of arterial stiffness with depressive symptoms and depressive disorders was assessed using logistic regression and expressed as odds ratios with 95% confidence intervals. Age (continuously in years), gender, mean

arterial blood pressure (in mm Hg) and heart rate were controlled for in all analyses. We assessed measures of arterial stiffness in quartiles of the distribution. Common carotid distensibility and pulse wave velocity were also entered into the logistic regression model as a continuous variable divided by one standard deviation. For analyses on depressive disorders according to DSM-IV criteria screen positive subjects who did not fulfil the criteria were excluded. Additionally we performed an analysis excluding subjects on anti-depressant medication without depressive symptoms from the reference group.

To check for confounding we added all potential confounders to the basic model. In further analysis we additionally adjusted for measures of atherosclerosis. A change in estimate may indicate that part of the effect of arterial stiffness can be attributed to atherosclerosis. Subsequently we studied whether any relation between atherosclerosis and depression was independent of arterial stiffness. We also performed analyses stratified for gender. Finally, the relationship between blood pressure and depression was assessed by logistic regression. In an additional analysis we checked whether this association was curvilinear by entering a quadratic term in the model.

## RESULTS

Of the 234 screen positive subjects with a psychiatric work-up 96 had a depressive disorder as defined by the DSM-IV. The remaining subjects either had an anxiety disorder or other psychiatric disease (n=20), or did not meet criteria for an Axis I psychiatric disorder (n=118, subclinical depressive symptoms). Table 1 presents characteristics of the reference subjects, the screen positive subjects with depressive symptoms and the subjects with DSM-IV depressive disorders. Female gender, low cognitive score, smoking, low diastolic blood pressure, use of antidepressant medication and aortic calcifications were more frequently observed in subjects with depressive symptoms.

Depressive symptoms as defined by a score of 16 and above on the Center for Epidemiology Studies Depression scale were significantly associated with a decrease in distensibility of the common carotid artery; the association with pulse wave velocity was borderline significant. Subjects with increased arterial stiffness (upper quartiles) were more likely to have depressive symptoms. Table 2 shows the odds ratios per quartile of arterial stiffness adjusted for age,

**TABLE 1: Characteristics of the study subjects**

Variables	Reference subjects: no depressive symptoms (n=3454)	Depressive symptoms <sup>†</sup> (n=250)	Depressive disorders <sup>†</sup> (n=96)
Age, years, mean (range)	72.0 (61-101)	73.5** (61-93)	72.7 (61-87)
Gender, % female	56.5	73.2***	71.9**
Primary education only, %	46.8	55.6	53.1
MMSE-score, mean ( $\pm$ SD)	27.7 (2.0)	26.9* (2.7)	26.5** (3.3)
History of stroke, %	2.8	6.0**	5.2
Smoking current smoker, %	15.5	19.6*	19.8
ex-smoker, %	51.0	42.0	40.6
Peripheral arterial disease, %	16.7	20.3	18.5
Antidepressant medication, %	2	10***	13***
Diabetes mellitus, %	8.6	10.4	8.3
Carotid plaques, %			
0	30	29	26
1-4	50	52	54
5-8	17	18	16
$\geq 9$	3	2	4
Aortic calcifications, %			
<1 cm	22	18*	19
1-2,5 cm	35	30	32
2,5-5 cm	38	49	44
>5 cm	4	4	5
Heart rate, mean ( $\pm$ SD)	75 (14)	77 (15)	75 (13)
Total cholesterol, mmol/l, mean ( $\pm$ SD)	5.8 (1.0)	5.9 (0.9)	5.9 (1.0)
HDL-cholesterol, mmol/l, mean ( $\pm$ SD)	1.4 (0.4)	1.5 (0.4)	1.4 (0.4)
Body mass index, kg/m <sup>2</sup> , mean ( $\pm$ SD)	26.8 (4.0)	26.9 (4.9)	26.4 (3.8)
Diastolic blood pressure, mmHg, ( $\pm$ SD)	75 (11)	73* (11)	73 (12)
Systolic blood pressure, mmHg, ( $\pm$ SD)	143 (21)	142 (22)	141 (23)
Pulse pressure, mmHg, mean ( $\pm$ SD)	68 (17)	69 (18)	68 (19)
Distensibility coefficient of common carotid artery, 10 <sup>-3</sup> /kPa, mean ( $\pm$ SD)	10.6 (4.4)	9.5* (3.9)	9.2* (3.7)
Pulse wave velocity, m/s, mean ( $\pm$ SD)	13.5 (3.0)	13.9 (3.4)	14.3** (3.6)

\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001; ANCOVA or logistic regression adjusted for age and gender were appropriate; carotid plaques and aortic calcifications were tested for overall significance and not per category.

SD = Standard deviation, MMSE = Mini Mental State Examination

<sup>†</sup> The category depressive symptoms includes all subjects with a Center of Epidemiological Studies Depression scale (CES-D) score  $\geq 16$ . The category depressive disorders includes only the cases with major or minor depression and dysthymia.

**TABLE 2: The relation between quartiles of arterial stiffness and depression expressed as odds ratios**

Measure of arterial stiffness	Number of reference subjects	Depressive symptoms*			Depressive disorder*		
		Number of cases	OR (95% CI)	p-value	Number of cases	OR (95% CI)	p-value
Distensibility coefficient of common carotid artery (range in 10 <sup>-3</sup> /kPa)							
Quartile I (< 7.4)	687	66	1.9 (1.1; 3.2)	p=0.03	31	3.1 (1.3; 7.4)	p=0.01
Quartile II (7.4-9.9)	693	61	1.9 (1.2; 3.1)	p=0.01	21	2.2 (1.0; 4.9)	p=0.06
Quartile III (9.9-12.9)	702	52	1.6 (1.0; 2.6)	p=0.04	22	2.2 (1.0; 4.8)	p=0.05
Quartile IV (≥12.9)	722	31	1.0 (reference)		10	1.0 (reference)	
Pulse wave velocity (range in m/s)							
Quartile I (<11.4)	796	48	1.0 (reference)		13	1.0 (reference)	
Quartile II (11.4-13.2)	804	41	0.9 (0.6; 1.4)	p=0.58	18	1.5 (0.7; 3.2)	p=0.26
Quartile III (13.2-15.2)	779	63	1.4 (0.9; 2.2)	p=0.10	27	2.5 (1.2; 5.3)	p=0.01
Quartile IV (>15.2)	776	67	1.5 (0.9; 2.4)	p=0.10	27	2.6 (1.2; 5.8)	p=0.02

Logistic regression adjusted for age, gender, mean arterial blood pressure, heart rate, smoking, history of stroke and cognitive score. CI = confidence interval; OR = odds ratio.

Overall p values for categories of carotid distensibility were 0.07 and 0.08 for depressive symptoms and depressive disorders, respectively, for pulse wave velocity these were both 0.05.

\* The category depressive symptoms includes all subjects with a CES-D score ≥ 16. The category depressive disorders includes only the cases with major or minor depression and dysthymia.

**TABLE 3: The association between measures of arterial stiffness and depression adjusted for atherosclerosis (odds ratios and 95% CI per standard deviation in measure of arterial stiffness)**

Measure of arterial stiffness	Model	Depressive symptoms*			Depressive disorder*		
		Number of cases	OR (95% CI)	p-value	Number of cases	OR (95% CI)	p-value
Common carotid artery distensibility decrease (per SD)	Model 1 <sup>†</sup>	210	1.27 (1.03; 1.57)	0.03	83	1.44 (1.03; 2.03)	0.03
	Model 2 <sup>‡</sup>	201	1.29 (1.04; 1.60)	0.02	79	1.46 (1.03; 2.07)	0.03
	Model 3 <sup>§</sup>	176	1.19 (0.94; 1.49)	0.147	67	1.34 (0.92; 2.00)	0.13
Pulse wave velocity increase (per SD)	Model 1	219	1.19 (1.00; 1.40)	0.05	85	1.48 (1.16; 1.90)	0.0002
	Model 2	210	1.20 (1.01; 1.44)	0.04	81	1.48 (1.14; 1.90)	0.0004
	Model 3	183	1.12 (0.92; 1.36)	0.24	67	1.34 (1.00; 1.76)	0.05

CI = confidence interval; OR = odds ratio; SD = standard deviation

\* The category depressive symptoms includes all subjects with a CES-D score  $\geq 16$ . The category depressive disorders includes only the cases with major or minor depression and dysthymia.

<sup>†</sup> Logistic regression adjusted for age, gender, mean arterial blood pressure, heart rate, smoking, history of stroke, pulse pressure, diabetes, total and HDL-cholesterol, body mass index, antidepressant medication and cognitive score. Subjects without depressive symptoms were used as the reference group.

<sup>‡</sup> As model 1, but additionally adjusted for total carotid plaque score and peripheral artery disease.

<sup>§</sup> As model 1, but additionally adjusted for aortic atherosclerosis.

gender, blood pressure heart rate, smoking, history of stroke and cognitive score. Entering any other covariate into the models did not alter the estimates. The analyses using the categorical variables did not suggest substantial threshold effects.

In the analyses shown in table 3 arterial stiffness is used as a continuous variable. Odds ratios for depressive symptoms were 1.27 (95% confidence interval [CI]: 1.03, 1.57,  $p=0.03$ ) per standard deviation decrease in carotid distensibility, and 1.19 (95% CI: 1.00, 1.40,  $p=0.05$ ) per standard deviation increase in aortic pulse wave velocity. Table 3 also shows that this association was independent of peripheral arterial disease and atherosclerosis as measured by carotid plaques, but controlling for aortic calcifications reduced the point estimates. The association of both measures of arterial stiffness with DSM-IV depressive disorders was stronger than with depressive symptoms (Tables 2 and 3). Further, we observed only a modest reduction in estimates for depressive disorders when we adjusted for atherosclerosis (Table 3). Excluding subjects with antidepressant medication did not change the results (data not shown). To further explore our data we subsequently checked whether the relation of aortic calcifications with depressive symptoms (odds ratio for moderate and severe calcifications: 1.60, 95% CI: 1.05, 2.35;  $p=0.03$ ) was independent of arterial stiffness. We observed a substantial reduction of the association after adjustment for carotid-femoral pulse wave velocity as well as after adjustment for common carotid artery distensibility. The respective odds ratios of aortic calcifications did not reach significance anymore (1.30, 95% CI: 0.83, 2.04,  $p=0.24$  and 1.41, 95% CI: 0.93, 2.14,  $p=0.10$ ).

Subjects with low diastolic blood pressure had an increased risk to be depressed. However, this relation reached significance only for depressive symptoms and not for depressive disorders. Odds ratios were 0.88 per 10 mmHg (95% CI: 0.78, 0.99,  $p=0.04$ ) and 0.84 (95% CI: 0.70, 1.02,  $p=0.07$ ), respectively. Furthermore, we also computed the odds ratios of systolic blood pressure (0.94 per 10 mmHg, 95% CI: 0.89, 1.00,  $p=0.06$  for depressive symptoms and 0.92, 95% CI: 0.84, 1.02,  $p=0.12$  for depressive disorders). We found no curvilinear associations and observed no difference in pulse pressure between subjects with and without depression (data not shown).

## DISCUSSION

This large population based study showed that arterial stiffness is associated with depressive symptoms in the elderly. The strongest associations were found in subjects who fulfilled criteria for a DSM-IV depressive disorder. Participants with a reduced common carotid artery distensibility or an increased carotid-femoral pulse wave velocity were more likely to have a depressive disorder after the effects of age, sex, history of stroke, smoking and cognitive function were controlled for.

Some limitations of the present study need to be discussed. Firstly, the prevalence of subjects with depressive symptoms in this study (7.8%) was relatively low. However, it is within the range (2.8% to 35%) reported in a recent review<sup>16</sup> and is comparable to the prevalence in the US (9.0%) observed by Blazer (1991).<sup>17</sup> Further, subjects with depressive disorders were slightly under-represented in the analytical sample because they were less likely to visit the research center. This reduced the power to detect significant associations, but we think it is unlikely that the observed relation between arterial stiffness and depression was due to this selection effect.

Secondly, in analyses with arterial distensibility adequate correction for blood pressure is of utmost importance.<sup>12</sup> Higher blood pressure stretches the arterial wall and makes arteries less distensible. Because of the association of mean arterial blood pressure with stiffness we adjusted all analyses for this variable. However, high blood pressure also leads to structural changes in the arterial wall and therefore is an important determinant of arterial stiffness. Possibly, adjustment for mean arterial pressure is an over-correction. This might have attenuated the association between arterial stiffness and depressive disorder.

Strength of the present large, population-based study is the psychiatric work-up in subjects who were screen positive on the depression scale. We were able to determine in which group depressive symptoms were due to depressive disorders and perform analyses along the worsening spectrum of affective illness. Moreover, misclassification of disease is unlikely to have influenced our results. Studies in psychiatric epidemiology are frequently based on measurements of depressive symptoms only and include an inhomogeneous group of subjects with subclinical depressive symptoms and other psychiatric diseases.

One possible explanation for the association of arterial stiffness with

depressive disorder is the relation between arterial stiffness and atherosclerosis.<sup>18</sup> Atherosclerosis has been suggested as a risk factor for depression in late life and depressive symptoms itself are related to subsequent cardiovascular disease.<sup>19,20</sup> The vascular depression hypothesis postulates that structural changes in the brain due to atherosclerosis are of primary importance in late life depression.<sup>21</sup> However, indicators of extracerebral vascular changes have not been studied in this context. Research on depressive symptoms as a risk factor for cardiac morbidity, on the other hand, has concentrated on altered heart rate variability, platelet function, immunological factors and associations with other cardiac risk factors such as smoking.<sup>22</sup> The few studies investigating atherosclerosis have reported conflicting results and have not included a diagnosis of depression.<sup>23,24</sup> Likewise, the association found in the present study is not explained by atherosclerosis alone. Neither correction for carotid plaques nor correction for peripheral arterial disease changed the point estimates. Subclinical atherosclerosis as indicated by carotid plaques may actually reduce stiffness.<sup>25</sup> In our study only the adjustment for aortic calcifications reduced the observed association between arterial stiffness and depressive symptoms. However, aortic calcifications may reflect arterial stiffness as well as atherosclerosis. The presence of atheromatous plaques in the aorta is strongly correlated with vessel stiffness.<sup>26</sup> Moreover, the association of aortic calcifications with depressive symptoms found in this study was not independent of arterial stiffness. We may thus have overadjusted by adding aortic calcifications to the model. Adjusting for carotid plaques and peripheral arterial disease was probably adequate to control for the effect of atherosclerosis.

Another possible factor that could explain our findings is increased pulse pressure. Arterial stiffness leads to an increase in systolic blood pressure and simultaneously a decrease in diastolic blood pressure, hence increased pulse pressure. A high pulse pressure is a strong risk factor for cerebrovascular disease.<sup>27</sup> However, we did not observe a relation between pulse pressure and depressive symptoms. Instead, both a low diastolic and a low systolic blood pressure were associated with depressive symptoms. This could be explained by the fact that a high degree of medical comorbidity is reported in elderly depressives, which is frequently associated with low mean blood pressure.<sup>7,8,28</sup>

This is a cross-sectional study that can not demonstrate the chronology of the observed relationship. The etiological arrow may go from arterial stiffness

to depressive symptoms as well as the other way round. However, the present study sheds light on a possible mechanism underlying the association between vascular pathology and depression. Further studies on the mechanism may help identify the elderly depressed patients in whom vascular disease is important and who might benefit from specific interventions.<sup>1</sup> To measure pulse wave velocity may be the most practical approach in view of technical convenience, the cost of equipment and manpower, whereas measurement of aortic calcifications involves x-rays. If arterial stiffness plays a role, ACE inhibitors and calcium channel blockers, which decrease stiffness, may prevent late onset depression. Furthermore, drugs that increase endothelial nitric oxide and thus arterial compliance are of potential benefit. In this context a link between blood pressure, arterial stiffness and vascular depression is especially relevant. On the one hand, intensified antihypertensive treatment as a secondary prevention of vascular depression is discussed in the USA.<sup>29</sup> On the other hand, a sizeable proportion of doctors in European countries treat a "hypotensive syndrome". Patients with fatigue, depressive symptoms and low blood pressure are prescribed ephedrine, derivatives of ergot or amphetamine.<sup>7,8</sup>

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3.3

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## Cerebral haemodynamics

**ABSTRACT**

*Background:* Evidence from epidemiological and neuroimaging studies suggests that cerebrovascular disease is associated with depressive disorders in the elderly, but the extent to which it contributes to the pathogenesis of late life depression is unclear.

*Objective:* To investigate the relation between cerebral haemodynamics and depression in a population based study, using transcranial Doppler ultrasonography.

*Methods:* Cerebral blood flow velocity and CO<sub>2</sub> induced vasomotor reactivity in the middle cerebral artery were measured in 2093 men and women who participated in the Rotterdam study. All subjects were screened for depressive symptoms using the Center of Epidemiological Studies Depression scale, and those with a score of 16 or over had a psychiatric work up. In a semistructured interview, diagnoses of depressive disorders according to the DSM-IV and subthreshold depressive disorder were established. Analyses of covariance controlled for age, sex, stroke, cognitive score, and cardiovascular risk factors were used to compare means of haemodynamic variables.

*Results:* Subjects with depressive symptoms had reduced blood flow velocities (mean difference, -2.9 cm/s; 95% confidence interval (CI), -5.0 to -0.8; p = 0.008) and lower vasomotor reactivity (mean difference -0.5%/kPa; 95% CI, -1.0 to -0.05; p = 0.03). Blood flow velocity was reduced most in subjects suffering from a DSM-IV depressive disorder (mean difference, -4.9 cm/s; 95% CI, -8.5 to -1.4; p = 0.006). The overall reduction in vasomotor reactivity was accounted for by subjects with subthreshold depressive disorder.

*Conclusions:* Depression in late life is associated with cerebral haemodynamic changes that can be assessed by transcranial Doppler ultrasonography. The observed reduction in cerebral blood flow velocity could be a result of reduced demand in more seriously depressed cases with a DSM-IV disorder, whereas reduced CO<sub>2</sub> induced cerebral vasomotor reactivity is a possible causal factor for subthreshold depressive disorder.

## INTRODUCTION

The “vascular depression” hypothesis postulates that in late life vascular pathology contributes significantly to the pathogenesis of depression.<sup>1</sup> Evidence from epidemiology, genetics, and neuropsychology suggests that cerebrovascular changes may lead to depressive disorders.<sup>2-6</sup> Strong evidence comes in particular from neuroimaging studies.<sup>2,7</sup> These studies have focused on magnetic resonance imaging (MRI) hyperintensities, but single photon emission tomography (SPECT) and positron emission tomography (PET) have also been used to provide information on the function of the brain in depressive disorders.<sup>8-10</sup> In studies using MRI, white matter hyperintensities in the basal ganglia and the frontal lobes were reported. These lesions are attributed to vascular disease, but other causes cannot be ruled out.<sup>1</sup> In clinical studies employing SPECT, regional differences in cerebral blood flow were reported between subjects with depressive disorders and clinical controls.<sup>11-14</sup> It remains unclear whether low blood flow is a result of depression, reflects cerebrovascular disease, or marks neuronal loss predisposing to depression.<sup>15</sup>

With transcranial Doppler ultrasonography it is possible to assess the intracranial circulation directly. Aaslid et al first described a non-invasive Doppler ultrasound technique in 1982, which took advantage of the relatively thin temporal bone.<sup>16</sup> Transcranial Doppler is a useful tool for detecting the haemodynamic changes resulting from cerebrovascular impairment, despite the limitation that the temporal bone often cannot be passed by ultrasound.<sup>17,18</sup> Changes in both vasomotor reactivity and blood flow velocity are commonly measured in the basal cerebral arteries by transcranial Doppler. Vasomotor reactivity is reduced in patients with cerebral microangiopathy,<sup>17</sup> while changes in cerebral artery blood velocities reflect changes in blood flow and brain tissue perfusion.<sup>19-21</sup>

The Rotterdam study provided an opportunity to examine the relation between cerebral haemodynamics and depression in a population based sample of older adults. We conducted this study to evaluate whether cerebrovascular impairment, as measured by transcranial Doppler, is related to depression. Our *a priori* hypothesis was that subjects with depressive disorders have a reduced flow velocity and reduced vasomotor reactivity.

## METHODS

### Subjects

The investigation was conducted as part of the Rotterdam study, a population based cohort study ongoing since 1990, in which all inhabitants aged 55 and over in a suburb of Rotterdam were invited to participate.<sup>22</sup> In the third survey we added assessments of depressive symptoms and cerebral haemodynamics to the protocol. Measurements were conducted between 1997 and 1999. Screening for depressive symptoms was carried out during the home interview part of the survey, in which 4730 subjects participated.

In 3101 consecutive participants we attempted to perform transcranial Doppler ultrasonography as part of the standard clinical investigation at the research centre. No transcranial Doppler measurements were carried out in the remainder ( $n = 1629$ ), owing to the unavailability of a technician. In 990 subjects (32%), transcranial Doppler was undertaken but no results were obtained. In most cases this was because of window failure on both sides ( $n = 771$ ) or because restlessness or discomfort prevented the study being done ( $n = 36$ ). In 183 participants haemodynamic measurements could not be carried out for other reasons, such as ambiguous flow direction or lack of time.<sup>19,23</sup> Subjects in whom no transcranial Doppler data were obtained were older ( $p < 0.001$ ) and more likely to be female ( $p < 0.001$ ). The prevalence of psychiatric symptoms in the analytical sample was found to be lower than in the study population as a whole (5.5% v 7.8%). A further 18 subjects were excluded because they did not have complete screening for depression. The final study sample consisted of 2093 participants in whom adequate depression and haemodynamic indices were obtained.

The Rotterdam Study was approved by the medical ethics committee of Erasmus University Medical School. After complete description of the study to the subjects, written informed consent was obtained.

### Depression assessment

Depressive disorders were assessed using a two step procedure. First, participants completed the Dutch version of the original Center for Epidemiology Studies Depression scale (CES-D) during the home interview. The CES-D is a 20 item self reported measure of symptoms experienced in the past week, scored on a scale of 0 to 3 points. The validity of the CES-D has been well established.<sup>24</sup> We used a score of  $\geq 16$  as a cut off point, as this has been found to

have a high sensitivity for major depression in a random sample of older subjects in the Netherlands.<sup>25</sup> Previous studies have verified that a score of 16 and above on the CES-D represents clinically significant depressive symptoms.<sup>26</sup>

In a second step, screen positive subjects had a psychiatric work up. They were evaluated by the Dutch version of the present state examination (PSE-10), a semistructured psychiatric interview included in the *Schedules for Clinical Assessment in Neuropsychiatry*.<sup>27</sup> All interviews were conducted by two experienced clinicians. Psychiatric disorders were classified according to the DSM-IV criteria, with an algorithm based on the PSE-10 scores. The diagnostic categories include minor depression, as defined in the appendix of DSM-IV.

Of the 2093 subjects included in the analyses, 116 (5.5%) were screen positive for depression as measured by the CES-D. A psychiatric work up was performed in 111 of these participants (95.6%). Four subjects refused to participate in this evaluation, and one screen positive subject could not be reached. A depressive disorder as defined by the DSM-IV criteria was established in 42 cases. The remaining subjects were either classified as having anxiety disorders or other psychiatric disease ( $n = 8$ ) or did not meet criteria for an axis I psychiatric disorder ( $n = 61$ , subthreshold depressive disorder).

To define late onset depression we used the data from the baseline interview with a physician. All subjects in the present analysis responded to the questions about psychiatric history. Participants who reported a history of depression before the age of 60 were considered to be suffering from early onset depression.

### **Transcranial Doppler assessment**

Transcranial Doppler ultrasonography was done using a Multi-Dop X-4 instrument (DWL, Sipplingen, Germany), and the cerebral blood flow velocity (cm/s) was measured in the middle cerebral artery on both sides if possible. End diastolic, peak systolic, and mean cerebral blood flow velocity were recorded automatically.

CO<sub>2</sub>-induced cerebral vasomotor reactivity measurements were done as follows. The cerebral blood flow velocity was measured continuously and the participants first breathed room air through an anaesthetic mask, tightly fitted over mouth and nose, until a steady expiratory end tidal CO<sub>2</sub> was obtained. Next, participants inhaled a mixture of 5% carbon dioxide in 95% oxygen for two minutes. Cerebral vasomotor reactivity was defined as the percentage increase in cerebral blood flow velocity occurring during inspiration of 5% CO<sub>2</sub>,

divided by the absolute increase in end tidal  $\text{CO}_2$  in the same period (%kPa). End tidal  $\text{pCO}_2$  (kPa) was recorded continuously with a  $\text{CO}_2$  analyser (Multi-nex, Datascope, Hoevelaken, Netherlands). End expiratory  $\text{CO}_2$  was assumed to reflect arterial  $\text{CO}_2$ . TCD-8 DWL special software (VMR- $\text{CO}_2$ ) was used. All transcranial Doppler data were stored on hard disc for off-line analysis. The mean of the right and left haemodynamic variables was used for analyses if both middle cerebral arteries could be insonated adequately. A one sided haemodynamic variable was used if there was unilateral window failure.

### **Measurements of other covariates**

The following were considered to be possible confounding variables: age, sex, education, cognitive function (measured by the mini mental state examination (MMSE)), antihypertensive treatment, and antidepressant treatment. Education was measured on an ordinal scale and later dichotomised into low and high. Information on current antidepressant or antihypertensive drug treatment was obtained during the home interview. The following cardiovascular risk factors were assessed: stroke, smoking, systolic and diastolic blood pressure, diabetes mellitus, and total cholesterol. A history of stroke was obtained from all subjects through direct questioning and computerised linkage with general practitioner medical records. The history was considered positive when confirmed by a physician. Cigarette smoking was analysed in categories of current and former smoker. Sitting blood pressure was measured twice on the right arm with a random zero sphygmomanometer. Diabetes mellitus was defined as the use of insulin or oral blood glucose lowering drugs, or serum glucose concentrations of more than 11.0 mmol/l. Fasting blood samples were taken and serum total cholesterol was measured using an automated enzymatic procedure. The ankle to brachial index was used as an indicator of peripheral atherosclerosis. We assessed ankle to brachial index by taking the ratio of the systolic blood pressure measured at the tibial artery to that measured at the right arm. Subjects with an ankle to brachial index of less than 0.9 were considered to suffer from peripheral arterial disease.<sup>28</sup>

### **Statistical analysis**

The associations between haemodynamic variables and depressive disorders were addressed in three ways.

First, analysis of covariance (ANCOVA) was used to calculate means of the screen positive subjects and the reference group, adjusted for age and sex.



