Immunometabolic dysregulation is associated with reduced cortical thickness of the anterior cingulate cortex

Laura S. van Velzen, Lianne Schmaal, Yuri Milaneschi, Marie-José van Tol, Nic J.A. van der Wee, Dick J. Veltman, Brenda W.J.H. Penninx

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Abstract

Background
Immunometabolic dysregulation (low-grade inflammation and metabolic dysregulation) has been associated with the onset and more severe course of multiple psychiatric disorders, partly due to neuroanatomical changes and impaired neuroplasticity. We examined the effect of multiple markers of immunometabolic dysregulation on hippocampal and amygdala volume and anterior cingulate cortex thickness in a large sample of patients with depression and/or anxiety and healthy subjects (N = 283).

Methods
Interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-a), c-reactive protein (CRP), triglyceride levels and HDL-cholesterol and genomic profile risk scores (GPRS) for immunometabolic dysregulation were determined in peripheral blood and T1 MRI scans were acquired at 3T. Regional brain volume and cortical thickness was assessed using FreeSurfer. Covariate-adjusted linear regression analyses were performed to examine the relationship between immunometabolic dysregulation and brain volume/thickness across all subjects.

Results
Multiple immunometabolic dysregulation markers (i.e. triglyceride levels and inflammation) were associated with lower rostral ACC thickness across all subjects. IL-6 was inversely associated with hippocampal and amygdala volume in healthy subjects only. GPRS for immunometabolic dysregulation were not associated with brain volume or cortical thickness.

Conclusions
Multiple serum, but not genetic immunometabolic dysregulation markers were found to relate to rostral ACC structure, suggesting that inflammation and metabolic dysregulation may impact the ACC through similar mechanisms.
Introduction

Chronic psychological stress has been shown to disrupt homeostasis in the body and alter various physiological stress systems, including the immune-inflammatory system and the hypothalamus-pituitary-adrenal (HPA)-axis (see review by Epel 2009). Prolonged dysregulation of these systems, also referred to as increased allostatic load, may result in immunometabolic dysregulation, which consists of systemic low-grade inflammation and metabolic dysregulation. Metabolic dysregulation is often examined in the context of the metabolic syndrome, which includes metabolic risk factors such as hyperglycemia, abdominal obesity, elevated blood pressure, increased triglyceride levels and decreased HDL-cholesterol. Systemic low-grade inflammation and the metabolic syndrome are closely linked (see review by Choi et al. 2013), in part because adipose tissue is an important source of pro-inflammatory cytokines, and in part because the metabolic syndrome and inflammation are partly regulated by similar genetic pathways (Kraja et al. 2014).

Several meta-analyses have shown that chronic low-grade inflammation and the obesity-related components of the metabolic syndrome (abdominal obesity, low HDL-cholesterol and hypertriglyceremia) are associated with psychiatric disorders, including depression (Howren et al. 2009; Dowlati et al. 2010; Xu et al. 2011; Pan et al. 2012; Penninx et al. 2013), schizophrenia (Mitchell et al. 2013; Miller et al. 2014; Fernandes et al. 2015) and bipolar disorder (Vancampfort et al. 2013; Dargél et al. 2015; Vancampfort et al. 2015) and immunometabolic dysregulation may also contribute to the chronicity of a psychiatric disorder (Vogelzangs et al., 2014). Longitudinal studies have suggested that obesity and its closely linked immunometabolic dysregulation both predict onset of depression or depressive symptoms (Roberts et al. 2003; Baune et al. 2012), indicating that immunometabolic dysregulation may be a risk factor for developing a psychiatric disorder, although some studies also suggest that depression may in turn contribute to increased immunometabolic dysregulation (Luppino et al. 2010).

