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Testing the estrogen hypothesis of schizophrenia: Associations between cumulative estrogen exposure and cerebral structural measures

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ABSTRACT

Background: Bone mineral density (BMD), as an indicator of cumulative estrogen exposure, may be reduced in female patients with psychotic disorder (van der Leeuw et al., 2013), possibly reflecting reduced cerebral exposure to estrogen and alterations in neuroprotective effects. To the degree that BMD is a marker of cumulative (endogenous) estrogen exposure, we hypothesized that BMD would be positively associated with cerebral gray and white matter indices.
Methods: Dual X-ray absorptiometry (DEXA) and magnetic resonance (MRI) scans were acquired in fourteen female patients diagnosed with a psychotic disorder. BMD was expressed in total BMD (g/cm^2), Z- and T-scores. Cerebral cortical thickness (CT) (as indicator of gray matter status) and fractional anisotropy (FA) (as indicator of white matter integrity) were measured and served as the dependent variables in multilevel random regression models. BMD measures were the independent variables.
Results: Femoral BMD measures were positively associated with CT at trend significance (total BMD: $B = 0.266$, 95% CI: −0.019–0.552, $p = 0.067$; Z-score: $B = 0.034$, 95% CI: 0.001–0.067, $p = 0.046$; T-score: $B = 0.034$, 95% CI: 0.000–0.068, $p = 0.052$). There were no significant associations between femoral BMD measures and FA.
Conclusions: The data suggest that in women with psychotic disorder, alterations in the neuroprotective effect of estrogen (as measured by BMD) impact cortical gray matter, but not white matter integrity. These findings merit further investigation and, if replicated, would lend support to the estrogen hypothesis of schizophrenia.

1. Introduction

Gender differences in the incidence and course of schizophrenia have been ascribed to neuroprotective effects of endogenous estrogen in females. In early adulthood, incidence rates of schizophrenia are lower in women than in men, and the age at onset of schizophrenia occurs three to four years later in women (Riecher-Rossler and Hafner, 1993, 2000; Hafner et al., 1998; Halbreich and Kahn, 2003; Markham, 2011). However, when estrogen levels decline around menopause, women display a second peak in the incidence of schizophrenia, which is absent in men (Riecher-Rossler and Hafner, 1993; Hafner et al., 1998; Abel et al., 2010). In the premenopausal period, female patients generally tend to fare better than their male counterparts, displaying a less severe course of symptoms, with a superior response to antipsychotic (AP) treatment and better social outcome (Seeman, 1983, 1996; Hafner et al., 1998; Salem and Kring, 1998; Hafner, 2003; Abel et al., 2010; Markham, 2011). Also, symptom variability during the menstrual cycle has been reported. Amelioration of symptoms is associated with a rise in estrogen and more clinical admissions take place during low estrogen phases (Riecher-Rossler et al., 1994; Seeman, 1996; Huber et al., 2004).

There are two (related) estrogen hypotheses of schizophrenia: 1) the hypoestrogenism or deficiency hypothesis which describes (chronic) gonadal dysfunction in women with schizophrenia, and 2) the protection hypothesis which states that estrogen exerts a relative protection against schizophrenia in premenopausal women (Riecher-Rossler and Hafner, 1993; Riecher-Rossler, 2002). The neuroprotective mechanism of estrogen in the human brain is complex. It includes structural effects such as the conservation of neurons, stimulation of growth and synaptogenesis, as well as effects at the receptor level, i.e. preservation of neurotransmitter receptors and modulation of neurotransmission (Brann et al., 2007; Boerma et al., 2010; Liu et al., 2010; Azcoitia et al., 2011; Kulkarni et al., 2012; McEwen et al., 2012).