## RESULTS

Table 1 compares the demographic characteristics and confounding variables of the 116 participants who were screen positive and the 1977 participants who were screen negative for depression. Age, sex, education, a history of major depression before age 60, previous stroke, and cognitive function were all associated with current depressive symptoms. However, the cardiovascular risk factors smoking, diabetes, systolic and diastolic blood pressure, and total cholesterol were not related to depressive symptoms.

Subjects with depressive symptoms as determined by the CES-D had a lower mean cerebral blood flow velocity (age and sex adjusted mean difference, -2.9 cm/s; 95% confidence interval (CI), -5.0 to -0.8;  $p = 0.007$ ) and reduced vasomotor reactivity (age and sex adjusted mean difference, -0.7 %/kPa; 95% CI, -1.2 to -0.2;  $p = 0.008$ ). Table 2 shows the relation between cerebral haemodynamic variables and depressive symptoms, with additional adjustment for education, history of stroke, antidepressant and antihypertensive drug treatment, cognitive function, and cardiovascular risk factors.

In a further analysis, screen positive subjects were classified according to the severity of the depressive symptoms (table 3). Subjects with subthreshold depressive disorder and DSM-IV depressive disorders were included as dis-

**TABLE 2: Association between cerebral hemodynamic parameters and depressive symptoms\***

	Non-depressed (n=1977)		CES-D score $\geq 16$ (n=116)	
	mean	mean	adjusted difference (95% CI)	p-value
Blood flow velocity (cm/s)				
- end diastolic	32.5	29.6	-2.3 (-3.9; -0.7)	$p=0.005$
- mean	50.5	47.8	-2.9 (-5.0; -0.8)	$p=0.008$
- peak systolic	86.5	82.5	-4.2 (-7.6; -0.7)	$p=0.02$
Vasomotor reactivity (%/kPa)	3.9	3.1	-0.5 (-1.0; -0.05)	$p=0.03$

\*Analysis of covariance with hemodynamic parameters entered as continuous variables and adjusted for age, gender, education, cognitive function, smoking, systolic and diastolic blood pressure, antidepressant and antihypertensive medication, total cholesterol, diabetes mellitus, and history of stroke.

Values represent unadjusted means and adjusted differences (95% CI), pairwise comparison with non-depressed reference group.

**TABLE 3: Association between cerebral hemodynamic parameters and depression\***

	Non-depressed (n=1929)		Subthreshold depressive disorder (n=59)		Depressive disorders (n=40)	
	mean		mean	adjusted difference	mean	adjusted difference
Blood flow velocity (cm/s)						
- end diastolic	32.5		30.6	-1.0 (-3.1; 1.2)	27.8	-4.0 (-6.6; -1.4)
- mean	50.5		48.5	-2.0 (-4.8; 0.9)	44.9	-4.9 (-8.5; -1.4)
- peak systolic	86.5		84.1	-4.0 (-8.7; 0.8)	79.5	-6.8 (-12.5; -1.0)
Vasomotor reactivity (%/kPa)	3.9		2.7	-0.9 (-1.6; -0.2)	3.4	-0.3 (-1.1; 0.6)

\*Analysis of covariance with hemodynamic parameters entered as continuous variables and adjusted for age, gender, education, cognitive function, smoking, systolic and diastolic blood pressure, antidepressant and antihypertensive medication, total cholesterol, diabetes mellitus, Subjects with stroke were excluded.

Values represent unadjusted means and adjusted differences (95% CI), pairwise comparison with non-depressed reference group, same covariates.

**TABLE 4: The relation between tertiles of cerebral hemodynamic parameters and depression expressed as odds ratios**

Tertile	Number of subjects	Odds ratios* (95% CI)		
		CES-D score $\geq 16$ (n=116)	Subthreshold depressive disorder† (n=59)	Depression† (n=40)
Mean blood flow velocity (range in cm/s)				
1st (54.4-112)	692	1.0 (reference)	1.0 (reference)	1.0 (reference)
2nd (44.8-54.3)	697	1.2 (0.7;2.0)	1.1 (0.6;2.1)	1.2 (0.5;2.9)
3rd (14.8-44.7)	703	1.9 (1.2;3.1)	1.5 (0.8;2.7)	2.6 (1.2;5.8)
End diastolic blood flow velocity (range in cm/s)				
1st (35.6-83.5)	694	1.0 (reference)	1.0 (reference)	1.0 (reference)
2nd (28.1-35.5)	692	1.0 (0.6;1.7)	0.8 (0.4;1.6)	1.2 (0.5;2.8)
3rd (8.0-28.0)	705	1.8 (1.1;2.9)	1.2 (0.7;2.3)	2.5 (1.1;5.5)
Peak systolic blood flow velocity (range in cm/s)				
1st (93.6-170)	695	1.0 (reference)	1.0 (reference)	1.0 (reference)
2nd (77.0-93.5)	692	1.3 (0.8;2.1)	1.2 (0.6;2.2)	1.2 (0.5;2.7)
3rd (37.0-77.0)	706	1.7 (1.0;2.7)	1.5 (0.8;2.7)	2.0 (0.9;4.3)
Vasomotor reactivity (range in %/kPa)				
1st (4.48-22.9)	669	1.0 (reference)	1.0 (reference)	1.0 (reference)
2nd (2.67-4.47)	674	2.2 (1.3;3.8)	2.8 (1.3;6.4)	1.6 (0.7;3.5)
3rd (0.05-2.66)	671	2.0 (1.2;3.5)	3.0 (1.3;6.7)	1.2 (0.5;2.7)

Note: the numbers of cases with subthreshold depressive disorder and depressive disorder do not add up to 116 because 5 subjects had no psychiatric work-up and 12 screen positive subjects had other psychiatric diseases or a previous stroke.

\*Logistic regression adjusted for age, gender, cognitive function.

† Subjects with depression or a history of stroke excluded from analysis.

‡ Subjects with subthreshold depressive disorder or a history of stroke excluded from analysis.

tinct groups. The results showed a consistent pattern for end diastolic, mean, and peak systolic blood flow velocity. Blood flow velocity of subjects with depressive disorders was significantly lower than in the reference group. The mean values of subjects with a subthreshold depressive disorder lay in between. A different pattern was observed for vasomotor reactivity. Subjects with subthreshold depressive disorder had a lower vasomotor reactivity than non-depressed reference subjects, whereas there was no clear cut difference between subjects with depressive disorders and the reference group. Very similar estimates were observed if we excluded subjects with a history of stroke or those taking antidepressant drugs, rather than adjusting for those variables.

Table 4 shows the odds ratios for depressive symptoms per tertile of haemodynamic variables adjusted for age, sex, and cognitive function. The particular contributions of subjects with subthreshold depressive disorder and subjects with DSM-IV depressive disorders are also presented in the table. It can be seen that more subjects with depressive disorders were found in the lowest tertile of blood flow velocity. For vasomotor reactivity, the middle and the lower tertiles were associated with an increased risk of subthreshold depressive disorder.

The observed relations between depressive status and cerebral haemodynamic variables were not altered after we controlled for a history of major depression before age 60 and peripheral arterial disease. There were more subjects with peripheral arterial disease in the group with subthreshold depressive disorder (28.8%) and the group with depressive disorders (25.0%) than in the reference group (16.5%). However, the relation between blood flow velocity or vasomotor reactivity and depression was neither explained by nor modified by peripheral arterial disease (data not shown).

## DISCUSSION

This study shows that haemodynamic changes as assessed by transcranial Doppler ultrasonography are associated with depressive symptoms. Both cerebral blood flow velocity and vasomotor reactivity were found to be lower in subjects with depressive symptoms, after the effects of age, sex, education, history of stroke, cognitive function, and cardiovascular risk factors were controlled for. These results support the view that cerebrovascular impairment

may be a cause of depressive symptoms in the elderly. To our knowledge this study is the first to report results of transcranial Doppler ultrasound measurements and depression. Most studies using transcranial Doppler ultrasonography in psychiatric settings have looked at dementia or panic disorders.<sup>20,29-33</sup>

The strength of the present large population based study is the psychiatric work up in subjects who were screen positive on the CES-D. A previous study in an elderly Dutch population reported high sensitivity using the same cut off point, and misclassification of disease is thus unlikely to have influenced our results.<sup>25</sup> Furthermore, we were able to determine in which group depressive symptoms were caused by depressive disorders. The prevalence of depressive symptoms in the Rotterdam study (7.8%) falls within the range reported in a recent review of community prevalence of depressive symptoms in the elderly and is comparable with the prevalence of 9.0% reported in the USA.<sup>26</sup>

The term "vascular depression" was introduced by Alexopoulos.<sup>1</sup> He postulated that geriatric depression encompasses a high percentage of patients with cerebrovascular disease. While there are few clinical differences between early and late onset depression,<sup>7</sup> the hypothesis has been supported by studies showing that persons with late onset depression have more neuroradiological abnormalities than non-depressed individuals.<sup>34</sup> This finding could only partially be replicated in a population based study. Steffens et al reported hyperintensities in the basal ganglia, but other white matter lesions were not related to depressive symptoms as measured by a shortened version of the CES-D.<sup>2</sup>

Different mechanisms for altered cerebral blood flow velocity as measured by transcranial Doppler have been postulated.<sup>35</sup> Reduced blood flow may reflect altered cerebral metabolism, an intrinsic property of the vascular smooth muscle, or a neuronal dysfunction of sympathetic nerve fibres. Metabolic autoregulation is probably of key importance and explains the increased flow velocity during cognitive activity.<sup>36,37</sup> As reduced cognitive activity is a well recognised symptom of depressive disorder, a reduction of blood flow velocity might be an epiphenomenon of depression. The decreased blood flow velocity in our study could reflect the diminished demand in depressive states and does not necessarily support the vascular hypothesis.

Vasomotor reactivity, on the other hand, is probably a good indicator of microangiopathy. A reduced vasomotor reactivity indicates that the cerebral arterioles are unable to dilate in order to compensate for increased demand.<sup>38</sup>

In patients with stroke or transient ischaemic attacks reduced vasomotor reactivity has often been reported.<sup>17,39</sup> In a subset of 73 patients with MRI scans who participated in this study, we previously observed that vasomotor reactivity was related to deep subcortical and periventricular white matter lesions.<sup>40</sup> However, in the present study we did not confirm our hypothesis that reduced vasomotor reactivity is associated with depressive disorders. The observed impairment of vasomotor reactivity in subjects with depressive symptoms was accounted for by cases with subthreshold depressive disorder. If this is not a chance finding, it suggests that cerebral microangiopathy may be less important in the more severely diseased and more often cause subthreshold depressive disorder. Interestingly, a recent neuropathological necropsy study also found no evidence of microvascular disease either locally or generally in the brain of depressed patients.<sup>41</sup>

### **Limitations**

Some limitations of the study must be discussed. This was a cross sectional study and it cannot show whether the observed association with cerebrovascular changes precipitates or results from the depressive symptoms. Furthermore, we should consider whether selection influenced the outcome of the study. In the first place, transcranial Doppler measurements were not performed in all subjects participating in the third survey of the Rotterdam study; however, this omission was entirely random so it did not introduce bias. Second, transcranial Doppler measurements were unsuccessful in nearly one third of the subjects, and participants in whom transcranial Doppler measurements were unsuccessful were on average older and more often female. This was expected, as temporal bone acoustic thickness increases with age and in postmenopausal women,<sup>42</sup> and this adversely affects the transmission of ultrasound. Hyperostosis, which is predominantly found in women, is also thought to preclude transcranial Doppler measurements.<sup>43</sup> As old age and female sex are positively associated with depressive symptoms, persons with mood disorders were underrepresented in our study. This selection may have impeded the detection of modest associations. However, we think it unlikely that the observed relations between cerebral haemodynamic variables and depressive symptoms were a result of selection. There is no indication that haemodynamic changes differ in women or very old depressed subjects.

## Conclusions

We have shown that depressive symptoms are associated with changes in both blood flow velocity and vasomotor reactivity. Our finding of reduced vasomotor reactivity suggests that vascular pathology may be a causal factor in subjects with subthreshold depressive disorder but not in DSM-IV depressive disorders. Furthermore, our data indicate that reduced cerebral blood flow velocity could be caused by reduced demand in depressed subjects and does not necessarily reflect microangiopathy.

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Cerebral small vessel disease

## **ABSTRACT**

*Context:* Vascular pathology may contribute to late-onset depression. Cross-sectional studies showed that elderly patients with depression more frequently have cerebral infarcts and white matter lesions on MRI. The relationship between cerebrovascular disease and depressive disorders has not been studied in the general population longitudinally.

*Objective:* To prospectively examine the association between symptomatic and asymptomatic brain infarcts, white matter lesions and the risk of depressive disorders.

*Design and Setting:* The Rotterdam Scan Study, a large population-based prospective cohort study conducted in the Netherlands.

*Participants:* 1077 elderly people, aged 60 to 90 years, who all underwent MRI of the brain in 1995-1996. Presence of infarcts and severity of white matter lesions at baseline was scored. Participants were followed for on average 3.6 years.

*Main Outcome Measure:* Depressive disorders, assessed by psychiatric work-up of participants with CES-D score  $\geq 16$  and monitoring of medical records of all 1077 participants for the development of depressive disorders during follow-up.

*Results:* Seventy-eight participants developed a depressive disorder during follow-up. The presence of brain infarcts at baseline nearly doubled the risk of depressive disorders (odds ratio 1.8, 95% confidence interval 1.1-3.1). This risk increase of depressive disorders was restricted to participants with baseline infarcts who acquired new infarcts during follow-up. Severity of subcortical white matter lesions was associated with an increased risk of incident depressive disorders (odds ratio per ml increase in subcortical lesions 1.08, 95% confidence interval 1.00-1.17).

*Conclusions:* The presence of brain infarcts and white matter lesions increases the risk of depressive disorders in elderly people. Our findings support the hypothesis that cerebrovascular disease plays an important role in late-onset depression.

## INTRODUCTION

Late-onset depression may have a different etiology than early-onset depression. It has been postulated that vascular factors may play a role in the onset of depression in elderly people.<sup>1</sup> Epidemiological and neuroimaging studies have shown that cerebrovascular lesions are more frequently present in depressive elderly patients than in controls.<sup>2,3</sup> We showed earlier that white matter lesions on MRI were associated with depressive symptoms.<sup>4</sup> Furthermore, one third of hospitalized stroke patients develop a major depression.<sup>5</sup> These findings support the 'vascular depression' hypothesis<sup>6</sup> that cerebrovascular disease contributes to the pathogenesis of late-onset depression. However, most of these studies were cross-sectional and hospital-based. Recently, a longitudinal population-based study found that cerebrovascular disease was related to depressive symptoms.<sup>7</sup> Whether cerebrovascular disease increases the risk of depressive disorders in the general population has not been studied prospectively. We therefore examined the association between brain infarcts, white matter lesions and the risk of depressive disorders in an elderly population-based follow-up study. Because the risk of depression may be related to lesion location,<sup>5,8</sup> we additionally investigated if this relationship was influenced by different locations of these cerebrovascular lesions on MRI.

## METHODS

### Participants

The Rotterdam Scan Study was designed to study causes and consequences of brain changes in the elderly. The study design has been described in detail.<sup>9</sup> In 1995-1996, we randomly selected participants aged 60 to 90 years in strata of age (5 years) and sex from two large ongoing population-based studies, the Zoetermeer Study and the Rotterdam Study.<sup>10,11</sup> A total of 1077 non-demented elderly participated in our study (overall response 63%). The medical ethics committee of the Erasmus Medical Center approved the study and each participant gave informed consent.

Baseline examination in 1995-1996 comprised a structured interview, screening for depressive symptoms, physical examination, blood sampling, and neuropsychological tests at the research center, as well as a cerebral

MRI scan. All 1077 participants were continuously monitored after baseline for mortality and major morbidity including depressive and other psychiatric disorders, dementia, stroke, and TIA. Follow-up was virtually complete until March 1st, 2000 (99.4%; of 7 participants we had no information on depression). In 1999-2000, we re-examined 787 of the participants at the research center with a protocol similar to the baseline examination (response 81%). Participants who were non-eligible (n=104) or those who refused the second examination (n=186) were significantly older and less educated compared to those who did participate; those who refused had also more depressive symptoms at baseline. In total, 668 underwent a second MRI scan in 1999-2000 (response 70%).

### **Cerebral infarcts and white matter lesions**

All participants underwent MRI of the brain in 1995-1996. We made axial T1-, T2-weighted, and proton-density scans on 1.5 Tesla MRI scanners (for participants from Zoetermeer: MR Gyroscan, Philips, Best, the Netherlands and for participants from Rotterdam: MR VISION, Siemens, Erlangen, Germany). The slice thickness was 5 or 6 mm with an interslice gap of 20%. In 1999-2000, participants underwent a second MRI using the MR VISION with the same sequences and protocol as at baseline.

The presence of infarcts was rated similarly at baseline and at follow-up. Infarcts were defined as focal hyperintensities on T2-weighted images, 3 mm in size or larger. Proton-density scans were used to distinguish infarcts from dilated perivascular spaces. Lesions in the white matter also had to have corresponding prominent hypointensities on T1-weighted images, in order to distinguish them from cerebral white matter lesions. A single trained physician (S.E.V.) scored infarcts both on baseline and second MRI, including their location and size. An intrarater study (n=110) for detecting infarcts showed good agreement ( $\kappa=0.80$ ).<sup>12</sup> We obtained a history of stroke and transient ischemic attack (TIA) by self-report, and by checking medical records in all 1077 participants. An experienced neurologist (P.J.K.) subsequently reviewed the medical history and scans and categorized the infarcts as silent or symptomatic. We defined silent brain infarcts as evidence of one or more infarcts on MRI, without a history of a (corresponding) stroke or TIA. Participants with both symptomatic and silent infarcts were categorized in the symptomatic infarct group.

White matter lesions were considered present if visible as hyperintense on

proton-density and T2-weighted images, without prominent hypointensity on T1-weighted scans. Two raters scored periventricular and subcortical located white matter lesions independently. Both intrareader and interreader studies (n=100) showed a good to excellent agreement ( $\kappa=0.79-0.90$ ,  $r=0.88-0.95$ ). A detailed description of the scoring method has been reported previously.<sup>9</sup> Briefly, severity of periventricular white matter lesions was rated semi-quantitatively at three regions (grade range 0-9). A total volume of subcortical white matter lesions was approximated based on number and size of lesions in the frontal, parietal, occipital, and temporal lobes (volume range 0-29.5 ml).

### **Assessment of depressive disorders**

We assessed whether participants had a psychiatric history in 1995-1996 by interview and by checking medical records and indications of prescribed drugs in all 1077 participants. We also screened the participants for depressive symptoms with a validated Dutch version of the original Center for Epidemiologic Studies Depression (CES-D) scale (range 0-60).<sup>13,14</sup> A history of depression was defined as a depressive episode before the baseline examination lasting for more than two weeks, diagnosed by a psychiatrist, clinical psychologist, or general practitioner and treated with antidepressant medication.

Information on persistent and incident depressive disorders was obtained in two ways. Firstly, we re-examined 787 participants for depressive symptoms with the CES-D scale in 1999-2000. Participants with a CES-D score of 16 or more were considered screen-positive,<sup>14</sup> and received a psychiatric work-up. A psychiatrist visited these participants at home and evaluated them with the Dutch version of the Present State Examination, a semi-structured psychiatric interview included in the Schedules for Clinical Assessment in Neuropsychiatry.<sup>15</sup> Psychiatric disorders including major depression, minor depression, and dysthymia were classified according to the DSM-IV criteria. Six participants were screen-positive on the CES-D scale, but received no psychiatric work-up and were subsequently excluded. Secondly, we continuously monitored the medical records of all 1077 participants at the general practitioner's office and the Regional Institute for Ambulatory Mental Health to obtain information on depressive episodes. Depressive disorders were diagnosed either by our screening and psychiatric work-up or by information from medical records if diagnosed by a psychiatrist, clinical psychologist, or general practitioner and treated with antidepressant medication. Persistent depression was defined as a depressive disorder during follow-up, with depressive symptoms

(CES-D score  $\geq 16$ ) or antidepressant medication at baseline. If participants did not have depressive symptoms or antidepressant medication at baseline, the depressive disorder during follow-up was considered to be incident.

### **Other measurements**

Neuropsychological tests at baseline included the Mini-Mental State Examination (MMSE) (score range 0-30) as a measure of global cognitive function. All participants were followed for the development of dementia, which was diagnosed according to standardized criteria.

### **Data analysis**

We first examined the cross-sectional association between the presence of brain infarcts and depressive symptoms at baseline (defined as CES-D  $\geq 16$  or antidepressant medication at baseline) by estimation of the odds ratio and 95% confidence interval with multiple logistic regression analyses. For the longitudinal analyses, we excluded participants of whom we had no information on depression ( $n=7$ ), those who were screen-positive but received no psychiatric work-up ( $n=6$ ), and those who were diagnosed with another psychiatric disorder than a depressive disorder during follow-up ( $n=8$ ). We examined whether the presence of brain lesions on MRI was predictive of a depressive disorder, persistent or incident, with multiple logistic regression analyses. In all analyses we adjusted for age, sex, education, and baseline MMSE score. We used separate models for presence of symptomatic and silent brain infarcts, and for severity of periventricular and subcortical white matter lesions. For the analyses with brain infarcts, the reference group comprised participants without brain infarcts and no distinction was made between participants with one or more infarcts on MRI. Periventricular and subcortical white matter lesions were analyzed in tertiles of their distribution, and continuously if the association was linear. In addition, we examined the risk of incident depressive disorders by excluding all participants on antidepressant medication and those with depressive symptoms at baseline, defined as CES-D score of 16 or over ( $n=92$ ). In order to investigate the risk of incident late-onset depression, we additionally excluded all participants with a history of early-onset depression ( $n=21$ ). Early-onset depression was defined as a depressive episode with an onset before the age of 60 years. Because depressive symptoms often precede dementia and this might confound the associations of interest, we repeated the analyses after exclusion of participants who

became demented during follow-up (n=34).

We further investigated the relationship between location of MRI lesions and risk of depressive disorders. According to some studies, patients with basal ganglia lesions in the left hemisphere have a higher risk of post-stroke depression.<sup>5,8</sup> We examined if the relationship with the risk of depressive disorders was different for infarcts located in the left basal ganglia compared to infarcts located elsewhere. In this respect, we also looked at subcortical white matter lesions in the frontal, parietal, occipital, and temporal lobes in relation to the risk of depressive disorders.

Finally, we explored the contribution of incident brain infarcts on the second MRI to the onset of depressive disorders during follow-up, because we hypothesized that a new infarct might result in depression.

## RESULTS

The baseline characteristics of the study population are shown in Table 1. Brain infarcts on baseline MRI were associated with depressive symptoms at baseline in cross-sectional analysis (adjusted odds ratio 1.9, 95% confidence

**TABLE 1. Baseline characteristics of all participants.**

	All participants n=1077
Age, years	72.2 ± 7.4
Women	555 (52%)
Primary education only	375 (35%)
History of depression	67 (6%)
Use of antidepressant medication	23 (2%)
CES-D score, range 0-60	5.9 ± 6.2
CES-D score ≥ 16	79 (7%)
MMSE score, range 0-30	27.4 ± 2.2
Brain infarcts on MRI:	259 (24%)
Symptomatic	42 (4%)
Silent	217 (20%)
Periventricular white matter lesions, grade	2.4 ± 2.2
Subcortical white matter lesions, ml	1.4 ± 2.9

Values are unadjusted means ± standard deviation or number of participants (percentages).

interval 1.2-3.0). Participants with symptomatic infarcts more often had depressive symptoms than those with silent brain infarcts when compared with participants without infarcts on baseline MRI, although this difference was not statistically significant (adjusted prevalence odds ratio for symptomatic infarcts 2.9, 95% confidence interval 1.2-7.0 and for silent infarcts 1.7, 95% confidence interval 1.0-2.8).

Seventy-eight participants (7%) were diagnosed with a depressive disorder during a mean follow-up of 3.6 years, of whom 30 had a persistent and 48 had an incident depressive disorder. In 39 participants the depressive disorder was assessed by information from medical records and in 39 participants the depressive disorder was diagnosed during re-examination, of whom 18 had a major depression, 18 a minor depression, and 3 dysthymia. The mean age of the 78 participants with a depressive disorder during follow-up was 72.1 years and 55 (71%) were women. Twenty-six of these 78 participants had a history of a treated depression before baseline. Twenty-seven (35%) of them had one or more brain infarcts present on baseline MRI, of whom 23 had silent brain infarcts only. They also had more severe periventricular and subcortical white matter lesions than participants without a depressive disorder during follow-up.

The presence of brain infarcts at baseline nearly doubled the risk of a persistent or incident depressive disorder (Table 2). Risks were similar for participants with symptomatic and those with silent brain infarcts, although the risk increase for symptomatic infarcts was not significant due to small numbers. The risk of a depressive disorder increased linearly with increasing periventricular and subcortical white matter lesions, which was borderline significant (Table 2). When analyses were restricted to those participants without antidepressant medication and depressive symptoms at baseline, the relationship between brain infarcts and the risk of depressive disorders disappeared (Table 2). Subcortical white matter lesions remained associated with incident depressive disorders, whereas the association with periventricular lesions diminished (Table 2). Additional exclusion of participants with early-onset depression in history did not change any of the results (data not shown). None of the above risk estimates changed after exclusion of participants who developed dementia during follow-up, of whom 6 were diagnosed with a depressive disorder before the dementia diagnosis (data not shown).

Participants with basal ganglia infarcts in the left hemisphere at baseline (n=62) had a nearly threefold-increased risk of depressive disorders

**TABLE 2. Association between presence of brain infarcts (symptomatic and silent) and white matter lesions (periventricular and subcortical) on baseline MRI and the risk of depressive disorders during follow-up, estimated by odds ratios (OR) with 95% confidence intervals (CI) adjusted for age, sex, education, and cognitive function.**

	Persistent or incident depressive disorder* OR (95% CI)	Incident depressive disorder† OR (95% CI)
Infarcts (yes/no):		
All infarcts	1.8 (1.1-3.1)	1.0 (0.5-2.1)
Symptomatic infarcts	1.9 (0.6-5.8)	1.4 (0.3-6.2)
Silent infarcts	1.8 (1.0-3.1)	1.0 (0.4-2.1)
White matter lesions:		
Periventricular (per grade increase)	1.11 (0.99-1.24)	1.09 (0.95-1.25)
Subcortical (per ml increase)	1.06 (0.99-1.13)	1.08 (1.00-1.17)

\* Analyses are based on 1056 participants with complete follow-up of depressive disorders.