Immunometabolic dysregulation may increase vulnerability for developing a stress-related psychiatric disorder or contribute to chronicity of the disorder by inducing neuroanatomical changes and impairments in neuroplasticity (Tamashiro et al. 2015). Previous neuroimaging studies have shown an inverse relationship between markers of immunometabolic dysregulation (e.g. pro-inflammatory cytokines IL-6 and TNF-a and increased body mass index) with volume of the hippocampus (Cherbuin et al., 2015; Frodl et al., 2012; Marsland et al., 2008; Sudheimer et al., 2014), a region that has been implicated in the pathophysiology of multiple psychiatric disorders (van Erp et al. 2015; Schmaal et al. 2015). Immunometabolic dysregulation has also been linked to structure of other brain regions, however. Obesity, which is characterized by increased immunometabolic dysregulation, has been inconsistently linked to decreased prefrontal, parietal, temporal
and cingulate cortex volume and amygdala volume (Brain Development Cooperative Group 2012; Alosco et al. 2014) and reduced cortical thickness of the frontal, parietal and temporal cortex (Dieset et al. 2015). Thus, it appears that immunometabolic dysregulation is associated with alterations in brain volume and cortical thickness. However, previous studies have not examined multiple markers of immunometabolic dysregulation, did not correct for lifestyle factors or have had modest sample sizes.

In this study, we examined the effect of multiple markers of immunometabolic dysregulation (abdominal obesity, interleukin-6, c-reactive protein, tumor necrosis factor-alpha, triglyceride levels and HDL-cholesterol level) on structure of brain regions associated with psychological and physiological stress (i.e. hippocampus, amygdala and anterior cingulate cortex) in a large group of participants from the Netherlands Study of Depression and Anxiety. As the effect of immunometabolic dysregulation on brain structure is a general pathophysiological mechanism, we expected a similar inverse relationship between immunometabolic dysregulation and brain structure in both healthy subjects and patients with depression and/or anxiety disorders. Therefore in this study, we examined this relationship across patients and controls, which creates more variation and may increase the chance of finding an association.

Whether genetic risk variants for the immunometabolic dysregulation markers under study relate to brain structure has not yet been studied. Therefore we made genomic profile risk scores (GPRS) of three immunometabolic markers (c-reactive protein, body mass index and triglyceride levels), based on three large-scale recent genome wide association meta-analyses (Speliotes et al. 2010; Teslovich et al. 2010; Dehghan et al. 2011) and examined the relationship between these GPRS and brain structure. Based on previous findings, we hypothesized that immunometabolic dysregulation markers are associated with reduced regional brain volume and cortical thickness. We further speculated that GPRS scores are also inversely related to regional brain volume and cortical thickness suggesting that genetic vulnerability for immunometabolic dysregulation has a detrimental effect on brain structure. Finally, in secondary analyses, we explored whether these associations were different in patients and controls.

Materials and methods

Participants

The Netherlands Study of Depression and Anxiety (NESDA) is a longitudinal study, which examines the course of depression and anxiety in a total of 2981 participants. Patients with depressive and/or anxiety disorders as well as subjects without a lifetime psychiatric diagnosis were included in this study. Subjects were recruited from the community, general
practitioners and specialized mental health care institutions (for details please see Penninx et al. 2009).

A subgroup of participants was asked to participate in the NESDA neuroimaging study (N = 301). Inclusion criteria for the imaging study were a DSM-IV diagnosis of major depressive disorder (MDD) and/or anxiety disorder (social anxiety disorder and/or panic disorder and/or generalized anxiety disorder) in the six months preceding the interview for patients and no history of psychiatric disorders for controls. These diagnoses were established using the Composite International Diagnostic Interview (CIDI version 2.1) (Wittchen 1994). Depression severity was assessed using the Inventory of Depressive Symptomatology (IDS; Rush et al., 1996). Mean IDS score in patients was 23.3. 27.9% of all subjects had mild symptoms (scores 14-25), 22.1% had moderate symptoms (scores 26-38), 6.2% had severe symptoms (scores 39-48) and 2.5% had very severe symptoms (scores 49-84).

Exclusion criteria for the NESDA neuroimaging study for all subjects were: i) abuse or dependency of drugs or alcohol in the past year ii) general MRI contraindications iii) presence or history of a severe internal or neurological disorder. Patients were excluded if they used psychotropic medication other than stable use of SSRIs or infrequent benzodiazepines and healthy controls were excluded if they used psychoactive medication.