The dysconnectivity hypothesis of schizophrenia proposes that altered structural connectivity may represent a key pathological mechanism in schizophrenia (Friston, 1998; Konrad and Winterer, 2008). The estrogen and dysconnectivity theories are possibly linked. Recently,

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Peper et al. (2011b) reviewed the available literature reporting on the potential association between sex steroid hormones, white matter (WM) indices and functional connectivity in the human brain, and concluded that gonadal hormones appear to organize and activate structural connections within the brain. Animal studies have shown that sex steroid hormones play an essential role in myelination (Peper et al., 2011b). In humans, sex steroids have been associated with structural brain development during puberty, exerting effects on both white (Asato et al., 2010; Herting et al., 2011; Peper et al., 2011a) and gray matter (Neufang et al., 2009; Peper et al., 2009, 2011a).

Other evidence for associations between estrogen and gray matter comes from the work of Goldstein et al. (2001, 2002) showing disruption of the normal sexual dimorphism in schizophrenia, particularly in the cortex. In addition, hormonal fluctuations during the menstrual cycle (Protopopescu et al., 2008; Pletzer et al., 2010) and hormonal contraceptive use (Pletzer et al., 2010) have been associated with gray matter changes in women of childbearing age. In the context of estrogen therapy (ET) and its effect on the aging brain in postmenopausal women, both higher and lower gray matter concentrations in specific cortical areas have been suggested (Boccardi et al., 2006; Robertson et al., 2009; Lord et al., 2010). Animal models of multiple sclerosis have demonstrated prophylactic effects of estrogen with regard to gray matter atrophy (Mackenzie-Graham et al., 2012). Thus, disparate research fields have provided clues as to the relationship between estrogen and cerebral structure at different life stages. However, studies investigating cumulative estrogen exposure and brain structure in patients with psychotic disorder have, to the best of our knowledge, not been conducted.

Previously, we used dual X-ray absorptiometry (DEXA) to assess bone mineral density (BMD) as a marker of cumulative estrogen exposure (Clemons and Goss, 2001), and found that reduced femoral BMD was associated with being female and having a psychotic disorder, but not with familial risk for psychotic disorder. This suggests that unique environmental factors, contributing to primary low estrogen levels in women, may impact the risk of developing a psychotic disorder, in support of the estrogen (deficiency) hypothesis of schizophrenia (van der Leeuw et al., 2013).

The aim of the current study was to investigate the potential association between endogenous estrogen exposure (as indexed by BMD, corrected for exogenous estrogen exposure such as contraceptives) and gray and white matter indices in this female patient group. Using magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI), cortical thickness (CT) and white matter integrity (represented by fractional anisotropy (FA)) were assessed respectively. We hypothesized that both CT and FA would be positively associated with BMD, reflecting the neuroprotective effects of estrogen in schizophrenia.

2. Methods and materials

2.1. Subjects

Data was collected in the context of an ongoing multicenter longitudinal study (Genetic Risk and Outcome of Psychosis, G.R.O.U.P.) in the Netherlands. In selected representative geographic areas in the Netherlands and Belgium, patients presenting consecutively at mental health services either as outpatients or inpatients were recruited for the study. Patients between the ages of 16 and 50 years, with a diagnosis of non-organic, non-affective psychosis according to DSM-IV criteria, were included (Korver et al., 2012). Sufficient command of the Dutch language was mandatory. A complete description of the recruiting protocol of the MRI sub-study is provided by Habets et al. (2011).

As mentioned in the introduction, the aim of the current study was to investigate associations between BMD and brain measures in female patients with a psychotic disorder, as a reduction in BMD (possibly reflecting low endogenous estrogen levels) was previously found in female ($n = 16$), but not in male ($n = 46$) patients (van der Leeuw et al., 2013). A T1-weighted structural MRI scan was obtained in 14, and an additional DTI scan in 13 of the original 16 female patients. Thus, the present study sample comprised 14 female patients.

The sample included eight patients who were diagnosed with schizophrenia, one patient with schizoaffective disorder, four patients with a diagnosis of psychotic disorder not otherwise specified and one patient with a diagnosis of brief psychotic disorder. Patients were genetically unrelated. The mean illness duration was 6.6 years.