† Analyses are restricted to 969 participants without antidepressant medication and without depressive symptoms (CES-D score < 16) at baseline.

compared with participants without infarcts on MRI (adjusted odds ratio 2.9, 95% confidence interval 1.4-6.2). This was higher than for participants with brain infarcts located elsewhere when compared with those without infarcts (adjusted odds ratio 1.5, 95% confidence interval 0.8-2.7), although these risk estimates were not significantly different. The risk of depressive disorders was similar for subcortical white matter lesions in the frontal lobes as for lesions in the parietal, occipital, and temporal lobes (data not shown).

Participants with infarcts on baseline MRI who acquired new infarcts during follow-up (n=35) had a threefold-increased risk of depressive disorders compared with those without infarcts and those with baseline infarcts only (adjusted odds ratio 3.4, 95% confidence interval 1.3-8.9). These participants had twice the risk of incident depressive disorders, although no longer significant, after exclusion of participants with antidepressant medication or depressive symptoms at baseline (adjusted odds ratio 1.9, 95% confidence interval 0.5-7.2).

## DISCUSSION

We found that elderly people with brain infarcts, both symptomatic and silent,

have twice as frequent a depressive disorder compared with those without infarcts in the general population. Brain infarcts on baseline MRI did not predict the risk of incident depressive disorders in people without depressive symptoms at baseline, nor did periventricular white matter lesions. Severity of subcortical white matter lesions predicted the risk of incident depressive disorders, both in persons with and without depressive symptoms at baseline.

The strengths of this study are the large number of participating elderly people and its prospective population-based design. Furthermore, we hardly had any losses to follow-up. However, a potential methodological limitation of our study is misclassification. Despite good agreement, we may have systematically over- or underrated infarcts or white matter lesions on MRI. Misclassification may also have occurred in the diagnosis of depressive disorders during follow-up. Participants tend to underreport depressive symptoms and physicians probably underdiagnose depressive disorders, which will have resulted in an underestimation of the true number of events. However, in addition to information from medical records we actively screened participants for the presence of depressive symptoms, and this will have reduced the underdiagnosis of depression. Furthermore, the raters and neurologist who identified and classified white matter lesions and infarcts and the psychiatrist who diagnosed the depressive disorders were blinded to all other data. If anything, this non-differential misclassification will have attenuated the associations and will not have introduced a major bias in our study.

We report an increase of persistence and incidence of depressive disorders in elderly people with brain infarcts and severe white matter lesions on MRI in the general population. We found that both people with symptomatic infarcts and those with asymptomatic lesions, i.e. silent brain infarcts or white matter lesions, had an increased risk of a depressive disorder. This suggests that in elderly people depression may be a direct consequence of vascular brain damage and is not only a psychological reaction to physical disability or perceived disease. Ischemic damage of striatal and prefrontal cortical systems may disrupt neurotransmitter circuitry involved in mood regulation.<sup>16</sup> Several hospital-based studies found that 30% of the stroke patients develop a depression,<sup>5</sup> and this is three times higher than the 10% of the participants with symptomatic brain infarcts that acquired a depressive disorder in our study. We have to keep in mind that participants with symptomatic brain infarcts in our study have less severe stroke symptoms than patients in hospital-based studies. Furthermore, our participants with symptomatic infarcts may have

experienced their stroke or TIA years before the baseline examination and it could well be that people who developed a depression directly after their stroke did not participate in our study. Therefore, our study cannot unravel the psychological impact of experiencing a stroke on the development of depression after a stroke. Still, the increased risk in neurologically asymptomatic participants with silent brain infarcts and white matter lesions suggests that brain lesions themselves play a role in late-onset depression, extending our earlier cross-sectional findings.<sup>4</sup> We found that severity of subcortical white matter lesions did increase the risk of incident depressive disorders, while the presence of brain infarcts did not. This is in line with the Cardiovascular Health Study that recently reported that MRI infarcts were associated with persistence of depressive symptoms, whereas white matter lesions were associated with worsening of depressive symptoms.<sup>7</sup> Infarcts are acute events and perhaps a depressive disorder develops directly after an infarct has occurred due to total cell loss in the infarcted area. White matter lesions on the other hand may represent a more gradual process of brain damage that might result in a depressive disorder after the damage has reached a certain threshold. This implies that many people with baseline infarcts already have a depression at baseline, which is supported by our cross-sectional findings and those of the Cardiovascular Health Study.<sup>17,18</sup> Our finding that the risk of depressive disorders was confined to people with baseline infarcts who acquired new infarcts during follow-up supports this. As for lesion location, we found a stronger relationship of depression with subcortical than with periventricular white matter lesions. But the frequency of depressive disorders did not differ for any specific location of these subcortical lesions. This can be explained by the involvement of many brain structures, with subcortically located connecting fibers, in mood regulation. The risk of depressive disorders seemed to be higher for people with basal ganglia infarcts in the left hemisphere, but this was not significantly different from that for persons with elsewhere located infarcts.

Silent brain infarcts, of which the majority is lacunar,<sup>12,19</sup> and white matter lesions reflect mainly small-vessel disease.<sup>20</sup> Both are related to an increased stroke risk<sup>21</sup> and are thought to be risk factors for dementia and cognitive decline.<sup>22</sup> Depressive symptoms often precede the development of dementia.<sup>23</sup> The relationship between depressive symptoms and silent brain infarcts disappeared after adjustment for physical disability and cognitive impairment in the Cardiovascular Health Study.<sup>18</sup> In our study, the risk increase of

depressive disorders with the presence of brain lesions remained both after adjustment for cognitive function and after exclusion of participants who developed dementia during follow-up.

In conclusion, we found an increased risk of depressive disorders in elderly people with cerebrovascular lesions in the general population. The associations were present for symptomatic and asymptomatic brain lesions. This supports that vascular brain damage plays an important role in late-onset depression. Whether prevention of brain infarcts and white matter lesions will diminish the number of depressive disorders in elderly people needs to be examined.

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## **4. Inflammatory, nutritional and metabolic factors in late life depression**



4.1

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Inflammatory proteins

**ABSTRACT**

*Background:* Clinical studies suggest that depression may be accompanied by an activation of the inflammatory response system. We investigated the relation of cytokine interleukin-6 (IL-6) and the acute phase proteins  $\alpha$ 1-antichymotrypsin (ACT) and C-reactive protein (CRP) with depression in a population-based study.

*Methods:* We screened 3,884 adults at age 60 and older living in a district of Rotterdam for depressive symptoms, and performed a psychiatric work-up on those who were screened positive. All subjects had blood drawn. We compared levels of inflammation markers adjusted for smoking, stroke, functional disability and cognitive score for 263 cases with depressive symptoms (including 106 subjects with depressive disorders) and 461 randomly selected reference subjects.

*Results:* Age- and gender-adjusted analyses suggested associations of ACT and CRP with depressive disorders, but these associations disappeared after adjustment for additional confounding factors. Even after adjustment, increased levels of IL-6 were related to depressive disorders (odds ratio per standard deviation increase IL-6 = 1.47; 95% confidence interval = 1.15 – 1.89). Further analyses demonstrated that this relation was mainly due to the subjects with high IL-6 concentrations, suggesting a threshold effect.

*Conclusions:* IL-6 levels were strongly increased in some subjects with depressive disorders, possibly indicating a specific immunological process. However, the associations of acute phase proteins with depression in this population-based study could be explained by confounding.

## INTRODUCTION

Clinical studies have shown that depression is associated with increased levels of circulating cytokines and acute phase proteins.<sup>1-4</sup> However, critics argue that the increased levels of acute phase proteins such as C-reactive protein (CRP) are not specific for depression but mainly reflect the effects of other associated variables such as hospitalization, tobacco dependence, and co-morbidity.<sup>4-6</sup> Indeed, some studies have not been able to reproduce the findings of immune activation.<sup>7-9</sup>

The present study explored the relation of cytokine IL-6 and the acute phase proteins CRP and  $\alpha$ 1-antichymotrypsin (ACT) with depressive disorders. IL-6 is a central mediator of the acute phase response and CRP is the classic acute phase reactant.<sup>10,11</sup> Our aim was to investigate whether the reported associations with depression can be found in a population-based study after controlling for a variety of demographic and medical variables.

## METHODS

### Study population

This study is based on the Rotterdam Study, an ongoing population-based cohort study in which all inhabitants age 55 and over, living in a defined geographic area of Rotterdam, had been invited to participate.<sup>12</sup> The baseline response rate was 78%. The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants. In the third survey (1997-1999) we added assessment of depressive symptoms to the study protocol. Of the 4703 persons who participated in that survey, 3,884 visited the research center and had venous blood samples drawn. In the present analysis we compared inflammatory proteins in 263 cases with depressive symptoms and 461 randomly selected reference subjects.

### Depression assessment

Depressive disorders were assessed using a two-step procedure. First, participants completed the Dutch version of the original Center for Epidemiological Studies Depression scale (CES-D) during a home interview.<sup>13,14</sup> We used a score of 16 as a cut-off, which indicates clinically significant depressive symp-

toms.<sup>15</sup> Secondly, screen-positive subjects had a psychiatric work-up using the Present State Examination, a semi-structured psychiatric interview.<sup>16</sup> All interviews were conducted by two experienced clinicians. Psychiatric disorders were classified according to the Diagnostic and Statistical Manual of Mental Disorders-IV criteria. The diagnostic categories include major depression, dysthymia and minor depression.

Of the 3884 subjects who visited the research center, 263 (7%) were screen-positive for depressive symptoms. Of these, 250 (95%) had a psychiatric work-up. In total, 106 cases had a depressive disorder. These subjects were classified as having major depression (n=47), dysthymia (n=13) or minor depression (n=46). The remaining 144 subjects were diagnosed as having another psychiatric disorder (n=29), or they did not meet criteria for an Axis I psychiatric disorder (n=115, subclinical depressive symptoms). Eight subjects declined to undertake the interview, and five subjects could not be reached.

### **Blood specimens**

Overnight fasting blood was collected, put on ice directly, and centrifuged at 2000g for 10 min. Plasma was separated and dispensed into two 1.5 ml aliquots and then frozen within three hours at -80°C. Both ACT and CRP were assessed by means of a nephelometric method (BN 100, Dade Behring, Marburg, Germany). The IL-6 concentrations were determined with quantitative enzyme-linked immunosorbent assay with a test kit from R&D systems (Minneapolis, USA). The intra-assay and interassay coefficients for all measurements were < 5% and < 8%, respectively.

### **Other measurements**

The following variables were considered as possible confounding variables: age, sex, education, stroke, cognitive function (as measured by the Mini Mental State Examination), smoking, body mass index, antidepressant drug use and functional disability. Education was measured on an ordinal scale and later dichotomized at the median of the sample into low and high education. A history of stroke was obtained through direct questioning and computerized linkage with general practitioner medical records. Smoking was coded as number of cigarettes currently smoked per day and in categories of current, former and never smoker. Functional status was assessed using the Stanford Health Assessment Questionnaire.<sup>17</sup> In an additional analysis we adjusted for atherosclerosis as measured by the intima-media thickness in the

carotid arteries.<sup>18</sup>

### Statistical analysis

We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) of inflammation markers for depressive symptoms, depressive disorders and major depression. Inflammation markers were entered into separate models as a continuous variable (divided by its standard deviation). To further explore the relation we checked whether there was a threshold effect. For this, we calculated the odds ratios for each decile of the inflammation marker. We plotted the odds ratios at the median of each decile using the unit of inflammation markers on the x-axis.

Age (continuous) and sex were controlled for in all analyses. To check for confounding we added potential confounders to the basic model. If this changed the effect estimate meaningfully the variables were included in further analyses.<sup>19</sup>

**TABLE 1: Selected characteristics of the study subjects, Rotterdam Study, 1997-1999**

	Non-depressed (n=461)	Depressive symptoms* (n=263)	Depressive disorder* (n=106)
Age (yrs) (mean, range)	72.4 (61-101)	73.7 (61-97)	73.6 (61-97)
% female	59	74	73
Primary education only (%)	49	57	57
Body mass index (kg/m <sup>2</sup> ) (mean, SD)	27.7 (8.3)	27.9 (9.4)	27.9 (10.6)
Smoking			
-cigarettes per day (mean, range)	1.7 (0-30)	2.7 (0-30)	2.8 (0-25)
-current smoker (%)	15	19	21
-ex-smoker (%)	50	42	41
History of stroke (%)	2	7	8
MMSE-score (mean, SD)	27.6 (2.2)	26.8 (2.9)	26.2 (3.7)
Functional disability (%)	30	56	60
Antidepressant medication (%)	2	11	17
Interleukin-6 (pg/ml)	3.19	3.80	4.56
$\alpha$ 1-Anti-chymotrypsin (mg/dl)	39.6	40.3	41.5
C-reactive protein (mg/l)	3.08	3.36	3.80

CES-D = Center of Epidemiologic Studies Depression scale; MMSE = Mini Mental State Examination; BMI = Body Mass Index.

\*The category 'depressive symptoms' includes all subjects who were screen positive, the category 'depressive disorder' includes only the subjects with major or minor depression and dysthymia.

## RESULTS

Table 1 presents characteristics of the reference subjects, the cases with depressive symptoms, and the subgroup of cases with depressive disorders.

No strong associations between inflammation markers and depressive symptoms were observed (Table 2). The odds ratios for DSM-IV depressive disorders were higher than those for depressive symptoms. Adjustment for general confounders did not change the association with IL-6: Control for atherosclerosis had a moderate effect, but even after this adjustment, subjects with increased levels of IL-6 were more likely to have a depressive disorder. In contrast, the odds ratios associated with ACT and CRP decreased substantially after adjustment. Age, sex, smoking, stroke, functional disability and atherosclerosis were entered into the model; no other covariate altered the estimates. Furthermore, we compared the subjects with major depression and

**TABLE 2: The association between inflammatory proteins and depression, Rotterdam Study, 1997-1999**

Inflammatory proteins	Depressive symptoms* (263 cases)		Depressive disorder* (106 cases)	
	Odds ratio	95% CI	Odds ratio	95% CI
Interleukin-6, per SD				
Model 1 <sup>†</sup>	1.23	0.99, 1.53	1.46	1.15, 1.85
Model 2 <sup>‡</sup>	1.18	0.94, 1.47	1.47	1.15, 1.89
Model 3 <sup>§</sup>	1.11	0.86, 1.43	1.38	1.04, 1.83
α1-Anti-chymotrypsin, per SD				
Model 1	1.12	0.95, 1.32	1.21	0.98, 1.50
Model 2	0.99	0.83, 1.18	1.08	0.86, 1.37
Model 3	0.93	0.77, 1.11	0.93	0.72, 1.20
C-reactive protein, per SD				
Model 1	1.06	0.91, 1.23	1.16	0.96, 1.39
Model 2	1.01	0.86, 1.18	1.09	0.90, 1.33
Model 3	0.97	0.80, 1.16	1.00	0.76, 1.29

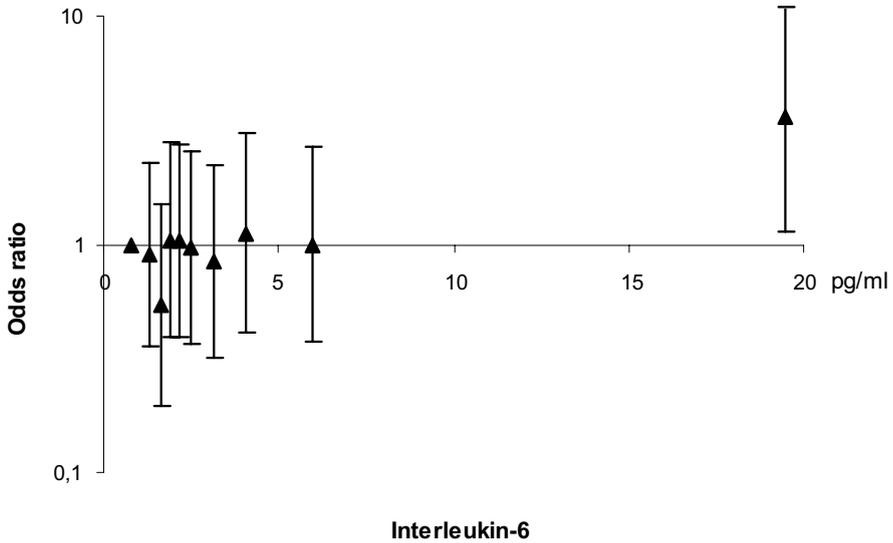
SD = standard deviation; CI = confidence interval

\*The category 'depressive symptoms' includes all subjects who were screen positive, the category 'depressive disorder' includes only the subjects with major or minor depression and dysthymia.

<sup>†</sup> Model 1: Logistic regression analyses adjusted for age and gender; subjects (n=461) without depressive symptoms were used as the reference group. Inflammation markers were analyzed as a continuous variable divided by one standard deviation.

<sup>‡</sup> Model 2: As model 1 and additionally adjusted for smoking, history of stroke and functional disability.

<sup>§</sup> Model 3: As model 2 and additionally adjusted for atherosclerosis as measured by intima-media thickness in the carotid arteries. These analyses were based on 96 subjects with depressive disorders, 240 subjects with depressive symptoms and 433 reference subjects due to missing atherosclerosis data.



**Figure 1: The association between deciles of interleukin-6 and depressive disorders.**

Logistic regression analysis adjusted for age, gender, history of stroke, smoking and functional status. Odds ratios for depressive disorders (triangles) and 95% confidence intervals (vertical lines) are plotted at the median of the interleukin-6 decile.

the reference group. Results were essentially the same as for the group of depressive disorders as a whole (data not shown).

Figure 1 shows the odds ratios of deciles of IL-6. Over a broad range of IL-6 levels an association between IL-6 and depressive disorders was hard to discern. However, visual inspection suggested a threshold at high levels of IL-6; subjects in the upper decile of IL-6 (above 9.3 pg/ml) were at a high risk to have a depressive disorder. If the upper decile of IL-6 is chosen as a cut-off (dichotomous variable), the odds ratio was 3.9 (95% CI = 1.5 – 9.9).

## DISCUSSION

In this population-based study of elderly subjects we found that high levels of cytokine IL-6 were associated with depressive disorders. After controlling for confounders, the acute phase proteins ACT and CRP were not increased in depressed subjects.

Some methodologic issues of the present study need to be considered.

Firstly, residual confounding may explain our findings. Cardiovascular risk, medical co-morbidity or treatment may be incompletely controlled for.<sup>4,20</sup> Secondly, the prevalence of subjects with depressive symptoms in this study (7%) was relatively low and this could impede the detection of modest associations. However, it is comparable to the community prevalence of depressive symptoms in the elderly reported by Blazer et al. in the US (9%) in a study that also used the CES-D.<sup>15</sup> A strength of the study is the psychiatric work-up; hence, misclassification of disease is unlikely to have influenced our results.

For many years the finding that increased levels of acute phase proteins may occur in association with depression primarily emanated from one research group studying hospitalized patients and healthy controls.<sup>1,21-23</sup> Several confounding factors such as severity of symptoms, co-morbidity and tobacco dependence might have accounted for the heterogeneity in observations reported by other investigators.<sup>4</sup> In the present study smoking status as measured by cigarettes currently smoked per day, as well as stroke, were strongly related to the acute phase proteins. Only a weak association of ACT and CRP with depressive disorders remained after adjustment, whereas IL-6 may be independently related to depression. Our findings concerning the ACT and CRP are in contrast to some previous observations, but these were comparisons of clinical samples with healthy controls.<sup>8,21,24,25</sup> On the other hand, a recent population-based study of IL-6 reported a moderate association with depressive symptoms as measured by the CES-D.<sup>26</sup> Further research is needed to establish whether only specific inflammatory proteins are elevated in depression or whether our results were a chance finding.

The observed relation between IL-6 and depression in the present study was not linear. To our knowledge previous studies did not investigate the nature of the association with IL-6. However, our results are compatible with observations in experimental studies. Pollmächer and coworkers<sup>27</sup> stimulated an IL-6 response by applying endotoxins intravenously. Negative emotions were observed at IL-6 levels more than 20 times higher than those in our study. In contrast, moderately increased levels had a positive effect on mood. In another study endotoxin also elicited a dose-dependent effect of on sleep.<sup>28</sup> Low doses enhanced sleep and high levels resulted in a disrupted sleep pattern typical for psychiatric illnesses. Thus there may be a threshold above which IL-6 has a negative effect on mood, and high concentrations could directly cause the depressed affect in a small group of community dwelling subjects. Furthermore, part of the relation between inflammation markers

and depression was explained by atherosclerosis. Inflammation is related to atherosclerosis, and atherosclerosis in turn is a possible cause of depression in the elderly.<sup>29,30</sup> However, the present study cannot demonstrate whether atherosclerosis actually is part of the causal pathway between inflammation and depression. Cardiovascular disease and depression could be related independent of inflammatory processes, in which case atherosclerosis must be seen as a confounder.

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4.2

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Plasma fatty acid composition

## **ABSTRACT**

*Background:* It has been hypothesized that n-3 polyunsaturated fatty acids (PUFAs) are involved in mood regulation, but epidemiological evidence for such a link in the general population is lacking.

*Objective:* This study examined whether community dwelling elderly persons with depression have a different fatty acid composition than the non-depressed.

*Design:* As part of the Rotterdam Study we screened 3,884 adults aged 60 years and over for depressive symptoms. Screen positive subjects had a psychiatric interview to diagnose depressive disorders. All eligible subjects had blood drawn and concentrations of plasma phospholipids were determined. We compared percentage of n-3 and n-6 PUFAs and their ratio between 264 cases with depressive symptoms, including 106 subjects with depressive disorders, and 461 randomly selected reference subjects. We also investigated whether atherosclerosis or inflammatory response as measured by C-reactive protein (CRP) underlie the relationship between fatty acid composition and depression.

*Results:* Subjects with depressive disorders had a higher ratio of n-6/n-3 PUFAs, but differences in individual PUFAs were mostly small. However, the depressed subjects with normal CRP concentrations had a substantially altered fatty acid composition; n-3 PUFA percentage was lower and n-6/n-3 ratio higher in subjects with depressive disorders as compared to controls (5.2% vs. 5.9%,  $p=0.02$  and 7.2 vs. 6.6,  $p=0.01$ , respectively). This relation was not due to atherosclerosis.

*Conclusions:* In community dwelling persons fatty acid composition is related to depression. Because this was not secondary to inflammation, atherosclerosis or possible confounders this association suggests a direct impact of fatty acid composition on mood.

## INTRODUCTION

The long chain polyunsaturated fatty acids (PUFAs) fall into two main families: omega-3 (n-3) and omega-6 (n-6). The n-3 PUFAs are derived from fish and some plants, the n-6 PUFAs mainly from vegetable oil. The principal precursors of the n-3 and n-6 PUFAs cannot be endogenously synthesized from carbohydrates. The specific concentrations of the n-3 and n-6 PUFAs in blood or cell membranes thus reflect dietary intake.<sup>1</sup> Long term changes of dietary habits in Western societies are believed to have altered the ratio between n-6 and n-3 PUFAs. In ecological studies it has been postulated that the rise in the incidence of coronary heart disease and depressive disorders in the last century was related to a diet change, which was characterized by a slightly increased intake of total and saturated fats, and a two- to threefold increase in oils from seeds.<sup>2-5</sup>

Different mechanisms may underlie the relation found in the ecological studies. Fatty acid composition determines the biophysical properties of neuronal membranes and influences neurotransmission.<sup>6</sup> Higher n-3 PUFA concentrations lead to higher membrane fluidity, which in turn increases serotonin transport.<sup>3,7</sup> These biochemical mechanisms connect fatty acids to the current receptor- and neurotransmitter-based hypothesis of depression. But other mechanisms are also discussed, dietary intake of n-3 PUFAs decreases the risk of atherosclerosis,<sup>8</sup> whilst increased n-6 PUFAs result in an overproduction of prostacyclins and inflammatory markers.<sup>9</sup> Both inflammation and atherosclerosis have been associated with depression and could link fatty acids and depression.<sup>10-12</sup>

A few small hospital studies have investigated the relation between the fatty acid composition and depressive disorders.<sup>10,13-16</sup> They reported low n-3 PUFA concentrations in subjects with depression as compared to healthy controls. However, since the studies thus far have been restricted to psychiatric patients, it is unknown whether fatty acid composition affects mood in the general, community dwelling population. Various factors such as chronic diseases, cigarette smoking or cholesterol levels are related to both depression and fatty acid composition.<sup>17</sup> These confounders might explain the associations observed in earlier studies. The present population-based study investigates the relation between fatty acid composition of plasma phospholipids and depressive disorders in elderly persons controlling for a number of demographic and biological variables. Nutrient intake in elderly persons usually

shows less day-to-day variation<sup>18</sup> and fatty acid concentrations presumably reflect long-term intake under the assumption of fairly consistent dietary patterns.

## **SUBJECTS AND METHODS**

### **Study population**

This study is based on the Rotterdam Study, an ongoing population based cohort study for which all inhabitants aged 55 and over of a suburb of Rotterdam were invited in 1990 to 1993.<sup>19</sup> A total of 7983 men and women (78% percent of those eligible) entered the study. The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants. In the third survey we added assessment of depressive symptoms to the study protocol. Measurements took place between March 1997 and December 1999 and included a home interview and a visit to the research center. Of the 4703 persons who participated in the home interview, 3884 visited the research center and had venous blood samples drawn. The 819 subjects who were not seen at the research center were on average older (77.5 versus 72.3 years), more likely to be female (70 % versus 58 %) and had more depressive symptoms (12.2% versus 6.8%, overall prevalence 7.8%). In the present analysis we compared fatty acid composition between all subjects with depressive symptoms and randomly selected reference subjects.

### **Depression assessment**

Depressive disorders were assessed using a two step procedure. First participants completed the Dutch version of the original Center for Epidemiologic Studies Depression scale (CES-D) during the home interview. The CES-D is a 20-item self-reported measure of symptoms experienced in the last week. Each item is scored on a scale of 0 to 3 points. The criterion validity of the CES-D version is well established.<sup>20</sup> We used a score of 16 as a cut-off, which had a very high sensitivity for major depression in a random sample of older subjects in the Netherlands.<sup>21</sup> Moreover, previous studies have verified that a score of 16 and above on the CES-D represents clinically significant depressive symptoms.<sup>22</sup> As a second step, screen positive subjects had a psychiatric work-up using the Dutch version of the Present State Examination (PSE-10), a

semi-structured psychiatric interview included in the Schedules for Clinical Assessment in Neuropsychiatry (SCAN).<sup>23</sup> The psychometric properties of the SCAN have been studied in Dutch patients. An excellent agreement on the syndrome level and a substantial test-retest reliability were found.<sup>24</sup> One or other of two experienced clinicians conducted all interviews. Psychiatric disorders were classified according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria with an algorithm based on the PSE-10 scores. The diagnostic categories include major depression and dysthymia in addition to minor depression (as defined in the appendix of the DSM-IV).

