Oestradiol moderates the association of visceral fat on brain structure and cognitive function in women

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List of Abbreviations

VAT	visceral adipose tissue
AD	Alzheimer's disease
BMI	body mass index
SCAT	subcutaneous adipose tissue
MRI	magnetic resonance imaging
CT	computed tomography
HPA	hypothalamic-pituitary-adrenal
GM	grey matter
WM	white matter
RPCTs	randomized placebo-controlled trials
fMRI	functional magnetic resonance imaging
HRT	hormone replacement therapy
PET	positron emission tomography
WHI	Women's Health Initiative
LIFE	Health Study of the Leipzig Research Centre for Civilization Diseases
CERAD	Consortium to Establish a Registry for Alzheimer's disease
CRP	C-reactive protein
PCOS	polycystic ovary syndrome

I. Introduction

1. Sex-specific prevalence rates of obesity and dementia and the link between both pathologies

Accumulating evidence from neuroimaging studies supports a link between obesity, accelerated brain ageing and cognitive decline (Raji et al., 2010). This is problematic given that in 2016, 39% of adults were overweight and 13% were obese, worldwide (World Health Organisation, 2017b). Rates of dementia are equally as alarming, with around 50 million people in the world currently diagnosed and the predicted number of people with dementia to be 82 million by 2030 (World Health Organisation, 2017a). Obesity is already a well-established risk factor for atherosclerosis, cardiovascular and metabolic pathologies (Mokdad et al., 2003), with visceral adipose tissue (VAT) playing a critical role in developing metabolic syndrome (Britton et al., 2013; Mathieu et al., 2008). But a growing amount of studies report that central obesity, particularly during midlife (Kivipelto et al., 2005), is a major risk factor for brain atrophy (Debette et al., 2010; Hamer & Batty, 2019), cognitive decline and dementia in later life (Whitmer et al., 2008). The rates, pathophysiology and clinical manifestation of dementia show prominent sex differences (Kim et al., 2015; Podcasy & Epperson, 2016). For example, the most common form of dementia is Alzheimer's disease (AD), which women have an almost twofold increased lifetime risk for as men (Seshadri et al., 1997). Additionally, although vascular and multi-infarct dementia is more common in men (Meyer et al., 1988), metabolic risk factors such as obesity have stronger adverse associations on women's cognitive brain functioning (Appelros et al., 2009) as well as executive functioning (Schwartz et al., 2013) compared to men. In line with this, women show a higher susceptibility for a symptomatic manifestation of AD pathology than men (Barnes et al., 2005). Together, these findings highlight the need for sex-specific investigations of the associations between specific fat deposit patterns as potential risk factors for cognitive decline in later life.

2. Sex differences in body fat distribution: implications for *in vivo* imaging studies

The most common metric used to determine obesity status is body mass index (BMI), which is calculated by dividing an individual's weight (in kilograms) by height (in meters squared). While this approach does allow for a non-invasive, cheap and fast measurement, it cannot distinguish between muscular and skeletal tissues or visceral and subcutaneous fat tissues (Poirier, 2007). Recent technological advancements have facilitated the use of modern imaging techniques to directly quantify subcutaneous adipose tissue (SCAT) and VAT volumes using magnetic resonance imaging (MRI) or computed tomography (CT). Directly quantifying fat tissue, as opposed to using proxies such as BMI, may be essential for *in vivo* studies of human metabolic health as recent investigations suggest VAT as an unique

risk factor for developing metabolic syndrome, independent of overall obesity (Carr et al., 2004; Wajchenburg, 2014). Fat tissue volumes quantified by CT or MRI provide specific information about fat storage distribution (Abate et al., 1994; Machann et al., 2005; Seidell et al., 1990; Thomas et al., 1998). But MRI has the additional benefit of not exposing the person to ionising radiation, which can increase the risk of adverse long-term effects on human tissues, such as cancer (World Health Organisation, 2011). Using T1-weighted MRI sequences, fat tissue displays hyperintense (=white) with better contrasts to water dominated areas compared to the T2-weighted sequences, in which fat tissue displays with lower contrasts to other tissues. In T1 images, muscles, bones and air display less intense than fat, which allows for an accurate and reproducible differentiation between fat tissue and other tissue compartments. Therefore, for human *in vivo* studies, MRI is a reliable and non-invasive imaging tool to differentiate and quantify between different adipose tissue volumes.

Critically, there are known sex differences in fat accumulation and distribution patterns. Women accumulate lower rates of VAT and higher rates of SCAT during the premenopausal lifetime, with the highest volume of VAT located more caudal compared to men (Kvist et al., 1988; Machann et al., 2005). In addition, women with healthy premenopausal ovarian hormone production accumulate more adipose tissue in the subcutaneous gluteofemoral region and not in the abdominal cavity (Kissebah et al., 1985). The menopausal transition with the loss of ovarian hormones in women is associated with an excessive accumulation of VAT, most prominently starting during perimenopause (Lovejoy et al., 2008). Because VAT can contribute to a dysregulation in the hypothalamic-pituitary-adrenal (HPA) axis, higher amounts of VAT can result in a higher stress response and abnormal cortisol secretion (Andrew et al., 2005). Altogether, a strong influence of ovarian hormones on body fat and metabolic profiles in women is likely.

3. Sex-specific associations between visceral adipose tissue, brain structure and cognitive function: evidence from cross-sectional neuroimaging studies

Cross-sectional neuroimaging studies provide novel insight into associations between obesity and brain structure. However, it is not possible to infer causality within a cross-sectional study design. Structural MRI of the brain is a well established non-invasive imaging procedure to visualise grey and white matter anatomy *in vivo*. T1-weighted imaging sequences have been reported to provide the best contrast between grey matter (GM, less intense) and white matter (WM, more intense) (Wang et al., 2014). Optimised protocols for segmentation of GM and WM in the obtained images as well as multi-modal analysis of GM volume, cortical thickness and cortical surface area can then be applied (Andersson et al., 2007; Fischl et al., 2002; Good et al., 2001). Through the use of large sample sizes and

multivariate models, cross-sectional neuroimaging studies can provide essential information for future longitudinal or interventional study designs.

Previous cross-sectional neuroimaging studies have revealed an inverse association between visceral adiposity and alterations in brain structures related to cognitive performance (Debette et al., 2010; Isaac et al., 2011; Kurth et al., 2013; Raschpichler et al., 2013). Recent studies report a negative association between central obesity with total brain volume (Debette et al., 2010), hippocampal volume (Isaac et al., 2011; Jagust et al., 2005; Zade et al., 2013) left prefrontal cortical thickness (Isaac et al., 2011; Kurth et al., 2013), as well as a positive association of central obesity with increased WM hyperintensities (Jagust et al., 2005; Zade et al., 2013). Higher VAT is associated with subclinical WM lesion load in middle-aged adults prior to detectable cognitive changes (Pasha et al., 2015). Moreover, lower GM volume and cortical thickness have been associated with central obesity in the cerebellum (Raschpichler et al., 2013), hypothalamus, prefrontal regions as well as in anterior temporal and inferior parietal cortices (Kurth et al., 2013), with greater effect sizes in men compared to in women (Kurth et al., 2013). Interestingly, a recent report from the UK Biobank study replicated the findings of lower GM volumes in centrally obese subjects compared to overweight participants who were not classified as centrally obese (Hamer & Batty, 2019). Veit et al. (2014) observed an association between higher VAT and reduced cortical thickness in the left occipital area, the left inferior temporal cortex, and the left precentral and inferior parietal area independent from BMI. Veit and colleagues did not report a sex-specific interaction in the negative association between VAT and cortical thickness, but this could be explained by the sample size (N=72) and a limited age-range of the participants (age-range=19-50 years) (Veit et al., 2014). In summary, although the cross-sectional nature of these neuroimaging studies does not allow for any causal interpretation, these findings indicate that VAT may be a key risk factor for accelerated brain atrophy in cognition-related brain areas, thus calling for future research.

4. Elevated visceral adipose tissue in midlife as a risk factor for cognitive decline in later life: evidence from longitudinal studies and a focus on women

Longitudinal observational studies allow for investigation of individual trajectories of endogenous sex hormones, adipose tissue accumulation and brain ageing. Evidence from such longitudinal studies supports the hypothesis that obesity and greater amounts of VAT in midlife represent a critical risk factor for cognitive decline and dementia in later life (Hassing et al., 2009; Pedditizi et al., 2016; Whitmer et al., 2005). Lovejoy et al. (2008) found fluctuation and decline of endogenous oestradiol levels during perimenopause to be associated with increased accumulation of VAT. Subsequently, an increase in waist circumference, total body fat and VAT has been observed during perimenopause (Lovejoy et

al., 2008), which may be associated with heightened risk for developing dementia in later life (Kivipelto et al., 2005).

The potential interplay between sex hormones and adipose tissue accumulation is of critical interest, as higher VAT is also associated with greater risk for dementia (Cereda et al., 2007). In line with these findings, additional longitudinal data suggest that central obesity, especially in midlife, is associated with higher risk for dementia in later life independent of diabetes or any cardiovascular comorbidities (Whitmer et al., 2008; Whitmer et al., 2005), with women being at a greater risk than men (Whitmer et al., 2007; Whitmer et al., 2005). Interestingly, the authors suggest central adiposity to play a key role in the negative association of obesity on cognition in women (Whitmer et al., 2005). Furthermore, higher VAT has been linked to lower verbal memory, independent of SCAT, as well as lower executive functioning (Isaac et al., 2011; Schwartz et al., 2013). In summary, these studies indicate that women may be more sensitive to the adverse effects of VAT on cognitive functioning during mid-life (Isaac et al., 2011; Schwartz et al., 2013), and thus uniquely susceptible to develop dementia in later life.

Given the evidence of significant sex differences in (i) abdominal fat accumulation and distribution (Machann et al., 2005), (ii) metabolic properties of adipose tissue compartments (White & Tchoukalova, 2014), (iii) brain structure (Horstmann et al., 2011; Luders & Toga, 2010; Witte et al., 2010), and (iv) development of neurodegenerative and neuropsychiatric diseases (Bao & Swaab, 2010), including dementia and AD (Podcasy & Epperson, 2016), it is likely that sex hormones play a fundamental role in the interaction between VAT, brain structural ageing, and loss of cognitive function.

5. Ovarian hormones, specifically oestradiol, as potential neuroprotective factors for brain structure integrity

Evidence from both rodent and human studies provides support for beneficial effects of ovarian hormones on brain structure and functioning (Peper et al., 2011). Oestrogen specifically have been identified as potential neuroprotective hormones with anti-inflammatory properties and favourable effects on body fat composition (Pozzi et al., 2006). Oestradiol, the most potent oestrogen (Thomas & Potter, 2013), strengthens the robustness of the HPA-axis and exhibits anti-inflammatory properties within the central nervous system (Straub, 2007).

5.1 Evidence from animal studies

Animal models indicate that oestradiol can induce synaptogenesis and an increase in synaptic spine density (Fester et al., 2012), as well promote dendritic spine density in the hippocampus, a brain region implicated in memory and cognitive functioning (Woolley & McEwen, 1994). Furthermore, the activation of microglia by lipopolysaccharides can be inhibited by oestrogen, subsequently lowering the inflammatory response (Vegeto et al., 2001). In cortical explant cultures, oestradiol has been shown to protect cells from ischemia-associated cell death (Wilson et al., 2000). These neuroprotective effects have been further validated in a lesion stroke model revealing that oestradiol enhances neurogenesis after ischemic stroke (Suzuki et al., 2007). Specifically in female rats, oestradiol also regulates adipose tissue deposition (Pedersen et al., 1991; Wade & Gray, 1978). In summary, animal models provide consistent evidence suggesting neuroprotective properties of oestradiol. The translation of these findings into human models and clinical translation, however, has proven challenging.

5.2 Evidence from human interventional studies

Interventional study designs are required to establish a causal effect of oestradiol on cognitive function. Here, the body of evidence can be divided into observational interventional studies and randomized placebo-controlled trials (RPCTs). RPCTs represent the current gold standard for obtaining causal effects of substances on certain endpoints, while observational interventional studies can aid in defining such endpoints. This section provides a brief review of both observational interventional studies and RPCTs that investigate the effects of exogenous oestradiol administration on cognitive performance.