For the current study, we excluded twelve participants due to poor image quality. Six participants were additionally excluded due to daily use of major anti-inflammatory medication (prednisone, flucloxacillin, amoxicillin, mesalazine and azathioprine), leaving a total of 283 subjects. The Ethical Review Boards of the three participating centers (i.e. Academic Medical Center Amsterdam, University Medical Center Groningen and Leiden University Medical Center) approved this study and all subjects provided written consent.

**Imaging**

MRI images were acquired on 3T Philips MR scanners (Philips, Best, The Netherlands) at three participating imaging centers (Leiden University Medical Center, Amsterdam Medical Center and University Medical Center Groningen). In Amsterdam, a SENSE-6 channel head coil was used, while the other sites used a SENSE-8 channel head coil. Anatomical images were acquired using a sagittal three-dimensional gradient-echo T1-weighted sequence (TR: 9 msec; TE: 3.5 msec; matrix: 256_256; voxel size: 1 mm³; 170 slices). As inter-center variability is has not explicitly been examined, we corrected for this by adding scan site (coded as two dummy variables) as a covariate in all analyses.

Volumetric segmentation and cortical reconstruction was performed using FreeSurfer image analysis suite (version 5.3; Martinos Center for Biomedical Imaging, Harvard-MIT, Boston, MA; http://surfer.nmr.mgh.harvard.edu/). Freesurfer includes motion correction and averaging, Talairach transformation, removal of non-brain tissue,
segmentation of subcortical structures and cortical regions, intensity normalization and
cortical reconstruction. A visual inspection of all subcortical structures and cortical regions
was performed, using a quality assessment protocol developed by the ENIGMA consortium
(http://enigma.ini.usc.edu/protocols/imaging-protocols/).

We focused on volume of the hippocampus and amygdala and cortical thickness of
the rostral and caudal anterior cingulate cortex (ACC) (see supplemental Figure 1), based on
previous studies that examined the relationship between immunometabolic dysregulation
markers and brain structure and function (Frodl and Amico 2014; Willette and Kapogiannis
2015) and the role of these areas in psychological stress (Dedovic et al. 2009; McEwen et al.
2015). As we did not have a priori hypotheses that the pathophysiological mechanisms of
immunometabolic dysregulation may be different for the left and right hemispheres and
given the fact that we did not have power to test separately per hemisphere, we calculate
the mean volume or thickness across hemispheres and used this in subsequent analyses.

Markers of immunometabolic dysregulation

(Abdominal) obesity
Body mass index (BMI) was calculated by dividing weight in kilograms by height in
centimetres squared. Waist circumference (WC) was measured in centimetres at the
midpoint between the lowest front rib and highest point of the pelvis. BMI and WC are highly
correlated measures of obesity, however WC is a more specific measure of abdominal
obesity.

Inflammation
Circulating plasma levels of interleukin-6 (IL-6), c-reactive protein (CRP) and tumor necrosis
factor-alpha (TNF-α) were assessed in blood in duplicate (Vogelzangs et al. 2012). Fasting
blood samples were obtained and kept frozen at -80°. Plasma levels of CRP were measured
by an in-house high-sensitivity enzyme-linked immunosorbent assay (ELISA) based on
purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). Intra- and
inter-assay coefficients of variation were 5% and 10% respectively and the lower detection
limit was 0.1 mg/l.
Plasma IL-6 levels were measured in duplicate by a high sensitivity ELISA (PeliKine Compact
ELISA, Sanquin, Amsterdam, The Netherlands), with intra- and inter-assay coefficients of
variation 8% and 12% and lower detection limit of 0.35 pg/ml.
Plasma TNF-α levels were assayed using a high-sensitivity solid phase ELISA (Quantikine HS
Human TNF-α Immunoassay, R&D systems, Minneapolis, MN, USA), intra- and inter-assay
coefficients of variation were 10% and 15% respectively and the lower detection limit was
0.10 pg/ml. Due to the non-normal distribution, IL-6, TNF-α and CRP were LN-transformed
prior to further analysis. A combined inflammation score was calculated as the standardized sum of the three standardized LN-transformed inflammation markers. Due to missing values, IL-6 and TNF-α levels were available for 278 out of 283 subjects, while CRP and the combined inflammation score were available for 277 participants.