Of the 3884 subjects who visited the research center and had blood taken, 264 (6.8%) were screen-positive on the CES-D and were included in the analysis of depressive symptoms. Nine subjects refused the subsequent psychiatric interview, and five screen positive subjects could not be reached. Psychiatric disorders and duration of symptoms were thus defined in 250 (95.1%) participants according to DSM IV criteria. Psychiatric work-up revealed that 106 cases had a depressive disorder as defined by the Diagnostic and Statistical Manual of Mental Disorders-IV. The remaining 144 subjects were diagnosed as having either an anxiety (n=20) or another psychiatric disorder (n=9) or did not meet criteria for an Axis I psychiatric disorder (n=115, subclinical depressive symptoms).

### **Selection of reference subjects**

A sample of 461 participants ranging in age from 61 to 101 years, served as the reference group. They were randomly selected from the participants of the Rotterdam Study who were screen-negative for depression. A large number of controls increase statistical efficiency but financial constraints did not permit determination of fatty acid composition in all subjects. We did not match on possible confounding variables in order to be able to study the effect of several of these variables on the relation between fatty acids and depression.

### **Blood specimens**

Overnight fasting blood was collected under standardized conditions in a 10 ml tube of citrate-anticoagulated blood, put on ice directly and centrifuged at 2000g for 10 min. Plasma was separated and dispensed into two 1.5 ml aliquots and frozen within three hours at -80°C until analyzed. The concentration of fatty acids originating from the phospholipid fraction of plasma

was determined. The total lipid extracts of plasma were prepared according to a standard method.<sup>25</sup> The plasma lipids were extracted from 0.25 ml plasma with chloroform and methanol after addition of internal standard (Nonadecanoyl 1,2-Diacyl-*sn*-Glycero-3-Phosphocholine, Avanti Polar Lipids Inc Alabaster, USA). Thereafter, solid phase extraction was used to separate the phospholipids from the other lipid classes in the extract by NH<sub>2</sub> columns (Bond Elut 500mg 3ml, Varian, Middelburg, the Netherlands). After separation, the phospholipids are methylated via reaction with 14% boron trifluoride in methanol at 100°C for one hour. Butylated hydroxytoluene was added as anti-oxidant to all organic solvents. Fatty acid methyl esters present in the phospholipid fraction were determined via high-resolution capillary gas-liquid chromatography (Shimadzu GC17A chromatograph, Shimadzu Benelux 's-Hertogenbosch, The Netherlands) with split injection (1:15) and a flame ionization detector using a 50m fused silica column (CP-SIL 88 for FAME, 0.25mm ID, 0.2 µm film, Chrompack, Varian, Middelburg, the Netherlands). The temperature program was as follows: an initial temperature of 160°C for 10 min, followed by a temperature increase of 3.2°C/min up to 190°C, a 15-min isotherm period, a temperature increase of 5.0°C/min up to 230°C, a 7-min isotherm period.

Fatty acid amounts present in the phospholipid fraction were quantified based on the amount of 19:0 internal standard fatty acid methyl ester recovered, and expressed as mg/l plasma and percentage of total fatty acids (based on the mg/l). Total phospholipid fatty acid concentrations did not differ between depressed and reference subjects (1139 mg/l v 1140 mg/l). Therefore, a difference in percentage of individual fatty acids between the groups also reflects a difference in absolute amounts. The data were transferred electronically to minimize transcription errors.

### **Other measurements**

The following variables were considered as possible confounding variables: age, gender, level of education, history of stroke, cognitive function as measured by the Mini Mental State Examination, disabilities in activities of daily living and the cardiovascular risk factors cigarette smoking, blood pressure, serum total and HDL cholesterol. Education was measured on an ordinal scale and later dichotomized at the median of the baseline sample into low and high education. A history of stroke was obtained from all subjects through direct questioning and computerized linkage with general practitio-

ner medical records. We assessed cholesterol concentration in fasting blood by an automated enzymatic procedure. Cigarette smoking was scored in categories of current, former and never smoker. Functional status was assessed using the Disability Index of the Stanford Health Assessment Questionnaire.<sup>26</sup> This measure reflects the consequences of disease in terms of functional performance and activities.

We also measured the inflammation marker C-reactive protein (CRP) by a nephelometric method and intima-media thickness as an indicator of atherosclerosis. The latter was assessed by recording ultrasonographic images of the common carotid artery; in the analyses we used the average of near and far wall measurements of both left and right side.

### **Statistical analysis**

We used the individual fatty acids percentages (% of total fatty acids) in the analyses. Further, we calculated the ratio between n-6 and n-3 PUFAs as well as the ratios between arachidonic acid to eicosapentaenoic acid (AA/EPA) and arachidonic acid to docosahexaenoic acid (AA/DHA). Previous work focussed on AA/EPA, whereas DHA is believed to be important in mental function.<sup>10,15</sup> The association of fatty acids with depressive symptoms, sub-clinical depressive symptoms and depressive disorders was quantified with analysis of covariance (ANCOVA). Fatty acid percentages and ratios were entered into separate models as continuous variables. Equal variance of fatty acid percentages in case and referent subjects was tested with Levene's Test for Equality of Variances. No significant differences in variance were found. Age (continuous) and gender were controlled for in all analyses. To check for confounding we added potential confounders to the basic model. If this changed the effect estimate meaningfully (defined as more than 5%) the variables were included in further analyses.<sup>27</sup> In the tables we present the unadjusted means and the fully adjusted mean differences.

Finally, we investigated whether C-reactive protein and intima-media thickness were intermediates or effect modifiers of the association between fatty acid ratios and depression. To study possible intermediates we examined whether associations changed when these variables were entered into the model as covariates and calculated the non-parametric correlation (Spearman's) of CRP and intima-media thickness with fatty acids. Effect modification was formally tested by putting the product terms into the model. Furthermore, we stratified the analyses by the median of CRP.

We used SPSS for Windows 9.0 in all our analyses.

## RESULTS

The characteristics of the reference subjects and the cases with depressive disorders are presented in table 1. Subjects with depressive disorders were more likely to be female, to have had a stroke and had lower activities of daily living and cognitive scores.

We found no difference in plasma phospholipid fatty acid composition between the reference group and the 264 subjects that had depressive symptoms as determined by the CES-D (data not shown). The comparisons of the 115 subjects with subclinical depressive symptoms and the 106 cases who fulfilled criteria for a DSM-VI depressive disorder with the reference subjects are shown in table 2. Subjects with subclinical depressive symptoms had the same percentages of fatty acids in phospholipids and the same ratios as the non-depressed reference subjects.

In the analyses restricted to persons with DSM-IV depressive disorders and the reference subjects we observed moderate differences in average percentages of only two individual PUFAs. Mean arachidonic acid percentage was higher and docosahexaenoic acid percentage was lower in subjects with depressive disorders. However, we observed a borderline significant difference in the ratio of n-6/n-3 as well as the AA/EPA and the AA/DHA ratios. This was due to the higher percentage n-6 and less n-3 PUFAs depressed subjects had compared to reference subjects.

The results shown in table 2 were adjusted for age, gender, smoking, blood pressure and activities of daily living score. Neither adjusting for any other of the possible confounders that we measured, CRP nor intima-media thickness changed the results. Thus there was no evidence that the inflammation marker CRP or atherosclerosis as measured by intima-media thickness mediate the relation between fatty acids and depressive disorders. Moreover, all Spearman's correlation coefficients for the relation of both variables with the fatty acids were below 0.01 except for percentage saturated fatty acids. However, a formal test for interaction demonstrated that the relation between n-6/n-3 ratio and depressive disorders depended on the CRP concentration (interaction term included in ANCOVA,  $p=0.02$ ). The difference in n-6/n-3 ratio between depressed and non-depressed subjects increased with lower

**TABLE 1: Selected characteristics of the study subjects**

	No depressive symptoms	Subthreshold depressive symptoms <sup>1</sup>		Depressive disorders	
	n=461	n=115	p-value <sup>2</sup>	n=106	p-value <sup>2</sup>
Age, years <sup>3</sup>	72.5 (61-101)	73.9 (61-93)	0.10	73.7 (61-97)	0.16
Gender (% female)	58.6	77.3	0.01	72.6	0.05
Primary education only (%)	48.8	58.2	0.44	56.6	0.52
MMSE <sup>4</sup> -score <sup>3</sup>	27.6 (2.2)	27.2 (2.1)	0.55	26.2 (3.7)	0.001
History of stroke (%)	2.0	7.8	0.01	7.5	0.03
Smoking					
- current smoker	15.0	17.3	0.21	20.8	0.06
- ex-smoker	49.5	44.3	0.49	40.6	0.16
Blood pressure					
- systolic	144 (21)	141 (20)	0.17	139 (24)	0.03
- diastolic	75 (12)	72.2 (11)	0.06	73 (12)	0.23
Total cholesterol (mmol/L)	5.9 (0.9)	5.8 (1.0)	0.37	5.8 (1.0)	0.40
HDL cholesterol (mmol/L)	1.4 (0.4)	1.4 (0.4)	0.12	1.4 (0.4)	0.88
Activities of daily living score <sup>5</sup>	0.5 (0.5)	0.7 (0.6)	0.01	0.8 (0.6)	0.001
C-reactive protein (mg/l)					
- mean	3.1 (4.6)	3.1 (5.2)	0.95	3.8 (5.3)	0.16
- median	1.5	1.6		1.6	

<sup>1</sup> Subjects with subthreshold depressive symptoms were screen positive on the Center of Epidemiological Studies Depression Scale but did not fulfil the DSM-IV criteria for depressive disorders or for any other psychiatric disease.

<sup>2</sup> p values were calculated performing analysis of covariance or logistic regression adjusted for age and gender where appropriate.

<sup>3</sup> Values are unadjusted means and ranges for age, and unadjusted means and standard deviations for MMSE-score, blood pressure, cholesterol, activities of daily living score and C-reactive protein.

<sup>4</sup> MMSE = Mini Mental State Examination, HDL = high density lipoprotein.

<sup>5</sup> Higher scores indicate a higher degree of functional disabilities in activities of daily living.

concentrations of CRP. Therefore we stratified the analysis between fatty acids and depressive disorders at the median of CRP (1.5 mg/l). We found no significant difference in fatty acid composition between non-depressed and depressed subjects with a CRP concentration above 1.5 mg/l (data not shown). The analysis of subjects with a CRP concentration below 1.5 mg/l is

**TABLE 2: Association of fatty acids and fatty acid composition in plasma phospholipids with depression<sup>1</sup>**

Percentage fatty acids	Non-depressed (n=461)		Subthreshold depressive symptoms <sup>2</sup> (n=115)		Depressive disorders (n=106)		
	mean	mean	adjusted difference (95% CI)	p-value	mean	adjusted difference (95% CI)	p-value
Saturated fatty acids	45.9	46.1	0.1 (-0.2; 0.4)	0.4	46.1	0.1 (-0.3; 0.4)	0.7
Monounsaturated	11.8	11.9	0 (-0.4; 0.4)	1.0	11.8	-0.1 (-0.5; 0.3)	0.7
Polyunsaturated (PUFA)							
n-6							
Linoleic (18:2)	21.8	21.8	0.1 (-0.4; 0.7)	0.7	21.6	-0.1 (-0.7; 0.6)	0.7
Dihomogammalinolenic (20:3)	3.3	3.3	-0.1 (-0.2; 0.1)	0.4	3.3	0 (-0.2; 0.1)	0.7
Arachidonic (AA; 20:4)	9.0	8.7	-0.2 (-0.7; 0.2)	0.3	9.3	0.5 (0; 1.0)	0.05
Total n-6 PUFA	35.3	35.1	-0.2 (-0.7; 0.4)	0.6	35.6	0.4 (-0.2; 1.1)	0.2
n-3							
Eicosapentaenoic (EPA, 20:5)	0.9	0.9	0 (-0.1; 0.1)	0.6	0.8	0 (-0.2; 0.1)	0.4
Docosapentaenoic (22:5)	0.9	1.0	0 (-0.1; 0.1)	0.8	0.9	0 (-0.1; 0.1)	0.3
Docosahexaenoic (DHA, 22:6)	3.7	3.7	0 (-0.3; 0.2)	0.9	3.5	-0.2 (-0.5; 0)	0.05
Total n-3 PUFA	5.8	5.9	0 (-0.3; 0.3)	0.9	5.5	-0.3 (-0.7; 0)	0.06
Ratios							
n-6/n-3 PUFA	6.6	6.4	-0.1 (-0.7; 0.4)	0.7	6.9	0.4 (0; 0.8)	0.05
AA/EPA	13.0	11.7	-0.9 (-2.4; 0.7)	0.3	14.1	1.6 (0; 3.2)	0.06
AA/DHA	2.7	2.6	-0.1 (-0.3; 0.2)	0.5	2.9	0.3 (0; 0.5)	0.04

<sup>1</sup> Analyses of covariance with percentage fatty acid entered as continuous variables and adjusted for age, gender, smoking, systolic blood pressure and activities of daily living. Subjects with subthreshold depressive symptoms and subjects with depressive disorders were compared with non-depressed subjects. Values represent unadjusted means and adjusted differences (95% CI).

<sup>2</sup> Subjects with subthreshold depressive symptoms were screen positive on the Center of Epidemiological Studies Depression Scale but did not fulfil the DSM-IV criteria for depressive disorders or for any other psychiatric disease.

**TABLE 3: Association of fatty acids and fatty acid composition in plasma phospholipids with depression in subjects with low C-reactive protein concentrations<sup>1</sup>**

Percentage fatty acids	Non-depressed (n=232)	Depressive disorders (n=51)		
	mean	mean	adjusted difference (95% CI)	p-value
Saturated fatty acids	45.8	46.1	0.3 (-0.2; 0.7)	0.2
Monounsaturated	11.6	11.5	-0.1 (-0.7; 0.5)	0.6
Polyunsaturated (PUFA)				
n-6				
Linoleic (18:2)	22.1	22.4	0.3 (-0.6; 1.2)	0.6
Dihomogammalinolenic (20:3)	3.2	3.2	-0.1 (-0.3; 0.1)	0.5
Arachidonic (AA; 20:4)	9.0	9.3	0.3 (-0.4; 1.0)	0.4
Total n-6 PUFA	35.6	36.1	0.6 (-0.3; 1.4)	0.2
n-3				
Eicosapentaenoic (EPA, 20:5)	0.9	0.7	-0.2 (-0.4; 0)	0.05
Docosapentaenoic (22:5)	1.0	0.9	0 (-0.1; 0)	0.1
Docosahexaenoic (DHA, 22:6)	3.7	3.2	-0.4 (-0.8; -0.1)	0.02
Total n-3-PUFA	5.9	5.2	-0.7 (-1.2; -0.1)	0.01
Ratios				
n-6/n-3 PUFA	6.6	7.6	0.9 (0.3; 1.6)	0.006
AA/EPA	13.2	16.4	3.3 (0.9; 5.7)	0.007
AA/DHA	2.7	3.1	0.4 (0.1; 0.7)	0.02

<sup>1</sup>Analyses of covariance with percentage fatty acid entered as continuous variables and adjusted for age, gender, smoking, systolic blood pressure and activities of daily living. Values represent unadjusted means and adjusted differences (95% CI). Low C-reactive protein was defined as values below 1.5 mg/l (median).

presented in table 3. Depressed subjects had a significantly lower n-3 PUFA percentage and both ratios showed an altered fatty acid distribution as compared to the reference subjects with CRP below 1.5 mg/l.

To further investigate whether a substantially different fatty acid composition is seen in all depressed subjects with a normal CRP, we repeated the analyses and excluded all subjects (cases and referents) with a CRP above the normal range (upper limit 5 mg/l). Depressed subjects with normal CRP concentrations had significantly lower total n-3 PUFA percentage and a higher n-6/n-3 ratio in plasma phospholipids than the non-depressed subjects with normal CRP concentrations (mean percentage: 5.2 vs. 5.9,  $p = 0.02$ , mean ratio: 7.2 vs. 6.6,  $p = 0.01$ ).

## DISCUSSION

This population-based study showed that fatty acid composition is associated with depressive disorders in the elderly after adjustment for demographic and biological variables. Subjects with depressive disorders had a moderately higher ratio of n-6/n-3 PUFAs, but differences in individual fatty acids were mostly small. However, depressed subjects with a normal CRP had a much more substantial shift in fatty acid composition. No difference in fatty acid composition was found between subjects with subclinical depressive symptoms and reference subjects.

Some methodological issues of the present study need to be discussed before we can interpret the findings. First, this is a cross-sectional study and cannot demonstrate whether depression precedes or follows from altered fatty acid composition. Although depressed subjects generally have less appetite it is not very likely that our results reflect this. Diet change would have to selectively affect percentage n-3 PUFAs and n-6/n-3 ratio but leave percentage saturated and monounsaturated fatty acids unaffected. However, the possibility that the intake of PUFAs changed after the onset of depression (e.g. eating less oily fish) cannot be ruled out.

Secondly, the prevalence of subjects with depressive symptoms in this study (7.8%) was relatively low, but within the range (2.8% to 35%) reported in a recent review.<sup>28</sup> It is also comparable to the prevalence in the US (9.0%).<sup>22</sup> Further, subjects with depressive disorders were slightly underrepresented in the analytical sample because they were less likely to visit the research center. However, we think it is unlikely that the observed relation between fatty acids and depression was due to a selection effect.

Strengths of the present study are its size, the population base and the psychiatric work-up in subjects who were screen positive on the depression scale. We determined which depressive symptoms were due to depressive disorders. Therefore, misclassification of disease is unlikely to have influenced our results. Also, we controlled for numerous confounders. Furthermore, we were able to separate the effects of smoking and atherosclerosis. To our knowledge only three hospital studies investigated the n-6/n-3 ratio comparing depressed subjects with controls. Maes et al. repeatedly found a shift in the n-6/n-3 ratio<sup>10,16</sup>, in a recent study by Peet et al. the n-3/n-6 ratio did not differ between cases and controls.<sup>13</sup> In the latter study concentrations of both total n-3 and n-6 PUFAs were higher in controls as compared to depressed subjects.

Others examined fatty acid composition along the worsening spectrum of depressive disorders, compared depressed to other psychiatric inpatients or focussed on n-3 PUFAs only.<sup>14,15,29</sup>

Several mechanisms may be responsible for the observed association between fatty acid composition and depression. There is a large body of evidence showing that fatty acid composition influences the biophysical properties of neuronal membranes.<sup>30</sup> Via this pathway fatty acids have an impact on receptor function, neurotransmitter re-uptake and signal transmission. In animal models of depression it has been shown that diet can influence membrane properties, n-3 PUFA enriched food for example augmented serotonin receptor sensitivity.<sup>3</sup>

Furthermore, a low ratio of n-6/n-3 PUFAs reduces the risk for vascular disease, presumably by affecting platelet aggregation, blood pressure or direct atherogenic effects.<sup>8,31,32</sup> According to the vascular depression model vascular factors contribute significantly to the pathogenesis of depression. However, in the present study we found no indication that atherosclerosis is an intermediate between fatty acid composition and depression. The same holds for the immune activation. Our data suggest that acute phase proteins do not link fatty acids and depression, unless CRP is not specific enough to measure the immune process. Immune activation is associated with depression and an unfavorable n-6/n-3 ratio most probably stimulates the production of proinflammatory cytokines and other signs of the inflammatory response system.<sup>9</sup> It is assumed that high n-6 PUFA concentrations (especially arachidonic acid) can increase the production of proinflammatory prostaglandins whereas high n-3 PUFA concentrations (especially eicosapentaenoic and docosahexaenoic acid) may inhibit the formation of prostaglandine E<sub>2</sub>.<sup>10,33</sup> Indeed, the differences in PUFAs between depressed and reference subjects in our study were mainly due to the fatty acids associated with immune response. However, if immune modulation underlies the relationship we should have found more subjects with depression among those with both a high n-6/n-3 PUFA ratio and a high CRP.

Rather, the association between fatty acids and depression became stronger with lower CRP concentrations. Different explanations for this interaction are possible. The acute phase response signaled by CRP is characterized by lipolysis.<sup>34</sup> Fatty acids are mobilized from adipocytes and hepatocytes. It has been shown that the acute phase high-density lipoproteins have a different phospholipid composition than normal ones.<sup>35</sup> This suggests that the

serum phospholipid fatty acid composition in the present study could be less informative with respect to long term dietary intake in the presence of high CRP. Another possible explanation is, that high CRP concentrations identify a subpopulation with more health problems, which in turn are associated with depressive disorders in late life. Biological risk factors for depression such as fatty acid composition could be relatively less important. On the other hand, low CRP values indicate the more healthy subjects and in them the relation of depression with fatty acid composition may not be diluted by other health related factors.

In summary, we showed a different fatty acid composition in community dwelling subjects with depressive disorders. Our data suggest that this is not secondary to smoking or cardiovascular risk factors and not linked to depression by atherosclerosis or the inflammatory marker CRP. The findings make it more credible that relatively low concentrations of n-3 PUFAs have a direct impact on mood disorder. Specific diets might thus influence mood. Ultimately, clinical trials are necessary to prove whether diet change or fatty acid supplementation may play a role in prevention or treatment of depressive disorders. So far a positive effect of supplementation with the n-3 PUFAs has only been shown for the short-term course of bipolar and recurrent depression.<sup>36-39</sup>

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4.3

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Vitamin E

**ABSTRACT**

*Objective:* To investigate the reported association between low vitamin E levels and depressive symptoms in a population based study.

*Methods:* The study is based on a cohort of 3,884 adults aged 60 years and over who participated in the third survey of the Rotterdam Study, were screened for depressive symptoms with the Center of Epidemiological Studies Depression Scale and from whom blood was drawn. All screen positive subjects had a psychiatric work up. Blood levels of vitamin E were compared between 262 cases with depressive symptoms and 459 randomly selected reference subjects. All analyses were stratified by sex, and adjusted for age, cholesterol, cognitive score, smoking, dietary supplement use, marital status, living alone, and functional disability score.

*Results:* Vitamin E levels in men with depressive symptoms were lower than in non-depressed men after adjusting for age, whereas no such difference was found in women. This association in men was substantially weakened after controlling for biological factors, and disappeared with additional adjustment for nutritional behaviour and social factors. No differences were observed when the analyses were restricted to cases with depression as defined in the Diagnostic and Statistical Manual of Mental Disorders IV.

*Conclusions:* After control for several biological and behavioural factors relating to health we found no association between low vitamin E levels and depressive symptoms or depression in the elderly.

## INTRODUCTION

Vitamin E is a dietary compound that functions as an antioxidant preventing lipid peroxidation.<sup>1,2</sup> High levels of antioxidants have been reported to slow processes related to atherosclerosis, ageing, and selective neuronal damage.<sup>3,4</sup> The role of oxidative stress in neurological diseases has received much attention in experimental studies.<sup>5</sup> Depression, like dementia and stroke has been associated with impaired antioxidant defences, neuronal damage and vascular changes.<sup>6</sup>

On the basis of this argument the relationship of vitamin E and depression was investigated in two recent epidemiological studies by Maes et al.<sup>7</sup> and Shibata et al.<sup>8</sup> The first was a cross-sectional study in a psychiatric setting, whereas the latter was a population based study in the elderly with cross-sectional and longitudinal analyses. Both studies reported an association between low vitamin E levels and depressive symptoms, although in the Japanese study this association was only observed in men. The relationship between vitamin E and depression, however, may also be explained by other biological and behavioural factors. Nutrition, cognitive and physical function are possible variables that are related to depression and might influence Vitamin E levels. Both quoted studies considered only biological variables and their approach may not have been adequate.

The aim of the present study was to examine the relation of vitamin E and depression with adequate control for possible confounding.

## METHODS

### Study population

This study is based on the Rotterdam Study, an ongoing population-based cohort study in the elderly. The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants. Measurements for the present study took place during the third examination, between March 1997 and December 1999 in which 4,730 persons participated. Of these, 3,884 visited the research centre and had fasting venous blood samples drawn. In the present study, levels of vitamin E were compared between 262 cases with depressive symptoms and 459 randomly selected reference subjects.

### **Depression assessment**

Depressive symptoms were assessed using the Dutch version of the original Center for Epidemiology Studies Depression scale (CES-D). We used a cut-off ( $\alpha$  score of 16), which had a very high sensitivity for major depression in a random sample of older subjects in the Netherlands.<sup>9</sup> Screen positive subjects had a psychiatric work-up as a second step. They were evaluated with the Dutch version of the Present State Examination (PSE-10)<sup>10</sup> and psychiatric disorders were classified according to the Diagnostic and Statistical Manual of Mental Disorders-IV.

Of the 3884 subjects who visited the research centre and had blood taken, 265 (6.8 percent) were screen-positive on the CES-D. Psychiatric disorders were defined in 249 (94 percent) of these participants. Six subjects were lost to follow-up or refused to participate in the psychiatric work-up. These subjects were excluded in the analyses that took the psychiatric work-up into account. Furthermore, blood samples of three screen-positive subjects could not be retrieved.

### **Laboratory assessment**

Vitamin E was determined in plasma using a high pressure liquid chromatography method.<sup>11,12</sup> The intra-assay and interassay coefficients were 3.7 and 3.2 percent, respectively.

### **Other measurements**

The following variables were considered as possible confounders: age, gender, cholesterol levels, cognitive score, smoking, antidepressant drug use, dietary intake of antioxidants, dietary supplement use, marital status, living alone, level of education, and functional disability score. Dietary intake of antioxidants and dietary supplement use were assessed with a semiquantitative food frequency questionnaire at baseline examination (1990-1993) only. Subjects who used supplement preparations that contained at least one of the antioxidants  $\beta$ -carotene, flavonoids, vitamin C or vitamin E, were classified as users of antioxidative supplements. The data for all other measurements was obtained during the third survey. Cholesterol was measured as total cholesterol and HDL cholesterol in mmol/l, cognitive function was assessed with the Mini Mental State Examination. Functional status was assessed with the Disability Index of the Stanford Health Assessment Questionnaire which includes questions as whether subjects are able to do the shopping.<sup>13</sup> All remaining

variables were measured with a dichotomised categorical variable. Marital status expresses whether the participant is widowed and living status whether the participant lives in a home for the elderly.

### **Statistical analysis**

To assess the association between vitamin E and depressive disorders we used analysis of covariance. Vitamin E was entered as a continuous variable into the model. All analyses were also stratified for sex. To check whether confounding existed we compared the crude and the adjusted estimate of the association. The confounding variables were included if this affected the estimates.<sup>14</sup> The results are presented in different models with adjustment for confounding by biological factors, nutritional behaviour and social factors. Next, we performed an analysis of covariance making a distinction between subjects with depressive disorders and subjects with subclinical depressive symptoms. Finally, a regression model was used to evaluate whether a possible sex difference in vitamin E levels could be explained by the confounders measured.

## **RESULTS**

The mean age of the study population was 73.0 years (range 61 to 101). Sixty-four percent of the sample was female.