5.2.1 Observational studies

On the level of brain function, observational functional magnetic resonance imaging (fMRI) studies found beneficial effects of hormone replacement therapy (HRT) on brain networks responsible for working memory (Shaywitz et al., 1999), as well as higher metabolic activity in regions of the brain associated with cognitive functioning and memory performance, namely the hippocampus, the temporal lobe and the inferior frontal cortex (Eberling et al., 2004). A positron emission tomography (PET) study showed an increase in cerebral blood flow in brain areas prone to early deterioration in AD (Karow et al., 2010; Schroeter et al., 2009), like the temporal lobe, hippocampus and parahippocampal regions, as well as better memory performance in HRT users compared to nonusers (Maki & Resnick, 2000). A study combining fMRI and PET revealed different activation patterns between HRT and non-HRT groups during memory tasks, with HRT users showing better performance on neuropsychological tests of figural and verbal memory (Resnick et al.,1998). Moreover, a

study in perimenopausal women who started HRT use before their final menstrual periods focused on the hippocampus as the *a priori* region of interest and found that early HRT use was associated with enhanced verbal recognition and higher activity of the hippocampus and parahippocampal gyrus later in life (Maki et al., 2011). Lord et al. (2008) came to comparable results, showing higher hippocampal volumes in the HRT group compared to the non-HRT group (Lord et al., 2008). Interestingly, this study found a significant negative correlation between the duration of oestradiol-treatment and hippocampal volumes (Lord et al., 2008). In summary, these studies indicate beneficial effects of early HRT during the menopausal transition on brain structure and function. In contrast, the evidence suggests that long-term HRT can also reduce hippocampal volumes, a brain region associated with memory and cognition. To shed light into reasons for inconsistent modes of action of oestradiol administration, RPCTs are mandatory.

5.2.2 Randomized placebo-controlled trials

The randomized, double-blind, placebo-controlled Women's Health Initiative (WHI) trials conducted by the National Institute of Health investigated effects of HRT on age-related changes in cognition in women (Anderson et al., 1998). The WHI study found that HRT use by women did not positively affect cognitive functioning and actually seemed to increase the risk for developing dementia (Espeland et al., 2004; Rapp et al., 2003; Shumaker et al., 2003). Further RPCTs including postmenopausal women already suffering from cognitive impairment or dementia found no beneficial effects of HRT on cognition (Henderson et al., 2000; Mulnard et al., 2000; Wang et al., 2000). It is worth noting, however, that these studies did not examine perimenopausal or early postmenopausal women. In regards to the WHI trials specifically, the criticism was raised that the average age of the women in the study were older and post-menopause; the potentially harmful effects of HRT on cognitive function were observed in participants aged 65 years and older. Furthermore, many of these participants had increased BMI, a critical aspect for cardiovascular and cognitive health. In contrast, one study focussing on perimenopausal and early postmenopausal women found beneficial effects of HRT on executive function, and higher blood oxygen level dependent responses in prefrontal regions during verbal recall tasks (Joffe et al., 2006). In summary, the majority of recent RPCTs found no favourable associations between HRT and cognitive function in women (Henderson et al., 2000; Rapp et al., 2003; Shumaker et al., 2003). But additional studies in younger well-characterised populations are required as HRT has been shown to be associated with healthier patterns of executive function in perimenopausal and recently postmenopausal women (Joffe et al., 2006).

The literature remains divided on the potential benefits and risks of HRT on cognitive ageing. Altogether, the body of evidence suggests timing-dependent effects of oestrogen on cognitive function and dementia risk in later life. Henderson et al. (2005) concluded that women could benefit from a lower risk of early AD onset associated with HRT use only during early menopause and not during older age (Henderson et al., 2005). This conclusion is further supported by Whitmer et al. (2011) who included over 5000 participants and demonstrated that women using HRT only during midlife have a 26% reduced risk of developing AD, whereas HRT in late-life was associated with a 46% increase of dementia-risk (Whitmer et al., 2011). Indeed, HRT during perimenopause shows beneficial effects on memory and verbal performance compared to perimenopausal women without HRT (Maki et al., 2011). Together, these studies indicate that timing of HRT plays a key role for potential beneficial effects on cognition.

5.3 Implications for understanding hormonal transition as neurological transition states

In summary, different lines of evidence support beneficial effects of exogenous oestradiol administration during perimenopause and early postmenopause on women's fat accumulation patterns, metabolism, brain ageing and cognitive functioning. Current guidelines suggest HRT as a therapeutic option for treatment of climacteric symptoms during the menopausal transition if women are within 10 years of menopause or younger than 60 years of age (Rozenberg et al., 2013). However, harmful effects of HRT have also been reported, especially for older postmenopausal women (> 60 years of age), women with heightened cardiovascular risk and women who already suffer from AD (Rozenberg et al., 2013). Identifying the right timing of oestradiol administration may be key for maximising the potential beneficial effects of HRT on women's health.

Given the findings that (i) ovarian hormones affect body fat composition (Pedersen et al., 2004) and that (ii) central obesity in midlife is a major risk factor for cognitive decline in later life (Hassing et al., 2009; Whitmer et al., 2008), as well as (iii) a potential protective role of oestradiol on body and central nervous system metabolism (Fester et al., 2012; Wilson et al., 2000), it is important to investigate how endogenous hormone fluctuations and adipose tissue accumulation interact during natural hormone transition states to influence brain health and cognitive functioning (Georgakis et al., 2016; Lovejoy et al., 2008; Rocca et al., 2007).

6. Hormonal and metabolic profiles during perimenopause that may predispose to unhealthy brain ageing

The menopausal transition, which occurs during midlife, may be one of such critical neurological time windows in women (Brinton et al., 2015; Palacios et al., 2010). The onset of menopause is defined as twelve months after the final menstrual period whilst the time period of three years before the onset of menopause is defined as perimenopause (Harlow et al., 2012). Ovarian hormone levels change in this period due to a loss in ovarian functioning (Brinton et al., 2015). Lower oestradiol levels are already observeable three years before the onset of menopause (Lovejoy et al., 2008). The drop of oestradiol during the menopausal transition has been associated with changes in body fat accumulation and distribution (Svendsen et al., 1995; Toth et al., 2000). Women accumulate lower rates of VAT and higher rates of SCAT during the premenopausal lifetime (Kvist et al., 1988; Machann et al., 2005). During perimenopause, when endogenous oestradiol levels fluctuate and decline erratically, women tend to accumulate excessive VAT (Lovejoy et al., 2008). This redistribution of body fat may predispose women to an adverse metabolic profile with increased inflammatory reactivity (Piché et al., 2008), and thus heightened risk for cortical atrophy and cognitive decline in later life (Hassing et al., 2009; Veit et al., 2014).

Evidence of the negative effects of early-onset of menopause introduced by oophorectomy on cognition further supports the link between endogenous ovarian hormone depletion and increased risk of dementia (Rocca et al., 2007). Overall, sustained levels of endogenous oestradiol during perimenopause may help to support a more beneficial premenopausal subcutaneous fat phenotype and could have neuroprotective effects in later life.

Because of potential neuroprotective properties of oestradiol (Fester et al., 2012; Woolley & McEwen, 1994), HRT in women has been suggested as a potential opportunity for lowering the risk of developing dementia in later life (Leblanc et al., 2001). Findings of exogenous oestradiol administration on brain structure and cognitive performance remain controversial, having been reported as both advantageous as well as disadvantageous. As mentioned above, results from the WHI trials showed an increased risk for developing dementia with HRT in women aged 65 years of age or older (Shumaker et al., 2003). While these results are informative, the negative findings published from these trials unfortunately attenuated some of the scientific interest in researching potential neuroprotective properties of oestrogen.

As negative effects of HRT on cognition have been predominantly reported in older postmenopausal women (Resnick et al., 2009; Shumaker et al., 2003) and beneficial effects of HRT have mainly been shown in younger postmenopausal women (Joffe et al., 2006; Lord et al., 2008; Maki et al., 2011), the hypothesis of a window of opportunity for advantageous effects of exogenous oestrogen administration on cognition has been suggested (Maki, 2013; Sherwin, 2012). This conceptual theory proposes perimenopause as the best time for potential beneficial effects of HRT on women's brain ageing and lower risk for developing dementia in later life.

II. Rationale of the work

Modern medicine has increased human life expectancy with women on average at 81.4 years of age and men at 76.3 years of age (United Nations Development Programme, 2019). Given the growing older population, age-related medical disorders such as neurodegenerative disorders and cognitive decline are becoming one of the biggest health challenges that we face, with women at higher risk of developing dementia than men (Oeppen & Vaupel, 2002; Seshadri & Wolf, 2007; Xie et al., 2008). Higher prevalence rates of AD in women cannot solely be explained due to higher life expectancy of women, as researchers found support that women display faster progression of AD could be explained by a higher vulnerability of the postmenopausal female brain (Kim et al., 2015; Newhouse, 2014; Seshadri et al., 1997). To delay an onset of dementia and support healthy cognitive ageing as well as lower economic and social costs of treatment of the disease (Wimo et al., 2013), it is critical to clarify preventable risk factors, such as visceral fat, that expedite brain ageing (Hurd et al., 2013).

Cross-sectional 'big data' MRI studies identified a negative association between visceral adiposity and cognition related brain structure with significant sex differences in the distribution of fat storages (Debette et al., 2010; Hamer & Batty, 2019; Machann et al., 2005). Longitudinal observational studies further clarified that midlife-obesity in particular is associated with higher risk of developing dementia in later life (Whitmer et al., 2008; Whitmer et al., 2005) and that the loss of ovarian functioning during perimenopause is associated with exacerbated VAT accumulation (Lovejoy et al., 2008). Animal studies, interventional longitudinal studies as well as randomized placebo-controlled trials support neuroprotective properties of endogenous and exogenous oestradiol (Fester et al., 2012; Wolf & Kirschbaum, 2002; Woolley & McEwen, 1994), with beneficial effects on cognitive functioning (Eberling et al., 2003; Hogervorst et al., 2000; Leblanc et al., 2001). But other studies do not find evidence in support of positive effects of HRT on cognition, and report negative effects of HRT in older postmenopausal women, women who are already overweight or obese, or women who already suffer from mild cognitive impairment or dementia (Rapp et al., 2003; Resnick et al., 2006; Shumaker et al., 2003). Timing of HRT may play a key role in whether exogenous oestrogen have beneficial or adverse effects on cognition. Natural hormonal transition states during midlife, like the menopausal transition, have been suggested to be critical neurological transition states (Brinton et al., 2015). It has been shown that perimenopausal HRT could be beneficial for lowering the risk of developing dementia in later life (Maki et al., 2011), but HRT in older postmenopausal women has adverse effects on cognition (Resnick et al., 2009; Shumaker et al., 2003). Therefore, the critical time hypothesis has been established; suggesting a time window for beneficial effects of HRT on women's cognitive functioning in later life (Maki, 2013; Whitmer et al., 2011).

Although oestradiol and VAT appear to play opposing roles in regards to brain and cognitive ageing, it remains unclear how they may interact to influence brain health. It is thus imperative to investigate potential interactions between brain structure, VAT, oestradiol, and cognitive function in order to identify time periods in which women's cognitive health may best profit from hormone applications. Given that central obesity during midlife is associated with heightened risk for dementia in later life (Whitmer et al., 2008), changes of endogenous ovarian hormone levels during perimenopause are associated with changes in body fat composition (Lovejoy et al., 2008) and that adipose tissue displays substantial sex dimorphisms (Machann et al., 2005), we need to systematically study sex-specific risk trajectories for unhealthy brain and cognitive ageing. The current body of evidence investigating the interplay between these factors has limitations which subsequently raise the need for further research: (1) Most neuroimaging studies use BMI as an indicator for obesity (Karlsson et al., 2013; Marqués-Iturria et al., 2013; Mueller et al., 2012; Stanek et al., 2011; Xu et al., 2013), which is problematic given that this measure can not distuingish between different adipose tissues; (2) preliminary neuroimaging evidence suggests sex-specific interactions between central obesity and brain structure (Horstmann et al., 2011; Kurth et al., 2013), but we lack of studies focusing on sexual dimorphisms in associations between central obesity and cognition related brain areas; (3) a potential protective effect of oestradiol on the negative association between VAT and GM structure in brain areas implicated in higher cognitive function has not yet been investigated; and (4) we lack large population-based cohort studies that contain MRI-based brain and abdominal imaging as well as hormonal and cognitive function measurements.