Metabolic dysregulation
Blood samples were taken after an overnight fasting period. Triglyceride levels and HDL-cholesterol were determined according to routine, standardized laboratory methods. The maximum intra-assay variation coefficient for triglyceride levels was 1.5%, while the inter-assay variation was 1.8%.

Immunometabolic dysregulation score
The immunometabolic dysregulation (IMD) score was calculated for every subject as a measure of dysregulation severity. For every immunometabolic dysregulation marker (IL-6, TNF-a, CRP, triglyceride level, HDL-cholesterol, waist circumference and BMI), a score of 1 was given if the score for that subject was in the highest quartile. We then summed these scores to get the IMD score, which ranges from 0 to 7, for every subject.

Genomic profile risk scores (GPRS)
DNA extraction methods have been described in a previous study (Boomsma et al., 2008). Autosomal single nucleotide polymorphisms (SNPs) were genotyped on the Affymetrix 6.0 Human Genome-Wide SNP Array. Quality assessment was performed using methods previously described (Milaneschi et al. 2015), resulting in 435579 SNPs used for the current study.

GPRS were generated based on discovery genome-wide analysis study (GWAS) meta-analysis results from large international consortia (see Milaneschi et al. 2015). GWAS meta-analysis were previously performed by the GIANT consortium for BMI (≈ 120000 samples)(Speliotes et al. 2010), Teslovich et al. (2010)(≈ 100000 samples) for triglycerides and Dehghan et al. (2011) for C-reactive protein (≈70000 samples). For all three markers of immunometabolic dysregulation, four sets of independent SNPs were selected based on significance thresholds (p < 0.001, < 0.01, < 0.1, < 1) of the GWAS results. GPRS were then calculated as the number of scores alleles, weighted by effect sizes from the discovery statistics using PLINKv1.07 and were then standardized. The number of SNPs in the GPRS analyses according to the different significance thresholds can be found in supplemental table S1. GPRS significantly predicted immunometabolic dysregulation markers in our sample (see Table S2). Due to missing values, GPRS were available for 200 out of a total of
283 subjects. We considered there to be a significant and consistent effect if the same effect was seen in at least three out of four GWAS thresholds.

**Statistical analyses**

Unadjusted Pearson correlation coefficients were calculated to examine the relationship between different markers of immunometabolic dysregulation.

As we expect a similar negative association between immunometabolic dysregulation and brain structure in patients and controls, we examined this association across patients and controls, controlling for the presence of an affective disorder. Linear regression analyses were performed in SPSS 20 (IBM) to examine the relationship between BMI, inflammation and triglycerides on subcortical brain volume and cortical thickness of the ACC, adjusting for appropriate covariates (see below). All markers of immunometabolic dysregulation were tested in separate linear regression models. Furthermore, we examined the relationship between GPRS of BMI, CRP and triglycerides and brain structure using linear regression analyses using covariates. Significant associations were followed up by exploratory repeated measure analyses to examine whether findings were driven by the left or right hemisphere, i.e. whether there was a significant marker*hemisphere interaction effect. In the case of a significant interaction effect, post-hoc linear regression analyses stratified for hemisphere were performed.

To examine whether our findings may have been driven by patients or healthy subjects, in secondary analyses, linear regression analyses were performed to examine the interaction between markers of immunometabolic dysregulation and the presence of a psychiatric diagnosis on brain volume and thickness, while additionally controlling for the use of selective serotonin reuptake inhibitors (SSRI). Significant interactions were followed by stratified regression analyses to examine group differences.

To correct for multiple comparisons, we calculated an adjusted p-value using the modified false discovery rate (FDR) method of Benjamini and Yekutieli, which is described in Narum (2006). Due to the high correlation between the GPRS scores with different thresholds, we did not consider these tests to be independent and have therefore corrected for 44 tests (8 immunometabolic dysregulation markers*4 regions + 3 GPRS*4 regions = 44 tests). Results are now considered significant if p < 0.01143.

