The weight of subclinical vascular disease & neuroticism in late-life depression
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Waist circumference and Neutrophil Gelatinase-Associated Lipocalin in late-life depression

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Abstract

Background – Both visceral obesity and depression are associated with impaired health and excess mortality, possibly through overlapping pathophysiological mechanisms like adipose tissue derived inflammatory markers. These results, however, are primarily based on population-based surveys, often restricted to a young population and depression severity scales instead of patients with established diagnosis of depressive disorder.

Methods – We examined the relation between waist circumference and late-life depression using the baseline data of the Netherlands Study of Depression in Older persons (NESDO). Psychopathology has been assessed with Composite International Diagnostic Interview version 2.1.

Results – Adjusted for age, sex, education, lifestyle (smoking, alcohol, physical activity), drug use, cognition and chronic diseases as well as adjusted for body mass index (BMI), analysis of covariance showed that depressed older patients (n=376) had a significantly lower waist circumference (WC) compared to their non-depressed comparisons (n=130): estimated marginal mean (SE) = 93.9 (0.5) versus 97.8 (0.8) cm (F=15.9; df=1,467; p<.001). Multiple linear regression analyses within the depressed group showed that both depression severity (Inventory of Depressive Symptoms) as well as duration-related depression characteristics (age of onset, duration of illness, life-time comorbid dysthymia) were associated with the WC. Only the severity of depressive symptoms remained significant after further adjustment for the BMI. Interestingly, a recently discovered adipokine, Neutrophil Gelatinase-Associated Lipocalin (NGAL), was associated with late-life depression, but only in the subgroup of patients with a pathologically increased WC.

Conclusions – Population-based findings on the positive association between obesity and depressive symptoms can thus not be generalised to a clinical sample of depressed older patients. The impact of the WC on course and treatment outcome of late-life depression should be examined in clinical samples, taken into account the relative impact of the WC in proportion to the general level of obesity as indexed by the BMI and the role of adipokines.

Keywords

Waist circumference, Obesity, late-life Depression, Neutrophil Gelatinase-Associated Lipocalin
**Introduction**

Depression and obesity are two major risk factors for unfavourable health outcomes (Penninx et al, 2001; Everson-Rose et al, 2009). Meta-analysis of cross-sectional studies has confirmed a significant association between depression and obesity (de Wit et al, 2010). Subsequently, meta-analyses of longitudinal studies identified depression as a risk factor for weight gain, as well as obesity as a risk factor for the development of depression (Luppino et al, 2010). Current hypotheses about predisposition of depression to obesity include neuroendocrine disturbances in the Hypothalamic Pituitary Adrenal Axis (HPA-axis) (Deushle et al, 1998) changing lifestyle factors as eating patterns and reduced physical activity (Stunkard et al, 2003) and the use of antidepressants (Schwartz et al, 2004). Biological mechanisms like HPA-axis dysregulation (Pasquali 2012; van Reedt Dortland et al, 2012) but also psychological mechanisms like stigmatization, negative body image and disappointment about failing diets (Dixon et al, 2003) are hypothesized to predispose obese people to depression. In addition to these hypotheses, inflammatory pathways have gained increasing attention and may explain the bi-directional association between depression and obesity (Bremmer et al, 2008; Milaneshi et al, 2012; Daly et al, 2013).

Despite well conducted longitudinal studies on the relationship between depression and obesity, important clinical aspects remain unknown. Firstly, only three longitudinal studies in the above-described meta-analyses were conducted in an older cohort (Roberts et al, 2003; Sachs-Ericson et al, 2007; Vogelzangs et al, 2008). These three studies also suggested a bidirectional association between depression and obesity in later life, although only one of these studies reported statistically significant results (Roberts et al, 2003; Sachs-Ericson et al, 2007; Vogelzangs et al, 2008). More recent (and cross-sectional) population-based studies in older people have even reported a negative association, i.e. lower body weight in depression (Ho et al, 2008; Wong et al, 2011; Dong et al, 2012). Secondly, it is unknown whether depressive symptoms measured in population-based studies can be generalised to clinical samples of depressed patients. With one exception, all studies have been conducted in population-based samples, the vast majority in an adult population (de Wit et al, 2010; Luppino et al, 2010; Marijnissen et al, 2011; Arterburn et al, 2012). Recently, the first study in a clinical sample confirmed that depressive disorder is associated with an increase in abdominal obesity over time (Van Reedt Dortland et al, 2013). This study was limited to patients aged below 65 years. Whether these findings also account for older persons remains unclear. In late-life depression an absent or even inverse association might be hypothesized
as in later life the prevalence of physical frailty sharply increases with age (Collard et al, 2012) and physical frailty is associated with both underweight and depression (Fried et al, 2001; Collard et al, 2013).

Furthermore, it is important to consider the definition of obesity and depression. Depression has been specifically linked with visceral fat accumulation (Vogelzangs et al, 2008). A study on the prospective impact of the metabolic syndrome in adults has identified waist circumference as the most important component predicting the onset of depression in non-depressed persons and a protracted course in depressed patients (Vogelzangs et al, 2011). Despite the presence of diagnostic criteria, depressive disorder is a heterogeneous syndrome. Recently, in a large population based study among middle-aged and older persons, we found that depressive symptoms were positively associated with both body mass index and waist circumference. Interestingly, the cognitive-affective symptoms of depression were associated with both the increased body mass index and increased waist circumference, whereas the somatic-affective symptoms of depression were specifically associated with the increased waist circumference (Marijnissen et al, 2011; Marijnissen et al, 2013).