Addressing these current limitations (1-4) in the literature, we hypothesise (I) a negative association between VAT and cognition as well as between VAT and age-related GM networks, (II) sex-specific interactions in the association between VAT and GM networks, and (III) a moderating role of oestrogen in the association between VAT and GM networks. We further aim to investigate the perimenopausal age range (35-55 years) in women as a potential critical time window for changes in ovarian hormones, fat tissue, brain structure and cognition (IV).

To test these hypotheses, we combine measures of quantified VAT volume, multimodal neuroimaging analysis, cognitive testing and serum ovarian hormone levels (limitations 1-4) in a cross-sectional study of a German population-based cohort. In total, 974 participants (473 females, limitation 4) from the "Health Study of the Leipzig Research Centre for Civilization Diseases" (LIFE) were included in the final analyses. We used MRI-based fat tissue quantification as a direct measure of central adiposity (limitation 1). To ensure data quality, we first replicated previously reported sex-specific abdominal fat tissue distribution

and accumulation patterns (Machann et al., 2005). Further, the participants underwent MRI-based T1-weighted anatomical imaging of the brain. Linked independent component analysis was applied to GM volume, cortical thickness, and surface area to identify a GM network previously associated with accelerated brain ageing, cognitive decline and dementia (limitation 2 & 4) (Douaud et al., 2014; Groves et al., 2011; Groves et al., 2012). We calculated an individual pattern-measure per participant on this network, further indicated as network covariance (Groves et al., 2011, 2012), which reflects individual loading on the brain network. We used a standardized and reliable neuropsychological test for assessment of memory performance, developed by the Consortium to Establish a Registry for Alzheimer's disease (CERAD) (limitation 2 & 4, CERAD verbal episodic memory test) (Moms et al., 1989). To ensure the reproducibility of our GM network with the previously identified network, we used CERAD sum scores to confirm that the GM network was relevant to memory performance; additionally, we performed visual comparison, spatial correlation, as well as dual regression analyses to compare the previously identified network with ours (limitation 2 & 4) (for more information, see enclosed published article and discussion). We focused on sex-specific interactions between VAT and the GM network over the adult lifespan (limitation 2 & 4), including oestradiol serum levels to examine modulating effects in these associations (limitation 3). A detailed study protocol of the LIFE study has previously been published (Loeffler et al., 2015) and further information about the methods we applied can be obtained from the enclosed publication and supplemental material (Zsido & Heinrich et al., 2019).

The overall aim of this medical thesis is to contribute to the understanding of sex differences in visceral fat related accelerated brain ageing and cognitive decline. Furthermore, the present thesis explores a potential modulating role of oestradiol on the interaction of visceral fat and cognition-associated GM networks in a cross-sectional study of a German population-based cohort. Sex-specific analyses of the interaction between VAT and brain structure will provide additional evidence for the need of sex-linked clinical practice of neurodegenerative diseases. Establishing this interdisciplinary framework provides an important stepping stone to identify critical time windows during the lifespan in which sex-specific intervention strategies can be developed to maintain cognitive health in later life.

III. Published article

Association of estradiol and visceral fat with structural brain networks and memory performance in adults

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Association of Estradiol and Visceral Fat With Structural Brain Networks and Memory Performance in Adults

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Abstract

IMPORTANCE Changes in estradiol during aging are associated with increased dementia risk. It remains unclear how estradiol supports cognitive health and whether risk factors, such as midlife obesity, are exacerbated by estrogen loss.

OBJECTIVES To assess whether visceral adipose tissue (VAT) moderates the association between age and brain network structure and to investigate whether estradiol moderates the association between VAT and brain network structure.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional study of data from 974 cognitively healthy adults in Germany who participated in the Health Study of the Leipzig Research Centre for Civilization Diseases, a previously described population-based cohort study. Two moderation analyses were performed, including VAT as the moderator variable between age and brain network structure and estradiol as the moderator variable between VAT and brain network structure. The study was conducted from August 1, 2011, to November 23, 2014. Analyses were conducted from August 2017 to September 2018.

EXPOSURES Serum estradiol levels from fasting blood and visceral adipose tissue volume from T1-weighted magnetic resonance imaging (MRI).

MAIN OUTCOMES AND MEASURES Brain network covariance (individual loading on structural network derived from T1-weighted MRI) and memory performance (composite score from the Consortium to Establish a Registry for Alzheimer Disease [CERAD] verbal episodic memory test on learning [score range, 0-30], recall [score range, 0-10], and recognition [score range, 0-20]).

RESULTS Final analyses included data from 473 women (mean [SD] age, 50.10 [15.63] years) and 501 men (mean [SD] age, 51.24 [15.67] years). Visceral adipose tissue was associated with an exacerbation of the negative association of aging with network covariance for women (interaction term β = −0.02; 95% bias-corrected bootstrap CI, −0.03 to −0.01; P = .001) and men (interaction term β = −0.02; 95% bias-corrected bootstrap CI, −0.03 to −0.01; P < .001). Estradiol level was associated with a reduction in the negative association of VAT with network covariance in women (interaction term β = 0.63; 95% bias-corrected bootstrap CI, 0.14-1.12; P = .01), with no significant association in men. In the female midlife subgroup (age range, 35-55 years, when menopause transition occurs), low estradiol levels were associated with lower memory network covariance (Cohen d = 0.61; t_{80} = 2.76; P = .007) and worse memory performance (Cohen d = 0.63; t_{76} = 2.76; P = .007).

(continued)

Key Points

Question Does estradiol mitigate the negative association of visceral fat with structural brain networks and cognitive health?

Findings In this cross-sectional study of a German population-based cohort of 974 adults, higher estradiol levels were associated with increased structural brain network covariance and a reduction in the negative association of visceral fat with network covariance, but only for women. In women, higher estradiol levels were associated with better structural network covariance and cognitive performance during midlife.

Meaning Assessing visceral adipose tissue and hormone profiles, particularly in women during midlife, may be essential for promoting a healthy brain aging trajectory.

+ Supplemental content

Author affiliations and article information are listed at the end of this article.

Abstract (continued)

CONCLUSIONS AND RELEVANCE This study reports a novel association between VAT, estradiol, and structural brain networks as a potential mechanism underlying cognitive decline in women. These findings appear to highlight the need for sex-specific strategies, including VAT and hormonal screening during midlife, to support healthy cognitive aging.

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Introduction

Neuroimaging evidence suggests an association between age-related brain atrophy, cognitive decline, and obesity.[1] Emerging data linking obesity to increased cognitive impairment in old age are concerning given that approximately 39% of the world's adult population are overweight and 13% are obese.[2] Visceral adipose tissue (VAT) is a known risk factor for vascular and metabolic diseases,[3-5] but the results of some studies[6,7] suggest that elevated VAT may also impair cognitive function. Behaviorally, increased VAT is associated with reduced verbal memory, attenuated attention, and lower executive function. Expanded VAT is further linked to brain atrophy, such as decreased hippocampal volume, cortical thickness, and total brain volume.[6,8] Consequently, researchers are starting to view visceral obesity in midlife as a risk factor for dementia and depression in later life[9,10] independent of type 2 diabetes and cardiovascular comorbidities, with the findings of other studies[11,12] suggesting an even stronger association in women.

Expanded VAT is associated with systemic inflammatory biomarkers, proinflammatory cytokines,[13-15] and reduced levels of adipocyte-specific proteins with anti-inflammatory properties, for which substantial sex differences are reported.[13,16] Visceral adipose tissue is also associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and may contribute to cortisol regeneration[17] and vulnerability to stress-induced cortisol reactivity in women.[18] Moreover, if VAT compromises brain structural integrity owing to its association with systemic inflammation and HPA dysregulation, estradiol may have a protective role due to its anti-inflammatory properties and ability to strengthen HPA robustness.[19] This is in line with menopause transition risk models for neuropsychiatric disorders, which suggest that ovarian hormone fluctuations induce alterations in stress response pathways.[20] Further support for the moderating role of estradiol stems from the finding that estradiol replacement reduces metabolic syndrome symptoms in estradiol-depleted women.[21-23] Estradiol also has vasodilatory, antiapoptotic, and antioxidative effects, which could help preserve myelin architecture.[24-26] Although VAT and estradiol appear to have opposing roles in association with healthy brain aging, it remains unclear how they interactively alter brain network structure. This has serious implications for female cognitive decline in later life because women are potentially more sensitive to the effects of VAT on cognition.[7] Moreover, dementia in women is likely influenced by midlife obesity,[27] a time also characterized by rapid, unstable decreases in estradiol.[28] To investigate these associations in adults, we require integration of brain, abdominal, hormonal, and cognitive data.

We addressed these issues in a novel way by integrating measures of brain network structure, VAT, sex hormones, and cognitive function in a comprehensive data set of 974 participants (473 women) aged 19 to 79 years. We first characterized a structural brain network that shows accelerated degeneration with aging and, when compromised, has been associated with poor memory performance and vulnerability to unhealthy aging and disease.[29,30] After validating that this network correlated with memory performance, we explored how associations between VAT, estradiol levels, and structural brain network covariance differ between men and women. To our knowledge, this is the first study to examine how these factors interact to shed light on biological mechanisms underlying cognitive decline. In a secondary analysis, we investigated how estradiol levels are associated with network covariance and memory performance specifically in midlife women (age range, 35-55 years) because this is a crucial transition point when midlife obesity is a recognized risk

factor for dementia[27] and estradiol levels are known to fluctuate during the menopause transition.[28] Based on the research summarized above linking brain network structure, VAT, estradiol levels, and cognitive function, we hypothesized (1) that VAT would be associated with an amplification of the negative association of age with brain network structure and cognitive health and (2) that estradiol would be associated with a reduction in the negative association of VAT with structural network covariance and cognitive performance in women.

Methods

Participants

Data from 1159 participants were taken from a German population-based cohort study, the Health Study of the Leipzig Research Centre for Civilization Diseases (LIFE). Participants provided written informed consent after all procedures were explained. The protocol and informed consent forms were approved by the research ethics board of the University of Leipzig. Study design and assessment information were described previously[31] (eAppendix 1 in the Supplement). Excluded from analysis were 183 individuals owing to medication intake altering the central nervous system, immunosuppressive medication, previous stroke or other lesions, current diagnosed cancer or cancer treatment during the previous year, head tumors, epilepsy, multiple sclerosis, or Parkinson disease. One individual was excluded because of failure of the macro for fat tissue segmentation and another individual owing to missing anthropometric data needed for VAT normalization. In total, 974 participants were included in the final analyses, all of whom were confirmed to not have dementia during a neuropsychological assessment, including the Mini-Mental State Examination, by a trained study physician. Dates of the original cohort study were August 1, 2011, to November 23, 2014. Analyses were conducted from August 2017 to September 2018. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.[32]

Brain and Abdomen Imaging

Magnetic resonance imaging (MRI) data were acquired on a 3-T imaging system (Magnetom Verio; Siemens). It was equipped with a 32-channel head array coil and body coil for the transmit-receive coil of abdominal scans.

Abdominal Data Acquisition and Analysis

Magnetic resonance imaging was performed using an axial T1-weighted fast spin-echo technique with the following variables: echo time of 18 milliseconds per repetition time of 520 milliseconds, echo train length of 7; slice thickness of 5 mm, 5 mm between slices; scanning matrix of 320 × 306 pixels (no partial Fourier); and field of view of 500 mm ×375 mm, final voxel size of 1.6 mm ×1.6 mm ×5.0 mm, water saturation. Nine slices under and 10 slices above the umbilicus, diaphragm excluded, were segmented. The abdominal fat tissue segmentation was graphically evaluated using a macro to distinguish visceral or subcutaneous fat semiautomatically in ImageJ (https://imagej.nih.gov/ij/download/) by 4 raters (M.H., M.R., U.S., and a nonauthor) for accuracy. The results were inspected visually (slice by slice, identifying misclassified fat and nonfat voxels) and corrected for minor voxel misclassifications manually in 897 participants (eAppendix 2 in the Supplement).