**Covariates**

Potential variance due to age, sex, education level (in years), scan site, presence of an affective disorder (coded as a dummy variable: yes/no) and intracranial volume were corrected for in all regression analyses. In analyses with GPRS, we also adjusted for three
ancestry-informative principal components derived from GWAS data (Abdellaoui et al. 2013) in order to correct for potential population stratification. In secondary analyses the following variables were added as additional covariates: current SSRI use (coded as a dummy variable: yes/no), smoking (categorized as no smoker and current smoker), alcohol use (number of drinks per week), physical activity (in 1000 metabolic equivalent minutes per week), presence of chronic diseases (e.g. cardiovascular disease, diabetes, osteoarthritis, liver disease, cancer, ulcers, intestinal disorders, epilepsy, thyroid disease and chronic lung disease), statin use (ATC codes C10AA and C10B) and infrequent anti-inflammatory medication use (ATC codes M01A, M01B, A07EB and A07EC).

Results

Sample characteristics

A total of 283 participants were included in this study. Sample characteristics are presented in Table 1.

Immunometabolic dysregulation markers

There was a significant correlation between multiple markers of metabolic stress (see supplemental table S3).

Immunometabolic dysregulation and brain volume/thickness

All immunometabolic dysregulation markers did not differ between healthy subjects and subjects with an affective disorder (IL-6: $F(1,272) = 0.428, p = 0.513$; TNF-a$F(1,272) = 0.603, p = 0.438$; CRP:$F(1,272) = 1.255, p = 0.264$; triglyceride levels: $T(278) = 0.358, p = 0.721$; BMI:$T(281) = -1.164, p = 0.245$; waist circumference: $T(281) = -0.346, p = 0.730$; HDL-cholesterol: $T(279) = 1.947, p = 0.053$).

Hippocampal volume

Covariate adjusted linear regression analyses revealed an inverse relationship between IL-6 levels and hippocampal volume ($p = 0.023$; see Table 2), however, this association did not survive correction for multiple comparisons. Other immunometabolic dysregulation markers were not related to hippocampal volume (see Table 2).

Genetic profile risk scores for BMI were associated with hippocampal volume, but only for one threshold (i.e. inconsistent; see Table 3) and this finding did not remain significant after correction for multiple comparisons.
Table 1. Sample characteristics

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>N = 283</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; SD)</td>
<td>37.65 (10.18)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>33.6%</td>
</tr>
<tr>
<td>Education (years; SD)</td>
<td>12.80 (3.20)</td>
</tr>
<tr>
<td>Scan site (Amsterdam/Leiden/Groningen)</td>
<td>91/105/87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Structural neuroimaging measures</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ICV (liter; SD)</td>
<td>1.510 (0.183)</td>
</tr>
<tr>
<td>Mean amygdala volume (mm³; SD)</td>
<td>1637.26 (202.88)</td>
</tr>
<tr>
<td>Mean hippocampal volume (mm³; SD)</td>
<td>3993.92 (424.44)</td>
</tr>
<tr>
<td>Mean thickness rostral ACC (mm; SD)</td>
<td>2.57 (0.16)</td>
</tr>
<tr>
<td>Mean thickness caudal ACC (mm; SD)</td>
<td>2.39 (0.18)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunometabolic dysregulation markers</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>0.69 (0.48-1.21)</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>0.70 (0.5-1.0)</td>
</tr>
<tr>
<td>CRP (mg/ml)</td>
<td>1.16 (0.46-2.82)</td>
</tr>
<tr>
<td>Triglyceride level (mmol/l)</td>
<td>1.15 (0.67)</td>
</tr>
<tr>
<td>BMI</td>
<td>24.99 (4.60)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>86.27 (12.74)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.62 (0.40)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lifestyle</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking (% current smoker)</td>
<td>34.3%</td>
</tr>
<tr>
<td>Physical activity (MET-minutes)</td>
<td>3685 (3345)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease status</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence chronic disease (% yes)</td>
<td>20.8%</td>
</tr>
<tr>
<td>Presence affective disorder (% yes)</td>
<td>77.0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medication use</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-inflammatory medication (% yes)</td>
<td>1.8%</td>
</tr>
<tr>
<td>SSRI use (% yes)</td>
<td>27.2%</td>
</tr>
<tr>
<td>Statin use (% yes)</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