The waist circumference is often used as a proxy for visceral adipose tissue. Visceral fat is a metabolically active tissue secreting cytokine-family proteins, collectively called adipokines (Trujillo et al, 2006; Milaneschi et al, 2012; Zhao et al, 2013). Such adipokines are hypothesised to induce a chronic low-grade inflammatory environment, thus contributing to the negative health effect of obesity. Neutrophil Gelatinase-Associated Lipocalin (NGAL), also called Lipocalin-2, is a recently identified adipokine with high levels of expression and secretion in the white adipose tissue (Huang et al, 2012). Interestingly, induction of a peripheral inflammatory response as well as psychological stressors induces cerebral NGAL expression (Ip et al, 2011; Mucha et al, 2011), which consequently can reduce hippocampal synaptic spine density (Mucha et al, 2011). We recently showed that NGAL expression can induce a pro-apoptotic signaling cascade by attenuating Akt phosphorylation of the protein kinase B (PKB)/Akt pathway and sensitize neurons to beta-amyloid induced cell death (Naudé et al, 2012). Cellular signaling via Akt has been postulated as a key pathway involved in neuroplasticity in the hippocampus (Balu et al, 2012; Jin et al, 2012). Interestingly the PKB/Akt activity is also decreased in post mortem human brain tissue of suicide victims compared to non-depressed controls (Karege et al, 2011). These data collectively suggest that increased central nervous system NGAL levels can lead to reduced neuroplasticity. Plasma levels of NGAL appear to be increased in depressed older persons (Naudé et al, 2013) as well as obese people (Auget et al, 2010; Huang et al, 2012). Animal models have further shown that NGAL plays a functional role in systemic insulin sensitivity and glucose homeostasis (Wang et al, 2007). As with leptin (Milaneschi et al, 2012), the
presence of increased NGAL levels in obese persons may be considered as an indicator of NGAL resistance. If true, the risk for the onset of depression would be especially increased in persons with high levels of NGAL and visceral fat.

The primary objective of the present study was to examine the association between depression and waist circumference in an older (≥ 60 years) depressed sample and to explore several characteristics of late-life depression within this association. Our second objective is to explore the role of NGAL. Being an adipokine, we hypothesise that increased NGAL levels are associated with depression, especially in persons with an increased waist circumference as proxy for visceral obesity.

**Methods**

**Sample**
For the present study, we used the baseline assessment of the Netherlands Study of Depression in Older persons (NESDO) (see Comijs et al, 2011). NESDO is an on-going cohort study designed to examine the determinants of the course and consequences of depressive disorders in older persons, including 378 depressed and 132 non-depressed older persons. Recruitment of depressed older persons took place in five regions in the Netherlands from both mental health care institutes and general practitioners in order to include persons with late-life depression in various developmental and severity stages. Persons with a primary diagnosis of dementia, a Mini Mental State Examination-score (MMSE) under 18 (out of 30 points) (Folstein et al, 1975), and insufficient command of the Dutch language were excluded. The comparison group of non-depressed persons was recruited at the same general practices that recruited patients. A random sample of older people who scored less than four on the Geriatric Depression Scale during a visit to their GP was asked informed consent. Exclusion criteria were a lifetime diagnosis of depression, dementia or other serious psychiatric disorders, and insufficient command of the Dutch language.

Data collection of the baseline assessment started in 2007 and was finished in September 2010. The baseline assessment included written questionnaires, interviews and physical assessments. Interviews were audio taped to control the quality of the data. The ethical review boards of the participating institutes have approved this study. All participants gave informed consent after oral and written information about the study. Of the 510 participants in NESDO, 4 participants were excluded because of missing body mass index (n=1) and missing waist circumference (n=3). This left a final study sample of 506 people (376 depressed and 130 non-depressed).
With respect to the second objective, a further 12 participants (9 depressed, 3 non-depressed) were excluded due to refusing or failing blood withdrawal. Thus, analyses on NGAL/Lipocalin2 are based on 494 participants (367 depressed patients and 127 non-depressed controls).

Variables of interest

Depression diagnoses - The past 6-month diagnosis of depression and dysthymia according to DSM-IV-R criteria (APA, 2000) were assessed with the Composite International Diagnostic Interview (CIDI; WHO version 2.1; 12 month version, 1997). The CIDI is a structured clinical interview that is designed for use in research settings and has high validity for depressive and anxiety disorders (Wittchen et al, 1991; Kessler et al, 2010). As in NESDA (The Netherlands Study of Depression and Anxiety; Penninx et al, 2008), we added questions to determine the research DSM-IV diagnosis of current minor depression (Comijs et al, 2011). Among the depressed sample, 357/376 (94.9%) met criteria for a major depressive disorder (MDD) with the past 6 months, 20 (5.3%) for a current minor depression and 100 (26.6%) for dysthymia (in the past-six months). Due to double diagnoses, numbers do not add up to 100%.

Depression characteristics - Characteristics of depression will be categorised in those characteristics indicative of a prolonged or chronic course (duration related measures) and those indicative of a more severe episode (severity related measures) as indicated in Table 4.

Duration-related measures - Based on data from the CIDI-interview, we assessed additional age of onset of depression (age of the participant at the time of the first depressive episode), recurrence (presence of depressive episode prior to the current episode) and life-time dysthymia.

Severity related measures - Severity of depression was measured by the 30-item selfrating Inventory of Depressive Symptomatology (IDS), which has adequate psychometric properties (Rush et al, 1996). The sum score ranges from 0 to 60; the severity of depression can be classified as none (score range 0 through 12), mild (13 through 24), moderate (25 through 37), severe (38 through 47) and very severe (48 or higher). To examine different symptom dimensions of late-life depression, three symptom profiles of the IDS were used, a mood, motivation and somatic dimension. These three homogenous symptom dimensions were shown to have a good fit with exploratory and confirmatory factor analysis in the NESDO study (Hegeman et al, 2012).