Brain Data Acquisition and Analysis

We collected a 3-dimensional magnetization-prepared rapid acquisition with gradient echo (MPRAGE) sequence using the Alzheimer Disease Neuroimaging Initiative (ADNI) standard protocol with the following variables: inversion time of 900 milliseconds, repetition time of 2300 milliseconds, echo time of 2.98 milliseconds, flip angle of 9°, band width of 240 Hz per pixel, image matrix of 256 × 240 pixels, 176 partitions, field of view of 256 × 240 × 176 mm^3, sagittal orientation, 1 average. Voxel size was 1 × 1 × 1 mm^3, with no interpolation.[33,34] We processed structural T1-weighted data with FSL-VBM (FSL, version 5.0.9), an optimized voxel-based morphometry

protocol using FMRIB Software Library (FSL) tools. All structural images were brain extracted, gray matter segmented, and registered to the Montreal Neurological Institute (MNI) 152 standard space using nonlinear registration. A symmetric study-specific gray matter template was built from images of the study population. All native gray matter images were nonlinearly registered to this study-specific template and "modulated" to correct for local expansion or contraction due to the nonlinear component of the spatial transformation. Modulated images were smoothed with an isotropic gaussian kernel with a sigma of 4 mm (approximately 9.4-mm full width at half maximum). Before FSL-VBM processing, volumes were masked by the full brain–segmented volume output from FreeSurfer (FreeSurfer, version 5.3.0) to exclude nonbrain compartments. Brain structural information was derived from vertexwise cortical thickness, and surface area was calculated in FreeSurfer by an automated surface reconstruction scheme. We inspected all surface reconstructions for misplaced boundaries in FreeView (implemented in FreeSurfer) and manually corrected 142 cases. Cortical thickness and surface area maps were sampled from participant space to the common FsAverage template (163 842 vertices) and smoothed with a surface full width at half maximum of 10 mm. Linked independent component analysis was then applied to measures of gray matter volume, cortical thickness, and pial area (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLICA), decomposing the data into 70 independent components. For each component, we calculated an individual course per participant, further indicated as network covariance,[35,36] which reflects individual loading on the brain network.

Memory Testing

We used the 10-word verbal episodic memory test from the neuropsychological test battery of the Consortium to Establish a Registry for Alzheimer Disease (CERAD),[37] from which we calculated a composite score per participant according to previous studies[30,38-40] (eAppendix 3 in the Supplement). The composite score was from the CERAD verbal episodic memory test on learning (score range, 0-30), recall (score range, 0-10), and recognition (score range, 0-20). To assess performance independent of educational achievement, all memory performance scores used are unstandardized education residuals.

Hormone Measurement

Serum estradiol levels from fasting blood were assessed in a subsample of 390 participants (181 women). Estradiol level was measured by electrochemiluminescence immunoassay (ECLIA) (Cobas; Roche), with a sensitivity of 5.01 pg/mL and representative interassay coefficients of variation of 4.4% to 9.9% for the range of 85 to 110 pg/mL and 2.7% to 5.6% for the range of 501 to 555 pg/mL (to convert estradiol level to picomoles per liter, multiply by 3.671).

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 24 (IBM) and R 3.3.2 (R Foundation). Visceral adipose tissue values were height standardized and log-transformed. Estradiol levels were log-transformed. We performed a linear regression with memory network covariance as the independent variable and memory performance as the dependent variable, controlling for age. To investigate VAT accumulation rates, we generated regression models for women and men separately using age as the independent variable and VAT as the dependent variable for each 1-year age bin. To assess best fit, we compared R^2 values of linear, quadratic, and polynomial fit of third degree. To examine sex interactions in the association between VAT and memory network covariance, we performed a multiple linear regression with VAT as the independent variable and network covariance as the dependent variable. To assess the association between estradiol level and this network independent of age, we performed a linear regression with estradiol level as the independent variable and network covariance as the dependent variable using unstandardized age residuals of network covariance.

Moderation analyses were conducted separately per sex with the PROCESS macro (SAS Institute), a modeling program using an ordinary least squares–based path analytical framework to test for direct and indirect associations.[41] We tested regression pathways in a moderation model (first model) (PROCESS, version 3.0) (eFigure 1 in the Supplement). Variables were mean centered before analyses. A 95% bias-corrected bootstrap CI (BBCI), excluding zero and based on 10 000 bootstrap samples, was considered to be a robust result.[41] In the first model, we assessed significance and stability of the interaction of VAT and age in association with network covariance by defining age as the independent variable, network covariance as the outcome variable, and VAT as the moderator variable. In the second model, we defined VAT as the independent variable, memory network covariance as the outcome variable, and estradiol as the moderator variable.

Finally, we assessed a subgroup of 82 women in midlife (age range, 35-55 years, when menopause transition occurs). In these women, we performed an estradiol-level median split and compared the mean differences in memory network covariance and memory performance using independent-samples *t* tests. All testing was 2 sided, and $P < .05$ was considered statistically significant.

Results

In total, 974 participants were included in analyses (**Table**). The female sample (n = 473) had a mean (SD) age of 50.10 (15.63) years (age range, 20-78 years), and the male sample (n = 501) had a mean (SD) age of 51.24 (15.67) years (age range, 19-79) years.

Structural Brain Network Covariance and Memory Performance

We replicated a structural network linked to memory performance and cognitive decline[29] (**Figure 1**A, eAppendix 4 in the Supplement for network reproducibility analyses, and eFigure 2 in the Supplement for other networks). The network explains 17% of total variance in the imaging data, revealing a transmodal network of cortical and limbic gray matter regions. The network is composed of 57% gray matter volume (explaining 20% of total gray matter volume variance), 42% cortical thickness (explaining 29% of total cortical thickness variance), and 2% pial area (explaining 1% of total pial area variance). Higher individual structural covariance was associated with better memory performance (adjusted $R^2 = 0.21$; $\beta = 0.46$; $P < .001$) (Figure 1B). Data indicate an expected ceiling effect for memory performance in a healthy population.[42]

Sex Differences in VAT Volume Across Age

We obtained VAT volume information by segmenting visceral from subcutaneous fat in abdominal MRI (**Figure 2**A). Men and women displayed different trends in VAT accumulation. For men, a

Table. Study Population Demographic Characteristics

Variable	All	Women	Men	*P* Value
Age, y				
No. of participants	974	473	501	.26
Mean (SD)	50.69 (15.65)	50.10 (15.63)	51.24 (15.67)	
BMI				
No. of participants	974	473	501	<.001
Mean (SD)	26.47 (4.62)	25.87 (5.13)	27.04 (4.00)	
VAT, cm^3				
No. of participants	974	473	501	<.001
Mean (SD)	2188.40 (1462.27)	1569.93 (1059.40)	2772.30 (1548.57)	
Estradiol, pg/mL				
No. of participants	390	181	209	<.001
Mean (SD)	40.76 (73.68)	61.84 (103.89)	22.50 (9.61)	

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); VAT, visceral adipose fat tissue.

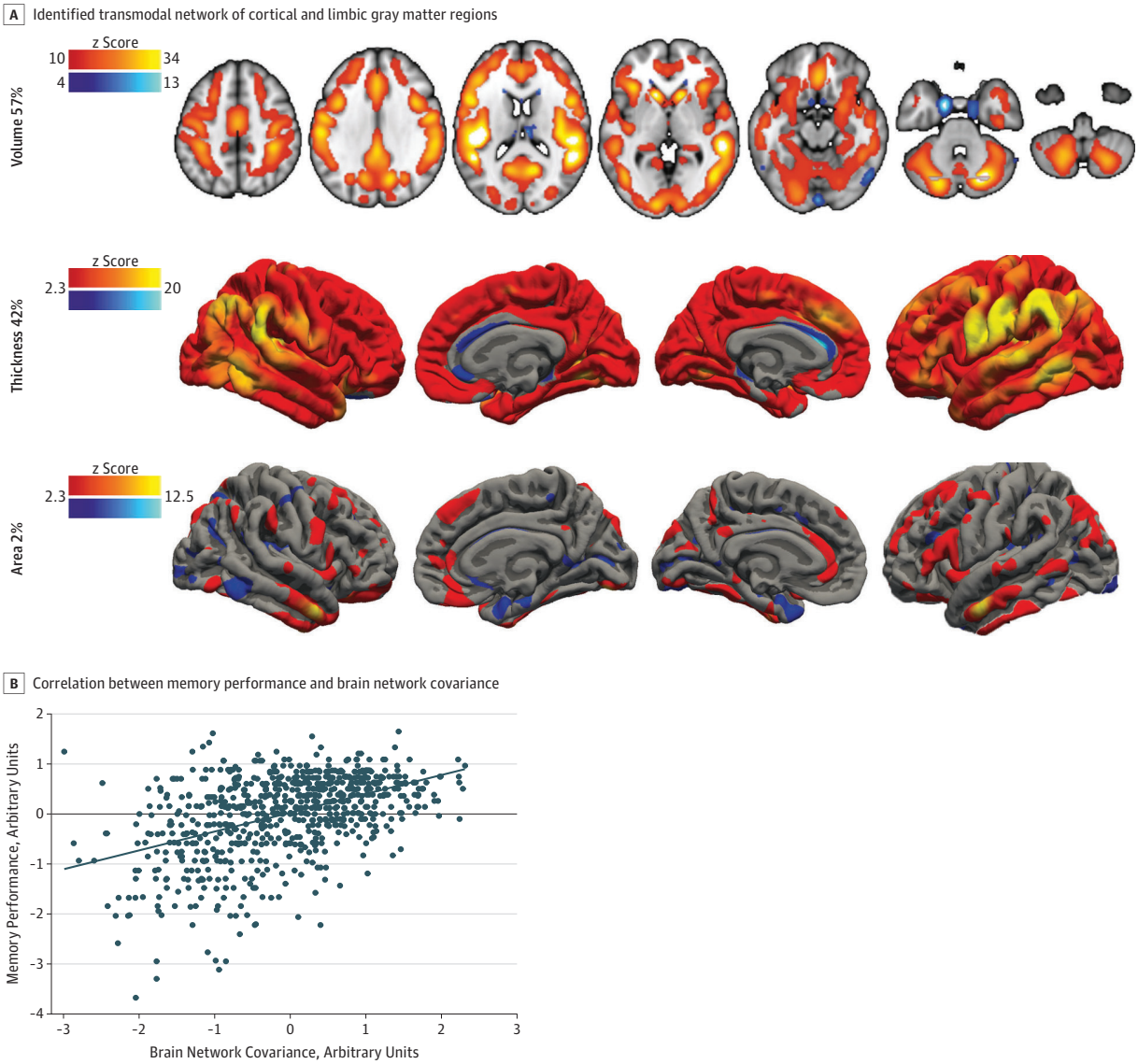
SI conversion factor: To convert estradiol level to picomoles per liter, multiply by 3.671.

quadratic model revealed the best fit (adjusted R^2 = 0.93; P < .001) compared with the linear model ($F_{1,58}$ = 101.56; P < .001) (Figure 2B). The quadratic function showed a concave progression with a steady slope decrease. For women, a polynomial model of third degree showed the best fit (adjusted R^2 = 0.85; P < .001) compared with the linear model ($F_{1,59}$ = 4.25; P = .02) and quadratic model ($F_{2,58}$ = 7.74; P = .007). The cubic curve progression began convex and became concave at the inflection point of 47 years. Therefore, men in this sample had the highest VAT to age ratio at an earlier age, and this decreased with age; in contrast, women had the highest VAT to age ratio during midlife.