We found lower levels in vitamin E in men with depressive symptoms as compared to men without depressive symptoms after controlling for age (Table 1). No such difference was found in women (Table 2). This association in men was substantially weakened after controlling for smoking, cognitive score and cholesterol levels. Additional adjustment for nutritional behaviour related variables further reduced the association and rendered it non-significant. (Table 1)

To further examine the relationship between depression and vitamin E we performed separate analyses for cases with depressive syndromes and subclinical depressive symptoms and excluded subjects with psychiatric co-morbidity. The fully adjusted difference for depressed men (n=28) was -0.09  $\mu\text{mol/l}$ ; 95% confidence interval (CI): -2.1, 1.9;  $p = 0.93$  if compared to men without depressive symptoms. Male subjects with subclinical depressive symptoms (n=35) had lower vitamin E levels. The difference was -0.96  $\mu\text{mol/l}$

**TABLE 1: Association between vitamin E levels and depressive symptoms in men (adjusted mean levels, adjusted differences and 95% confidence intervals)<sup>a</sup>**

	Vitamin E levels (µmol/l) in men				
	Reference group n=190	Subjects with depressive symptoms n=68	Difference	95% CI	p-values
Model 1 <sup>b</sup>	26.4	23.8	-2.6	-4.3, -1.0	p=0.002
Model 2 <sup>c</sup>	26.2	24.6	-1.6	-2.9, -0.3	p=0.02
Model 3 <sup>d</sup>	26.1	24.8	-1.4	-2.7, -0.02	p=0.05
Model 4 <sup>e</sup>	26.0	24.9	-1.1	-2.5, 0.2	p=0.11

<sup>a</sup> Analysis of covariance with vitamin E entered as a continuous variable.  
<sup>b</sup> Model 1: Ancova adjusted for age, values represent mean differences, pairwise comparison with non-depressed group.  
<sup>c</sup> Model 2: Ancova adjusted for age, smoking, cognitive score, total cholesterol and HDL cholesterol.  
<sup>d</sup> Model 3: As model 2 but additionally adjusted for dietary supplement use and functional disability score.  
<sup>e</sup> Model 4: As model 3 but additionally adjusted for marital status and living situation.

**TABLE 2: Association between vitamin E levels and depressive symptoms in women (adjusted mean levels, adjusted differences and 95% confidence intervals)<sup>a</sup>**

	Vitamin E levels (µmol/l) in women				
	Reference group n=269	Subjects with depressive symptoms n=194	Difference	95% CI	p-values
Model 1 <sup>b</sup>	28.4	28.7	0.2	-1.0, 1.4	p=0.70
Model 2 <sup>c</sup>	28.4	28.8	0.4	-0.5, 1.4	p=0.37
Model 3 <sup>d</sup>	28.4	28.9	0.5	-0.5, 1.4	p=0.33
Model 4 <sup>e</sup>	26.0	24.9	0.4	-0.6, 1.4	p=0.41

<sup>a</sup> Analysis of covariance with vitamin E entered as a continuous variable.  
<sup>b</sup> Model 1: Ancova adjusted for age, values represent mean differences, pairwise comparison with non-depressed group.  
<sup>c</sup> Model 2: Ancova adjusted for age, smoking, cognitive score, total cholesterol and HDL cholesterol.  
<sup>d</sup> Model 3: As model 2 but additionally adjusted for dietary supplement use and functional disability score.  
<sup>e</sup> Model 4: As model 3 but additionally adjusted for marital status and living situation.

(95%CI: -2.65, 0.74; p = 0.27) compared to men without symptoms.

The sex difference in vitamin E levels observed in our data set (see Table 1) was explained by the differences in cholesterol between men and women. If only gender is entered into the regression model the estimate for gender is highly significant (regression coefficient = 2.95; 95%CI: 2.0, 3.9; p < 0.001)

but it sharply drops and becomes non-significant if total cholesterol and HDL cholesterol are added (regression coefficient = 0.40; 95%CI: -0.40, 1.20;  $p = 0.33$ ).

## DISCUSSION

We found no indication for a relation between vitamin E levels and depressive symptoms in the present population-based study.

This is in contrast to the findings of Maes et al.<sup>7</sup> who observed marked differences in vitamin E levels in a clinical sample and the findings of Shibata et al.<sup>8</sup> in men with depressive symptoms in the Tokyo Metropolitan Institute of Gerontology study.

We considered several possible explanations for these discrepant findings. Firstly, the populations were not alike with respect to age and severity of depression. The average age of the cases in the study of Maes et al. was 54 years and although not explicitly mentioned we assume that all cases had a major depressive episode. However, in our study lower vitamin E levels were observed in less severely depressed men and women.

Secondly, Shibata et al. reported a positive association in the longitudinal but not in the cross-sectional analysis. They included the baseline depressive symptom score in the model of symptom change. This approach - although not uncommon in studies of the association between change in a risk factor and disease - produces biased results because of the phenomenon of regression towards the mean.<sup>15</sup> The larger the effect of the regression towards the mean and the weaker the association between baseline and change, the more bias is introduced.<sup>16</sup> In the study of Shibata et al., there was a significant cross-sectional association in an unadjusted model. Therefore, the association in their longitudinal analyses most likely results from the reported baseline association.

In both studies control for confounders may not have been adequate. Maes et al. used healthy controls and only adjusted for age, sex and LDL cholesterol. Shibata et al. controlled for age, education, total cholesterol and total cholesterol minus HDL-cholesterol. Neither of the studies adjusted for smoking, cognitive function, social or dietary factors.

Finally, one could argue that our adjustment for nutritional behaviour and social factors is an over-correction. Possibly, the elderly who are widowed or

who cannot go shopping on their own get depressed due to poor vitamin E intake. The authors of the present study, however, prefer the interpretation that these factors are independently associated with depression and indicate a poor diet with a low vitamin E intake. Therefore, the fully adjusted model is the most adequate one. Antioxidant dietary intake as measured by the food frequency questionnaire is not included because it did not confound the relation between vitamin E levels and depression in the present study. This does not mean that there is no relation between intake and blood levels. The differences in normal nutritional intake may be too subtle to be detected with the method used in this study.<sup>17,18</sup>

In summary, our findings do not support the hypothesis that depression and vitamin E levels are associated. In the present study, the observed differences in vitamin E levels were largely due to confounding by biological factors and variables related to nutritional behaviour or social factors.

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## 4.4

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Vitamin B<sub>12</sub>, folate  
and homocysteine

**ABSTRACT**

*Objective:* The associations of vitamin B<sub>12</sub>, folate, and homocysteine with depression were examined in a population-based study.

*Method:* The authors screened 3,884 elderly people for depressive symptoms. Subjects with positive screening results had psychiatric workups. Folate, vitamin B<sub>12</sub>, and homocysteine blood levels were compared in 278 persons with depressive symptoms, including 112 with depressive disorders, and 416 randomly selected reference subjects. Adjustments were made for age, gender, cardiovascular disease, and functional disability.

*Results:* Hyperhomocysteinemia, vitamin B<sub>12</sub> deficiency, and to a lesser extent, folate deficiency were all related to depressive disorders. For folate deficiency and hyperhomocysteinemia, the association with depressive disorders was substantially reduced after adjustment for functional disability and cardiovascular disease, but for vitamin B<sub>12</sub> this appeared independent.

*Conclusions:* The association of vitamin B<sub>12</sub> and folate with depressive disorders may have different underlying mechanisms. Vitamin B<sub>12</sub> may be causally related to depression, whereas the relation with folate is due to physical comorbidity.

## INTRODUCTION

Folate and vitamin B<sub>12</sub> are involved in the one-carbon metabolism necessary for the production of monoamine transmitters. Several case-control studies since the 1960s have shown high prevalences of folate and vitamin B<sub>12</sub> deficiency in depression.<sup>1</sup> More recently, the total plasma homocysteine level was shown to be a sensitive marker of folate and vitamin B<sub>12</sub> deficiency, and higher concentrations of homocysteine were observed in depressed patients.<sup>2</sup>

Thus far we know of only one population-based study; that study<sup>3</sup> confirmed that vitamin B<sub>12</sub> deficiency was associated with depressive symptoms. However, folate deficiency and hyperhomocysteinemia were not related to depressive symptoms. The study was restricted to physically disabled women, and no clinical diagnosis of depression was made. In this study we examined the associations of folate, vitamin B<sub>12</sub>, and homocysteine with depression in community-dwelling elderly subjects in the Rotterdam Study who had a clinical diagnosis of depression.

## METHOD

This study was conducted as part of the Rotterdam Study, a population-based study to which all inhabitants aged 55 years and over in a district of Rotterdam were invited in 1990–1993. In the third survey (1997–1999) we added assessments of depression to the study protocol. Of the 5,901 subjects who were invited, 4,730 participated in the home interview. Of these, 3,884 visited the research center and had overnight fasting blood samples drawn. Subjects were screened for depressive symptoms during the home interview with the Center for Epidemiology Studies Depression Scale (CES-D Scale).<sup>4</sup> This is a 20-item self-report measure of symptoms experienced in the last week and is scored on a scale of 0 to 3 points. Subsequently, subjects with positive results in the screening (a score of 16 or higher) had a psychiatric workup with the Present State Examination. Psychiatric disorders were classified according to DSM-IV criteria. Of the subjects who visited the research center and had blood taken, 278 (7.0%) had positive screening results, and 262 (94.2%) of these were given psychiatric workups. Of these subjects, 112 fulfilled the diagnostic criteria for depression, i.e., major or minor depression or dysthymia. The sub-

jects with depressive symptoms and disorders were compared with 416 comparison subjects randomly selected from the subjects with negative screening results. The study was approved by the Medical Ethics Committee of Erasmus University School. After complete description of the study to the subjects, written informed consent was obtained.

Serum folate and vitamin B<sub>12</sub> levels were measured by using an immunoassay. The total plasma homocysteine level was determined with high-performance liquid chromatography. To determine vitamin deficiency we used the following cutoffs, which correspond to the normal ranges of the assays and are described in the literature.<sup>3</sup> Folate deficiency was considered present when the serum folate level was less than 11.4 nmol/liter and the homocysteine level was higher than 13.9 mmol/liter. Vitamin B<sub>12</sub> deficiency was defined as a level less than 258 pmol/liter. We defined hyperhomocysteinemia as a plasma level above 15.0 mmol/liter.

The following variables were considered as confounders: age, gender, alcohol consumption, education, smoking, and cognitive function as measured by the Mini-Mental State Examination (MMSE). In additional analyses we also adjusted for functional status, blood pressure, and history of stroke and myocardial infarction. The latter were obtained through direct questioning and linkage with general practitioner records. Functional status was assessed with the Stanford Health Assessment Questionnaire.<sup>5</sup> We used logistic regression to assess the associations of hyperhomocysteinemia, vitamin B<sub>12</sub> deficiency, and folate deficiency with depressive disorders or symptoms. Furthermore, differences in mean levels were tested with analysis of variance. Finally, we analyzed the relation between self-reported loss of appetite and vitamin deficiencies. For this the subjects' score on the CES-D Scale item "I did not have any appetite" was entered in an age- and gender-adjusted model.

## RESULTS

The mean age of the study sample was 72.9 years (SD=7.1), 16% were smokers, 11% had had a myocardial infarction, and 4% had had a stroke. The subjects with depressive symptoms were more likely to be female (73% versus 58%,  $\chi^2=18.2$ ,  $df=1$ ,  $p<0.001$ ), had a higher age-adjusted functional disability score (mean=0.8, SD=0.6, versus mean=0.5, SD=0.4;  $F=47.7$ ,  $df=1$ ,  $694$ ,  $p<0.001$ ), and had a lower MMSE score (mean=27, SD=2, versus mean=28,

**TABLE 1: The relation of vitamin B<sub>12</sub> deficiency, folate deficiency and hyperhomocysteinemia to depressive symptoms and disorders among apopulation-based sample of elderly subjects in Rotterdam**

	Likelihood of depressive symptoms among subjects with depressive symptoms (N=278) and subjects without depressive symptoms (N=416) <sup>b</sup>		Likelihood of depressive disorders among subjects with depressive disorders (N=112) and subjects without depressive symptoms (N=416) <sup>c</sup>	
	Odds ratio	95% CI	Odds ratio	95% CI
<b>Nutritional abnormality and model (type of adjustment)<sup>a</sup></b>				
Vitamin B <sub>12</sub> deficiency (<258 pmol/liter)				
Model 1 (age and gender)	1.02	0.74; 1.42	1.69	1.10; 2.56
Model 2 (general confounders)	0.98	0.70; 1.36	1.64	1.05; 2.56
Model 3 (functional disability)	0.96	0.68; 1.35	1.63	1.03; 2.56
Model 4 (cardiovascular risk factors)	0.97	0.69; 1.37	1.63	1.03; 2.58
Folate deficiency (<11.4 nmol/liter plus homocysteine >13.9 µmol/liter)				
Model 1 (age and gender)	1.14	0.72; 1.80	1.52	0.85; 2.71
Model 2 (general confounders)	0.95	0.59; 1.53	1.49	0.83; 2.67
Model 3 (functional disability)	0.88	0.55; 1.44	1.06	0.56; 1.99
Model 4 (cardiovascular risk factors)	0.89	0.55; 1.44	0.97	0.51; 1.87
Hyperhomocysteinemia (>15.0 µmol/liter)				
Model 1 (age and gender)	1.55	0.95; 2.53	2.07	1.11; 3.83
Model 2 (general confounders)	1.44	0.87; 2.38	1.87	0.99; 3.54
Model 3 (functional disability)	1.25	0.75; 2.10	1.51	0.77; 2.97
Model 4 (cardiovascular risk factors)	1.16	0.69; 1.97	1.27	0.63; 2.58

<sup>a</sup> In model 2, general confounders included age, gender, education, smoking, alcohol intake, and score on the Mini-Mental State Examination. Model 3 was additionally adjusted for the score on the Stanford Health Assessment Questionnaire (5), a measure of disability in activities of daily living. Model 4 was additionally adjusted for cardiovascular risk factors: history of stroke, history of myocardial infarction, and systolic blood pressure.

<sup>b</sup> Depressive symptoms were defined as a score of 16 or higher on the CES-D Scale. The numbers of subjects with vitamin B12 deficiency, folate deficiency, and hyperhomocysteinemia were 95, 41, and 35, respectively.

<sup>c</sup> A depressive disorder was DSM-IV major or minor depression or dysthymia. The numbers of subjects with vitamin B12 deficiency, folate deficiency, and hyperhomocysteinemia were 49, 21, and 19, respectively.

SD=3;  $F=22.2$ ,  $df=1$ , 694,  $p<0.001$ ) than the nondepressed subjects.

Among the 278 subjects with depressive symptoms and the 416 comparison subjects, hyperhomocysteinemia, vitamin B<sub>12</sub> deficiency, and folate deficiency were not associated with the presence of depressive symptoms (Table 1). When we restricted the analysis to the 112 subjects fulfilling the DSM-IV criteria for depressive disorders, we found that subjects with vitamin B<sub>12</sub> deficiency were nearly 70% more likely than the comparison subjects to have a depressive disorder (Table 1). This association was independent of cardiovascular factors and functional status. When adjusting for only age and gender, we also observed a significant association of hyperhomocysteinemia and a nonsignificant association of folate deficiency with depressive disorders. However, these associations disappeared after correction for cardiovascular factors and functional status.

No substantial differences in mean levels of vitamin B<sub>12</sub> and folate were observed between the subjects with depressive disorders or symptoms and the reference subjects (data not shown). The subjects with depressive disorders had higher homocysteine levels (mean=11.7  $\mu\text{mol/liter}$ , SD=5.9) than the reference subjects (mean=10.6, SD=3.7); the age- and gender-adjusted difference was 1.1  $\mu\text{mol/liter}$ , and the 95% confidence interval (CI) was 0.3 to 2.0 ( $p=0.01$ ). However, adjustment for cardiovascular risk indicators and functional disability strongly decreased this difference, to 0.2  $\mu\text{mol/liter}$  (95% CI=-0.7 to 1.1,  $p=0.66$ ).

Self-reported loss of appetite was not associated with vitamin B<sub>12</sub> deficiency (odds ratio=1.03 per 1-point increase in symptom score, 95% CI=0.86 to 1.24,  $p=0.73$ ), whereas it was significantly related to hyperhomocysteinemia (odds ratio=1.32, 95% CI=1.03 to 1.70,  $p=0.03$ ); the relationship to folate deficiency fell short of significance (odds ratio=1.24, 95% CI=0.98 to 1.58,  $p=0.07$ ).

## DISCUSSION

This population-based study shows that elderly persons with vitamin B<sub>12</sub> deficiency are more likely to have a depressive disorder. Furthermore, we observed a significant relationship of hyperhomocysteinemia and a nonsignificant relationship of folate deficiency to depression that were due to physical comorbidity and cardiovascular risk factors in subjects with depression.

Some methodological issues of the present study need to be considered.

First, this was a cross-sectional study; it cannot demonstrate whether the observed association with vitamin deficiencies precedes or results from the depression. Most important, lack of appetite is a cardinal feature of depression and could explain the association. Indeed, we observed a relationship of self-reported loss of appetite with hyperhomocysteinemia and folate deficiency. However, no such relationship was found with vitamin B<sub>12</sub> deficiency, and thus, appetite loss cannot account for the respective findings. Second, the prevalence of depressive symptoms (7.0%) was relatively low. However, it falls within the range (2.8% to 35%) reported in a review of the prevalence of depression among elderly people.<sup>6</sup> A strength of this population-based study is the psychiatric workup of subjects who had positive screening results. Therefore, misclassification of disease is unlikely to have influenced our results.

The findings are consistent with the results of Penninx et al.<sup>3</sup>, who reported that physically disabled women with vitamin B<sub>12</sub> deficiency were twice as likely to have severe depressive symptoms. However, they observed no association between homocysteine or folate and depression. The present study strongly suggests that the higher rates of depressive disorders in subjects with low folate and high homocysteine levels are due to differences in cardiovascular factors and physical comorbidity. It is possible that earlier reports of a relation between folate deficiency and depression in psychiatric patients were confounded.<sup>1</sup> The cardiovascular risk profile of the patients with depression might have been different from those of comparison subjects. On the other hand, it can be argued that adjustment for cardiovascular factors and functional disability is overcorrection. First, functional disabilities in daily activities, which include difficulties in shopping, may reduce the vitamin intake. It is plausible that depression develops thereafter as a consequence of low intake. Second, late-life depression might result from vascular diseases due to hyperhomocysteinemia. Adjustment for cardiovascular factors is appropriate only if one studies the direct effects of hyperhomocysteinemia that might be due to alterations in monoamine metabolism. In this case one should control for the fact that depression may also result from health impairment or neuronal damage in patients with vascular disease.

The effects of adjustment indicate that the associations of vitamin B<sub>12</sub> and folate deficiency with depression have different underlying mechanisms. It has been reported<sup>3</sup> that serum folate is more sensitive to nutritional intake than vitamin B<sub>12</sub>. Moreover, in the present study, folate but not vitamin B<sub>12</sub> deficiency was related to loss of appetite. In summary, this study suggests that

clinicians need to be aware of possible hyperhomocysteinemia in combination with cardiovascular factors or functional disability in depressed patients. Furthermore, vitamin B<sub>12</sub> deficiency may be causally related to depression in the elderly. In view of the possible benefits of vitamin replacement, detection of this subgroup is important.

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Plasma pterins  
and folate

## **ABSTRACT**

*Background:* Tetrahydrobiopterin is a cofactor in the synthesis of serotonin. Numerous small clinical studies investigated the association between biopterin and depression with conflicting results. We studied the relation of plasma biopterin with depression in a population-based study taking neopterin and folate levels into account. High neopterin levels may signal impaired biopterin biosynthesis. High folate levels increase biopterin biosynthesis.

*Methods:* We screened 3,884 adults aged 60 years and over for depressive symptoms and performed psychiatric interviews in screen-positives. Plasma pterins were determined in 238 cases with depressive symptoms and 357 randomly selected non-depressed persons.

*Results:* In the non-depressed the neopterin/biopterin ratio was negatively associated with folate (-0.21 per standard deviation (SD), 95% confidence interval (CI)=-0.40, -0.02) but not in depressed subjects (0.01; 95%CI: -0.14, 0.17). Instead, in depressed persons high folate were accompanied by high neopterin concentrations (1.4 nmol/l per SD; 95%CI=0.41, 2.3). Furthermore, persons with low folate and a low neopterin/biopterin ratio had more depressive symptoms ( $p=0.004$  for interaction between folate and neopterin/biopterin ratio).

*Conclusions:* Our findings suggest differences in pterin metabolism between depressed and non-depressed persons. Regulation of the biopterin homeostasis is complex and immunological, hormonal and genetic factors are involved. However, in some persons low folate levels probably cause depression in case of impaired pterin synthesis.

## INTRODUCTION

Tetrahydrobiopterin (BH<sub>4</sub>) is present in probably every cell or tissue of higher organisms. The best-established function of BH<sub>4</sub> is that of a cofactor in the hydroxylation of phenylalanine, tyrosine and tryptophan.<sup>1</sup> These are rate-limiting steps in the synthesis of the monoamine neurotransmitters dopamine, serotonin and noradrenalin.

Neurotransmitter metabolism in depression has been a major area of psychiatric research over the past 40 years. On the basis of experimental evidence from pharmacological, pathoanatomical and imaging studies in humans and animals monoamine theories of depression have been postulated. According to the classic monoamine theory, functional deficiencies of catecholamines and serotonin in the brain play a crucial role in the pathogenesis of depression.<sup>2,3</sup> The theory has stimulated researchers to investigate the association of depression with potential markers of monoamine metabolism.<sup>4</sup> Against this background, numerous small clinical studies have tried to relate plasma concentrations of biopterins to depressive disorders in patients. However, the findings are conflicting; reduced and increased concentrations of biopterin in depressed patients have been reported.<sup>5-10</sup> More recently, neopterin and folates were investigated in this context.<sup>11,12</sup> Increased neopterin may signal a failure to synthesize biopterin.<sup>13,14</sup> Folates on the other hand increase the biosynthesis of biopterin and a salvage pathway for BH<sub>4</sub> depends on dihydrofolate reductase.<sup>1,15</sup>

We examined the relation of biopterin and neopterin with depression in a large population-based sample of older adults. In particular, we sought to investigate whether this relation was dependent on folate levels. Demographic, clinical, and functional characteristics that may influence the reporting of depressive symptoms were controlled for.

## SUBJECTS AND METHODS

### Study population

This study is based on the third examination round of the Rotterdam Study, an ongoing population based cohort study in a district of Rotterdam.<sup>16</sup> The Medical Ethics Committee of the Erasmus University approved the study and written informed consent was obtained from all participants. Measurements

took place between March 1997 and December 1999 and included a home interview and a visit to the research center. Of the 4703 persons over 60 years who participated in the home interview, 3,884 visited the research center, where blood was drawn. The 819 subjects who were not seen at the center were on average older (77.5 versus 72.3 years), more likely to be female (70 % versus 58 %) and had more depressive symptoms (12.2% versus 6.8%, overall prevalence 7.8%). From 3510 subjects blood samples were available for biochemical analysis. The 334 subjects in whom no or insufficient blood samples were available did not differ from the remainder in respect to age, gender and frequency of depressive symptoms. In the present analysis we compared plasma biochemical parameters between all non-demented subjects with depressive symptoms and 357 randomly selected non-demented reference subjects.

### **Depression assessment**

Depressive disorders were assessed using a two step procedure. First participants completed the Dutch version of the original Center for Epidemiologic Studies Depression scale (CES-D) during the home interview. The CES-D is a 20-item self-reported measure of symptoms experienced in the last week including a question on lack of appetite. Each item is scored on a scale of 0 to 3 points. The criterion validity of the CES-D version is well established.<sup>17</sup> We used a score of 16 as a cut-off, which had a very high sensitivity for major depression in elderly subjects in the Netherlands.<sup>18</sup> Moreover, previous studies have verified that a score of 16 and above on the CES-D represents clinically significant depressive symptoms.<sup>19</sup> As a second step, screen positive subjects had a psychiatric work-up using the Dutch version of the Present State Examination (PSE-10), a semi-structured psychiatric interview included in the Schedules for Clinical Assessment in Neuropsychiatry.<sup>20</sup> All interviews were conducted by one or other of two experienced clinicians. Psychiatric disorders were classified according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria with an algorithm based on the PSE-10 scores. The diagnostic categories include major depression, dysthymia and minor depression as defined in the appendix of the DSM-IV.<sup>21</sup>

Of the 3510 subjects eligible for the present study, 243 (6.9 %) were screen-positive on the CES-D. Five subjects with depressive symptoms were excluded because of dementia. The remaining 238 subjects were included in the analysis of depressive symptoms. Of these, nine subjects refused the subsequent

psychiatric interview, and five screen positive subjects could not be reached. Psychiatric disorders were thus assessed in 224 (94 %) participants. Psychiatric work-up revealed that 91 cases had a depressive disorder as defined by the Diagnostic and Statistical Manual of Mental Disorders-IV. The remaining 133 subjects were diagnosed as having depressive symptoms as part of anxiety or another psychiatric disorder (n=28) or did not meet criteria for an Axis I psychiatric disorder (n=105, subclinical depressive symptoms).

### **Selection of reference subjects**

A sample of 357 participants ranging in age from 61 to 101 years, served as the reference group. They were randomly selected from the non-demented participants of the Rotterdam Study who were screen-negative for depression. We did not match on possible confounding variables in order to be able to study their effect on the relation between pterins (neopterin and biopterin) and depression.

### **Blood specimens**

Overnight fasting blood was collected under standardized conditions in a 10 ml tube of ethylene diamine tetracetic acid (EDTA)-anticoagulated blood, put on ice directly and centrifuged at 2650g for 20 min. Plasma was separated, dispensed into two 1.5 ml aliquots and frozen within three hours at -80°C until analyzed. Plasma biopterin occurs as  $BH_4$  (active and main reduced form), as quinoid  $BH_2$  and oxidized biopterin. We measured total biopterin and neopterin (dihydroneopterin and neopterin) after acid oxidation of the reduced forms of both pteridines as described by Fukushima and Nixon (1980).<sup>22</sup> Plasma (0.4 ml) was oxidized in 0.1 ml 1 M trichloroacetic acid and 0.05 ml iodine solution (0.5% I<sub>2</sub>, 1%KI in 0.2 M trichloroacetic acid). After standing 60 min under reduced light, excess iodine was reduced by the addition of 20 µl of 1% ascorbic acid solution and the mixture was centrifuged at 12 000 x g for 15 min at 4°C. The supernatant (0.4 ml) was transferred to an amber glass vial and 10 µl was injected directly onto the analytical column using an HPLC system with an autosampler and a fluorescence detector as described previously.<sup>23</sup> The intra-assay and interassay coefficients for neopterin were 2.4% and 3.8%, respectively, and for biopterin 2.1% and 2.8%, respectively.