Data are shown as mean (standard deviation), median (IQR) or as frequencies. ACC: anterior cingulate cortex; BMI: body mass index; ICV: intracranial volume; CRP: c-reactive protein; HDL: HDL-cholesterol; IL-6: interleukin-6; SE: standard error; TG: triglyceride level; TNF-α: tumor necrosis factor-α; WC: waist circumference IQR: inter-quartile range; SD: standard deviation.
Table 2. Association between markers of immunometabolic dysregulation and brain structure

<table>
<thead>
<tr>
<th></th>
<th>Hippocampus volume</th>
<th>Amygdala volume</th>
<th>Rostral ACC thickness</th>
<th>Caudal ACC thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>SE</td>
<td>p</td>
<td>Beta</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.040</td>
<td>0.053</td>
<td>0.454</td>
<td>0.003</td>
</tr>
<tr>
<td>WC</td>
<td>-0.089</td>
<td>0.058</td>
<td>0.125</td>
<td>-0.059</td>
</tr>
<tr>
<td>TG</td>
<td>-0.010</td>
<td>0.052</td>
<td>0.853</td>
<td>0.015</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.117</td>
<td>0.051</td>
<td>0.023</td>
<td>-0.089</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.028</td>
<td>0.051</td>
<td>0.576</td>
<td>0.023</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.037</td>
<td>0.050</td>
<td>0.461</td>
<td>0.063</td>
</tr>
<tr>
<td>CIscore</td>
<td>-0.047</td>
<td>0.051</td>
<td>0.360</td>
<td>0.005</td>
</tr>
<tr>
<td>HDL</td>
<td>0.063</td>
<td>0.056</td>
<td>0.256</td>
<td>0.103</td>
</tr>
<tr>
<td>IMD score</td>
<td>-0.059</td>
<td>0.053</td>
<td>0.265</td>
<td>-0.044</td>
</tr>
</tbody>
</table>

Beta: standardized beta; BMI: body mass index; CIscore: combined inflammation score (IL-6, CRP and TNFα combined) CRP: c-reactive protein; HDL: HDL-cholesterol; IL-6: interleukin-6; SE: IMD score: immunometabolic dysregulation score: the number of markers that are dysregulated (highest quartile) per subject, standard error; TG: triglyceride level; TNF-α: tumor necrosis factor-α; WC: waist circumference. Printed in italic are results that are significant at p<.05 and in bold are results that survive multiple comparison correction. Results are corrected for age, sex, education level, presence of an affective disorder, scansite and intracranial volume.
Amygdala volume
Immunometabolic dysregulation markers and genetic profile risk scores for immunometabolic dysregulation were not related to amygdala volume (see Table 2 and Table 3).

Thickness of the caudal ACC
There was no association between immunometabolic dysregulation markers and caudal ACC thickness (see Table 2). Nor was there a significant relationship between genetic profile risk scores for immunometabolic dysregulation and caudal ACC thickness (see Table 3).

Thickness of the rostral ACC
BMI, waist circumference, triglyceride levels, CRP, TNF-a and IMD score, but not HDL-cholesterol were inversely associated with rostral ACC thickness (see Table 2), while this association was similar for all immunometabolic dysregulation markers, only the relationship between triglyceride levels and rostral ACC thickness and the association between the combined inflammation score and rostral ACC thickness survived correction for multiple comparisons (see Table 2). The association between the IMD score and rostral ACC thickness was also significant after correcting for multiple comparisons. These associations were not driven by the left or right rostral ACC (see supplemental Table S5), however, post-hoc linear regression analysis show that the association between TNF-a and rostral ACC thickness was driven by the right hemisphere (beta: -0.200; SE: 0.056, p <.001), and not the left hemisphere (beta: -0.008; SE: 0.055; p = 0.884). Genetic profile risk scores for immunometabolic stress were not associated with rostral ACC thickness (see Table 3).