Obesity - Waist circumference was measured at the level of the umbilicus (cm). According to the National Cholesterol Education Program (NCEP)-Adult Treatment Panel III, a waist
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Circumference above 88 cm and 102 cm is considered pathologically increased in females and males, respectively (Alexander et al, 2003). In addition, standardized assessments of weight (kg) and height (m) were used to calculate the body mass index (weight (kg) divided by the square of the height (m²)).

**NGAL measurement in plasma by ELISA** - Quantification of NGAL from plasma was performed via a constructed sandwich ELISA using human Lipocalin-2/NGAL ELISA capture antibody (R&D Systems), recombinant human Lipocalin-2/NGAL (R&D Systems) for the internal standard and biotinylated human Lipocalin-2/NGAL detection antibody (R&D Systems). Plasma was diluted 1:100. A blinded ELISA analysis was performed on coded samples. Briefly, plates (96 wells, Maxisorb, Nunc) were coated with the capture antibody (100 µl; 2 µg/ml) diluted in phosphate buffered saline (PBS, pH 7.4). After overnight incubation at room temperature, the coated plates were washed with Tris Buffered Saline (TBS) containing 0.05% Tween 20 (TBS/T) and nonspecific binding sites blocked by incubation with 300 µl of PBS containing 1% Bovine Serum Albumin (BSA) (PBS/BSA) for 2 hours at room temperature on a shaker. After washing, 100 µl of either the standards (recombinant human NGAL) or samples, diluted in PBS/BSA were added to the plates and incubated for 2 hours at room temperature on a shaker. Plates were washed six times and 100 µl of biotinylated human Lipocalin-2/NGAL detection antibody (100 ng/ml) diluted in PBS/BSA was added. After 2 hours on a shaker at room temperature, plates were washed six times and incubated with 100 µl Avidin-horseradish peroxidase (eBioscience) in PBS/BSA (1:1000) for 20 minutes on shaker at room temperature. Plates were then washed six times and 100 µl of substrate solution containing 1mg/ml of O-phenylenediamine (Sigma) in 0.05M citric acid sodium phosphate Buffer (pH 5.0) with hydrogen peroxide (0.06%) was added. The reaction was stopped by adding 100 µl of a 3N HCl solution. The absorbance was determined at 492 nm with background subtraction at 620 nm using an ELISA reader (Asys UVM 340, Biochrom, Cambridge, UK). The quantity of NGAL was estimated from the calibration curve which ranged from 78 to 5000 pg/ml. Samples were stored at -80 °C. Blood samples were collected in the morning to standardize for collection time. The intra- and inter-assay coefficients of variation were 3% and 5%, respectively (Comijs et al, 2011; see Naudé et al, 2013).

**High-sensitivity C-reactive protein** - In addition to NGAL levels, high-sensitivity plasma levels of CRP were measured in duplicate by an immunoturbidimetric assay (Tina-quant CRPHS, Roche Diagnostics, Mannheim, Germany) at the Clinical Chemistry department of the VU University Medical Center). Intra- and inter-assay coefficients of variation were 2% and 2%. As CRP levels were positively skewed, ln-transformed values
were used to normalize the distribution when included in the multivariate analyses.

**Covariates**

In addition to age, sex and years of education (first set of confounders), we *a priori* considered the following potential confounders based on their relationship with both depression and obesity.

The second set of confounders included lifestyle factors like smoking, use of alcohol, physical activity and use of antidepressants (Schwartz et al, 2004). Smoking was defined as currently smoking (yes/no). Based on the first two questions of the Alcohol Use Disorders Identification Test (AUDIT) (Babor et al, 2001; Aalto et al, 2011), we classified alcohol consumption in three categories according to the average number of units taken on a typical drinking day and the frequency of drinking: 1) no drinking, 2) moderate alcohol use, and 3) severe alcohol use. Severe alcohol use was defined as taking 5 – 10 units on a typical drinking day irrespective of the frequency of drinking or 3 or 4 units on a typical drinking day at least 4 or more days a week. Moderate alcohol use was defined as any alcohol use not being severe use. Physical activity was measured with the last-seven-days short-form (8-items) of the self-administered version of the International physical Activities Questionnaire (IPAQ). Psychometric properties of the long and short version of the IPAQ are acceptable (Craig et al, 2003). The physical activity was classified in three categories (minimal, moderate, high).

Medication use was assessed based on drug container inspection of all drugs used in the past month and classified according to the World Health Organization Anatomical Therapeutic Chemical classification (ATC) (WHO, 2012). Medication was only considered when taken on a regular basis (at least 50% of the time). For the present study, we took antidepressant drugs and anti-inflammatory drugs into account. Antidepressant medication included selective serotonin reuptake inhibitors (SSRIs) (N06AB), serotonin-norepinephrine reuptake inhibitors (SNRI) (N06AX16, N06AX21), tricyclic antidepressants (TCAs) (N06AA) and tetracyclic antidepressants (TeCA) (N06AX03, N06AX05, and N06AX11). Anti-inflammatory drugs included amino salicylic acid and similar agents (A07EC), anti-allergic agents (A07EB), systemically applied corticosteroids (H02A), anti-inflammatory and anti-rheumatic products (M01) and other analgesics and antipyretics (N02B).

The third set of confounders consisted of parameters of physical functioning and included cognitive functioning and the number of chronic diseases. Cognitive functioning was assessed by the Mini Mental State Examination (MMSE) (Folstein et al, 1975). The MMSE score ranges from 0-30, with higher scores indicating better cognitive functioning.