The concentration of the serotonin metabolite 5-HIAA (5-hydroxyindoleacetic acid) was analyzed by HPLC and electrochemical detection.<sup>24</sup> Intra-assay coefficient was 3.6% and interassay coefficient 4.2%.

Serum folate concentrations measured by an immunoassay (Roche Diagnostics, Mannheim, Germany) were available in all participants but for four subjects with depressive symptoms and five reference subjects.<sup>25</sup>

### **Other measurements**

The following variables were considered as possible confounding variables: age, gender, level of education, history of stroke and myocardial infarction, cognitive function as measured by the Mini Mental State Examination, disabilities in activities of daily living and cigarette smoking. Education was measured on an ordinal scale and later dichotomized at the median of the baseline sample into low and high education. A history of stroke or myocardial infarction was obtained from all subjects through direct questioning and computerized linkage with general practitioner medical records. Cigarette smoking was scored in categories of current, former and never smoker. Disabilities in activities of daily living were assessed using the score (continuous) of the Disability Index of the Stanford Health Assessment Questionnaire.<sup>26</sup> This measure reflects the consequences of disease in terms of functional performance and activities.

### **Statistical analysis**

First, we calculated the ratio between neopterin and biopterin. Subsequently, the associations of pterins, their ratio and 5-HIAA with depression were quantified with logistic regression. We estimated odds ratios and 95 % confidence intervals (CI) for depressive symptoms and depressive disorders. The plasma concentrations and the ratio were entered into separate logistic regression models as continuous variables (divided by their standard deviation).

Next, we included folate levels in our analyses of pterins and depression. First, we studied the association of folate with pterins and neopterin/biopterin ratio in depressed and non-depressed subjects using multiple regression analysis. Secondly, we examined whether there was an association between pterins and depression that depended on serum folate concentrations. For this aim, we entered folate concentrations and an interaction term between pterins and folates into the models that already contained pterins. Primarily, this was done using the continuous variables and p-values for the multiplicative interaction term are reported. Because the interaction between two continuous variables is difficult to interpret, we subsequently categorized folate concentration and compared neopterin/biopterin ratio with stratified analyses of

covariance.

Age (continuous) and gender were controlled for in all analyses. To control for confounding we added all measured potential confounders to the basic model. Finally, all analyses were rerun and additionally adjusted for lack of appetite to check whether associations found were a consequence of poor dietary intake in depressed subjects.

## RESULTS

The characteristics of the non-depressed persons and those with depressive symptoms and disorders are presented in table 1. Depressed subjects were more likely to be female, to have had a stroke or myocardial infarction, had higher levels of functional disability and lower cognitive scores.

Table 2 shows that in the unstratified analyses pterins, the neopterin/biopterin ratio and 5-HIAA concentrations were not associated with depres-

Variables	Non-depressed n=357	Depressive symptoms <sup>†</sup> n=238	Depressive disorders <sup>†</sup> n=91
Age, years, mean (range)	72.3 (61-101)	73.8 (61-93)	73.4 (61-97)
Gender, % female	59.4	73.5	72.5
Primary education only, %	50.1	55.9	57.1
MMSE-score, mean (SD)	27.8 (1.9)	27.1 (2.7)	26.7 (3.2)
History of stroke, %	2.2	6.3	8.8
History of MI, %	10.6	11.3	14.3
Smoking: current smoker, %	14.3	18.5	19.8
ex-smoker, %	35.7	42.0	42.9
Functional disability score	0.5 (0.5)	0.7 (0.6)	0.8 (0.7)
Neopterin (nmol/l)	21.1 (99.6)	21.6 (8.6)	22.7 (10.4)
Biopterin (nmol/l)	6.2 (1.4)	6.4 (2.5)	6.8 (3.9)
Neopterin/biopterin ratio	3.5 (1.7)	3.5 (1.4)	3.6 (1.6)

SD = Standard deviation, MMSE = Mini Mental State Examination, MI = myocardial infarction  
<sup>†</sup> The category depressive symptoms includes all subjects with a Center of Epidemiological Studies Depression scale (CES-D) score  $\geq$  16. The category depressive disorders includes only the cases with major or minor depression and dysthymia.

**TABLE 2: The association of pterins and 5-HIAA with depressive symptoms and depression, Rotterdam Study, 1997-1999**

	Depressive symptoms* (238 cases)		Depressive disorder* (91 cases)	
	Odds ratio	95% CI	Odds ratio	95% CI
Neopterin (per SD)	0.94	0.80, 1.10	1.02	0.83, 1.25
Biopterin (per SD)	1.02	0.90, 1.15	1.07	0.93, 1.23
Neopterin/biopterin ratio (per SD)	0.93	0.77, 1.13	1.01	0.79, 1.29
5-HIAA (per SD)	0.93	0.77, 1.12	1.05	0.82, 1.35

SD = standard deviation; CI = confidence interval; 5-HIAA = 5-hydroxyindoleacetic acid  
 \*The category 'depressive symptoms' includes all subjects who were screen positive, the category 'depressive disorder' includes only the subjects with major or minor depression and dysthymia.  
 †Logistic regression analyses adjusted for age, gender, cognitive score, smoking, history of stroke and myocardial infarct; subjects (n=357) without depressive symptoms were used as the reference group. Inflammation markers were analyzed as a continuous variable divided by one standard deviation.

sive symptoms or depressive disorders. The analyses presented were adjusted for age, gender, smoking, cognitive score, functional disability score, history of myocardial infarction and stroke.

In both depressed and non-depressed subjects there was a trend for higher folate levels to be associated with higher biopterin concentrations (Table 3). However, the relation between folates and neopterin differed substantially between depressed and non-depressed persons. In subjects with depressive symptoms and depressive disorders higher folate levels were accompanied by higher neopterin concentrations, whereas this was not the case in the non-depressed. In this group, higher folate concentrations were weakly related to lower neopterin levels. Finally, the neopterin/biopterin ratio was inversely related to folates in non-depressed but not in the depressed persons.

Next, we examined whether the relation between pterins and depression depended on serum folate concentrations. We found that in subjects with lower folate concentrations those with a lower neopterin/biopterin ratio were more likely to have a depression, whereas in subjects with higher folate levels a higher neopterin/biopterin ratio was associated with a higher risk of depression (p-values for interaction terms were 0.004 for depressive symptoms and 0.04 for depressive disorders in fully adjusted models). Interaction with folate levels was also found for neopterin (p = 0.02 and p = 0.08, respectively) but

**TABLE 3: Association between folate concentration and pterins in subjects with and without depressive symptoms (expressed as increase per standard deviation of folate level)**

Group of subjects	Biopterin nmol/l			Neopterin nmol/l			Neopterin/biopterin ratio		
	increase	95% CI	p-value	increase	95% CI	p-value	increase	95% CI	p-value
Non-depressed per SD of folate	0.14	-0.02, 0.31	0.08	-0.70	-1.8, 0.40	0.23	-0.21	-0.40, -0.02	0.03
Depressive symptoms* per SD of folate	0.28	-0.03, 0.60	0.08	1.4	0.41, 2.3	0.005	0.01	-0.14, 0.17	0.93
Depressive disorders* per SD of folate	0.46	-0.25, 1.17	0.2	2.0	0.32, 3.61	0.02	-0.01	-0.30, 0.25	0.81

SD=Standard deviation

Values for increase (betas) were obtained with multiple regression adjusted for age, gender, cognitive function, history of stroke and myocardial infarct, smoking, and functional disability score.

\*The category 'depressive symptoms' includes all subjects who were screen positive, the category 'depressive disorder' includes only the subjects with major or minor depression and dysthymia.

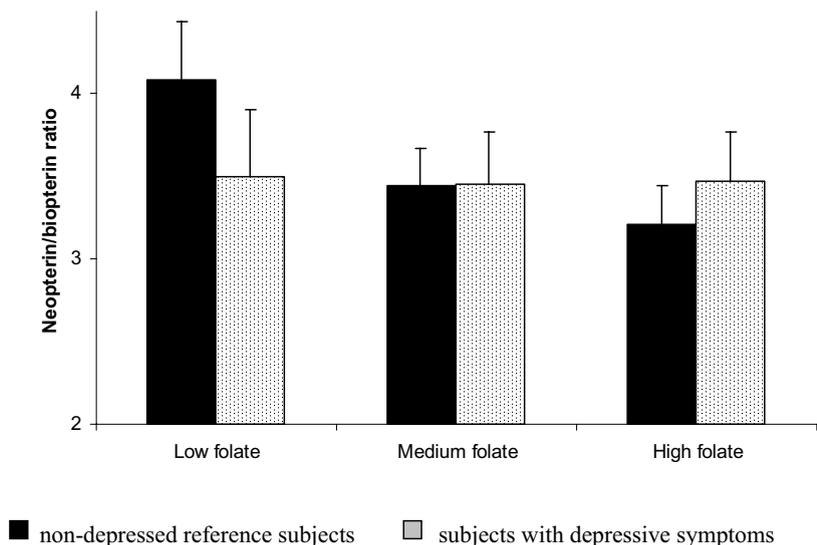


Figure 1: Association between plasma neopterin/biopterin ratio and depressive symptoms by tertiles of serum folate concentrations.

Mean plasma neopterin/biopterin ratio and upper limit of 95% confidence intervals adjusted for age, gender, smoking, history of stroke and of myocardial infarction, cognitive score, and functional disability score as calculated by analysis of covariance. Number of subjects with depressive symptoms were 81, 70 and 83 in first to third tertile and 111, 123 and 118 referent subjects, respectively. Logistic regression was performed to test the interaction between neopterin/biopterin ratio and tertiles of folate on depressive symptoms, overall  $p=0.01$ .

not for biopterin ( $p=0.6$  and  $p=0.9$ , respectively).

Because the interaction between two continuous variables is difficult to interpret we categorized folate concentration and compared neopterin/biopterin ratio with stratified analyses of covariance. We defined tertiles to have sufficient numbers of cases and referent subjects in each category. The interaction effects were tested with logistic regression and found to be comparable to the continuous model. Figure 1 shows that the neopterin/biopterin ratio between reference subjects and subjects with depressive symptoms differs in particular in subjects with low folate. Little differences were observed in the middle and upper tertile of folate concentration. The fully adjusted mean neopterin/biopterin ratios in the lower tertile were 3.5 and 4.1 for subjects with and without depressive symptoms, respectively, mean difference was 0.6, 95% confidence interval (CI): 0.1, 1.2,  $p=0.04$ . The respective results for depressive disorders (figure 2) were 4.1 and 3.3, adjusted mean difference = 0.8, 95% CI: 0-1.6,  $p=0.06$ .

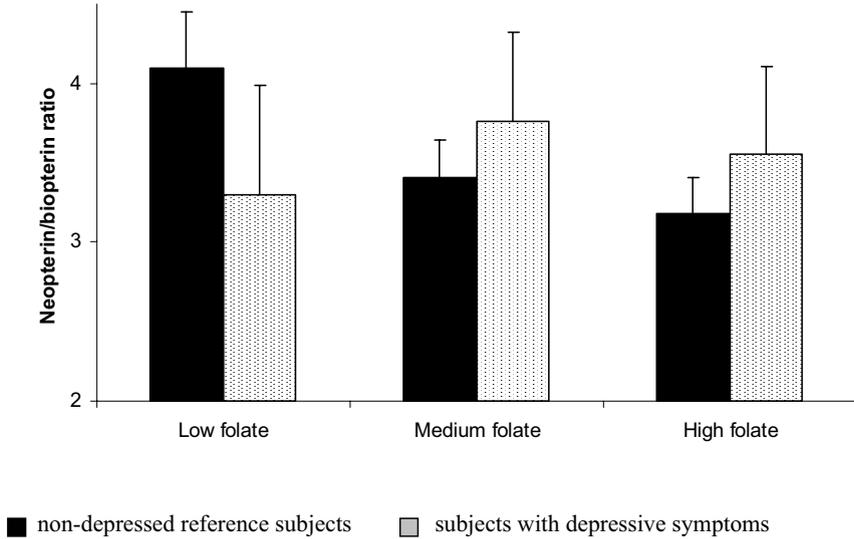


Figure 2: Association between plasma neopterin/biopterin ratio and depressive disorders by tertiles of serum folate concentrations.

Mean plasma neopterin/biopterin ratio and upper limit of 95% confidence intervals adjusted for age, gender, smoking, history of stroke and of myocardial infarction, cognitive score, and functional disability score as calculated by analysis of covariance. Number of subjects with depressive disorders were 37, 26 and 27 in first to third tertile and 111, 123 and 118 referent subjects, respectively. Logistic regression was performed to test the interaction between neopterin/biopterin ratio and tertiles of folate on depressive disorders, overall  $p=0.03$ .

Subsequently, we investigated whether 5-HIAA concentrations between subjects with and without depression differed according to folate levels. No differences were observed in the middle and upper tertile of folate concentration. Subjects with depressive symptoms and folate levels in the lower tertile had slightly lower 5-HIAA concentration than the reference subjects (4.1 log (nmol/L) vs 4.0 log (nmol/L), mean difference 0.08; 95% CI: -0.01, 0.18,  $p=0.08$ , values were logtransformed to achieve a normal distribution).

Finally, all analyses were rerun with additional adjustment for appetite. Results did not change.

## DISCUSSION

In this large population-based study no association was found between plasma levels of pterins and depression. However, the relation between pter-

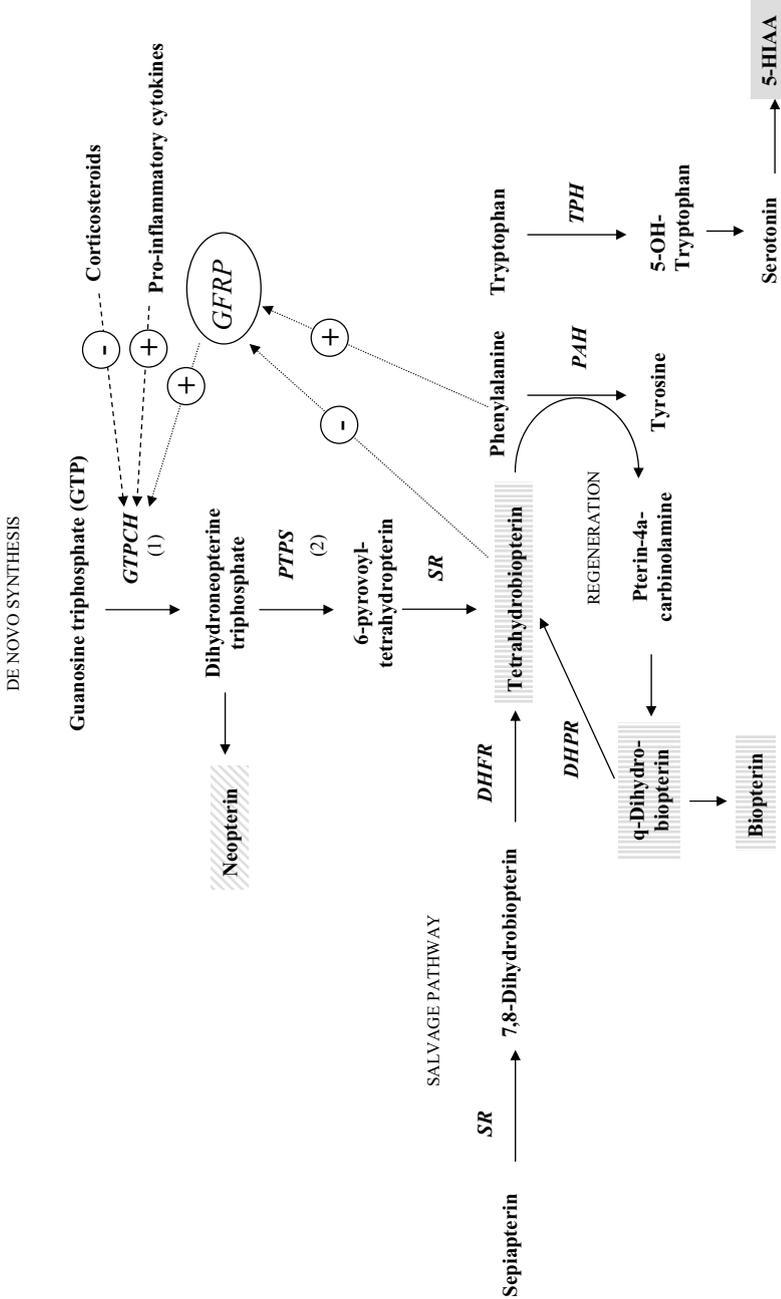
ins and folate concentrations was different for depressed and non-depressed persons. In depressed subjects higher folate concentrations were accompanied by higher neopterin concentrations, whilst in the non-depressed participants folate was negatively associated with the neopterin/biopterin ratio. The difference between the two groups was most obvious at relatively low folate levels. Persons with low folate and a lower neopterin/biopterin ratio were more likely to have depressive symptoms than those with a higher ratio.

The strengths of this study are the large number of elderly people participating and its population-based design. The size of the study enabled us to detect interactions. A methodological limitation is the cross-sectional design. It is not possible to conclude whether depression follows or precedes the altered pterin metabolism. Furthermore, we determined pterin concentrations in plasma and not cerebrospinal fluid. Because of the blood brain barrier our observations reflect peripheral biopterin metabolism much more than brain metabolism. However, lumbar puncture is not feasible in a population based study.

Previous research on pterins and depression yielded conflicting results. High and low plasma biopterin levels as well as high and low plasma neopterin/biopterin ratios in depressed patients have been reported.<sup>5-10,12</sup> Other investigators interested in immunological aspects of depression have focussed on neopterin measurements only.<sup>27-29</sup> Neopterin is released by activated macrophages and considered a marker of cell-mediated immunity. Some of these authors have reported increased plasma levels in patients as compared to normal controls.<sup>27,29</sup>

We found a relation between pterins and depression, but only when folate concentrations were taken into account. This may explain the conflicting observations on biopterin and neopterin/biopterin ratio in psychiatric research reported to date; interaction with folate concentrations was not considered. Previous studies examined the correlation between blood folate concentrations and urinary or cerebrospinal liquor biopterin in depressed patients.<sup>10,30,31</sup> However, an association between plasma neopterin or neopterin/biopterin ratio and folates in community dwelling persons has, to our knowledge, not been investigated.

Tetrahydrobiopterin ( $BH_4$ ) dependent enzymes include the phenylalanine, tyrosine, and tryptophan hydroxylases, the latter two being the rate-limiting enzymes for catecholamine and 5-hydroxytryptophan (serotonin) biosynthesis.<sup>1</sup> In the last ten years the molecular basis of biosynthesis and regeneration



**Figure 3 Biosynthesis of Tetrahydropterin**

GTPCH = guanosine triphosphate cyclohydrolase I; GFRP = GTPCH feedback regulatory protein; PTPS = 6-pyruvoyl-tetrahydropterin synthase; SR = sepiapterin reductase; DHFR = dihydropteridin reductase; DHPR = dihydropteridin reductase; PAH = phenylalanine-4-hydroxylase; TH = tyrosine-3-hydroxylase; 5-HIAA= 5-hydroxyindoleacetic acid. Levels of neopterin (////), tetrahydrobiopterin, q-dihydrobiopterin and biopterin (||||), and 5-HIAA (■) were determined in the present study. (1) and (2) refer to enzymes mentioned in the text.

of BH<sub>4</sub> has been unraveled.<sup>32</sup> The *de novo* biosynthesis of BH<sub>4</sub> is illustrated in figure 3. The first enzymatic step is the major controlling point for the biosynthesis (see 1 in figure 3). The activity of the involved enzyme (can be stimulated up to 100-fold.<sup>1</sup> The figure also shows some of the many regulators of this enzyme, e.g. BH<sub>4</sub> inhibits its activity (endproduct feedback).<sup>33</sup> The next step is rate-limiting because the activity of the involved enzyme in humans is low (see 2 in figure 3). Foliates are closely related to the pterin metabolism. Tetrahydrofolate is required to form the starting molecule of the biosynthesis (not shown). Furthermore, a salvage and a regeneration pathway for BH<sub>4</sub> exist. These are essential because *de novo* synthesis is not sufficient for controlling hepatic phenylalanine and brain monoamine neurotransmitter homeostasis.<sup>1</sup> The salvage pathway forms another link of folates with pterins as it depends on dihydrofolate reductase. In the brain dihydrofolate reductase concentrations are low and hence regeneration dominates. However, the location of BH<sub>4</sub> biosynthetic enzymes has not been studied extensively at the cellular level.<sup>1,13</sup> Experimental evidence also supports the importance of folates. Some patients with BH<sub>4</sub> deficiency benefit from folinic acid supplementation and BH<sub>4</sub> synthesis *in vitro* is stimulated by tetrahydrofolate addition.<sup>13,15</sup> Experimental studies suggest that a raised neopterin/biopterin ratio may imply a failure to synthesize BH<sub>4</sub>.<sup>14</sup> Furthermore, decreased biopterin levels have been described in the cerebrospinal fluid of Alzheimer and Parkinson patients.<sup>13</sup>

Our observations in non-depressed persons are compatible with the regulation of peripheral BH<sub>4</sub> homeostasis. In case of low folate levels *de novo* synthesis may be insufficient and the activity of the salvage pathway enzyme limited.<sup>1</sup> Consequently, biosynthesis would be stimulated via upregulation of the first enzyme. This increases the neopterin/biopterin ratio because the following enzymatic step is rate-limiting. However, we did not observe this in persons with depressive symptoms and disorders. Instead, the neopterin concentrations increased with higher folate levels. This suggests that low folate limit the biosynthesis of pterins in the depressed and that synthesis generally is upregulated as compared to the non-depressed.

Different explanations for an impaired or altered pterin metabolism in depressed subjects are possible. Many cell- and tissue-specific substances regulate the enzyme activity of biopterin synthesis at transcriptional, post-transcriptional level or via an interacting protein.<sup>33</sup> In humans pterin biosynthesis is controlled by hormones, cytokines and certain immune stimuli. Pro-inflammatory cytokines activate, whereas hormones such as corticoste-

roids inhibit biosynthesis.<sup>1,33,34</sup> Both the hormone and the immune system are implicated in the pathophysiology of depression.<sup>35,36</sup> Hence, the dysregulation of these systems could maintain or even precipitate a monoamine deficiency if folate levels are low. The subtle differences in 5-HIAA concentrations between depressed and non-depressed subjects with low folates in our study are compatible with this interpretation. We may not have found differences in plasma biopterin concentrations between depressed and non-depressed, because decreased BH<sub>4</sub> concentrations were concealed by normal total biopterin levels.<sup>7</sup>

Alternatively, an impaired regulation could signal a genetic disorder in pterin metabolism. Various rare, genetically determined abnormalities of the biopterin synthesis have been described.<sup>13</sup> The 6-pyruvoyl-tetrahydropterin synthase deficiency for example is characterized by extremely high neopterin and low biopterin concentrations. However, little is known about any polymorphisms associated with biopterin levels in the normal range. Clinical research has concentrated on identifying recessive traits in homozygote patients with extreme biopterin deficiencies.

Our findings could shed light on the association between low folate status and depressive disorders. A large number of clinical studies since the 1960s reported a high prevalence of serum folate deficiency in depressed patients, though the nature of this association has remained obscure.<sup>37</sup> Further, the association was not observed in this nor another population-based study after adjusting for confounders.<sup>25,38</sup> The present results suggest that the interaction between folate and pterin metabolism may be relevant in depression. Effects of low folate could be diluted in many populations because deficiencies become a problem only if pterin synthesis is impaired.

The findings provide initial evidence that there are differences in pterin metabolism between depressed and non-depressed persons. In some people depression could be due to insufficient biopterin synthesis when folate levels are relatively low. However, regulation of the biopterin homeostasis is complex and different mechanisms may underlie impaired metabolism. Modulations of enzymes by hormones or cytokines as well as genetic disorders need to be investigated in depressed persons.

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## **5. General discussion**



This thesis presents evidence on the role of biological factors in late life depression. All studies described in this work were based on the Rotterdam Study. This is an ongoing population-based cohort study among 7983 persons. In 1990 to 1993 all inhabitants aged 55 years or over living in a suburb in Rotterdam were invited to participate in the baseline examination. Screening for depressive symptoms was first performed in the second (1993-1995), but a psychiatric-work-up with a diagnostic interview was introduced only in the third examination (1997-1999). In total, 5901 persons of the baseline population were alive and invited for this third round. Of these, 4,730 (response rate 80%) participated in the home interview and 4603 (97%) persons completed the screening for depressive symptoms. Subsequently, 4214 persons underwent physical examinations at the research centre. In additional home visits one or the other of two experienced clinicians assessed the symptoms of screen-positive subjects. Of the 363 persons with depressive symptoms, 337 (93%) had a psychiatric interview.

The merits and limitations of the studies in this thesis have been described in the previous chapters. In the general discussion I will elaborate only the most salient methodological issues pertaining to the research. Then I will summarise the main findings. Finally, I will give a broad outlook on future studies needed.

## **1. STUDY DESIGN AND METHODOLOGICAL CONSIDERATIONS**

### **1.1. Definition and measurement of depression**

The criteria for depression of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM) have become the lingua franca for clinical research.<sup>1</sup> Nevertheless epidemiological research frequently fails to adequately assess depression, in particular when psychological factors are not the primary outcome under study.<sup>2-4</sup> Differences in definition have hampered comparison of results. Furthermore, comparability is difficult to achieve even when the well-defined and operationalised DSM criteria are employed. The diagnosis can be based on self-report, interview or observation and different instruments are available.<sup>5</sup>

In the current study we used a two-step procedure to assess depressive disorders. We screened participants with the Dutch translation of the original Center for Epidemiologic Studies Depression scale (CES-D). This version

includes items about positive emotions although these are not part of current concepts of depression.<sup>6</sup> Besides, two items produce a gender bias.<sup>7</sup> However, the CES-D is a frequently chosen self-report measure of symptoms and we were able to use an established cut-off score.<sup>8</sup>

Next, subjects with depressive symptoms had a psychiatric interview. We chose a semi-structured psychiatric interview, the WHO's Schedules for Clinical Assessment in Neuropsychiatry (SCAN).<sup>9</sup> As opposed to a fully structured interview this instrument retains the features of a clinical examination. The psychometric properties of the SCAN have been studied in Dutch patients. An excellent agreement on the syndrome level and a substantial test-retest reliability were found.<sup>10</sup>

Our approach enabled us to classify psychiatric disorders according to DSM criteria. In all our analyses we combined the diagnostic categories major depression, dysthymia and in addition, minor depression (as defined in the appendix of the DSM-IV). In the elderly, these syndromes have similar prognosis and consequences for well-being and disability.<sup>11,12</sup> Furthermore, the syndromes are difficult to delineate in the elderly; the validity of diagnostic criteria in this age group and certain standardised interviews can be questioned.<sup>13</sup>

The psychiatric interview made it possible to exclude subjects from our analyses in whom no depressive but an anxiety or another psychiatric disorder was diagnosed. Subjects who did not fulfil criteria for an Axis I disorder but had clinically relevant depressive symptoms could also be analysed. However, there is a confusion of terminology – the terms subthreshold or subclinical depression/depressive symptoms are used – and the group has an even more heterogeneous clinical presentation than the group of persons with depressive disorders.<sup>14</sup> Even more importantly, the concept is linked to particular screening instruments used mainly in population-based studies.