Prior to DEXA acquisition, participants were screened for the following exclusion criteria: 1) metabolic or endocrinologic disease, 2) dietary deficiency or eating disorder, 3) medication: corticosteroids, thyroxin, anti-epileptics, heparin, lithium, cytostatic agents, 4) (semi-) professional athletes, 5) polydipsia (>3 l/day), 6) pregnancy, and 7) hormonal (infertility) treatment. Exclusion criteria for MRI constituted: 1) head injury with loss of consciousness for a duration of more than 1 h, 2) meningitis of other neurological diseases that might affect brain structure or function, 3) cardiac arrhythmia requiring medical treatment, 4) severe claustrophobia, 5) (suspected) pregnancy, and 6) any metal foreign object in the body, including the presence of an intrauterine device.

2.2. Measures

2.2.1. Age at menarche and dysmenorrhea

Age at menarche and the occurrence of menstrual irregularity were assessed. Dysmenorrhea was specified as altered duration and/or frequency of menses or the absence of two or more menses during the previous three months. Amenorrhea was defined as the absence of menses for at least three months.

2.2.2. Use of contraceptive drugs (exogenous estrogen exposure)

Cumulative (lifetime) exogenous estrogen exposure in women was expressed in micrograms, as the product of daily dose and total days of use.

2.2.3. Substance use

Substance use was assessed using the composite international diagnostic interview (CIDI). Cannabis use was assessed as the reported lifetime frequency of use. Other drug use, such as stimulants, sedatives, opiates, cocaine, PCP, psychedelics, inhalants or other (e.g. ecstasy, poppers) was assessed in the same way. Alcohol use was defined as the average number of weekly consumptions during the previous 12 months. Tobacco use was defined as the number of cigarettes per day, in the past year.

2.2.4. Antipsychotic medication (AP) exposure

Current AP use was classified by type: "prolactin-raising" APs, in this study comprising first-generation APs, risperidone and amisulpride; or "prolactin-sparing" APs, comprising second or third-generation APs with the exception of risperidone and amisulpride. Subjects who were AP-free at the time of the investigations were placed in the prolactin-sparing group.

Previous AP use was assessed retrospectively by self-report. Best estimate lifetime (cumulative) AP exposure was determined by multiplying the number of days of AP use with the daily AP dose converted to haloperidol equivalents (in milligrams), and summing all periods of use. Lifetime exposure was calculated for all APs and separately for prolactin-raising APs.

2.3. DEXA acquisition and processing

DEXA scans were acquired at Maastricht University Medical Centre with a Hologic Discovery A (Tromp Medical, Castricum, the Netherlands) (NHANES and Ethnic Reference Data). DEXA scans were performed in two anatomical areas: the lumbar spine, vertebrae L2 through L4; and the proximal left femur, specifically the collum, trochanter major, intertrochanteric area and Ward's triangle. BMD measures

were expressed as total BMD in grams per square centimeter (g/cm^2), Z-scores and T-scores. The Z-score compares an individual's BMD with the mean BMD of a comparable population (with respect to gender, age and ethnicity). The T-score compares an individual's BMD to peak bone mass (PBM). Peak bone mass is the highest BMD an individual is expected to acquire during life. The T-score is used to diagnose osteopenia and osteoporosis. In the present study, the three femoral BMD measures were a priori used for further analysis, as these were reduced in female patients (van der Leeuw et al., 2013).