Sex Differences in VAT and Memory Network Association

We observed an interaction of VAT and sex on memory network covariance (F_3 = 163.37; adjusted R^2 = 0.33; P = .02): men showed a stronger negative association between VAT and network covariance (adjusted R^2 = 0.33; β = −0.57; P < .001) than women (adjusted R^2 = 0.29; β = −0.54;

Figure 1. Brain Network Covariance and Memory Performance



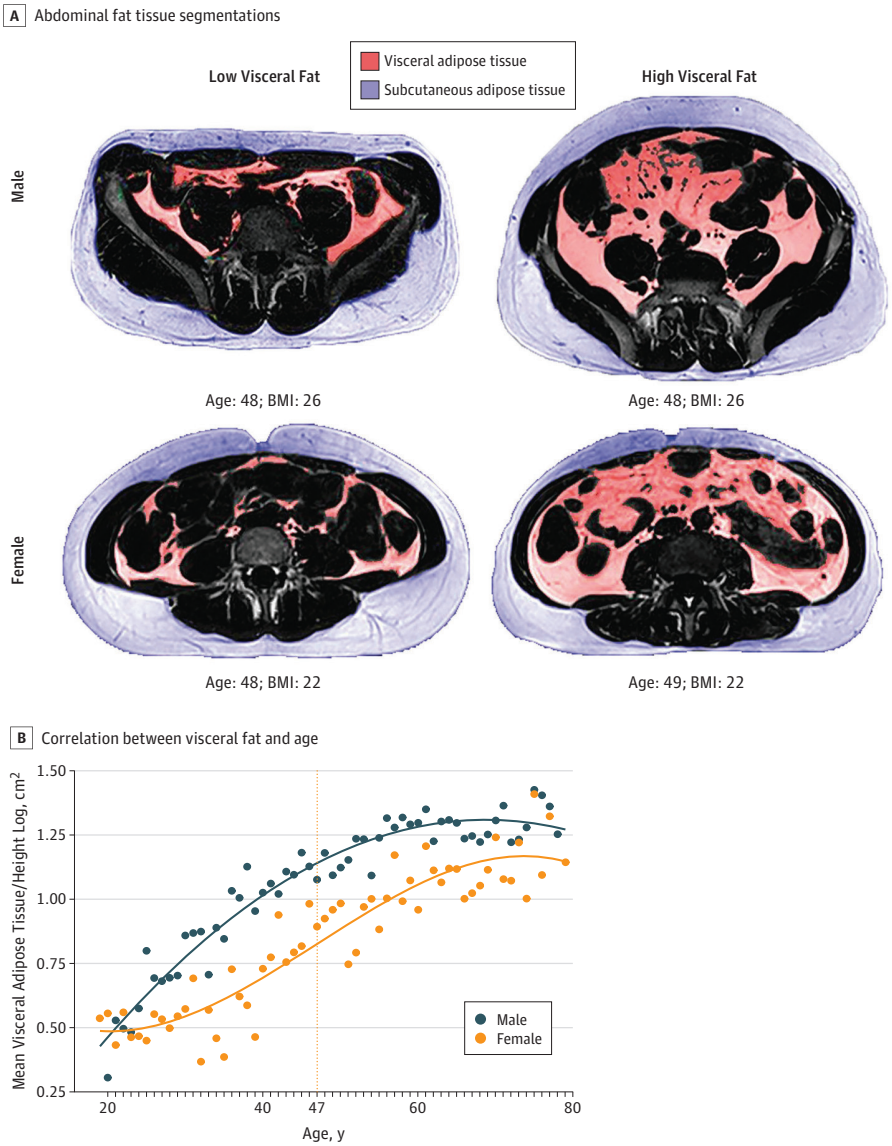
Color bars indicate z-scored positive (red/yellow) or negative (blue/light-blue) covariations within the network.

$P < .001$) (**Figure 3**A). We conducted a moderation analysis in both sexes (Figure 3B) and found that overall models for men ($F_{3,497} = 474.43$; $R^2 = 0.74$; $P < .001$) and women ($F_{3,469} = 322.31$; $R^2 = 0.67$; $P < .001$) were significant. The interaction of VAT and age was significant for women (interaction term β = −0.02; $t_{469} = 4.07$; 95% BBCI, −0.03 to −0.01; $P = .001$) and men (interaction term β = −0.02; $t_{497} = -4.83$; 95% BBCI, −0.03 to −0.01; $P < .001$), suggesting that VAT is a moderator of the association between age and network covariance in both sexes and is associated with an exacerbation of the negative association of aging with memory network covariance.

Sex Hormones and Memory Network Covariance

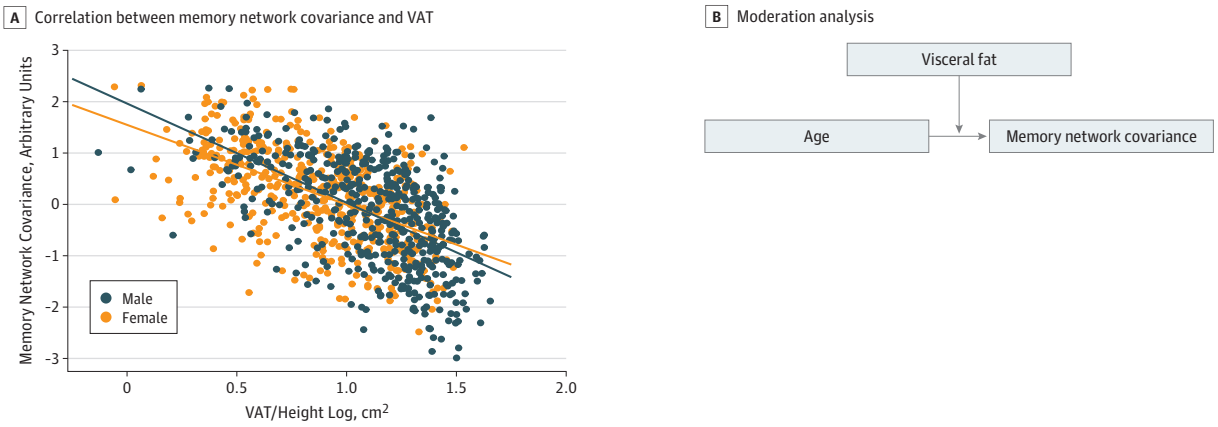
Linear regression analysis revealed a significant positive association between estradiol levels and memory network covariance in women after adjustment for age (adjusted $R^2 = 0.07$; $P = .002$) (**Figure 4**A). In the next set of moderation analyses (Figure 4B), we again found that overall models were significant for both men ($F_{3,205} = 30.13$; $R^2 = 0.31$; $P < .001$) and women ($F_{3,177} = 19.60$;

Figure 2. Sex Differences in the Association Between Visceral Adipose Tissue and Age



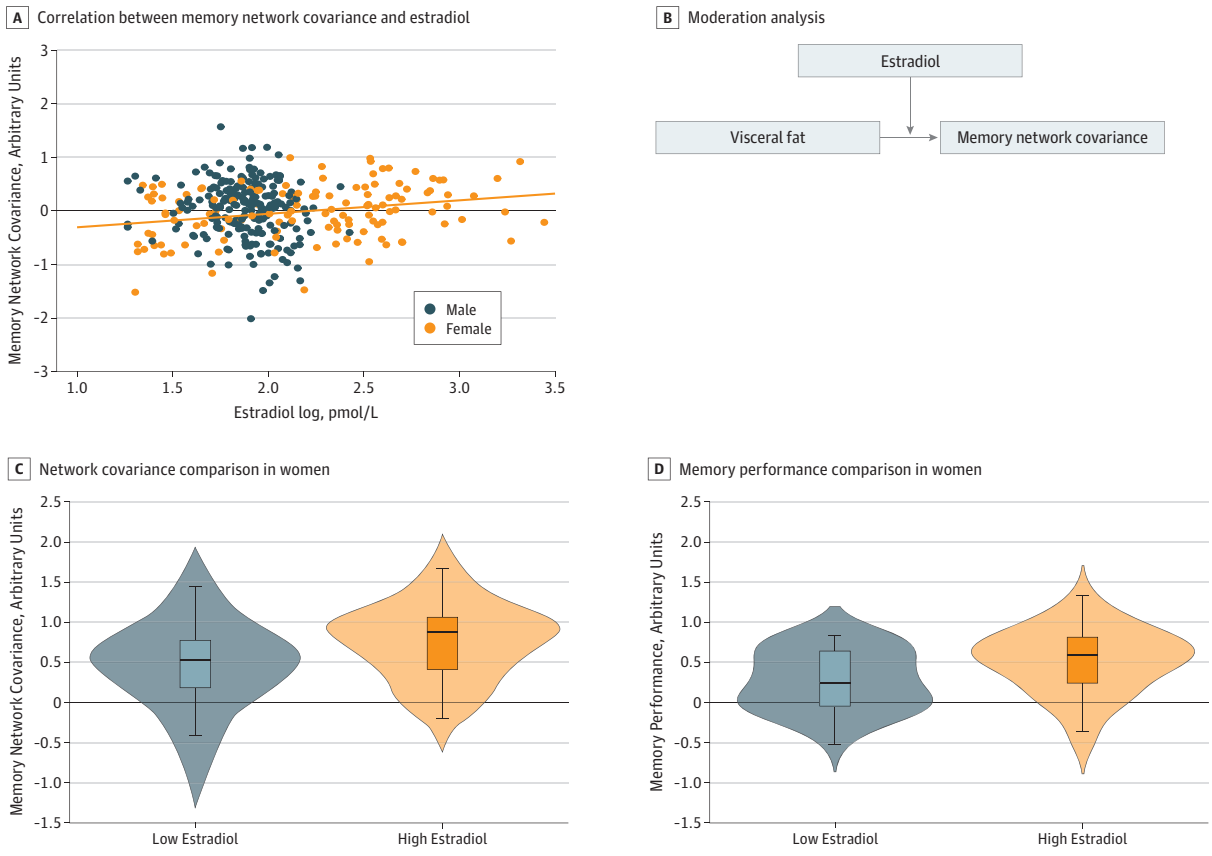
A, Representative visceral adipose tissue segmentations in 2 women and 2 men. Participants of the same sex, age, and body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) can have vastly different fat distribution profiles. B, Nonlinear correlation between visceral adipose tissue and age in women and men. For women, the dashed line shows the inflection point.

Figure 3. Visceral Adipose Tissue (VAT) and Structural Network Covariance



A, The plot shows simple slopes of VAT and structural network covariance for each sex, which differ significantly. The VAT values were height standardized and log-transformed. B, Moderation analysis of interaction of VAT and age on network covariance in both sexes.

Figure 4. Estradiol and Structural Network Covariance in Women Only



A, The plot shows a simple slope of estradiol and structural network covariance. B, Moderation analysis of interaction of visceral adipose tissue and estradiol on network covariance. C and D, The inner box plot shows the median (centers) and interquartile range (borders), with whiskers extending 1.5 times the interquartile range. The width of the shaded area shows the proportion of data located there. To convert estradiol level to picograms per milliliter, divide by 3.671.

R^2 = 0.25; P < .001). However, the interaction of VAT and estradiol level was significant only for women (interaction term β = 0.63; 95% BBCI, 0.14-1.12; t_{177} = 2.52; P = .01), suggesting that estradiol is a moderator of the association between VAT and network covariance in women and is associated with a mitigation of the negative association of VAT with memory network covariance.

Finally, in the female subgroup (age range, 35-55 years), the low estradiol and high estradiol groups did not differ by age or VAT. However, low estradiol level was associated with lower memory network covariance (Cohen d = 0.61; t_{80} = 2.76; P = .007) (Figure 4C) and worse memory performance (Cohen d = 0.63; t_{76} = 2.76; P = .007) (Figure 4D).

Discussion

To our knowledge, this is the first large, population-based study to investigate associations between structural patterns of brain aging, VAT as a metabolic risk factor for structural brain atrophy, and estradiol levels in adults of a broad age range. Our primary finding is that, while VAT was associated with increased risk for compromised brain network structure and cognitive impairment in both men and women, estradiol level was associated with reducing the negative consequences of VAT in women. Specifically, men had the highest VAT to age ratio at an earlier age; in women during midlife, VAT was associated with accelerated cognitive aging, and estradiol may protect the female brain against these structural patterns of atrophy, particularly during midlife. Our results have important clinical implications for developing sex-specific strategies to support healthy cognitive aging.

To effectively preserve cognitive abilities throughout life, it is imperative to consider sex-specific risk trajectories and identify biological mechanisms that may degrade or protect relevant brain network integrity. Integrating MRI-based VAT in large cross-sectional neuroimaging studies on cognitive function represents a novel approach to identify such a mechanism. We specifically included VAT as the main adiposity measure given the well-established role of VAT in conferring metabolic and inflammatory risk.[3-5,13] Assessing VAT volume by MRI rather than with more conventional and easily accessible anthropometric proxies, such as body mass index or waist to hip ratio, is even more critical given the findings indicating VAT as a unique risk factor for neurodegenerative processes.[10,43-45] Our analysis revealed substantial sex differences not only in VAT accumulation but also in the association between VAT and brain health: while both sexes showed a negative association of VAT with memory network covariance, this association was stronger in men.