The number of chronic diseases was assessed by means of a self-report questionnaire that has previously been used in NESDA (Penninx et al, 2008). The participants were
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asked whether they currently or previously had any of the following chronic diseases or disease events: cardiac disease (including myocardial infarction), peripheral atherosclerosis, stroke, diabetes mellitus, COPD (asthma, chronic bronchitis or pulmonary emphysema), arthritis (rheumatoid arthritis or osteoarthritis) or cancer, or any other disease. Compared to general practitioner information, the accuracy of self-reports of these diseases was shown to be adequate and independent of cognitive impairment (Kriegsman et al, 1996).

In case of associations tested with NGAL or hsCRP, having had a cold or fever in the previous week to blood withdrawal (yes/no) was also taken into account.

**Statistical methods**

Waist circumference was compared between depressed and non-depressed persons with ANCOVA adjusted for different sets of covariates (model 1: age, gender, level of education; model 2: additionally adjusted for cognitive functioning, chronic diseases and antidepressant drug use; model 3: additionally adjusted for lifestyle characteristics including smoking, alcohol use and physical activity). Subsequently, in model 4 the analysis was additionally corrected for body mass index in order to examine the impact of the waist circumference when adjusted for general obesity.

In order to examine a potentially moderating role of obesity in the association between NGAL and depression, multiple logistic regression analyses were conducted with depression (yes/no) as dependent variable adjusted for the covariates described above, added with having had a cold or fever in the previous week to blood withdrawal. Waist circumference and plasma NGAL levels will be included as independent variables. In these models, the interaction between NGAL and waist circumference will be tested and in case of significance, analyses will be presented separately for participants with and without a pathologically increased waist circumference.

Subsequently, multiple linear regression analyses in the depressed subgroup were conducted with waist circumference as dependent variable and depression severity characteristics (IDS sum score, IDS-mood subscale, IDS-motivation subscale and IDS-somatic subscale) as well as duration related characteristics (age of onset, duration of illness, life-time comorbidity with dysthymia) as independent variables in separate models. These analyses were fully adjusted for the covariates described above.

Finally, a moderating role of plasma NGAL in the association between obesity and depression characteristics will be examined by adding the interaction term between NGAL and a specific depression characteristic to the fully adjusted linear regression models described above.

Finally, all analyses were rerun replacing NGAL levels by hsCRP levels, in order to examine whether results were indeed specific for NGAL and not a none-specific
inflammatory reaction.
By convention, p-values below .05 will be considered statistically significant for individual variables, whereas p-values below .10 will be considered statistically significant for interaction terms. All analyses were performed in SPSS version 18.0 (Inc. Chicago).

Results

Obesity in depressed versus non-depressed persons
Table 1 presents the characteristics of the study population. Depressed patients and the non-depressed comparison group did not differ with respect to the body mass index (26.3 (SD=4.4) versus 27.0 (SD=4.1), t=1.6, df=504, p=.121), whereas depressed persons had a significantly lower waist circumference (93.5 (SD=13.0) versus 98.4 (SD=15.3), t=3.5, df=504, p=.001).

As shown in Table 2, the difference in waist circumference between depressed and non-depressed persons became more significant when adjusted for covariates, and also remained significant after adjustment for body mass index (estimated marginal mean (SE) = 93.9 (0.5) versus 97.8 (0.8); F=15.9; df=1,467; p<.001).

As these results were contrary to our hypothesis that an increased waist circumference would be found in depression post-hoc analyses were conducted excluding depressed patients that reported depression related weight loss in the past 2 years based on the corresponding CIDI question (n=131). These analyses yielded almost comparable results. In the fully adjusted models, depressed older persons still had a significantly lower waist circumference compared to the non-depressed comparison group (94.3 (SE=0.9) versus 100.2 (SE=1.3); F=13.0, df=1,340; p< .001), which also remained significant after adjustment for body mass index (95.0 (SE=0.6) versus 98.8 (SE=0.9); F=12.5, df=1,339; p< .001).

Impact of obesity on the association between NGAL and depression status
In a previous paper of the present sample, we already showed that depression is associated with increased NGAL levels (see Naudé et al, 2013). In order to examine whether the association between NGAL and depression status (yes/no) is conditional on the waist circumference, we examined the interaction term between NGAL level and waist circumference in a logistic regression model adjusted for all confounders, with depression status as the dependent variable. The interaction term between NGAL and waist circumference was significant irrespective of adjustment for body mass index. Without adjusting for body mass index the OR of waist circumference by NGAL was
1.001 [95% CI = 1.000 – 1.002], p=.093; when additional adjusting for body mass index the OR of waist circumference by NGAL was 1.001 [95% CI: 1.000 – 1.002], p=.072. Therefore, subsequent analyses will be presented separately for participants with nor-

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Depressed patients (n=376)</th>
<th>Non-depressed controls (n=130)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressive symptom severity (IDS) Mean (SD)</td>
<td>30.0 (13.0)</td>
<td>7.6 (5.7)</td>
<td>T=-18.9, df=496, p&lt;.001</td>
</tr>
<tr>
<td>Plasma NGAL levels (pg/ml) Mean (SD)</td>
<td>62.00 (23.30)</td>
<td>53.25 (21.29)</td>
<td>T=-3.7, df=492, p&lt;.001</td>
</tr>
<tr>
<td>High-sensitivity CRP levels Median (IQR)</td>
<td>1.88 (3.18)</td>
<td>1.59 (2.28)</td>
<td>T=-1.4, df=486, p=166*</td>
</tr>
</tbody>
</table>