2.4. MRI acquisition and processing

Magnetic resonance imaging scans were obtained at Maastricht University, the Netherlands, using an Allegra syngo MR A30 (Siemens, Erlangen, Germany) operating at 3.0 T. The following anatomical scan parameters were used: Modified Driven Equilibrium Fourier Transform (MDEFT) sequence; 176 slices, 1 mm isotropic voxel size, echo time 2.4 ms, repetition time 7.92 ms, inversion time 910 ms, flip angle 15°, total acquisition time 12 min 51 s; Magnetization Prepared Rapid Acquisition Gradient-Echo (MPRAGE; Alzheimer's Disease Neuroimaging Initiative) sequence 192 slices, 1 mm isotropic voxel size, echo time 2.6 ms, repetition time 2250 ms, inversion time 900 ms, flip angle 9°, and total acquisition time 7 min 23 s. The matrix size was 256 × 256 and field of view was 256 × 256 mm^2. The number of excitations was one. Two similar sequences were used because of a scanner update during data collection.

Microstructural anatomy was examined using diffusion tensor imaging with an echo-planar-imaging sequence (field of view 230 mm^2, TR 10800 ms, TE 84 ms, voxel size 1.8 × 1.8 × 1.8 mm^3, b-value 1000 s/mm^2, noise level 40, 85 slices, no overlap). As a result of the scanner update, two DTI sequences were used: one with 76 directions (of which 4 T2-weighted (B0) and 72 diffusion-weighted (B)), and one with 81 directions (8 × B0 and 73 × B). Total acquisition time of the DTI sequence was 15 min.

2.4.1. Cortical thickness measurement

Scans were processed and analyzed using Freesurfer stable release v5.0.0, http://surfer.nmr.mgh.harvard.edu (Dale et al., 1999; Fischl et al., 1999; Fischl and Dale, 2000; Fischl et al., 2002; Segonne et al., 2004).

To measure CT, the cerebral cortex was parcellated into units based on gyral and sulcal structure (Fischl et al., 2004; Desikan et al., 2006). Furthermore, a variety of surface-based data was created including maps of curvature and sulcal depth. This method uses both intensity and continuity information from the entire three dimensional MR volume in segmentation and deformation procedures to produce representations of CT, calculated as the closest distance from the gray/white matter boundary to the gray matter/CSF boundary at each vertex on the tessellated surface (Fischl and Dale, 2000). The maps are created using spatial intensity gradients across tissue classes and are not restricted to the voxel resolution of the original data, thus are capable of detecting sub-millimeter differences between groups. CT measurement procedures have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003; Salat et al., 2004). Individual CT values for each predefined region of interest (hereafter: ROI; adapted from the Desikan atlas (Desikan et al., 2006), 34 ROIs per hemisphere) were derived by FreeSurfer and exported to STATA version 12 (StataCorp). Thus, every individual had 68 CT measurements over the predefined ROIs in both hemispheres.

2.4.2. Diffusion tensor imaging analysis

Processing of DTI data was effectuated using tract-based spatial statistics (TBSS) v1.2 in FSL 4.1.6 (FMRIB Analysis Group, Oxford, UK, http://www.fmrib.ox.ac.uk/analysis/research/tbss). First, standard Siemens DICOM files were transformed into compressed NIFTI format using a custom built in-house software named GIANT (General Image ANalysis Tools developed by EHBMG). Raw data were corrected for head movement and eddy currents invoked during scanning. The B0 volume was skull-stripped using FSL's Brain Extraction Tool (Smith, 2002) and this served as a brain mask for all B volumes.

The next step was fitting a diffusion tensor model at each voxel using data output from the brain extraction, diffusion weighted data and gradient directions following a general linear model (FreeSurfer). After tensor fitting the process continued working on FA volumes, eroding them slightly.

Nonlinear registration aligned each FA volume to 1 × 1 × 1 mm standard FMRIB58_FA space. The standard FMRIB58_FA contains a template derived from high-resolution images of 58 participants in a well-aligned population (both males and females ranging between 20 and 50 years of age) (Smith et al., 2006).

After nonlinear transformation of the FA volumes into standard space, a mean FA skeleton from all participants per group was derived. The mean FA skeleton follows the major white matter tracts in each individual participant (normalized in MNI152 space) and provides a way to compare between (groups of) participants. The final step of the processing was setting the FA threshold using visual inspection of the FA skeleton, in the present study at a level of 0.25, to include major white matter tracts whilst removing small peripheral tracts that would cause excess interparticipant variability. In addition, this threshold setting avoided inclusion of regions that are likely to be composed of multiple tissue types or fiber orientations.