When studying a brain network implicated in cognitive decline, it is difficult to control for changes accompanying natural aging. To disentangle the associations of biological aging from the association of VAT with memory network covariance, we tested VAT as a moderator variable in the association between age and the memory network. Our analysis revealed a significant interaction of VAT and age on network covariance, suggesting that VAT is associated with accelerated brain aging. Our findings thus substantially extend prior research showing an association of VAT with brain tissue damage independent of age associations.[46] While those authors applied age correction in their analysis, the study was conducted in elderly individuals (mean age, 65 years); therefore, the possibility of age as a confounder for the sample could not be excluded.

Although multiple mechanisms may have contributed to the observed sex differences in the association between VAT and network covariance herein, differences in ovarian hormone states across the life span, particularly estrogen fluctuations, have been shown to influence cognitive aging processes.[47,48] Thus, we investigated the role of estradiol and identified a positive association between estradiol and network covariance in women after age correction. Although the effect size appears small, small associations can suggest strong support for a given phenomenon, particularly if they have substantial cumulative consequences.[49] Our findings indicate that ovarian aging goes beyond biological age-related brain changes and adds an additional layer to this process. This is important because of the prominent sexual dimorphism in neuropsychiatric disorder rates. A detailed understanding of these processes may provide critical insight into how to address the higher rates of depression[50] and dementia[51,52] in women. A subsequent moderation analysis revealed a

significant interaction of VAT and estradiol on network covariance in women, suggesting that estradiol is a significant moderator of the VAT-brain association in women. This association could be accounted for by several mechanisms. First, estradiol has anti-inflammatory properties, and systemic inflammation has been shown to occur in response to estradiol depletion in women (eg, after ovariectomy,[53] natural menopause,[54] or treatment with the antiestrogenic drug tamoxifen[55]). Second, VAT increases HPA axis dysregulation, which could harm the brain, and estradiol strengthens robustness of HPA activity. Third, estradiol has vasodilatory, antiapoptotic, and antioxidative effects that could have a neuroprotective role and help preserve myelin architecture.[24-26] The present study cannot identify what the mechanism is behind how VAT damages structural networks or how estradiol provides a potential protective association. However, our study results argue for longitudinal studies to assess changes in ovarian hormones, VAT accumulation, cognitive performance, and structural brain networks in women during perimenopause to provide insight into a transition period that may serve as the tipping point into neurodegenerative disease. Moreover, given growing evidence that fluctuations in ovarian hormones and their derived neurosteroids induce HPA axis dysregulation, thereby driving vulnerability to psychosocial stress and neuropsychiatric disease,[20] a future study could benefit from including markers of HPA axis function in conjunction with psychosocial stress evaluation.

Finally, we investigated the association of estradiol with cognitive health in women during midlife (age range, 35-55 years) because this is when we observed an inflection point in the curve for VAT ratios in women in our cross-sectional sample. It is also the age range when women typically experience rapid estradiol fluctuations and significant estradiol depletion during perimenopause.[28] We found less healthy patterns of memory network covariance and weaker memory performance in women with lower estradiol levels compared with women with higher estradiol levels during the perimenopausal age range. Because these women were VAT- and age-matched, our findings suggest that associations of ovarian aging with body and brain may extend beyond changes in body composition and brain network structure typically observed during biological aging.[56,57] This could explain why VAT had less of a negative association with network covariance in women than in men.

Limitations

It is important to acknowledge the methodological limitations of data-driven neuroimaging analysis, particularly regarding the interpretation of structural brain covariance changes in terms of cellular mechanisms.[29,58] Specifically, structural MRI is limited to probing information on a macroscopic scale, and extraction of morphological features of interest from MRI remains imperfect, thus making it difficult to disentangle the contributing cellular mechanisms. Despite these constraints, experts[29,58] still agree that this methodological approach represents a powerful tool to obtain unique insights into human brain organization. By combining this approach with cutting-edge abdominal adiposity imaging and assessment of sex hormone levels—and ultimately relating these multimodal measures to memory performance in a large sample—we provide a biologically relevant view of the consequences of VAT on brain network structure and cognitive abilities. Furthermore, not all measures herein (estradiol levels and educational achievement) were available for all enrolled participants owing to technical reasons; therefore, some analyses reported could only be conducted in subgroups. We also acknowledge that the CERAD verbal episodic memory test shows ceiling associations in young healthy participants, but the observation that the identified brain network in our sample is associated with memory performance and cognitive decline has also been demonstrated with other cognitive tests.[29] In addition, brain aging is a multifaceted process; while we applied strict screening criteria to exclude neurodegenerative or cerebrovascular disease and controlled for age and educational achievement, we cannot exclude that other variables could contribute to this process. Inherent to the cross-sectional design, no causal association can be inferred because all reported associations are correlational; therefore, future studies applying within-subject modeling are needed.

Conclusions

Given the dramatic increase in human life expectancy over the past century, age-related cognitive decline is rapidly becoming one of the biggest health challenges we face.[59-61] Herein, we identified sex-specific risk trajectories of brain and cognitive aging. We provide evidence for a detrimental association of VAT with structural brain networks important for memory and a potential protective association of estradiol with cognitive health in women through maintaining gray matter network integrity. These data underscore the need to consider adipose tissue and hormonal profiles during primary care visits in midlife to support healthy brain aging and maintain cognitive abilities in later life. Our findings highlight the perimenopausal transition as a window of opportunity to prevent accelerated brain aging and neurodegenerative disease development in women.

ARTICLE INFORMATION

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SUPPLEMENT.

eAppendix 1. Participant Recruitment
eAppendix 2. Abdomen: Data Acquisition and Analysis
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IV. Discussion and Outlook

1. Summary of findings

To our knowledge, this is the first cross-sectional study of a population-based cohort with adults of a broad age range to show sex differences in (1) abdominal fat distribution patterns, (2) VAT accumulation over the lifespan, (3) associations of VAT with a structural GM network critical for memory performance, and to reveal (4) endogenous oestradiol as a moderator of the association between VAT and the structural GM network covariance in women. Our results provide further support for lower rates of VAT in women compared to men, and for men to display their highest VAT-to-age ratios at an earlier age than women. Specifically, we found a negative association between VAT and the GM network covariance, with a stronger association in men. Our results suggest oestradiol as a moderator of this inverse association between VAT and the GM network covariance in that it appears to attenuate the negative consequences of VAT on the network integrity in women. When we specifically assessed the subgroup of women during midlife (35-55 years) when perimenopause occurs, higher oestradiol levels were associated with healthier patterns of the structural brain network as well as with better memory performance.

While the findings have been extensively reviewed in the published manuscript, this 'Discussion and Outlook' section intends to extend the discussion in regards to: methodological aspects of the MRI-based analyses, strengths and weaknesses of the work, suggestions for future study designs as well as clinical implications of sex-specific risk trajectories for unhealthy brain ageing.

2. Sex-specific accumulation patterns of visceral adipose tissue volume across age: a link to perimenopause

We observed sex differences in measures of abdominal fat distributions (VAT and SCAT), finding women to have lower amounts of VAT compared to men. These results are in line with previous findings (Lemieux et al., 1993; Machann et al., 2005) (details see Publication, Table). Moreover, our analyses display sex-specific patterns for highest VAT accumulation at different time-points. In our data, men showed the highest VAT-to-age ratio at an earlier age, while women showed the highest ratio at 47 years of age (details see Publication, Figure 2B). This may suggest that men accumulate VAT fastest at an earlier age and women accumulate VAT fastest during midlife. These findings support the conclusion that premenopausal women tend to store more fat in the gluteofemoral subcutaneous region than in the abdominal cavity. SCAT in the gluteofemoral region is associated with lower inflammatory properties compared to VAT accumulation within the abdominal cavity,

suggesting healthier metabolic patterns in premenopausal women (Fox et al., 2007). In addition, the findings indicate that midlife is associated with increased accumulation of VAT in women. Our data complement results from a previous study by Lovejoy et al. (2008) that focused on women during perimenopause and found that higher VAT accumulation during midlife is associated with the fluctuations in ovarian hormones that start approximately three years before the onset of menopause. The authors suggest that perimenopause represents a critical time window for accelerated VAT accumulation (Lovejoy et al., 2008), which may be associated with heightened risks for adverse metabolic and inflammatory profiles (Bays et al., 2008).

To conclude, changes in hormonal blood levels, especially decrease in oestradiol, are associated with lower lipolytic rates in the abdominal region promoting storage fat in the abdominal cavity (Poehlman et al., 1995; Toth et al., 2000). Additionally, the perimenopausal drop of oestradiol may trigger the generation of new fat cells, which produce aromatase. This enzyme catalyses the biosynthesis of oestrone and oestradiol (Simpson et al., 1997). Due to higher amounts of adipocytes and subsequent higher aromatase activity, the lack of endogenous oestrogen during perimenopause could be partially compensated but this is associated with higher volumes of fat tissue, especially VAT (Bélanger et al., 2002). The inflection point in the age dependent VAT accumulation curve for women at 47 years of age (details see Publication, Figure 2B) coincides with perimenopause and the time when oestradiol levels drop and ovarian functioning starts to decline. Although the onset of menopause is highly variable amongst women, the general onset has been postulated at 51 years of age (McKinlay et al., 1985). This timing fits in the hypothesis that perimenopause, a time with natural fluctuations of ovarian hormones, is associated with greater VAT gain. This may be critical for dementia risk in later life as we showed that VAT displays a negative association with a GM network linked to memory performance.

3. Sex differences in the negative association between visceral adipose tissue and a structural grey matter-network linked to cognitive function

We observed sex differences in the inverse association of VAT and GM volume and cortical thickness in a multimodal, age- and memory related structural GM network with a stronger association in men (details see Publication, Figure 3). The spatial map of the GM network includes cortical and subcortical regions, which typically reveal early structural changes in the course of dementia (Karow et al., 2010; Schroeter et al., 2009) (details see Publication, Figure 1A). This structural GM network has been previously linked to ageing and shows an overlap with specific spatial patterns associated with AD (Douaud et al., 2014). After testing that this network was indeed associated with memory performance in our own

dataset (details see Publication 1B), we found an inverse association between VAT and GM network covariance with a stronger effect for men (details see Publication, Figure 3). Previous research has raised VAT as a risk factor for brain atrophy and cognitive decline (Debette et al., 2010; Veit et al., 2014; Widya et al., 2015). Our findings extend prior research showing sex differences in the negative association between VAT and brain structure. Increased VAT has also been linked to the development of metabolic syndrome via promoting chronic low grade inflammation through higher levels of C-reactive protein (CRP), misbalanced adipocytokine-cytokine secretion, arterial hypertension, insulin resistance and an adverse lipoprotein profile (Després et al., 2008). These metabolic factors and VAT as an inflammatory tissue itself have been associated with heightened risk for developing AD and other dementias (Cereda et al., 2007; Kivipelto, 2001; Ott et al., 1999; Solomon et al., 2009). Moreover, human research links inflammation to cognitive impairment based on evidence linking increased CRP- (Sweat et al., 2008) and interleukin-6 levels (Gimeno et al., 2008), as well as VAT accumulation (Isaac et al., 2011) to attenuated cognitive performance. Interestingly, this effect has been predominantly observed in women in these studies. Therefore, the inflammatory response following excessive VAT accumulation may be greater in women, and thus brain integrity in women may be more susceptible to metabolic stress with associated heightened vulnerability for reduced cognitive functioning. It is therefore imperative to identify factors which protect the female brain from inflammation and oxidative stress throughout the lifespan.