Demographics
- Age Mean (SD) 70.7 (7.4) 69.9 (7.0) T=-1.1, df=504, p=.267
- Female sex n (%) 248 (66.0) 80 (61.5) χ²=0.8, df=1, p=.363
- Years of education Mean (SD) 10.5 (3.4) 12.5 (3.5) T=5.8, df=504, p<.001

Somatic status
- Global cognitive Mean (SD) 27.7 (2.0) 28.4 (1.6) T=3.3, df=503, p=.001
- No. of chronic diseases Mean (SD) 2.1 (1.5) 1.5 (1.1) T=-4.5, df=503, p<.001
- Use of antidepressants n (%) 272 (72.3) 3 (2.3) χ²=191, df=1, p<.001
- Waist circumference (cm) Mean (SD) 93.5 (13.0) 98.4 (15.3) T=3.5, df=504, p=.001
- Body Mass Index Mean (SD) 26.3 (4.4) 27.0 (4.1) T=1.6, df=504, p=.121

Life-style characteristics
- Alcohol use χ²=36.2, df=2, p<.001
  - No use n (%) 148 (40.0) 16 (12.7)
  - Moderate usage n (%) 189 (51.1) 84 (66.7)
  - Severe usage n (%) 33 (8.9) 26 (20.6)
- Smoking χ²=20.2, df=1, p<.001
  - Low n (%) 99 (26.5) 10 (7.7)
  - Medium n (%) 138 (38.8) 55 (43.3)
  - High n (%) 114 (31.2) 50 (39.4)
- Physical activity χ²=9.0, df=2, p=.011
  - Low n (%) 113 (31.0) 22 (17.3)
  - Medium n (%) 138 (38.8) 55 (43.3)
  - High n (%) 114 (31.2) 50 (39.4)

* T-test on ln-transformed values
mal and pathological increased waist circumference (Table 3). As shown in Figure 1, NGAL plasma levels are only elevated in depressed older patients with a pathologically increased waist circumference.

HsCRP levels (see Table 1 for unadjusted results) did not differ between patients and controls in the fully adjusted models (p=.482). Moreover, these results were not conditional on the WC as indicated by non-significant interaction terms between hsCRP and WC when predicting depression status (p=.684 not adjusted for BMI; p=.649 adjusted for BMI).

**Table 2** Differences in obesity measures by depression status (ANCOVA*).

<table>
<thead>
<tr>
<th>Measures of obesity</th>
<th>Depressed patients</th>
<th>Non-depressed controls</th>
<th>Statistics</th>
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<tbody>
<tr>
<td></td>
<td>(n=376)</td>
<td>(n=130)</td>
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<tr>
<td></td>
<td>Adjusted Mean (SD)</td>
<td>Adjusted Mean (SD)</td>
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<tr>
<td><strong>Body Mass Index (BMI)</strong></td>
<td></td>
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<tr>
<td>Model 1 (+socio-demographics)</td>
<td>26.6 (0.2)</td>
<td>27.2 (0.4)</td>
<td>F=5.02, df=1,501, p=.025</td>
</tr>
<tr>
<td>Model 2 (+physical functioning)</td>
<td>26.2 (0.2)</td>
<td>27.5 (0.4)</td>
<td>F=8.74, df=1,498, p=.003</td>
</tr>
<tr>
<td>Model 3 (+life-style)</td>
<td>26.2 (0.2)</td>
<td>27.5 (0.4)</td>
<td>F=7.63, df=1,468, p=.006</td>
</tr>
<tr>
<td>Model 4 (+BMI)b</td>
<td>93.9 (0.5)</td>
<td>97.8 (0.8)</td>
<td>F=15.92, df=1,467, p=.001</td>
</tr>
<tr>
<td><strong>Waist circumference (WC)</strong></td>
<td></td>
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<tr>
<td>Model 1 (+socio-demographics)</td>
<td>93.4 (0.7)</td>
<td>98.8 (1.2)</td>
<td>F=15.82, df=1,501, p&lt;.001</td>
</tr>
<tr>
<td>Model 3 (+physical functioning)</td>
<td>93.1 (0.7)</td>
<td>99.5 (1.2)</td>
<td>F=21.42, df=1,498, p&lt;.001</td>
</tr>
<tr>
<td>Model 4 (+life-style)</td>
<td>93.1 (0.7)</td>
<td>100.1 (1.3)</td>
<td>F=22.20, df=1,468, p&lt;.001</td>
</tr>
<tr>
<td>Model 4 (+BMI)b</td>
<td>93.9 (0.5)</td>
<td>97.8 (0.8)</td>
<td>F=15.92, df=1,467, p&lt;.001</td>
</tr>
</tbody>
</table>

- Model 1: Adjusted for age, gender and level of education (ANCOVA, shown are adjusted means (SEM))
- Model 2: Additionally adjusted for global cognitive functioning (MMSE), no. of chronic diseases, use of antidepressants
- Model 3: Additionally adjusted for lifestyle, i.e. alcohol use (no, moderate, severe), smoking (yes/no), physical activity (minimal, moderate, high).
- Model 4: Additionally adjusted for BMI

* Please note that differences are not controlled for antidepressant drug use (as only 3 controls use anti-depressants):
  - Firstly, among depressed patients, antidepressant drug use was not associated with obesity in fully adjusted models comparing depressed patients without and with antidepressants (for WC: EM(SE) = 93.4 (1.2) versus 93.6 (0.8), F=0.03; df=1,345; p=.875; for BMI (EM(SE) = 26.4(0.4) versus 26.4(0.3), F=0.01, df=1,345, p=.915).
  - Secondly, results did not differ by antidepressant drug class (SSRI, TCA, other); for WC: F=1.08, df=3.320, p=.359; for BMI: F=1.43, df=3.320, p=.235.

b The BMI was not significant anymore in this latter model.
Table 3  Odds of NGAL on depression status (yes/no).