Correction for additional confounding variables
After additional correction for SSRI use, smoking, alcohol use, physical activity, presence of chronic diseases, statin use and anti-inflammatory medication, all previously significant relationships between markers of immunometabolic dysregulation and thickness of the rostral ACC remained the same (see supplemental Table S4).
Table 3. Associations between GPRS for immunometabolic dysregulation and brain structure

### Association with hippocampal volume

<table>
<thead>
<tr>
<th>GPRS-BMI</th>
<th>GPRS-CRP</th>
<th>GPRS-triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta</td>
<td>SE</td>
<td>p</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>0.107</td>
<td>0.061</td>
</tr>
<tr>
<td>p &lt; 0.01</td>
<td>0.131</td>
<td>0.060</td>
</tr>
<tr>
<td>p &lt; 0.1</td>
<td>0.079</td>
<td>0.060</td>
</tr>
<tr>
<td>p &lt; 1</td>
<td>0.043</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Beta: standardized beta; BMI: body mass index; CRP: C-reactive protein; SE: standard error. Results are corrected for age, sex, education level, scansite, intracranial volume and population stratification. Printed in italic are results that are significant at p<.05 and in bold are results that survive multiple comparison correction.

### Interaction with psychiatric diagnosis

In all abovementioned analyses, we corrected for the presence of an affective disorder. However, in secondary analyses we examined the interaction between immunometabolic dysregulation markers and the presence of an affective disorder on brain structure (while correcting for age, sex, education level, scan site, intracranial volume and SSRI use) in order to examine whether our findings may have been driven by patients or healthy subjects (see Table S6 for results). While there was limited evidence for an effect of psychiatric diagnosis,
we did observe a significant interaction between IL-6 and presence of a psychiatric disorder on hippocampal volume (p = 0.008) and amygdala volume (p = 0.021; see Table S5). Post-hoc stratified analyses revealed that the expected inverse relationship between IL-6 and hippocampal volume that was observed in healthy controls (standardized beta: -0.373; p < .001), was absent in patients (standardized beta: -0.072; p = 0.229). Similarly, in healthy subjects, there was a significant inverse relationship between IL-6 levels and amygdala volume, however this effect did not remain significant after correcting for multiple comparisons. There were no immunometabolic dysregulation*affective disorder interaction effect on rostral ACC thickness (see Table S5), indicating that our abovementioned associations were similar in patients and controls.

Discussion

Immunometabolic dysregulation has been associated with psychiatric disorders and a more chronic disease course. Changes in brain structure as a result of immunometabolic dysregulation may underlie this relationship. The aim of this study was therefore to investigate the association between various markers of immunometabolic dysregulation, namely (abdominal) obesity, inflammation, decreased HDL-cholesterol and increased triglyceride levels, and brain structure in a large study sample. As the genetic component of metabolic stress may be more stable over time than the markers, we also examined the relationship between genetic vulnerability for immunometabolic dysregulation and brain volume/cortical thickness.

Multiple markers of immunometabolic dysregulation (i.e. BMI, WC, IL-6, TNF-a, CRP and triglyceride levels) were inversely associated with rostral ACC cortical thickness, and this effect remained significant after correcting for lifestyle factors and medication use. There appeared to be a load effect, indicating that the rostral ACC thickness was further reduced in individuals with severe immunometabolic dysregulation. While effects were similar for all markers, only association with triglyceride levels and IMD scores remained significant after correction for multiple comparisons. The rostral ACC, which consists of the pregenual ACC and a small part of the subgenual ACC, is part of the medial prefrontal cortex, an important region for emotion regulation and has been implicated in many stress-related psychiatric disorders (Drevets et al. 2008; Stevens et al. 2011). Previous studies have reported a positive association between immunometabolic dysregulation markers (IL-6 and TNF-a) and task related activity of the ACC (Harrison et al. 2009; Slavich et al. 2010). Obesity, which is characterized by increased immunometabolic dysregulation, is associated with prefrontal structural deficits, including in the anterior cingulate cortex (Raji et al. 2010; Alosco et al. 2014). Structural deficits in the anterior cingulate cortex have also been associated with psychological stress in humans (Ansell et al. 2012).
The strong correlation between the markers of immunometabolic dysregulation and the similar effect on rostral ACC thickness, suggest that these markers have an effect on brain structure through a similar pathway. One mendelian randomization study suggests that obesity leads to increased low-grade inflammation, which may result in neurodegeneration and altered neuroplasticity through increased release of serotonin, dopamine and norepinephrine (Anisman and Merali 2002; Slavich and Irwin 2014), alterations in glutamate metabolism, re-uptake and release (Leah McNally et al. 2008; Miller et al. 2013) and increased release of neurotoxic quinolinic acid (Leonard and Maes 2012; Savitz et al. 2015; Meier et al. 2016) in the brain. The anterior cingulate cortex may be vulnerable to the effect of immunometabolic dysregulation as stress-related upregulation of glucocorticoid receptors may be more pronounced in this region than in the hippocampus (Lee et al. 2014). Interestingly, the anterior cingulate and prelimbic regions also play a role in inhibition of stress-induced activation of the HPA-axis (Diporto et al. 1993; Herman et al. 2005). Immunometabolic dysregulation-related damage to the anterior cingulate may disrupt this inhibition, and may result in further increased physiological stress.