The Johns Hopkins University International Consortium for Brain Mapping (JHU ICBM)-DTI-81 white-matter atlas labels (Mori et al., 2008) were used to assign specific tract names. From all 38 JHU labeled white matter tracts, mean FA values were extracted and exported to STATA.

This study was approved by the standing ethics committee. All subjects provided written informed consent in accordance with the committee's guidelines.

2.5. Statistical analyses

Conform previous work in this sample (Habets et al., 2011), datasets were transformed from a wide to a long format resulting in a hierarchical structure, with 68 regional CT measures and 38 FA values (level 1) nested in respectively 14 and 13 subjects (level 2). Given the clustering of brain measures compromising statistical independence of the observations, multilevel random regression models were fitted (Goldstein, 1987) using the XTREG command in STATA (StataCorp, version 12). Separate analyses were conducted for CT and FA. CT and FA were the dependent variables, and BMD measures were the independent variables. Although BMD measures were acquired in both the lumbar spine and the proximal femur, the current analyses were conducted a priori with the femoral BMD measures (total BMD, Z-score, T-score), as the previously reported reduction in BMD was specific for the proximal femur (van der Leeuw et al., 2013).

Analyses were adjusted for the a priori hypothesized confounders: age at scan, age at menarche, dysmenorrhea, cumulative exogenous estrogen exposure, lifetime exposure to cannabis and current use of prolactin-raising AP (0 = no AP or prolactin-sparing AP, 1 = prolactin-raising AP). In separate analyses, cumulative prolactin-raising AP exposure was entered in the model replacing current prolactin-raising AP use.

Since lifetime AP exposure is a much debated potential confounder in neuroimaging research, sensitivity analyses were carried out using lifetime exposure to all AP as a covariate, instead of present and cumulative prolactin-raising AP use.

3. Results

3.1. Descriptive analyses

The mean age at the time of investigation was 27 years. There were six participants with dysmenorrhea. The rate of substance use in the

present sample was low (see Table 1). Three women did not use AP medication at the time of the investigation. Three women used olanzapine, two women used aripiprazole, one woman used quetiapine, three women used risperidone, one woman used amisulpride, and one woman used haloperidol. Hence, five patients used a prolactin-raising AP at the time of the study. None of the patients were AP-naïve and the majority (with the exception of one individual) had used a prolactin-raising AP at some point during their lives.

The use of additional psychotropic medication was minimal, and consisted of three participants with antidepressant use (of which two selective serotonin reuptake inhibitors (SSRI) and one with a serotonin and noradrenalin reuptake inhibitor (SNRI)).

The mean femoral BMD measures, as well as the mean CT and FA are shown in Table 2.

3.2. Associations between BMD and CT

BMD measures of the femur were positively associated with CT when correcting for current exposure to a prolactin-raising AP (total BMD: B = 0.266, 95% CI: −0.019–0.552, p = 0.067; Z-score: B = 0.034, 95% CI: 0.001–0.067, p = 0.046; T-score: B = 0.034, 95% CI: 0.000–0.068, p = 0.052) (Table 3). When correcting for lifetime exposure to prolactin-raising AP, the positive association was strengthened (total BMD: B = 0.429, 95% CI: 0.104–0.753, p = 0.010; Z-score: B = 0.049, 95% CI: 0.012–0.085, p = 0.009; T-score: B = 0.048, 95% CI: 0.011–0.086, p = 0.011) (Table 3). As described under the heading Statistical analysis, a sensitivity analysis was carried out with lifetime exposure to all AP replacing present or cumulative exposure to prolactin-raising AP as a covariate. The positive association between femoral BMD measures and CT remained (total BMD: B = 0.390, 95% CI: 0.091–0.689, p = 0.011; Z-score: B = 0.046, 95% CI: 0.012–0.080, p = 0.008; T-score: B = 0.046, 95% CI: 0.011–0.081, p = 0.010).