<table>
<thead>
<tr>
<th></th>
<th>Not adjusted for BMI OR [95% CI]a</th>
<th>p</th>
<th>Adjusted for BMIb OR [95% CI]a</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Whole sample (n=506)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>• Waist circumference</td>
<td>0.946 [0.925 – 0.967]</td>
<td>&lt;.001</td>
<td>0.928 [0.895 – 0.963]</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Normal wc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Waist circumference</td>
<td>0.897 [0.828 – 0.972]</td>
<td>.008</td>
<td>0.912 [0.834 – 0.998]</td>
<td>.045</td>
</tr>
<tr>
<td>• Plasma NGAL level</td>
<td>0.998 [0.977 – 1.020]</td>
<td>.843</td>
<td>0.999 [0.977 – 1.021]</td>
<td>.899</td>
</tr>
<tr>
<td>Pathologically increased wc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Waist circumference</td>
<td>0.959 [0.923 – 0.997]</td>
<td>.033</td>
<td>0.927 [0.878 – 0.978]</td>
<td>.006</td>
</tr>
<tr>
<td>• Plasma NGAL level</td>
<td>1.023 [1.006 – 1.041]</td>
<td>.007</td>
<td>1.025 [1.007 – 1.042]</td>
<td>.006</td>
</tr>
</tbody>
</table>

a All models are adjusted for age, sex, educational level, cold or fever in the week previous to blood withdrawal (yes/no), no. of chronic disease, global cognitive functioning (MMSE), smoking (yes, no), alcohol use (no, moderate, severe) and physical activity (low, moderate, high), antidepressant drug use (SSRI, TCA, Other), anti-inflammatory drugs (yes/no).
b The BMI was not significant anymore in any of these models.

Figure 1  Plasma NGAL levels by waist circumference and depression status.*

* Overall, fully adjusted ANCOVA: F=3.54, df=3,462; p=.015
**Characteristics of obesity in depressed persons**

Linear regression analyses in the depressed subgroup (n=376) showed that increased waist circumference was associated with both depression severity measures as well as duration related characteristics (Table 4). When additionally adjusted for body mass index, the strength of the association decreased, but the IDS sum score as overall severity measure remained significantly associated \((B=0.06 \ (0.03), \beta=0.06, \ p=.046)\), whereas the motivation subscale became significant \((B=0.26(0.13), \beta=0.06, \ p=.050)\).

**Does NGAL moderate the association between depression characteristics and obesity**

Among the subgroup of depressed older persons, NGAL is associated with the waist circumference in fully adjusted linear regression models \((B(SE)=0.082 \ (0.030), \beta=.15; \ p=.006)\).

**Table 4** Determinants of WC in depressed patients separate, fully adjusted linear regression models.*

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Waist Circumference (WC)</th>
<th>Not adjusted for BMI</th>
<th>Adjusted for BMI*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Depression severity measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• IDS sum score</td>
<td>0.11 (0.06) .11 .046</td>
<td>0.06 (0.03) .06 .046</td>
<td></td>
</tr>
<tr>
<td>• IDS, mood subscale</td>
<td>0.32 (0.14) .13 .023</td>
<td>0.12 (0.08) .05 .131</td>
<td></td>
</tr>
<tr>
<td>• IDS, motivation subscale</td>
<td>0.41 (0.23) .10 .077</td>
<td>0.26 (0.13) .06 .050</td>
<td></td>
</tr>
<tr>
<td>• IDS, somatic subscale</td>
<td>0.16 (0.17) .05 .355</td>
<td>0.09 (0.10) .03 .367</td>
<td></td>
</tr>
<tr>
<td><strong>Duration related disease characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Age of onset (in years)</td>
<td>-0.08 (0.04) -.13 .024</td>
<td>-0.01 (0.02) -.02 .579</td>
<td></td>
</tr>
<tr>
<td>• Age of onset (&lt; 60 years)</td>
<td>-4.04 (1.50) -.15 .007</td>
<td>-1.21 (0.85) -.04 .155</td>
<td></td>
</tr>
<tr>
<td>• Life-time comorbidity with dysthymia</td>
<td>4.40 (1.40) .16 .002</td>
<td>0.59 (0.81) .02 .469</td>
<td></td>
</tr>
<tr>
<td>• Duration of illness (years)</td>
<td>0.08 (0.04) .12 .024</td>
<td>0.02 (0.02) .02 .455</td>
<td></td>
</tr>
</tbody>
</table>

* All models are adjusted for age, sex, educational level, no. of chronic disease, global cognitive functioning (MMSE), smoking (yes, no), alcohol use (no, moderate, severe) and physical activity (low, moderate, high), antidepressant drug use (SSRI, TCA, Other), anti-inflammatory drugs (yes/no).