Due to the role of the ACC in emotion regulation, we speculated that chronic immunometabolic dysregulation may increase the risk for developing a stress-related psychiatric disorder or contribute to a more severe disease course through decreased ACC thickness and emotion regulation deficits. However, in this study we did not find evidence for increased immunometabolic dysregulation in patients with a depression and/or anxiety disorder. Furthermore, the detrimental effect of immunometabolic dysregulation on rostral ACC structure was not stronger in patients with depression and/or an anxiety disorder. Findings were also unaltered after correcting for severity of depression and anxiety (data not shown). However, the cross-sectional design of this study does not allow us to investigate whether immunometabolic dysregulation alters vulnerability for psychiatric disorders through possible changes in brain structure. Longitudinal research is needed to examine this. Due to the cross-sectional design, we also cannot rule out that reduced anterior cingulate thickness leads to decreased inhibition and increased feeding behavior, which may lead to obesity and immunometabolic dysregulation (Rolls 2008).

We were able to replicate previous findings by reporting an inverse relationship between IL-6 levels and hippocampal volume (Marsland et al., 2008; Frodl et al., 2012), but this relationship was observed in healthy subjects only and not in individuals with an affective disorder. The hippocampus plays a crucial role in memory, has numerous IL-6 receptors (Schöbitz et al. 1992) and is therefore vulnerable to the neurodegenerative effects of inflammation. It is unclear why IL-6 is not inversely associated with hippocampal volume in patients with an affective disorder, especially considering the fact we observed similar IL-6 levels in patients and controls. We speculate that hippocampal IL-6 receptors may be downregulated in patients following chronic stress, which may explain why similar IL-6 levels
are not associated with decreased hippocampal volume in this group. In rats, chronic stress has been shown to decrease IL-6 receptor expression (Shizuya et al. 1997; Miyahara et al. 2000), however evidence for this effect in humans is lacking. A second hypothesis is that the effect of IL-6 on hippocampal volume is mediated through hippocampal glucocorticoid receptors (GR), and there is evidence for decreased responsiveness to glucocorticoids (glucocorticoid resistance) in depression (Pariante and Miller 2001), which may cause decreased signaling downstream of the GR in depressed subjects. However, the interplay between IL-6 and the HPA-axis is complex and bidirectional and more research is needed to examine this.

Strengths of this study include the fact that multiple markers of immunometabolic dysregulation were assessed in a large study sample. Furthermore, this is the first study to examine the effect of genetic vulnerability for immunometabolic dysregulation on brain structure. Limitations however, are the cross-sectional design of this study, which does not allow us to speculate on causal mechanisms and the power to detect associations between GPRS and brain morphology may have been limited. More research is a larger sample is needed to confirm the absence of an association between these polygenic risk scores and brain structure. Furthermore, it should be recognized that the rostral ACC is a complex region that consists of several subregions with different functions (Palomero-Gallagher et al. 2008; Palomero-Gallagher et al. 2009).

To conclude, different markers of immunometabolic dysregulation have a similar detrimental effect on rostral ACC thickness in patients and in healthy subjects, and this effect is unrelated to genetic vulnerability for immunometabolic dysregulation. Longitudinal studies are needed to examine whether immunometabolic dysregulation causes vulnerability for developing a stress-related psychiatric disorder through decreased rostral ACC thickness.
References