3.3. Associations between BMD and FA

There were no significant associations between femoral BMD measures and FA (Table 3). Substitution of either covariate pertaining to prolactin-raising AP use with lifetime exposure to all AP did not alter the pattern of findings.

4. Discussion

The present study examined, for the first time — to the best of our knowledge, associations between cumulative endogenous estrogen exposure (to the extent that it is reflected by femoral BMD) and brain structure in psychotic disorder. Our findings tentatively suggest that higher cumulative exposure to estrogen is associated with increased cerebral cortical thickness. There was no evidence for a significant association between cumulative estrogen exposure and microstructural white matter integrity.

Table 1
Demographic characteristics (n = 14).

Demographic characteristic	Mean ± SD
Age at scan (years)	27.64 ± 8.48
Age at menarche	13.0 ± 0.96
Dysmenorrhea (n)	6
Lifetime exogenous estrogen exposure (μg)	29923 ± 43477
Smoking (cigarettes per day)	6.21 ± 8.01
Alcohol use (units per week)	0.92 ± 1.21
Cannabis use (number of times lifetime)	17.71 ± 34.60
Other drug use (number of times lifetime)	18.71 ± 44.97
Present exposure to PRL-raising AP (yes/no)	5/9
Cumulative (lifetime) exposure to PRL-raising AP (hal. eq.)	2262 ± 2506
Cumulative (lifetime) exposure to all AP (hal. eq.)	3006 ± 3197

SD: standard deviation; PRL-raising: prolactin-raising; AP: antipsychotic medication; hal. eq.: haloperidol equivalents (in milligrams).

Table 2
Mean femoral BMD, CT and FA.

	Mean ± SD
Total femoral BMD (g/cm^2)	0.923 ± 0.109
Z-score	−0.193 ± 0.984
T-score	−0.257 ± 0.971
CT (n = 14)	2.553 ± 0.375
FA (n = 13)	0.576 ± 0.086

SD: standard deviation.

4.1. Findings

4.1.1. Cumulative endogenous estrogen exposure and CT

Our findings showed positive associations between BMD measures and CT in female patients with a psychotic disorder. In other words, higher cumulative estrogen levels may be associated with increased CT, or lower estrogen levels may be associated with reduced CT. Assuming that the association is true, a deficiency in estrogen exposure may impact gray matter, which may be reversed by higher levels of estrogen that may induce or activate neuroprotective mechanisms. The results thus fit both the estrogen deficiency and protection hypothesis.

Accumulating clinical research has demonstrated emerging treatment perspectives of estrogen therapy (ET) and selective estrogen receptor modulators (SERMs) as adjunctive therapy (Kulkarni et al., 2010, 2012). Following an earlier, inconclusive Cochrane review (Chua et al., 2005), a quantitative 2012 review suggests that the use of estrogen as adjunctive treatment for schizophrenia could be effective in women (Begemann et al., 2012). Though this indicates an association between hypoestrogenism and psychotic disorders in women, the question remains whether hypoestrogenism is a cause or consequence of the disease (Aston et al., 2010; Markham, 2011), or its treatment.

Interpretation of the current study findings is complicated by the cross-sectional nature of the data, as well as the fact that no previous studies have been conducted combining (markers of) estrogen levels and structural brain alterations in psychiatrically ill individuals. Nevertheless, disrupted sexual dimorphisms in the cortex of patients with schizophrenia as demonstrated by MRI (Goldstein et al., 2002) were reported over a decade ago, which helped refuel the debate about sex differences in schizophrenia. It seems biologically plausible that estrogen, through its impact on brain morphology, contributes to the risk of – and the sex differences in – psychotic disorder. Indirect support for this comes from a study in healthy postmenopausal women, showing beneficial influence of ET on the cortical cerebral surface (Boccardi et al., 2006). However, other groups failed to discover an association between ET and total gray matter (Low et al., 2006; Ha et al., 2007), or reported a negative association (Greenberg et al., 2006). During adolescence, higher estrogen levels are related to an overall decrease in cortical gray matter (Peper et al., 2009, 2011a), demonstrating that the organizing and structural effects of estrogen may be age-dependent and influenced by other factors.