4. Oestradiol attenuates the negative association between visceral adipose tissue and a memory-related structural brain network in women

The present study provides evidence to suggest that endogenous oestradiol may act as such a protective factor for brain structure in women. The negative association between VAT and structural network covariance was stronger in men than in women. Hereby, oestradiol serum levels were associated with an attenuation of the negative association between VAT and the GM network covariance in women (details see Publication, Figure 4A & B). The moderating effect of endogenous oestradiol on the interaction between VAT and a structural network in women represents a novel finding within the research field of neuroimaging studies. Finally, oestradiol levels are positively associated with network covariance and memory performance in a subgroup of women at midlife independent from age (details see Publication, Figure 4C & D). This finding suggests that associations between ovarian ageing and metabolism may provide additional insight into observed ageing processes in the brain across the female lifespan. Given that during midlife, women start losing oestradiol and accumulate VAT faster, the course of interaction of these processes

during perimenopause may be critical to our understanding of how to maintain cognitive health of the female brain in later life.

5. Perimenopause as a critical neurological window

Our results add to the literature supporting perimenopause as a critical neurological transition state for women and indicate that depletion of endogenous ovarian hormones can have significant implications for brain ageing (Brinton et al., 2015). This may be an important aspect to consider in order to understand why women have higher prevalence rates in dementia (Prince et al., 2014). Our findings highlight the importance of sex-specific clinical management of these neuropsychiatric and neurodegenerative diseases.

6. Strengths

Strengths of the current study include the large and well-characterised sample (974 participants from the LIFE study, that recruited an age- and sex-stratified random sample of adults in Leipzig, Germany) and the multimodal approach, which incorporates MRI acquisitions of the abdomen and the head (Loeffler et al., 2015). We integrated brain analysis results with cognitive assessments to evaluate functional aspects of the structural GM network, replicating evidence that supports functional relevance to memory for this brain network. Moreover, we performed all analyses with MRI-based quantified measures of fat tissue within the abdominal cavity, and ensured validity of the fat segmentation algorithms through high interrater variability. This study is the first to combine analyses of MRI-based VAT volumes, multimodal structural brain imaging, cognitive testing and hormone measurements in a healthy population-based cohort controlling for educational status and age. Several structural neuroimaging studies in that field focused on either whole or local brain GM volume using voxel-based morphometry (VBM), (Debette et al., 2010; Hamer & Batty, 2019; Kurth et al., 2013; Pannacciulli et al., 2006) or GM thickness (Veit et al., 2014). In the present study, we go beyond this work by combining three different measures of GM, namely VBM-based GM volume, cortical thickness and cortical surface area using linked independent component analysis (see Groves et al., 2012 for further information). This combination of modern techniques for MRI segmentation algorithms and multiple regression analyses in a large dataset provides novel insight into the complex field of sex-specific brain ageing.

7. Limitations

7.1 Limitations of the study population

We acknowledge that there are several limitations to the study. Firstly, for our study sample, we used strict exclusion criteria such as neurodegenerative disorders, previous stroke or other brain lesions, current diagnosed malignant tumors, epilepsy or medication intake altering the central nervous system as well as immunosuppressive medication. While our findings clarify the critical role of endogenous oestradiol and brain structure in a well-powered sample of healthy participants, further studies in clinical populations such as dementia patients is required to transfer these findings to clinical management of the disease. Secondly, as we investigated endogenous oestradiol in healthy women, our results cannot be transferred to women with a hormonal imbalance or pathologic metabolic diseases: For example, women with polycystic ovary syndrome (PCOS) show lower executive function and need additional neural resources during a working memory task (Soleman et al., 2016). In parallel, women who underwent oophorectomy before the natural onset of menopause seem to have higher risk for developing cognitive impairment or dementia (Rocca et al., 2007). Future studies could focus on women with PCOS or who underwent surgical oophorectomy before the onset of menopause. Following, we can only partly transfer our findings on women taking exogenous synthetic ovarian steroid hormones, for example during hormone replacement therapy or hormonal contraception because these synthetic oestrogen are attributed to other biochemical properties than natural endogenous oestradiol or oestrone (Tazuke et al., 1992). Thirdly, our study sample does not cover the age range of puberty, as puberty also represents an important endogenous hormonal transition period.

We acknowledge that data of oestradiol measurement and educational status were not available for each participant due to technical reasons. Therefore some analyses could only be conducted in subgroups. Because of the cross-sectional nature of the LIFE study to date of our research, our participants underwent only one single assessment. However, an additional longitudinal observation of a subgroup of LIFE participants is currently underway with the possibility to reproduce and extend our findings.

7.2 Methodological limitations

Inherent to the cross-sectional design of our study, we are not able to infer any causal associations between the included measures. Our data-driven multimodal neuroimaging approach does not allow for an interpretation of underlying cellular mechanisms (Douaud et al., 2014). Algorithms for analyses of structural MRI remain imperfect, but in general the

applied methods are widely accepted as a methodological approach that provides a unique tool for investigating human brain integrity (Douaud et al., 2014). However, brain ageing is a multifactorial process. Even using strict exclusion criteria as well as controlling for age and educational status, we cannot exclude that more variables could infer with this process. Despite the methodological advantages of a linked independent component analysis which allows investigation of several modalities of brain MRI data simultaneously, we acknowledge the limitation of only using GM modalities. Because WM microstructural damage also represents an important risk factor for cognitive decline and other brain pathologies (Debette & Markus, 2010), future studies combining GM and WM for similar investigations are needed.

8. Outlook: Implications of this thesis for future research and clinical management

Cognitive decline and dementia are growing global health concerns given that, by 2050, the prevalence of AD is likely to reach about 152 million people (Patterson, 2018). Moreover, the risk for developing cognitive decline or dementia shows robust sexual dimorphisms (Prince et al., 2014; Seshadri & Wolf, 2007): Women have an almost twofold increased lifetime risk for developing AD compared to men (Seshadri & Wolf, 2007) and AD is the 6th leading cause of death in the United States with more women than men dying from AD (Heron, 2018). To maintain cognitive and mental health in later life, we must identify preventable risk factors which are associated with higher risk for cognitive decline.

Our results show that visceral fat is associated with higher risk of structural brain atrophy in cognition related areas across the lifespan, and that oestradiol may be protective for the female brain against these structural patterns of atrophy and cognitive decline, particularly during midlife. Perimenopause may thus serve as a neurological transition period for available intervention before a disease-susceptible state tips into an irreversible pathology. We provide evidence for prioritising sex-specific research including the assessment of hormonal and metabolic profiles during hormonal transition states. Identifying risk factors will improve clinical management and may ultimately help in the prevention of dementia risk. Even individual therapeutic options could be drawn from screening results, such as nutrition, hormone supplementation and lifestyle changes. Given that we observed that endogenous oestradiol is associated with healthier patterns of a cognition related area in perimenopausal women, more research is required to create individual prevention and therapy strategies from which women will profit most.

In summary, our sex-specific cross-sectional analyses provide first evidence for a protective role of oestradiol in the negative association of VAT and a memory-related structural brain network in women. For future studies, we recommend the use of longitudinal study designs for sex-specific analyses of the interaction of metabolic state, brain and

cognition. Such studies would benefit from including hormonal data, cognitive and neuropsychological testing, MRI-quantified VAT as well as modern neuroimaging techniques including different modalities of GM and WM. As our study assessed endogenous hormones only, future studies could extend these findings using interventional placebo-controlled randomized trials to explore how exogenous oestrogen interact with adipose tissue and brain areas serving memory and cognitive function during perimenopause. Future work could focus on individual menopausal trajectories of body and brain, for example by assessing several controlled time-points throughout the menopausal transition.

To conclude, the field of neuroscience research needs to continue working towards understanding of the link between the natural loss of ovary functioning during perimenopause, brain and cognitive ageing as well as metabolic mechanisms which may accelerate these ageing processes. Establishing sex-specific screenings of hormonal and metabolic profiles in midlife could be an important stepping stone towards promoting healthy cognitive ageing for women and men in later life.

V. Summary

Dissertation zur Erlangung des akademischen Grades Dr. med.

Titel:

Oestradiol moderates the association of visceral fat on brain structure and cognitive function in women

eingereicht von:

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The number of people with dementia worldwide is estimated to surpass 150 million by 2050, with twice as many women as men predicted to develop the disease, and higher morbidity rates shown for female patients (Patterson, 2018; Podcasy & Epperson, 2016). To prevent development of dementia and neurodegenerative disease in later life, we must identify sex-specific risk trajectories for accelerated brain ageing and cognitive decline in midlife.

Several longitudinal studies report midlife obesity to be a critical risk factor for developing dementia or Alzheimer's disease (AD) in later life (Hassing et al., 2009; Kivipelto et al., 2005; Whitmer et al., 2005). Central adiposity has been specifically associated with both structural grey matter (GM) loss and cognitive decline, initiated by inflammatory processes in visceral adipose tissue (VAT) (Cereda et al., 2007; Debette et al., 2010; Hamer & Batty, 2019; Isaac et al., 2011; Veit et al., 2014). Given the known sexual dimorphisms in abdominal fat distribution patterns (Machann et al., 2005), brain structure (Horstmann et al., 2011; Luders & Toga, 2010; Witte et al., 2010) and in prevalence and morbidity rates of dementia (Seshadri & Wolf, 2007), it is likely that sex hormones play a key role. Previous research hypothesised a critical window of vulnerability during the menopausal transition, when women simultaneously experience drops in ovarian hormones and excessive VAT accumulation, along with higher susceptibility of brain structure to inflammatory processes (Lovejoy et al., 2008; Maki, 2013). So far, we do not know whether ovarian hormones affect the relationship between VAT and brain structure in areas related to cognitive functioning, in part because we lack large cohort studies that include brain, abdominal and hormonal data in both men and women.

Thus, the first aim of this thesis is to clarify if higher VAT volume is associated with lower brain network covariance in a structural GM network previously linked to memory performance and accelerated brain ageing (Douaud et al., 2014; Kharabian Masouleh et al., 2017). We examined potential sex specific differences in that association, and explored whether oestradiol plays a protective role on the brain network. We further hypothesised that a subgroup of women during midlife (35-55 years) with higher oestradiol level display higher covariance of the structural GM network compared to age-matched women with lower oestradiol level.

To test our hypotheses, we examined whether ovarian hormones modulate the relationship between VAT and structural GM networks using linked independent component analysis of GM volume, cortical thickness, and cortical surface area in the large, well-characterised Leipzig Research Centre for Civilization Diseases sample (*N*=974). We found significant sex differences in VAT with a significant interaction between sex and age. A large-scale, age-related GM network linked to cognitive function revealed an inverse interaction with VAT, which was significantly stronger for men. In addition, oestradiol levels showed a positive association with the structural brain network in women.

The present data indicates a sex-specific interaction between visceral fat and structural GM networks linked to cognitive performance. Furthermore, we provide evidence for a protective role of oestradiol in maintaining the brain network organisation, especially during the perimenopausal age range (35-55 years). Our evidence supports a

perimenopausal vulnerability model for the female brain. Possibly due to the loss of ovarian hormone production during perimenopause, women start to experience a visceral-fat associated acceleration of cognition-related structural GM loss. This finding extends our understanding of the interplay between ovarian ageing and age-related brain changes.

Our research provides further support for the critical period hypothesis by identifying higher VAT accumulation during the perimenopausal transition as a potential risk factor for accelerated brain ageing and cognitive decline. In addition, our data suggest that oestradiol may have a neuroprotective role by possibly preventing VAT-induced atrophy in cognition-related brain areas.

These results highlight the necessity for sex-specific strategies, including VAT as well as hormonal screening during midlife, to promote healthy cognitive ageing. We extend previous findings for the perimenopausal transition as a neurological transition state and a window of opportunity to prevent accelerated brain ageing and neurodegenerative disease development in women.

Article included in this thesis:

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VII. Appendix

A. Supplemental material of the publication

Supplementary Online Content

Zsido RG, Heinrich M, Slavich GM, et al. Association of estradiol and visceral fat with structural brain networks and memory performance in adults. *JAMA Netw Open*. 2019;2(6):e196126. doi:10.1001/jamanetworkopen.2019.6126

eAppendix 1. Participant Recruitment

eAppendix 2. Abdomen: Data Acquisition and Analysis

eAppendix 3. Memory Testing

eAppendix 4. Reproducibility of Structural Network From Linked-Independent Component Analysis

eFigure 1. Conceptual and Statistical Diagram for Moderation Analyses

eFigure 2. Spatial Maps of Brain Networks

eReferences.