* The BMI was not significant in any of these models.
### Table 5  Association between NGAL and waist circumference by different classes of depression severity and by the presence of life-time dysthymia using multiple linear regression analyses.*

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Waist Circumference (WC)</th>
<th>B (SE)</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severity of depression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not additionally adjusted for BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• No/mild depression</td>
<td>-0.01 (0.04)</td>
<td>-.02</td>
<td>.775</td>
<td></td>
</tr>
<tr>
<td>• Moderate depression</td>
<td>0.04 (0.05)</td>
<td>.07</td>
<td>.449</td>
<td></td>
</tr>
<tr>
<td>• (Very) severe depression</td>
<td>0.16 (0.07)</td>
<td>.27</td>
<td>.021</td>
<td></td>
</tr>
<tr>
<td>Additionally adjusted for BMIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• No/mild depression</td>
<td>0.01 (0.03)</td>
<td>.02</td>
<td>.686</td>
<td></td>
</tr>
<tr>
<td>• Moderate depression</td>
<td>0.01 (0.03)</td>
<td>.01</td>
<td>.858</td>
<td></td>
</tr>
<tr>
<td>• (Very) severe depression</td>
<td>0.12 (0.03)</td>
<td>.19</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td><strong>Life-time history of dysthymia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not additionally adjusted for BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• No life-time dysthymia</td>
<td>&lt;0.01 (0.03)</td>
<td>.01</td>
<td>.906</td>
<td></td>
</tr>
<tr>
<td>• Life-time dysthymia</td>
<td>0.19 (0.06)</td>
<td>.34</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>Additionally adjusted for BMIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• No life-time dysthymia</td>
<td>0.02 (0.02)</td>
<td>.04</td>
<td>.366</td>
<td></td>
</tr>
<tr>
<td>• Life-time dysthymia</td>
<td>0.11 (0.03)</td>
<td>.20</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td><strong>Age of onset depression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not additionally adjusted for BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Early onset depression (&lt;60 years)</td>
<td>0.05 (0.04)</td>
<td>.08</td>
<td>.291</td>
<td></td>
</tr>
<tr>
<td>• Late-onset depression (≥60 years)</td>
<td>0.11 (0.05)</td>
<td>.23</td>
<td>.019</td>
<td></td>
</tr>
<tr>
<td>Adjusted for BMIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Early onset depression (&lt;60 years)</td>
<td>0.07 (0.02)</td>
<td>.11</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>• Late-onset depression (≥60 years)</td>
<td>0.07 (0.03)</td>
<td>.15</td>
<td>.025</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of illness (median split at 19 years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not additionally adjusted for BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Short duration of illness (≤19 years)</td>
<td>0.11 (0.04)</td>
<td>.24</td>
<td>.003</td>
<td></td>
</tr>
<tr>
<td>• Long duration of illness (&gt;19 years)</td>
<td>0.03 (0.05)</td>
<td>.04</td>
<td>.624</td>
<td></td>
</tr>
<tr>
<td>Adjusted for BMIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Short duration of illness (≤19 years)</td>
<td>0.08 (0.02)</td>
<td>.16</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>• Long duration of illness (&gt;19 years)</td>
<td>0.08 (0.03)</td>
<td>.12</td>
<td>.004</td>
<td></td>
</tr>
</tbody>
</table>

* All models are adjusted for age, sex, educational level, cold or fever in the week previous to blood withdrawal (yes/no), no. of chronic disease, global cognitive functioning (MMSE), smoking (yes, no), alcohol use (no, moderate, severe) and physical activity (low, moderate, high), antidepressant drug use (SSRI, TCA, Other), anti-inflammatory drugs (yes/no).

a The BMI was not significant in any of these models.
First, we examined which depression characteristics (see Table 4) that were associated with waist circumference interacted with plasma NGAL level. Not adjusted for the body mass index, NGAL interacted with the IDS sum score ($B(SE)=0.004$ ($0.002$), $\beta=0.35$, $p=.019$), IDS mood scale ($B(SE)=0.012$ ($0.004$), $\beta=0.36$, $p=.009$), IDS motivation scale ($B(SE)=0.015$ ($0.008$), $\beta=0.29$, $p=.046$), age of onset (continuous: $B(SE)=0.002$ ($0.001$), $\beta=.33$, $p=.086$; dichotomised: $B(SE)=0.12$ ($0.06$), $\beta=.43$, $p=.053$), with life-time dysthymia ($B(SE)= 0.16$ ($0.06$), $\beta =0.34$, $p=.009$), and with duration of illness ($B(SE)=-0.003$ ($0.001$), $\beta=-.30$, $p=.057$) when predicting the waist circumference in depressed older persons. Interaction between NGAL and other depression characteristics were not significant (all $p$-values $>.10$).

Adjusted for body mass index, five depression characteristics interacted with plasma NGAL levels when predicting waist circumference in depressed older persons. The significant interaction terms included NGAL by IDS total score ($B(SE)=0.003$ ($0.001$), $\beta=.27$, $p=.006$), NGAL by IDS mood subscale ($B(SE)=0.009$ ($0.003$), $\beta =.26$, $p=.003$), IDS motivation scale ($B(SE)=0.011$ ($0.005$), $\beta=0.20$, $p=.31$), IDS somatic scale ($B(SE)=0.007$ ($0.004$), $\beta=0.20$, $p=.051$) and finally NGAL by life-time dysthymia (($SE)=0.103$ ($0.040$), $\beta =.22$, $p=.011$). Table 5 presents the results split by depression severity, life-time dysthymia, age of onset and duration of illness. Results by depression severity will be presented for three severity groups, i.e. none or mild symptoms, moderate symptoms, and severe/very severe depressive symptoms, respectively.

Finally, a similar approach was applied with respect to hsCRP levels. However, all results appeared to be non-significant.