## **1.2. The population-based approach**

Numerous population-based surveys and cohort studies have looked at the prevalence, determinants and course of depressive disorders.<sup>15</sup> However, biological parameters were rarely measured. Further, the large cohort studies with detailed physical examinations mostly included only very limited psychological assessments.<sup>3</sup> The present study is one of the first attempts to investigate the relation of biological factors with depressive disorders in a population-based sample; so far they have been studied mostly in clinical

populations. Some differences between the two approaches will be highlighted in the following.

Firstly, the reference group is easier to define in population-based studies. We chose all non-depressed subjects or a random selection of these as the referent group. In clinical psychiatric epidemiology, the commonly chosen control group is the medical staff or healthy volunteers. The possibility of selection bias is obvious, but this is not a trivial problem to overcome. The lack of case-control studies of depression that refer to a well defined study base is illustrative of this design problem. Selecting an appropriate reference populations in clinical studies is a major challenge for psychiatric epidemiology.

Secondly, the depressive disorders encountered in clinical or population-based studies might differ. In the Netherlands, only 50% of cases with major depression receive some form of treatment in the course of 12 months,<sup>16</sup> in the US around 30%.<sup>17</sup> It is generally found that community cases have less severe disorders than those in treatment. That clinical severity is related to treatment is, of course, not surprising. However, in psychiatry the argument is taken further. For example, the US Substance Abuse and Mental Health Service Administration decided that treatment of community cases with mental illness according to DSM criteria may not be a medical necessity because many are not severely ill.<sup>18</sup> The question arises whether this has implications for research or reflects only a policy-oriented interpretation of epidemiological data. The aetiology of depression might be different in community dwelling persons.

We conducted a psychiatric work-up that enabled us to study cases with more severe depressive symptoms separately. In all studies we ran analyses that were restricted to cases with DSM-IV depressive disorders and non-depressed reference subjects. The results were consistent for all but one possible risk factor (the vasomotor reactivity of cerebral arteries). We found stronger associations along the worsening spectrum of depressive symptoms. It cannot be ruled out, but seems unlikely, that the observed associations do not exist in subjects with even more severe depression. Furthermore, we were able to replicate most associations found in clinical studies. That some associations disappeared after careful adjustment does not mean that the aetiology differs between community dwelling and clinical populations. Rather, it makes it more likely that some clinical studies were flawed. Thus our results can probably be generalised to clinical populations.

Thirdly, the size of the study population is generally larger in population-

based settings. Although not inherent to the design, clinical studies mostly do not exceed 50 depressed subjects, whereas the present population-based study included more than 250 subjects with clinically relevant depressive symptoms.

### 1.3. Confounding

Confounding is by far the most likely cause of spurious associations.<sup>19</sup> In essence, confounding is a problem of comparability.<sup>20,21</sup> It can arise when there are differences in the comparison populations other than the determinant under study. Any factor that correlates to the biological determinant and might have a different distribution between the depressed and non-depressed is a potential confounder. We did not match on any demographic variable or risk indicator in order to be able to study the effect of various variables on the relation between risk factors and depression. We did, however, select comparable groups. All participants were aged 60 and above and lived in one district of the city of Rotterdam. Further, we controlled for confounders by adjusting the data statistically. A comprehensive number of risk factors and indicators of depression were available for the analyses because the study was conducted in an ongoing cohort study. Nevertheless residual confounding remains an issue in all observational research. Moreover, I will argue, the possibility of confounding is particularly present in studies of depression and what is a confounder or an intermediate may be less straightforward.

The study on vitamin E (chapter 4.3) and depressive symptoms illustrates the complexity of confounding in psychiatric epidemiology. The relation between vitamin E and depressive symptoms was different in women and men and confounded only in the latter. Firstly, low cholesterol levels in depressed men biased the relation with vitamin E in plasma as this is bound to cholesterol. The smoking habits also differ between the non-depressed and depressed and had to be taken into account as they were related to vitamin concentrations. Next, we adjusted for frailty indicators such as functional disabilities and cognitive score, because depression and vitamin levels correlated with physical health. Finally, a third of the residual difference in vitamin E concentrations between depressed and non-depressed men could be explained by social factors. Widowhood and living alone, two markers of social support, were more frequent both in persons with depression and low vitamin E levels. Thus only by studying biological, social, demographic, medical and physiological factors could we control for confounding.

At the same time this example also shows the danger of overadjusting when one controls for confounding. Some factors could be an intermediate in the causal pathway between vitamin E and depression. Depression has reciprocal associations with many medical conditions and social variables. For example, there is some evidence that low vitamin E levels predispose to a number of somatic diseases (e.g. ischemic heart disease),<sup>22</sup> which in turn cause depression. Adjusting for functional disabilities is thus debatable. To a certain extent this problem was also inherent to the cross-sectional design of our study (see also discussion of the cross-sectional design).

Overadjustment can result not only from an intermediate. Bias through adjustment for confounders can also be introduced if a variable is causally related to the exposure but only correlated to the outcome.<sup>23</sup> This is conceivable in the current example. Functional disability could be a sequel of behavioural changes accompanying depression (e.g. smoking). Some functional disabilities could also be related to depression because the proclivity to complain may differ between depressed and non-depressed persons (information bias).

This kind of information bias is frequently discussed in observational studies on psychological factors and physical health. Very recently a study in Scottish men was able to demonstrate it.<sup>24</sup> The strong positive relation between psychological stress and coronary heart disease (as measured by hospital admissions for cardiovascular symptoms) disappeared when other outcome measures were used (cardiovascular mortality). In our study, we related depression to physiologic parameters only. These are considered objective measures of health status or health risk. Possible information bias is not likely to be due to the participants' perceptions. One could argue, however, that our assessment of depression is biased, some persons complain more than others. On the other hand, this is part of the depressive syndrome. Thus in my view, measurement of the confounder is the most prominent problem related to this type of information bias. For example, there could be differential misclassification of functional disability or poor cognitive score. This is another reason why adjustment is problematic.

Furthermore, what is judged to be confounding can occur by chance.<sup>25</sup> Strictly speaking this is not a problem of confounding bias. However, it is intimately related to the way epidemiologists control for confounding. In most of our studies, we followed the approach recommended in standard epidemiology textbooks and included only those variables that changed the measure

of association.<sup>26</sup> If variables happened to decrease the strength of association by chance this selection biased the estimate. In our study we tested various other indicators of social support, nutritional behaviour or comorbidity. If the retained variables do not have the postulated causal association with depression our analyses were overadjusted.

Finally, we may have missed potential confounders. This can never be ruled out in an epidemiologic study but it remains a pertinent issue in studies on vascular pathology, immunological activation and depression. All are related to countless chronic somatic conditions and residual confounding could explain our findings. However, even with complete data on all comorbid conditions it would be difficult to control for without means of data reduction.<sup>27</sup> Including a large number of potential confounders leads to overfitted models and results cannot be generalized anymore.<sup>28</sup> On the other hand, small effects of many confounders can add up (see again the example of vitamin E in men).

#### **1.4. Cross-sectional design**

Cross-sectional studies have a poor reputation in epidemiological research. Rothman summarises the critique: "the cross-sectional approach can be viewed as a case-control study with an excessively large control group, with information from an inappropriate time period, and with a biased ascertainment."<sup>29</sup>

The first point of criticism can be dealt with shortly. The present studies were conducted in the Rotterdam Study cohort. If assessment of biological factor was part of the routine examination in the third round we used all participating non-depressed subjects as reference. Otherwise, we restricted the analyses to depressed subjects and randomly selected referent subjects. Obviously, this is a more efficient, but not a more valid approach. Rather, a large control group can be an advantage if the confounding variables are substantially imbalanced.<sup>30</sup> An adequate overlap of the confounding variable will only be secured by matching or a large control group.

The current cross-sectional studies could be biased because subjects with a more chronic course of disease were more likely to be included as a case. The first step of our psychiatric work-up was based on an instrument screening for depressive symptoms in the last week. Thus subjects with short, self-limiting disease episodes and those treated successfully were included in the reference group. On the one hand, our results may be an underestimation of the

true association due to non-differential misclassification. We defined cases as those currently depressed and not asymptomatic subjects with a history of depression. In the first place a history of depression is difficult to establish. Secondly, this definition of depressive cases makes little sense if the risk factor is variable over time.

On the other hand, the associations with biological factors could be overestimated in our study due to selection bias. Chronically depressed cases were overrepresented. However, we aimed to investigate whether the associations differed in the less severely depressed by performing separate analyses. The subthreshold groups included many subjects with shorter episodes of depression. Moreover, one can consider this more a problem of external validity. Our results apply to the typical late life depression which frequently is chronic or characterised by long periods with residual symptoms.

Next, and most importantly, cross-sectional studies cannot demonstrate the temporal relation between risk factor and outcome, which is one of the most important criteria for causality.<sup>31</sup> Ideally, the association between biological factors and depression should be studied prospectively in a cohort free of depression at baseline. In the present cross-sectional study temporal sequences had to be inferred very cautiously. Firstly, because the outcome may have influenced by the determinant. Depression brings about major behavioural and physiological changes. Secondly, some of the determinants of interest may be very variable in a subject over time. Then it becomes disputable whether we can assume a cumulative effect caused the depressive symptoms.<sup>32</sup>

Both considerations most obviously bear on our studies that relate nutrient blood levels to depressive disorders. The respective findings will therefore be discussed exemplarily. Reduced appetite is a symptom of depression and thus low folate, vitamin B<sub>12</sub> or vitamin E levels may be a consequence of poor intake in the depressed. There is little longitudinal data on vitamin levels, but individual levels probably are not very stable.<sup>33</sup>

As was discussed before, part of the association between vitamin E and depressive symptoms in men disappeared after adjustment for nutritional behaviour and social factors. This gave some indication of the temporal relation underlying the relation. Depression probably preceded a reduced intake.

The nature of the association between depression and folate deficiency is less clear. In our study higher rates of depressive disorders were found in sub-

jects with folate deficiency but this was due to differences in cardiovascular factors and physical comorbidity between the groups. In fact, this does not rule out that folate deficiency precipitates depression. However, other causal and temporal relations may seem likely. Folate deficiency could cause morbidity, which in turn was prior to depressive symptoms. Alternatively, the reduced folate intake might be a result of disabilities that included difficulty to go shopping. Of course, depression could also have preceded a reduced intake of folates and morbidity.

Adjusting for disability or comorbidity did not change the strong relation of vitamin B<sub>12</sub> deficiency with depressive symptoms. This suggests that different mechanisms underlie the associations with depression. It makes it also more plausible that vitamin B<sub>12</sub> deficiency directly caused depression than that folate deficiency did.

In the ongoing data-collection the incidence of depressive episodes is ascertained and we aim to prospectively study biological factors in depression. However, I would like to stress that a longitudinal design may not be able to give simple answers either. Firstly, depression is an episodic disorder and as many as half of the incident cases will have had prior depressive episodes.<sup>12</sup> These prior episodes may have preceded and even caused the determinant of interest. It is hardly possible to exclude these subjects as a history of depression is difficult to establish in the elderly.<sup>34</sup> On the other hand, it may not be necessary if one wants to study what maintains depression or causes recurrences in late life. Secondly, it is a big challenge to determine incident cases and in particular the incidence time of depression. Few longitudinal studies were able to follow a sample intensively.<sup>12,35</sup> Frequent remeasurements or information from the interval period are exceptional. Thirdly, as noted before, many of the factors may not directly give rise to the depressive disorders. A common pathological process may underlie both depression and the biological factor, the causal pathway may be bi-directional or the depressive symptoms secondary to physical disease that affected the variable of interest. In all instances, the temporal relation will be insufficient to establish a causal relationship.

## 2. MAIN FINDINGS

### 2.1. Frailty and depression

Population-based studies of depression mostly rely on self-reported measures of physical health. Frequently, functional limitations and the number of chronic conditions are assessed by asking patients explicitly whether they have certain problems.<sup>36,37</sup> The result may be biased and more objective measures should be used to measure physical health.<sup>24</sup> In our study we quantified physical health by constructing a study-specific frailty index. A multivariate score function was derived using baseline information from the Rotterdam Study on all available variables related to mortality. Demographic factors, medical problems, functional disabilities and indicators of health service use were included. However, most variables used were based on physiologic measurements. The score function was then employed to assess the physical health of depressed and non-depressed persons with data from the third examination round.

We found a strong association between physical health and depressive symptoms in community dwelling older adults even when subjective measures of health were excluded. The frailty score of depressed persons was only moderately lower than that of persons with a history of a stroke. There was no indication that poorer health was related to more severe depressive disorders nor that a depressed subpopulation without somatic comorbidity can be distinguished in community-dwelling elderly. We concluded that a frailty index is an appropriate tool to study the health, defined as the mortality risk, and probably the etiology of late life depression.

### 2.2. Vascular factors and late life depression

Several lines of evidence suggest that vascular disease may predispose to depression in late life. This vascular depression hypothesis is supported by the strong association of symptomatic and silent brain infarcts to subsequent depression.<sup>38</sup> Subtle white matter and deep grey matter abnormalities were also found more frequently in depressed than in non-depressed persons.<sup>39</sup> Furthermore, prospective studies have reported that a prior history of depression is related to the subsequent development of ischaemic heart disease.<sup>40,41</sup> While generalised atherosclerosis remains the most likely explanation of these findings, a clear demonstration that depression is associated with vascular changes is still required.<sup>42</sup> In this thesis we studied the association of hemo-

dynamic changes in the brain, atherosclerosis and arterial stiffness at different locations in the body with depression. To our knowledge none of these parameters has been studied in relation to depressive disorders in community dwelling subjects. Additionally, we studied cerebral small vessel disease as measured by MRI scanning of the brain. This was a longitudinal study of an association reported in several cross-sectional studies.<sup>4,43</sup>

- *Generalised atherosclerosis* – Subjects with atherosclerosis were more likely to be depressed. We found that a combined measure of extracoronary atherosclerosis was related to both depressive symptoms and depressive disorders although at some of the different locations the association was only moderate and non-significant. Further, we observed a substantial relation of severe coronary and aortic calcifications with depressive disorders.

Many clinical studies have been performed in patients with pre-existing vascular disease. These studies generally show a high risk of comorbid depression on survival after a cardiovascular event.<sup>44</sup> To our knowledge the present study is the first to examine the relation between measures of atherosclerosis and depressive disorders in community dwelling subjects. Although it cannot establish the causal role of atherosclerosis it provides strong evidence that a generalized atherosclerotic process accompanies late life depression. Depression in late life and ischaemic heart disease could to a certain extent be manifestations of a common pathological process.

- *Arterial stiffness* – Our study showed an association between arterial stiffness and depression in the elderly. Persons with decreased distensibility of the carotid artery and those with increased carotid-femoral pulse wave velocity were more likely to have depressive symptoms. The association with arterial stiffness was stronger for depressive disorders meeting DSM-IV criteria. Control for atherosclerosis, as measured by the ankle-to-brachial index or presence of plaques in the carotid artery, did not change the associations. Neither correction for carotid plaques nor correction for peripheral arterial disease changed the point estimates. Only the adjustment for aortic calcifications reduced the observed association between arterial stiffness and depressive symptoms. However, the presence of atheromatous plaques in the aorta is strongly correlated with decreased aortic distensibility. Adjusting for carotid plaques and peripheral arterial

disease was probably adequate to control for the effect of atherosclerosis. Thus our data suggest that the relation between vascular factors and depression may partly be caused by arterial stiffness.

- *Cerebral hemodynamics* – We found that depression in late life is associated with cerebral hemodynamic changes as assessed by transcranial Doppler ultrasonography. Subjects with depressive symptoms had a lower blood flow velocity and less CO<sub>2</sub>-induced vasomotor reactivity in the middle cerebral artery. Low blood flow may reflect an intrinsic property of the vascular smooth muscle or cerebral metabolism. Metabolic autoregulation determines the flow velocity changes during cognitive activity.<sup>45</sup> As reduced cognitive activity is a well-recognized symptom of depressive disorder, the low blood flow velocity in our study could be due to the diminished demand in depressive states and does not necessarily support the vascular hypothesis. Vasomotor reactivity, on the other hand, is a good indicator of microangiopathy.<sup>46</sup> Less vasomotor reactivity signals that the cerebral arterioles are unable to dilate in order to compensate increased demand. Hence, vasomotor reactivity is a possible causal factor for depressive symptoms.
- *Cerebral small vessel disease* – A total of 1077 non-demented elderly individuals underwent MRI images of the brain and assessment of depressive symptoms as part of the Rotterdam Scan Study in 1995 to 1996.<sup>43</sup> Brain infarcts as well as the periventricular and subcortical white matter lesions visible on the MRI were rated. All persons were continuously monitored for mortality and major morbidity including depression. In 1999 to 2000, a total of 787 subjects were re-examined at the research centre.

This made it possible to examine longitudinally whether cerebrovascular disease contributes to the pathogenesis of late-onset depression. We found that elderly people with brain infarcts, both symptomatic and silent, have a depressive disorder twice as frequently as those without infarcts. This suggests that ischaemic brain damage itself may lead to depressive symptoms. Brain infarcts on baseline MRI did not predict incident depressive disorders in people without depressive symptoms at baseline, nor did periventricular white matter lesions. However, severity of subcortical white matter lesions predicted incident depressive disorders, both in persons with and without depressive symptoms at baseline. This supports

the notion that vascular pathology plays an important role in the onset of late-life depression.

### **2.3. Inflammatory, nutritional and metabolic factors in late life depression**

Clinical and experimental studies suggest that depression is accompanied by immune dysfunction and more particularly by an enhanced production of pro-inflammatory cytokines.<sup>47,48</sup> Cytokines modulate depressive symptoms, regulate the stress response and contribute to neurotoxicity. On this basis researchers have postulated a macrophage theory of depression and suggested that cytokines are involved in the pathophysiology of depression.<sup>49</sup> This theory takes into account the altered levels of endocrine, nutritional and metabolic factors observed in depressed persons. We measured the inflammatory proteins interleukin-6 (IL-6),  $\alpha$ 1-antichymotrypsin (ACT) and C-reactive protein as well as neopterin. Neopterin is produced by macrophages and considered an immune marker - apart from being related to the serotonin metabolism. Plasma fatty acid composition, which may influence the expression of immune markers was also determined. High n-6 polyunsaturated fatty acids (PUFA) concentrations can increase whereas high n-3 PUFA concentrations may inhibit the formation of proinflammatory prostaglandines.

- *Inflammatory proteins* – We investigated the relation of cytokine IL-6 and the acute phase proteins ACT and CRP with depression in the Rotterdam Study. Higher levels of IL-6 were observed in persons with depressive disorders than in persons without depressive symptoms. This finding is in line with a previous population-based and a number of clinical studies.<sup>50,51</sup> Age- and gender-adjusted analyses of our data also suggested associations of ACT and CRP with depressive disorders, but these disappeared after adjustment for number of cigarettes smoked per day and stroke. For many years the finding that increased levels of acute phase proteins may occur in association with depression primarily emanated from one research group studying hospitalised patients and healthy controls.<sup>47,52</sup> Several confounding factors such as severity of symptoms, co-morbidity and tobacco dependence might have accounted for the heterogeneity in observations reported by other investigators. In the present study only IL-6 was independently related to depression. Furthermore, part of this relation was explained by atherosclerosis. Most probably, not only a direct effect of cytokines in the brain plays a role, but inflammation is a marker

of a generalized pathophysiological process. Further analyses also demonstrated that the association between inflammation markers and depression was not linear but suggested a threshold effect. This is compatible with observations in experimental studies that applied endotoxins intravenously to stimulate an IL-6 response.<sup>53</sup> Negative emotions were observed at IL-6 levels more than 20 times higher than those in our study, whereas moderately increased levels had a positive effect on mood. Thus there may be a threshold above which IL-6 has a negative effect on mood, and high concentrations could directly cause the depressed affect in a small group of community dwelling subjects.

- Plasma fatty acid composition* – We studied the plasma fatty acid composition in persons with depressive symptoms, including those with depressive disorders, and randomly selected reference persons. In particular, we compared the n-3 and n-6 plasma polyunsaturated (PUFA) percentages of total fatty acids in blood and their ratio. Subjects with depressive disorders according to the DSM-IV criteria had a higher ratio of n-6/n-3 PUFAs, but differences in individual PUFAs were mostly small. So far fatty acid composition has been investigated only in a few small studies of depressed patients which reported large differences between depressed and non-depressed persons.<sup>54,55</sup> In our study we found that the relation between plasma fatty acids and depression depended on the level of inflammation marker CRP. The depressed subjects with normal CRP concentrations had much lower n-3 PUFA percentage and higher n-6/n-3 ratio as compared to the non-depressed subjects. There were no differences between depressed and non-depressed persons with high CRP. We assume that CRP indicates physical comorbidity and that differences may be easier to detect in a relatively healthy group. Three mechanisms could explain the importance of fatty acids. Fatty acid composition influences the biophysical properties of neuronal membranes. Via this pathway fatty acids have an impact on receptor function, neurotransmitter re-uptake and signal transmission.<sup>56</sup> Furthermore, a low ratio of n-6/n-3 PUFAs reduces the risk for vascular disease, presumably by affecting platelet aggregation, blood pressure or direct atherogenic effects.<sup>57</sup> Finally, plasma fatty acids modulate immune activation.<sup>58</sup> However, in our study adjustment for neither atherosclerosis nor CRP changed the results. This is most compatible with the postulated direct impact of fatty acid composition on mood.

- *Vitamin E* – Vitamin E is a dietary compound that functions as an antioxidant preventing lipid peroxidation. High levels of antioxidants have been reported to slow processes related to atherosclerosis, ageing, and selective neuronal damage.<sup>69</sup>

Vitamin E levels in men with depressive symptoms were lower than in non-depressed men after adjusting for age, whereas no such difference was found in women participating in the Rotterdam Study. The association in men was weakened after controlling for biological factors such as cholesterol. It disappeared after adjustment for nutritional behaviour such as supplement use and social factors such as marital status and living alone. In one previous study only men,<sup>70</sup> in another women and men with low vitamin E status were more likely to be depressed.<sup>71</sup> Probably, these were spurious findings because important confounders were not adjusted for.

- *Vitamin B<sub>12</sub>, folate and homocysteine* – Folate and vitamin B<sub>12</sub> are involved in the one-carbon metabolism necessary for the production of monoamine transmitters. Several case-control studies since the 1960s reported a high prevalence of folate and vitamin B<sub>12</sub> deficiency in depression.<sup>67</sup> More recently, total plasma homocysteine was shown to be a sensitive marker of folate deficiency and raised concentrations of homocysteine were observed in depressives.<sup>68</sup>

Similarly, we found that hyperhomocysteinemia, folate and vitamin B<sub>12</sub> deficiency were more frequent in persons with depressive disorders. However, for folate deficiency and hyperhomocysteinemia the estimates were substantially reduced after adjustment for functional disability and cardiovascular disease, only for vitamin B<sub>12</sub> the effect was independent of these factors. The association of vitamin B<sub>12</sub> and folate with depressive disorders may have different underlying mechanisms. Vitamin B<sub>12</sub> could be causally related to depression, whereas the relation with folate was due to physical comorbidity.

- *Pterins* – Biopterin, neopterin and folate were studied in depressed and non-depressed persons participating in the Rotterdam Study. Tetrahydrobiopterin (BH<sub>4</sub>) is a cofactor in the synthesis of the monoamine neurotransmitters dopamin, serotonin and noradrenalin.<sup>59</sup> Neopterin, also considered an immune marker, may signal a failure to synthesise the active biopterin.<sup>60</sup> Folates, on the other hand, increase the biosynthesis of biopterin

and a salvage pathway for  $\text{BH}_4$  depends on dihydrofolate reductase. Previous research on pterins and depression yielded conflicting results. Higher and lower plasma biopterin levels were found in depressed subjects as compared to controls.<sup>61-63</sup> Other investigators reported higher and lower plasma neopterin/biopterin ratios in depression.<sup>64</sup>

We found that the relation between plasma pterins and folate concentrations was not the same in depressed and non-depressed persons. In depressed subjects higher folate concentrations were accompanied by higher neopterin concentrations. In the non-depressed the neopterin/biopterin ratio was associated with folate levels. The differences between the two groups were most obvious at relatively low folate levels. Persons with low folate and a low neopterin/biopterin ratio were most likely to have depressive symptoms. This may explain the conflicting findings of other groups.

Further, our observations in the non-depressed are compatible with the regulation of peripheral  $\text{BH}_4$  homeostasis.<sup>59</sup> With lower folates concentration  $\text{BH}_4$  levels decrease and biosynthesis is stimulated via upregulation of an enzyme. Because the subsequent enzymatic step is rate-limiting the intermediary product accumulates. Neopterin is derived from this intermediary. Thus the neopterin/biopterin ratio rises. In persons with depressive symptoms and disorders this was not the case, which suggests an impairment of the metabolism. This could be due to pathophysiologic regulation of biopterin synthesis that is controlled by hormones and immune stimuli.<sup>65</sup> Both the hormone and the immune system are implicated in the pathophysiology of depression. Hence, the dysregulation of these systems could maintain or even precipitate a monoamine deficiency especially if folate levels are low. Alternatively, an impaired regulation could signal a genetic disorder in pterin metabolism.<sup>66</sup>

The findings provide initial evidence that there are differences in pterin metabolism between depressed and non-depressed persons. It is premature to speculate over a causal role of pterins in depression. The regulation of the biopterin homeostasis is complex and different physiological mechanisms may underlie impaired metabolism. Modulations of enzymes by hormones or cytokines as well as genetic disorders need to be investigated in depressed persons.

### 3. OUTLOOK

This thesis was devoted to biological correlates of depression in community dwelling subjects. However, due to the cross-sectional design causality could only be inferred tentatively from the observed relationships. Prospective follow-up studies are, notwithstanding the possible problems inherent to studies on depression, a better tool to detect aetiologic mechanisms.