4.1.2. Cumulative endogenous estrogen exposure and FA

We did not find an association between BMD and FA. The literature on associations between estrogen and cerebral white matter is limited. In the developing brain, white matter increase is minimal in girls compared to boys, and white matter maturation may be primarily under the influence of luteinizing hormone (which is positively associated with white matter in puberty) (Peper et al., 2011a). In one study, estrogen was positively related to FA in boys, yet negatively related to FA in girls (Herting et al., 2011). One reason for the absence of an association between estrogen and FA in the current study, may be that other gonadal hormones constitute the principal hormonal effect on white matter, e.g. progesterone elicits the production of myelin and oligodendrocytes (Peper et al., 2011b). Results from studies investigating the effect of ET on white matter in the aging brain are inconsistent. Low et al. (2006) reported inconclusive findings with regard to the

Table 3
Associations between femoral BMD and structural brain measures.

	Total BMD	Z-score	T-score
	B (95% CI), p-value	B (95% CI), p-value	B (95% CI), p-value
CT (n = 14)			
Correction for:			
Present exposure PRL-raising AP	0.266 (−0.019–0.552), 0.067	0.034 (0.001–0.067), 0.046	0.034 (0.000–0.068), 0.052
Cumulative exposure PRL-raising AP	0.429 (0.104–0.753), 0.010	0.049 (0.012–0.085), 0.009	0.048 (0.011–0.086), 0.011
FA (n = 13)			
Correction for:			
Present exposure PRL-raising AP	0.002 (−0.134–0.138), 0.973	0.000 (−0.015–0.015), 0.993	0.000 (−0.015–0.015), 0.992
Cumulative exposure PRL-raising AP	0.012 (−0.287–0.311), 0.937	−0.001 (−0.027–0.026), 0.970	−0.001 (−0.028–0.026), 0.941

B's represent unstandardized regression coefficients of the multilevel regression analyses. CI: confidence interval; PRL-raising AP: prolactin-raising antipsychotic medication.

effect of ET on white matter volume, while Ha et al. (2007) found significantly greater white matter volumes in estrogen users.

4.2. Influence of AP on brain measures and BMD

AP exposure may be associated with both brain measures and BMD and may thus act as a potential confounder. With regard to the effect on brain structure, some studies found, in fairly large samples, no effect of duration of AP use or illness duration on white matter microstructure (Kanaan et al., 2009) or gray matter (Leung et al., 2011). In contrast, a large prospective study (van Haren et al., 2011) found that cortical thinning is a progressive process and is in part mediated by the use of AP. A recent meta-review (Shepherd et al., 2012) reported that structural brain changes (including cortical volume) may be affected by AP, though the effect of AP exposure may itself be confounded by disease progression (Ho et al., 2011).

In the current study, we initially corrected for the present use of a prolactin-raising AP or cumulative exposure to prolactin-raising AP, in line with our hypothesis that cerebral estrogen exposure may be affected by hyperprolactinemia-induced hypogonadism secondary to prolactin-raising AP. When using lifetime exposure to all AP instead of current or lifetime prolactin-raising AP use in the analyses, the positive associations between BMD and CT remained, and the absence of associations between BMD and FA was confirmed.