**Discussion**

**Main findings**

Depressed patients aged 60 years and over were significantly less obese than the non-depressed comparison group (see Table 2). Population-based findings of a positive correlation between obesity and depressive symptoms can thus not be generalised to patients suffering from late-life depression. The difference with respect to the waist circumference remained even significant after adjustment for the body mass index. Interestingly, the association between increased Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels and depression, recently reported by our group (Naudé et al, 2013) appears to be driven by a pathologically increased waist circumference. Increased NGAL levels may thus be only relevant in a subgroup of depressed older patients.
Obesity in late-life depression

Although most studies report a positive association between obesity and more severe depressive symptoms (as confirmed by meta-analyses), absence or even inverse relationships in this association as found in our study have been reported before (Ho et al, 2008; Wong et al, 2011; Dong et al, 2012). A closer look at these contradictory findings, shows that a negative association between depression and obesity has primarily been reported in an older, Asian population (Ho et al, 2008; Wong et al, 2011; Dong et al, 2012). In eastern cultures, obesity is regarded as a positive characteristic. As 95% (464/489) of our participants are from a north-European ancestry, this cannot be an explanation for our findings. Our sample further differs from population-based studies by having included a relatively severe group of depressed patients meeting the DSM-IV-TR criteria for depression or dysthymia. This is in contrast to population-based studies where severe cases are underrepresented by selection bias (Marijnissen et al, 2011) and results are generally based on depression severity scales (de Wit et al, 2010; Luppino et al, 2010). The only study on a clinical sample (aged 18-65 years old) thus far has confirmed the meta-analytic results of population-based studies (van Reedt Dortland et al, 2013). As this study has applied a similar study design as we did (see Penninx et al. 2008; Comijs et al, 2011), our results cannot simply be explained by having a clinical sample of depressed patients. Our data thus suggest that the relationship between obesity and depression may be different across the lifespan. Although tentative, it may be that depressed older persons have more often lost weight due to comorbid physical frailty or somatic comorbidity, which in itself is associated with depression (Andrew et al, 2007; Collard et al, 2013). Moreover, even aging in general has already been associated with nutritional deficiencies that may also contribute to depressive symptoms (de Boer et al, 2013). Another explanation could still be significant weight loss during the actual depressive episode. Nonetheless, this is unlikely as our post-hoc analyses excluding the patients with significant and unintended weight loss in the past two years yielded similar results. Moreover, this explanation also does not explain why the waist circumference is specifically affected relative to the body mass index.

Within the depressed group, characteristics of a more severe depressive episode as well as a longer history of depression were associated with a higher waist circumference. When adjusted for body mass index, only the association between waist circumference and overall depression severity remained significant, in contrast to the depression duration related characteristics which all lost significance. This may indicate that variance in waist circumference adjusted for general obesity may specifically affect actual depression severity. A possible explanation may lie in the metabolic activity of the visceral fat tissue (Trujillo et al, 2006). Interestingly, inflammatory markers have been specifically related with features of sickness behaviour, including general
weakness and fatigue (Dantzer et al, 2008).

**Adipokines and late-life depression**

Depression has increasingly been linked with visceral fat accumulation (Vogelzangs et al, 2008) as well as low-grade systemic inflammation (Dowlati et al, 2008; Howren et al, 2009). Visceral fat tissue is metabolic active by the secretion of both pro- and anti-inflammatory cytokines, collectively called the adipokines. Although NGAL has not been studied previously in the association between obesity and depression, comparable results have been found for other adipokines. In older men, high leptin has been associated with an increased onset of depressive symptoms in the presence of abdominal obesity (Milaneshi et al, 2012). The fact that NGAL exerts its effects primarily in case of a pathologically increased waist circumference, contributes to its role as an adipokine. Adipokines have indeed been specially associated with visceral obesity. For example, plasma levels of adiponectin, a protective cytokine for vascular health, are lowered in case of visceral obesity resulting in negative health effects independent of the body mass index (Mathieu et al, 2009; Taylor et al. 2010; Matsuzawa et al, 2012).

**Methodological considerations**

The strengths of our study are the large number of older persons suffering depression and the comprehensive assessment of depression characteristics and confounding factors. However, some limitations should be acknowledged for proper interpretation. Firstly, the cross-sectional study design precludes causal interpretations of the findings. Secondly, plasma NGAL levels cannot be translated directly to increased NGAL expression in adipocytes. Nonetheless, a recent study in 90 obese women showed that plasma NGAL levels were associated with visceral NGAL protein levels ($r=-.43$, $p=.044$), while the latter were associated with visceral NGAL mRNA levels ($r=-.59$, $p=.004$) (Auguet et al, 2010). The direct association between plasma levels and adipocyte mRNA, however, has not been reported. Thirdly, the comparison group was recruited from persons visiting the GP and especially obese persons do have a higher disease burden. This might have partly contributed to our finding of lower level of obesity in late-life depression. Fourthly, there is a small chance (one of 20) that we found some spurious findings (type one error) due to multiple comparisons. However, if one tests for the significance of an association using variables that are mutually correlated, the Bonferroni correction is even too conservative (Perneger, 1998). Therefore, we have not made the Bonferroni correction, but have chosen to present all individual statistics and p-values. Finally, we did not apply Computer Tomography at the level of the fourth lumbar vertebra as the gold standard for quantification of visceral fat (Weber-Haman et al, 2006).
Final conclusion and clinical implications
We conclude that population-based findings among older persons cannot be generalised to a clinical sample of depressed patients. Acknowledging the age-specific effects, we argue for further longitudinal studies specifically in depressed older persons. Such studies might be able to identify depressed subgroups with an unfavourable prognosis with respect to their physical health status. In these studies, the whole array of adipokines should be tested as well as the relative impact of the waist circumference in proportion to the general level of obesity as indexed by the body mass index.
References


Collard RM, Comijs HC, Naarding P et al. (2013) Physical frailty: Vulnerability of patients suffering from late-life depression. Aging Mental Health; Sep 3 [Epub ahead of print].


Waist circumference and NGAL in late-life depression


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Part two

Atherosclerosis and neuroticism