A prominent issue in geriatric psychiatry in need of longitudinal research are vascular factors. Most studies in this field are associative rather than causal. Moreover, the few existing prospective studies suffer from the short follow-up time and were able to study the persistence of depressive symptoms only.<sup>72</sup> If the evolving evidence on vascular depression is substantiated this has wide implications. It suggests the possibility of a treatment more akin to the management of cardiac disease or hypertension.

The same holds for the nutritional factors studied in this thesis. They offer the chance to find new treatments or ways of prevention. Studies presented in this thesis suggest that nutritional factors may play a role in the onset or maintenance of depressive symptoms in the elderly. However, it is premature to speculate on clinical implications. Prospective population-based studies are needed to demonstrate the relation of vitamin B<sub>12</sub>, folate and polyunsaturated fatty acids with depression. Ultimately, only clinical trials can prove the efficacy of supplements or dietary change. Even apparently robust findings from observational studies in nutritional epidemiology have not been confirmed by randomized clinical trials.<sup>33,73,74</sup>

Longitudinal studies are also necessary to show how depression increases the risk of cardiac morbidity and mortality. A number of epidemiological studies have reported a history of depression to be associated with subsequent ischaemic heart disease.<sup>3</sup> However, the nature of this association is unclear. The possible mechanisms include (1) depression is a marker of pre-existing, sub-clinical coronary disease, (2) side effects of antidepressant drugs, (3) poor adherence to medical treatment regimens, (4) behavioural changes that increase the effect of cardiac risk factors such as smoking or a poor diet, (5) depression is part of a pathological process encompassing generalised atherosclerosis or inflammation, (6) neuroendocrine function, and (7) altered autonomic tone. Of particular potential interest is cortisol excretion as an indicator of the hypothalamic-pituitary axis. Both hypo- and hyperactivity has been reported but many studies were restricted to severely depressed

in-patients.<sup>75</sup> The advance of saliva sampling made it easier for researchers to study the cortisol rhythm in community dwelling subjects.<sup>76</sup> Another promising lead is altered autonomic tone. Depressed patients display signs, such as elevated heart rate, decreased heart rate variability, and increased physiological reactivity to environmental stressors, which may indicate a predisposition to cardiovascular disease.<sup>77</sup> Furthermore, thyroid function must also be considered as an integral part of the neuroendocrine system and thus studied out of aetiologic and prognostic interest.<sup>78,79</sup>

For many decades psychiatric and social epidemiologists considered only a limited number of biological variables such as stress hormones.<sup>80</sup> Since the beginning of the 1990s this has changed, vascular factors, immunological markers and metabolic parameters are reconsidered. However, it is still a common mistake to regard one mechanism only. It is not unreasonable to try to measure all variables in one cohort study.<sup>3</sup> Biological mechanisms must be studied jointly and only combined analyses will permit to assess what are independent or intermediate factors. Psychiatric researchers are finally conducting large studies in close collaboration with basic science, radiology, medicine, and epidemiology.

Neuroimaging is becoming increasingly important as a means to investigate the pathophysiology of depression. MRI has now been used in a number of population-based studies and the modern concept of vascular depression is intrinsically related to this technique.<sup>39,72,81,82</sup> Structural imaging studies may be able to show volume loss or neuronal damage in specific brain areas of depressed persons. Functional MRI not only provides excellent spatial resolution but adds information on brain metabolism and blood flow.<sup>83</sup> The procedures require considerably less cooperation than before from the study subjects, but so far the costs and time constraints preclude the use in large populations.<sup>84</sup> Thus valid case-control designs are called for.

Genetics is perhaps the most frequently named area of future psychiatric research.<sup>85</sup> The rapidly evolving field of genetic epidemiology, however, will struggle with the limitations that psychiatric nosology remains at a syndromic level. Non-specific behavioural symptoms rather than pathophysiology define depressive disorders. Psychiatrists do not regard depressive disorders as a single entity, but no consensus exists which subgroups to differentiate.<sup>5</sup> Consequently, links between the broad spectrum of depressive syndromes and a single genetic polymorphism will be difficult to establish. I believe that behavioural genetic research in the elderly will book little progress unless

more sensitive designs are employed (e.g. population isolates and twin registries) or the limitations of psychiatric nosology are overcome.

Thus another challenge for future research lies in dealing with the heterogeneity of depressive syndromes. Different approaches are possible. One path traditionally pursued by biological psychiatrists is to employ physiological variables as a marker of subgroups. Dexamethasone suppression test,<sup>86,87</sup> latency to rapid eye movement sleep<sup>88</sup> and hypercortisolism have been used.<sup>89</sup> This approach could be rewarding if scientists realized more than in the past that the underlying physiological measures are continuous variables. Hence, sophisticated statistical methods may be needed to identify subgroups. Secondly, pure depressive disorder – persons without severe comorbidity – may be of particular interest not only in clinical trials.<sup>90</sup> In the individual case it is pointless to allocate depressive syndromes exclusively to categories such as endogenous or reactive to a somatic condition. However, our study on frailty in late life depression suggested that somewhat more homogenous strata of community dwelling subjects can be defined. Thirdly, researchers may want to focus on specific behaviours and emotions.<sup>91,92</sup> Thorough psychiatric assessment permits analyses of subjects with melancholic features or psychomotor retardation. Finally, psychiatric co-morbidity could be taken into account more. Depression frequently coexists with anxiety, somatization or sleeping disorders.<sup>90</sup> Depression with these co-occurring disorders has different risk factors and takes a different course.<sup>93</sup> It may have been fruitless to define a distinct clinical category of mixed anxiety-depressive disorder, but one could investigate biological factors related to anxiety-depression comorbidity.<sup>94</sup>

In any case, none of the suggested approaches can be pursued without adequately assessing depression and related psychiatric disorders. Furthermore, researchers should also use DSM categories to ensure comparability, although in the past the success of this classification system may have obfuscated the search for causes. Epidemiological research will otherwise allow no meaningful interpretation. Unfortunately, investigators have introduced conditions which are not clearly related to current psychiatric concepts. They were termed vital exhaustion,<sup>95</sup> hopelessness,<sup>2</sup> depressive traits,<sup>96</sup> complicated grief<sup>97</sup> or lack of emotional well-being.<sup>98</sup> All of these conceptions are characterized by symptoms of depression. However, the investigators failed to adequately assess depression, because it was not the primary psychological risk factor studied.

## EPILOGUE

More than anything the studies presented in this thesis have generated hypotheses for future research. Some biological factors emerged as intriguing candidates for etiologic research. Our studies made out possible risk factors for depression. However, I regard it as equally important that we demonstrated a complex or confounded relation between other biological factors and depression. We also proved that a population-based approach is of value for biological psychiatry. There is no question that large studies are needed to integrate physiological findings with social and psychological variables. Thus in the ongoing follow-up round of the main study we collect longitudinal data on depression and biological variables and additionally assess other psychiatric disorders, social support, quality of life, functional disability and grief in late life.

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## 6. Summary Samenvatting



## 6.1 SUMMARY

Depression in late life is emerging as a major health problem. Because of the increase in the proportion of elderly in the population and the high prevalence of depression it imposes a severe burden on public health. Biological factors are closely related to depression in the elderly and have been studied for more than hundred years. Clinical studies showed that factors such as vascular pathology, immune activation or nutritional deficiencies play a role in the aetiology and pathogenesis of late life depression. The aim of the present thesis was to examine the associations between biological factors and depression in a large sample of community-dwelling elderly. The basic idea of this thesis is that it is possible to learn something about possible causes and consequences of depression in the elderly by well controlled studies of correlates. To investigate these issues, we used data from the third examination in the Rotterdam Study. This is a large ongoing population-based study of the total population aged 55 years and over in the district of Ommoord in Rotterdam.

In **chapter 2**, we constructed a quantitative measure to assess physical health in the elderly. A study-specific frailty score was calculated using baseline information from the Rotterdam Study on all variables related to mortality. We showed that physiological measurements, which can be assessed more objectively, generally performed better in mortality risk assessment than self-reported information. Using the frailty score we found a strong association between physical health and depressive symptoms in community dwelling older adults even when subjective measures of health were excluded. The frailty score of depressed persons was only slightly lower than that of persons with a history of a stroke.

In **chapter 3**, studies of the relation between vascular factors and depression are described. **Chapter 3.1** shows that subjects with atherosclerosis were more likely to be depressed. We found that an overall measure of extra-coronary atherosclerosis was related to depressive disorders. Further, we observed a strong relation of severe coronary and aortic calcifications with depressive disorders, the relative risks of depressive disorders were 2.0 and 3.7, respectively. Although this study cannot establish the causal role of atherosclerosis it provides evidence that a generalised atherosclerotic process accompanies late life depression. In **chapter 3.2** the results pertaining to associations between arterial stiffness and depressive disorders in the elderly are presented. Persons with decreased distensibility of the carotid artery and

those with increased carotid-femoral pulse wave velocity were more likely to have depressive symptoms. The association with arterial stiffness was stronger for depressive disorders meeting DSM-IV criteria. Control for atherosclerosis, as measured by the ankle-to-brachial index or presence of plaques in the carotid artery, did not change the associations. In the following we examined depressed and non-depressed persons with functional and structural imaging techniques of the brain. A study of cerebral hemodynamics as assessed by transcranial Doppler ultrasonography is presented in **chapter 3.3**. We found that depression in late life is associated with cerebral hemodynamic changes. Subjects with depressive symptoms had a lower blood flow velocity and less CO<sub>2</sub>-induced vasomotor reactivity in the middle cerebral artery. However, the low blood flow may reflect reduced cognitive activity and does not necessarily indicate vascular pathology in depressed persons. Low vasomotor reactivity, on the other hand, is a good indicator of microangiopathy and signals that the cerebral arterioles are unable to dilate in order to compensate increased demand. We also investigated cerebral small vessel disease in a large group of persons who underwent MRI images of the brain and were followed up to assess incident depression. This study is presented in **chapter 3.4** and shows that elderly people with brain infarcts, both symptomatic and silent, have a depressive disorder twice as frequently as those without infarcts. This suggests that ischaemic brain damage itself may lead to depressive symptoms. Furthermore, we found that severity of subcortical white matter lesions predicted incident depressive disorders, both in persons with and without depressive symptoms at baseline. The studies in chapter 3 all support the notion that vascular pathology plays an important role in the onset of late-life depression.

**Chapter 4** contains five studies in which the relation of inflammatory, nutritional and metabolic factors with late life depression is examined. First, we investigated the plasma levels of cytokine interleukin-6 (IL-6) and the acute phase proteins  $\alpha$ 1-antichymotrypsin and C-reactive protein (CRP) in depressed and non-depressed persons. A current theory suggests that depression may be precipitated by an immune dysfunction. In **chapter 4.1** we report that only IL-6 was independently related to depression, whereas the relation between acute phase proteins and depression disappeared after adjustment for number of cigarettes smoked per day and stroke. Furthermore, part of the relation between IL-6 and depression was explained by atherosclerosis. Most probably, cytokines are a marker of a generalised pathophysiological process

and do not only signal a direct immune activation in the brain of depressed persons. Next, we studied the plasma fatty acid composition in persons with depression and randomly selected non-depressed persons. In chapter 4.2, we compared the n-3 and n-6 plasma polyunsaturated (PUFA) percentages of total fatty acids in blood and their ratio in depressed and non-depressed persons. Plasma fatty acid composition can influence the expression of immune markers. We found that persons with depression had a higher ratio of n-6/n-3 PUFAs than the non-depressed, but differences in individual PUFAs were mostly small. Further, we observed that depressed subjects with normal CRP concentrations had a much lower n-3 PUFA percentage and higher n-6/n-3 ratio as compared to the non-depressed subjects with normal CRP levels. We assume that CRP indicates physical comorbidity and that differences may be easier to detect in a relatively healthy group. In chapter 4.3 we describe the relation of plasma vitamin B<sub>12</sub>, folate and homocysteine with depression. Folate and vitamin B<sub>12</sub> are necessary for the production of monoamine neurotransmitters, whereas total plasma homocysteine is a sensitive marker of folate deficiency. Like other investigators before, we found that hyperhomocysteinemia, folate and vitamin B<sub>12</sub> deficiency were about twice as frequent in persons with depressive disorders as in the non-depressed. However, for folate deficiency and hyperhomocysteinemia the estimates were substantially reduced after adjustment for functional disability and cardiovascular disease, only for vitamin B<sub>12</sub> the effect was independent of these factors. This suggests that vitamin B<sub>12</sub> could be causally related to depression, whereas the relation with folate is due to physical comorbidity. In chapter 4.4 we focus on another nutritional factor, vitamin E. This is a dietary compound that functions as an antioxidant. High levels of antioxidants possibly slow processes related to atherosclerosis, ageing, and selective neuronal damage. The plasma vitamin E levels in men with depressive symptoms were lower than in non-depressed men participating in our study, whereas no such difference was found in women. However, the association in men was weakened after controlling for biological factors such as cholesterol and disappeared after adjustment for nutritional behaviour such as supplement use and social factors such as living alone. This strongly suggests that the association previously observed in men was a spurious finding. In chapter 4.5 the relation between plasma concentrations of biopterin, neopterin and folate with depression is examined. Tetrahydrobiopterin (BH<sub>4</sub>) is a cofactor in the synthesis of the monoamine neurotransmitters. Neopterin may signal a failure to synthesise the active biop-

terin, whereas folates increase the biosynthesis of biopterin. We found that the relation between plasma pterins and folate concentrations was not the same in depressed and non-depressed persons. In depressed subjects higher folate concentrations were accompanied by higher neopterin concentrations. In the non-depressed only the neopterin/biopterin ratio was associated with folate levels. Our observations in the non-depressed are compatible with the regulation of peripheral  $\text{BH}_4$  homeostasis. However, in persons with depression this was not the case, which suggests an impairment of the metabolism. This could be due to an altered regulation of biopterin synthesis, which is controlled by hormones and immune stimuli, in depressed persons.

In **chapter 5**, the general discussion, some of the methodological issues involved in the studies are discussed. The chapter focuses on the assessment of depressive symptoms in population-based studies, the design and possible bias by confounding. Furthermore, our findings are reviewed. The last part of this chapter provides a more general view on opportunities for population-based studies of late life depression. Particular emphasis lies on the limitations and chances of future research that includes assessment of biological factors.

## 6.2 SAMENVATTING

Depressie op oudere leeftijd is een toenemend gezondheidsprobleem. Omdat het percentage ouderen in de bevolking stijgt en depressie veel voorkomt bij ouderen, drukt depressie steeds zwaarder op de gezondheidszorg. Biologische factoren zijn vaak in verband gebracht met depressie op oudere leeftijd en de relatie wordt al meer dan honderd jaar onderzocht. Klinische studies hebben aangetoond dat factoren zoals vasculaire pathologie, immuun activatie, of voedingsdeficiënties een rol spelen in de etiologie en pathogenese van depressie op oudere leeftijd. Het doel van dit proefschrift was om het verband te bestuderen tussen biologische factoren en depressie in een grote steekproef van ouderen in de algemene bevolking. Het uitgangspunt is dat men iets kan leren over mogelijke oorzaken en gevolgen van depressie op oudere leeftijd door correlaties te bestuderen. Hiertoe maakten wij gebruik van gegevens uit de derde onderzoeksronde van de Erasmus Rotterdam Gezondheid en Ouderen (ERGO) studie. Dit is een grootschalig, langlopend bevolkingsonderzoek onder de totale bevolking van 55 jaar en ouder in de wijk Ommoord te Rotterdam.

In **hoofdstuk 2** wordt de constructie beschreven van een kwantitatieve maat om de fysieke toestand van ouderen vast te stellen. Een 'frailty score' werd berekend op basis van alle beschikbare determinanten binnen de ERGO studie die gecorreleerd waren aan mortaliteit. We konden aantonen dat fysiologische maten, welke objectief vastgesteld kunnen worden, beter waren in het voorspellen van het mortaliteits risico dan zelf gerapporteerde informatie. Met behulp van deze 'frailty score' vonden we een sterk verband tussen fysieke gezondheid en symptomen van depressie in ouderen, ook als subjectieve gezondheidsmaten werden geëxcludeerd. De 'frailty score' van depressieve personen was slechts iets lager dan die van personen die een herseninfarct hadden doorgemaakt.

In **hoofdstuk 3** worden studies naar de relatie tussen vasculaire factoren en depressie beschreven. In **hoofdstuk 3.1** wordt aangetoond dat personen met arteriosclerose een grotere kans hebben om depressief te zijn. We vonden dat een gecombineerde maat voor extracoronaire arteriosclerose gerelateerd was aan depressieve stoornissen. Verder zagen we een sterke relatie tussen ernstige coronaire en aorta calcificaties en depressie: relatieve risico's op een depressieve stoornis waren 2.0 en 3.7, respectievelijk. Hoewel deze studie niet een causaal verband kan aantonen, levert het wel bewijs dat een gegenera-

liseerd atherosclerotisch proces samengaat met depressie op oudere leeftijd. In **hoofdstuk 3.2** worden de resultaten gepresenteerd betreffende de associatie tussen vaatwandstijfheid van slagaders en depressieve stoornissen bij ouderen. Personen met een afgenomen distensibiliteit van de halsslagader en degenen met een toegenomen polsgolfsnelheid tussen de halsslagader en de liesslagader hadden een grotere kans op het hebben van depressieve symptomen. De associatie met vaatwandstijfheid was sterker voor een depressieve stoornis volgens de DSM-IV criteria. Correctie voor arteriosclerose, bepaald door de enkel-arm index of de aanwezigheid van plaques in de halsslagader, gaf geen verandering in de associatie. Vervolgens onderzochten we depressieve en niet-depressieve personen met behulp van functionele en structurele beeldvormende technieken van de hersenen. Een studie naar de hemodynamica van de hersenen, gemeten door transcraniële Doppler echografie, is beschreven in **hoofdstuk 3.3**. We vonden dat depressie op oudere leeftijd is geassocieerd met hemodynamische veranderingen in de hersenen. Personen met depressieve symptomen hadden een lagere stroomsnelheid van het bloed en een mindere CO<sub>2</sub>-geïnduceerde reactivatie van de middelste cerebrale arterie. Echter, de verminderde doorstroming kan een afname in cognitieve activiteit weerspiegelen en is niet noodzakelijkerwijs een indicatie voor vasculaire pathologie bij depressieve personen. Anderzijds, een afgenomen reactivatie is een goede voorspeller voor microangiopathie en duidt op een onvermogen van cerebrale arteriolen om te verwijden ter compensatie van een toegenomen vraag. Tevens hebben we afwijkingen in de kleine bloedvaten van de hersenen onderzocht binnen een grote groep personen die een MRI scan hadden ondergaan en vervolgens in de tijd werden vervolgd op het optreden van depressie. Deze studie, beschreven in **hoofdstuk 3.4**, toont aan dat personen met een herseninfarct, zowel symptomatisch als niet-symptomatisch, twee maal zo vaak een depressieve stoornis hebben als personen zonder herseninfarct. Deze bevinding suggereert dat ischemische schade aan de hersenen op zichzelf tot depressieve symptomen kan leiden. Verder vonden we dat de ernst van subcorticale witte stof afwijkingen voorspellend is voor het optreden van een depressieve stoornis, zowel in personen met als zonder depressieve symptomen aan het begin van de studie. Alle studies in hoofdstuk 3 ondersteunen het idee dat vasculaire pathologie een belangrijke rol speelt bij depressie op oudere leeftijd.

**Hoofdstuk 4** bevat vijf studies waarin de relatie tussen ontsteking, voeding, metabole factoren, en depressie op oudere leeftijd wordt bestudeerd.

Ten eerste onderzochten we de bloedspiegels van de cytokine interleukine-6 (IL-6) en de acute fase eiwitten  $\alpha$ 1-antichymotrypsine en C-reefctief proteïne (CRP) in personen met en zonder depressie. Een huidige theorie suggereert dat depressie wordt onderhouden door een immuun stoornis. In **hoofdstuk 4.1** rapporteren we dat alleen IL-6 onafhankelijk gerelateerd was aan depressie, terwijl de relatie tussen acute fase eiwitten en depressie verdween na correctie voor roken en het doorgemaakt hebben van een herseninfarct. Daarnaast kon een deel van de relatie tussen IL-6 en depressie worden verklaard door arteriosclerose. Waarschijnlijk zijn cytokines een uiting van een gegeneraliseerd pathofysiologisch proces en niet een kenmerk van selectieve immuun activatie in de hersenen van depressieve personen. Vervolgens bestudeerden we de samenstelling van vetzuren in plasma van personen met depressie en een willekeurig geselecteerde groep niet-depressieve personen. In **hoofdstuk 4.2** vergeleken we het percentage n-3 en n-6 meervoudig onverzadigde vetzuren (MOVZ) ten opzichte van het totale vetzuur gehalte en hun ratio in depressieve en niet-depressieve personen. De samenstelling van vetzuren in het plasma kan de expressie van immuun eiwitten beïnvloeden. We vonden dat personen met depressie een hogere n-6/n-3 MOVZ ratio hadden dan niet depressieven, maar verschillen in individuele MOVZ waren klein. Ook zagen wij dat depressieve personen met een normale CRP concentratie een veel lager n-3 MOVZ gehalte en een hoger n-6/n-3 ratio hadden in vergelijking met niet-depressieve personen met een normaal CRP gehalte. We veronderstellen dat CRP een indicatie is voor fysieke comorbiditeit en dat een verband makkelijker aan te tonen is in een relatief gezonde groep. **Hoofdstuk 4.3** beschrijft de relatie tussen plasma vitamine B<sub>12</sub>, foliumzuur en homocysteïne, en depressie. Foliumzuur en vitamine B<sub>12</sub> zijn noodzakelijk voor de productie van monoamine neurotransmitters, terwijl homocysteïne een gevoelige indicator is voor foliumzuur deficiëntie. Evenals andere onderzoekers vonden wij dat veel homocysteïne en een tekort aan foliumzuur en vitamine B<sub>12</sub> ongeveer twee maal zo vaak voorkwamen bij personen met een depressieve stoornis als bij niet-depressieve personen. Echter, in het geval van foliumzuur deficiëntie en hyperhomocysteïne werd deze risico schatting beduidend zwakker na correctie voor functionele stoornissen en cardiovasculaire aandoeningen, terwijl alleen voor vitamine B<sub>12</sub> het effect onafhankelijk was van deze factoren. Dit suggereert dat vitamine B<sub>12</sub> mogelijk causaal gerelateerd is aan depressie, terwijl de relatie met foliumzuur het gevolg is van comorbiditeit. In **hoofdstuk 4.4** richten we ons op een andere voedingsfactor, vitamine E.

Dit is een voedingsbestanddeel met een anti-oxidatieve werking. Een hoge concentratie anti-oxidanten kan mogelijk processen als arteriosclerose, veroudering, en selectieve neuronale schade vertragen. Het vitamine E gehalte in mannen met depressieve symptomen bleek lager te zijn dan in mannen zonder depressie, terwijl geen verschil werd gevonden bij vrouwen. Echter, de associatie bij mannen werd zwakker na correctie voor dieet gedrag, zoals het gebruik van supplementen, en sociale factoren, zoals alleen wonen. Dit is een sterke aanwijzing dat de eerder beschreven associatie bij mannen een onjuiste bevinding was. In **hoofdstuk 4.5** is depressie onderzocht in relatie tot het plasma gehalte aan biopterine, neopterine en foliumzuur. Tetrahydrobiopterine ( $\text{BH}_4$ ) is een cofactor in de synthese van monoamine neurotransmitters. Neopterine duidt op een stoornis in de vorming van het actieve biopterine, en foliumzuur verhoogt de biosynthese van biopterine. Wij vonden dat de relatie tussen plasma concentraties van pterines en foliumzuur niet hetzelfde waren in depressieve en niet-depressieve personen. In personen met depressie gingen hogere foliumzuur concentraties gepaard met hogere neopterine concentraties. In niet-depressieven was alleen de neopterine/biopterine ratio geassocieerd met het foliumzuur gehalte. Onze observaties bij niet-depressieven zijn in lijn met de regulatie van het  $\text{BH}_4$  homeostase. Echter, dit was niet het geval bij personen met een depressie, wat een stoornis in dit metabolisme suggereert. Dit zou het gevolg kunnen zijn van een verstoorde regulatie van biopterine synthese, welke gecontroleerd wordt door hormonen en immuun stimuli, bij depressieve personen.

**Hoofdstuk 5** bevat de algehele discussie van het proefschrift waarin enkele methodologische aspecten van de studies worden besproken. Het hoofdstuk richt zich op de diagnostiek van depressieve symptomen in bevolkingsonderzoeken, het ontwerp van de studie, en mogelijke bias door confounding. Verder worden alle bevindingen samengevat. Het laatste deel van dit hoofdstuk geeft een bredere kijk op de mogelijkheden van bevolkingsonderzoek naar depressie op oudere leeftijd. De nadruk ligt hierbij op de beperkingen en uitdagingen voor toekomstig onderzoek op het gebied van biologische factoren.

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## **ABOUT THE AUTHOR**

Henning Tiemeier was born on December 28, 1966 in Hamburg, where he grew up and passed secondary school in 1986. In the same year he was drawn in to military service for 15 months.

From 1987 to 1995 he studied Medicine, Sociology and Psychology at the Rheinische Friedrich-Wilhelms-Universität in Bonn, Germany and the University of Manchester, United Kingdom. He obtained both his medical degree and a Master of Arts in Sociology in 1995. Subsequently, he completed his residency at the University Hospital of Epileptology, Bonn (head: Prof. Dr. C.E. Elger). In 1997 he received a doctorate in medicine for research on the effect of slow-wave sleep deprivation on plasma catecholamines. This work was conducted at the Department of Psychiatry, University Bonn Medical Center (promotor: Prof. Dr. H.-J. Möller). After relocation of his family to the Netherlands in 1997 he was a househusband for 8 months until he joined the Netherlands Institute of Mental Health and Addiction (Trimbos Instituut), Utrecht as a research scientist. For two years he worked on the evaluation of clinical practice guidelines for depression under the guidance of Prof.dr. H. Rigter. In 1999 he started the work described in this thesis at the Department of Epidemiology & Biostatistics (head: Prof.dr. A. Hofman) of the Erasmus Medical Center in Rotterdam. In 2000 he obtained a Master of Science in Clinical Epidemiology at the Netherlands Institute of Health Sciences in Rotterdam. From 2001 to 2002 he was co-ordinator of the Rotterdam Study. Since May 2002 he holds a part-time position as lecturer in Child and Adolescent Psychiatry at the Sophia Children's Hospital, Erasmus Medical Center (head: Prof.dr. F.C. Verhulst); since January 2003 also a part-time position as lecturer in Psychiatric Epidemiology at the Department of Epidemiology & Biostatistics.

He is married to Dr. Michaela Hofbauer and has two children, Gesa and Marleen.

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Selectie bias.

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