With regard to the relation between AP and BMD, the majority of studies are suggestive of a negative association (Crews and Howes, 2012). In our original BMD study (n = 16), we found a negative main effect of prolactin-raising AP on lumbar BMD, but not femoral BMD (van der Leeuw et al., 2013). In addition, due to a reduction in sample size (and concomitant effect size reduction) when excluding female patients who used prolactin-raising APs, we were unable to exclude the possibility of AP exposure as a partial explanation for reduced BMD in female patients. However, the specific association between BMD and CT (and not FA) in the present study, corrected for present and lifetime prolactin-raising AP use and cumulative exposure to all AP, is at least suggestive of an independent cerebral effect of primary estrogen reduction in female patients with psychotic disorder.

4.3. Methodological considerations

Certain limitations in our study design need to be acknowledged.

First, bone mineral density was used as an indirect indicator of cumulative (endogenous) estrogen exposure and has disadvantages because it is influenced by many hormonal and non-hormonal factors and should thus be interpreted as a proxy marker. Nevertheless, the most important potential confounding factors were addressed. A clear advantage of BMD is that it reflects cumulative exposure to estrogen, while the direct measurement of estrogen in serum or urine is a momentary assessment. Although we could not consider the influence of the phase of the menstrual cycle on brain morphology as recommended by Pletzer et al. (2010), we adjusted for several hormonal factors such as age at menarche, dysmenorrhea, lifetime contraceptive use and current and lifetime prolactin-raising AP use.

Second, the sample size of the study was small, suggesting that our findings are best interpreted as hypothesis-generating. The sample size did not preclude the detection of positive findings in CT. However, it is possible that our study lacked power to detect an effect in FA. To date, other neuroimaging studies investigating estrogen effects on brain morphology in schizophrenia have not been performed and the present hypothesis-generating study therefore requires replication with larger sample sizes, to corroborate or challenge the results. The presented cross-sectional data is part of a longitudinal study. Follow-up data may provide further clues to the relationship between lifetime estrogen exposure and brain alteration (over time) in psychotic disorder.

Third, it is possible that the effects of estrogen are region-specific. The present study was not powered to examine whether the impact of BMD varied with specific CT or FA regions (i.e. BMD × region interaction). To discern regions of interest, an experimental treatment study using ET or SERMs may offer additional insights.

Fourth, our study is limited by the fact that our subjects were not first-episode AP-naïve patients. Inherent to the inclusion criteria of the GROUP study, individuals with a *relatively* recent onset of psychotic disorder were included (mean illness duration of 6.6 years). Thus, by implication, none of our patients were AP-naïve and the majority had used a prolactin-raising AP at some point in their lives. Nevertheless, the pattern of results remained consistent after extensive correction for AP use. Ideally, replication should be performed in a sample that has been exposed to neither AP nor contraconceptive drugs.

4.4. Conclusions

Reduced BMD (reflecting low cumulative estrogen levels) in female patients with a psychotic disorder may be associated with decreased cortical thickness, suggesting that a diminished neuroprotective effect of estrogen may contribute to cerebral gray matter alterations, indirectly supporting both aspects (deficiency and protection) of the estrogen hypothesis of schizophrenia. There was no evidence for an association between BMD and white matter integrity.

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The sponsors had no further role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Christine van der Leeuw performed the literature searches and data analyses, and wrote the first draft of the manuscript. Petra Habets and Patrick Domen collected the data and processed the imaging data. Ed Gronenschild and Stijn Michielse assisted in the pre- and postprocessing of the imaging data. Marinus van Kroonenburgh supervised the acquisition and interpretation of the DEXA scans. Jim van Os designed the study, wrote

the protocol and supervised the project. Machteld Marcelis designed the study, wrote the protocol, coordinated and supervised all aspects of the study. All authors contributed to and approved the final manuscript.

Conflict of interest

Jim van Os is or has been an unrestricted research grant holder with, or has received financial compensation as an independent symposium speaker from, Lilly, BMS, Lundbeck, Organon, Janssen, GlaxoSmithKline, AstraZeneca, Pfizer and Servier. Machteld Marcelis has received financial compensation as an independent symposium speaker from Lilly and Janssen. All other authors report no biomedical financial interests or potential conflicts of interest.

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