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. Participant Recruitment. Participant recruitment is previously reported in detail[1]. Briefly, the LIFE study is a population-based cohort study that examined approximately 10,000 randomly selected participants from Leipzig, Germany. The study was conducted by the Leipzig Research Centre for Civilization Diseases. The baseline examination was conducted from August 2011 to November 2014. All participants underwent a core assessment including questionnaires, structured interviews, physical examinations, and biospecimen collection. A subset of these participants additionally completed MRI-based head (>2400) and abdominal scans (>1000).

The sample compromised of age and gender-stratified sample of Leipzig residents. Address lists of randomly selected citizens were given by the resident's registration office. Citizens were sent an invitation letter (information leaflet about the study, a response form, postage-paid return envelope) and a reminder letter (if no response within four weeks). Non-responders were contacted by phone. Participation rate was 33%.

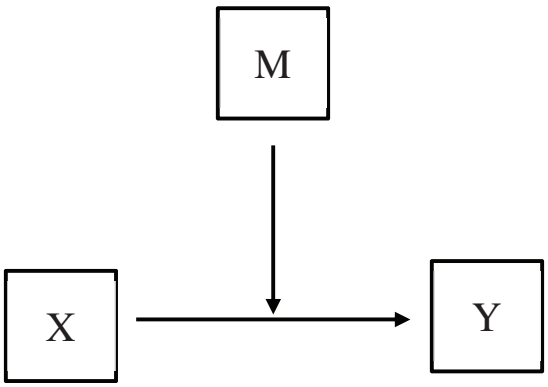
All participants included in the final analyses completed a neuro-psychological assessment with a trained study physician, including the Mini-Mental State Examination (MMSE) to confirm inclusion of cognitively healthy, non-demented participants. We used a clinical cut-off score of 24[2,3], although we acknowledge that the validity of this cut-off score for dementia is still debated in literature.

eAppendix 2. Abdomen: Data Acquisition and Analysis. MR imaging was performed using an axial T1-weighted fast spin-echo technique with the following parameters: TE=18 ms/TR=520 ms, echo train length 7; slice thickness 5 mm, 5-mm gap between slices; scanning matrix 320×306 (no partial Fourier); field of view 500 mm×375mm, final voxel size 1.6 mm×1.6 mm×5.0 mm, water saturation. To avoid breathing artefacts, all participants were asked to hold their breath for 18 seconds, during which 5 slices were recorded. Images were recorded from feet-to-head direction with 5 cm table shift after each acquisition, beginning 10 cm below the umbilicus and finishing in the liver region. All participants were scanned in the supine position with their arms above the abdominal region. We used 20 slices from each participant that included the umbilical region as the center and excluded the diaphragm. We segmented 9 slices under and 10 slices above the umbilicus. This ensured that the umbilical region was included but adipose tissue beyond the diaphragm was excluded in each participant. The graphic evaluation of the abdominal fat tissue was done with ImageJ (https://imagej.nih.gov/ij/download/). We used a macro that quantifies fat pixels semi-automatically using a threshold/histogram algorithm and distinguishes between visceral and subcutaneous adipose tissue, and used manual delineation when necessary. In short, the segmentation algorithm is based on image intensities whereas conventional automated thresholding evaluates fat from non-fat tissue. Using interpolation, the macro calculates adipose tissue volumes as the sum of fat pixels x slice thickness (*ie*, slice+gap). Visceral fat was defined as adipose tissue within the abdominal cavity; subcutaneous fat was defined as adipose tissue between the skin and musculoskeletal tissues. Intramuscular fat was excluded from the analyses. After applying the algorithm, the evaluator visually inspected the results slice-by-slice in order to identify misclassified voxels (fat versus non-fat voxels). Manual corrections for misclassifications were fixed in 897 participants. The most common cases involved myofascial fat tissue being misclassified as subcutaneous fat, and vertebral bodies being misclassified as visceral fat (this is because both have higher intensities in T1-weighted images). To ensure the accuracy of our adipose tissue segmentation method, four different raters applied the segmentation macro on the participants.

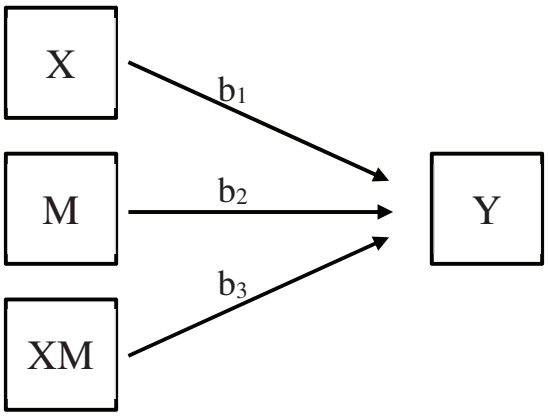
eAppendix 3. Memory Testing. We assessed participants' memory performance using the neuropsychological test battery of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD)[4]. We focused on the 10-word list for the verbal episodic memory test, from which we calculated three sum scores: "learning" was the number of correctly named words out of three consecutive learning trials, "recall" was the number of correctly recalled words from the list after a delay of approximately 5 minutes (during which participants performed a figure copy task), and "recognition" was the number of correctly recognized words from a list of 20 mixed words presented after the recall trial. All test scores were z-transformed. A composite score for the cognitive domain of memory performance was calculated per participant according to previous studies[5-8] as the mean of the three sub-scores. If a sub-score was missing, the composite score was calculated based on the average of the remaining available sub-scores.

eAppendix 4. Reproducibility of Structural Network from Linked-Independent Component Analysis. We visually compared the spatial maps of each component with the structural gray matter network previously linked to memory performance and cognitive decline ([9]; template available at http://www.fmrib.ox.ac.uk/analysis/LIFO+AD+AOS/) and chose the component with the highest accordance. We applied adaptive thresholding to visualize the spatial maps of our network to see where the highest covariations existed (resulting in z values: >14.1247 for positive and <-2.2584 for negative covariations) (see [10] for further details). We then computed voxel-by-voxel spatial cross-correlations with our network and the provided template to ensure the similarity of both spatial maps. Significance of the spatial correlation was calculated using a Monte Carlo approach with randomly generated and smoothed 1000 Gaussian noise images and comparing the strength of our observed correlation with empirically generated null-distribution from 1000 cross-correlations between each of these noise maps and our network (r=0.69 for the raw spatial map, r=0.65 for the thresholded spatial map). Furthermore, we performed a dual regression on the template network with our network using the four-dimensional dataset of gray matter images in a spatial regression against the template network (r=0.74, p <0.001). Finally, we performed a dual regression on our thresholded network and correlated these weights with the weights of the dual regression on the template network (r=0.79, p<0.001). To confirm that our network was the best fit out of the 70 components, we chose the 10 networks based on the elbow in a scree plot of the relative amount of total variance explained per component; beyond explaining the most variance within our imaging data across participants, our network also explained the most age-related variance (R^2_{adj}=0.70, p<0.001) and had the strongest association with memory performance (R^2_{adj}=0.21, p<0.001). Both associations survived correction for multiple comparisons. These analyses confirmed a high comparability of linked-independent component analysis results in this structural network and the previously identified network, indicating reproducibility of results in this method. Spatial maps of the other networks can be viewed in **eFigure 2**.

eFigure 1. Conceptual and Statistical Diagram for Moderation Analyses



Conceptual Diagram. X is the independent variable. Y is the outcome variable. M is the moderator variable.

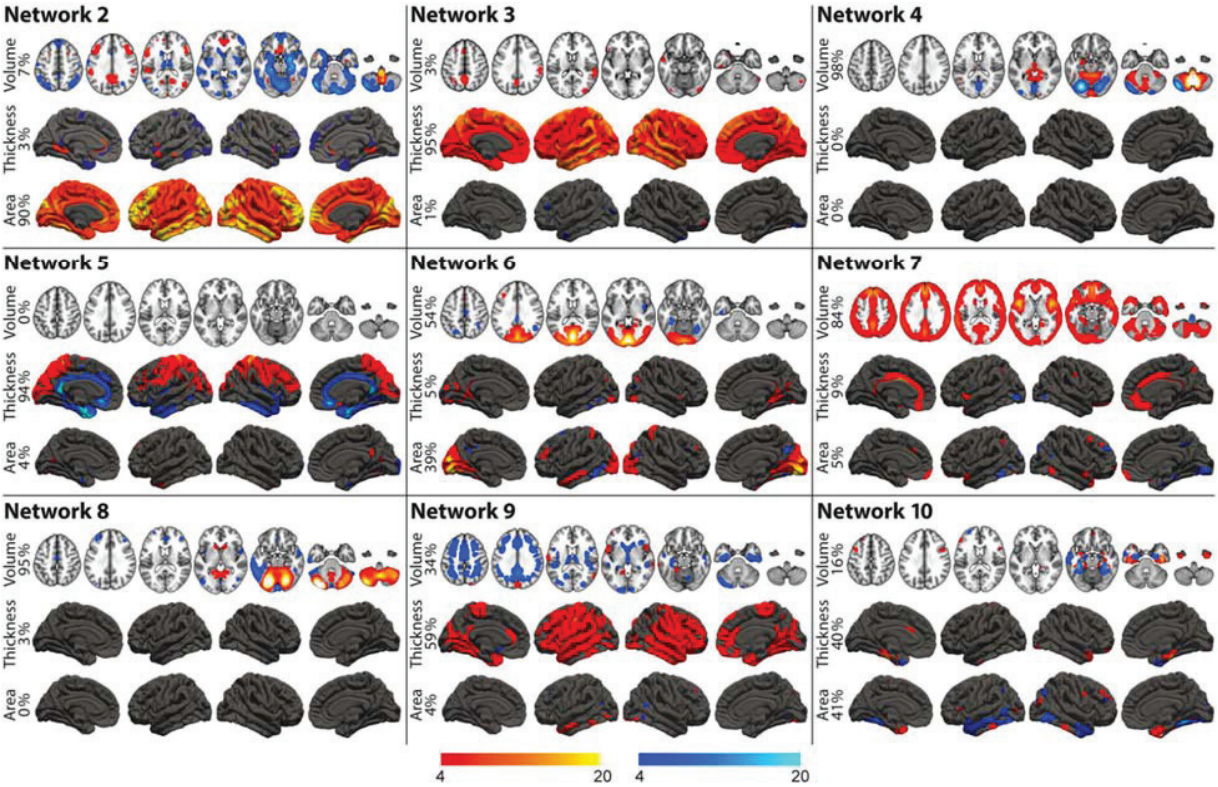


Statistical Diagram. X is the independent variable. Y is the outcome variable. M is the moderator variable. b_1, b_2, b_3 are the beta coefficients.

Conditional effect of X on Y = $b_1 + b_3M$

Diagrams adapted from Hayes AF. ***Introduction to mediation, moderation, and conditional process analysis: A regression-based approach.*** **2nd ed: Guilford Publications; 2017.**

eFigure 2. Spatial Maps of Brain Networks



We assessed the spatial maps of the network components resulting from the linked independent component analysis. We chose the top ten networks based on the elbow in a scree plot of the relative amount of total variance explained by each component. The first network explains the most variance and is displayed in Figure 1A. The other nine components are displayed here, along with the contribution of each modality (gray matter volume, cortical thickness, and pial area) to the generation of the network. To confirm specificity of the first network to memory performance (Figure 1B), we assessed the relationship between the other nine networks and memory performance. Our network also explained the most age-related variance (R^2_{adj}=0.70, p<0.001) and had the strongest association with memory performance (R^2_{adj}=0.21, p<0.001). Both associations survived correction for multiple comparisons.

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B. Declaration of authenticity

Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

3.1.21
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Datum

.....
Unterschrift

C. Author contributions to the publication
Erklärung zum eigenen Beitrag des Promovenden zur Publikation

*"Association of estradiol and visceral fat with structural brain networks and memory performance in adults" by Rachel G. Zsido (**RGZ**)*& Matthias Heinrich (**MH**), George M. Slavich (**GMS**), Frauke Beyer (**FB**), Shahrzad Kharabian Masouleh (**SKM**), Jürgen Kratzsch (**JK**), Matthias Raschpichler (**MR**), Karsten Mueller (**KM**), Ulrike Scharrer (**US**), Markus Löffler (**ML**), Matthias L. Schroeter (**MLS**), Michael Stumvoll (**MS**), Arno Villringer (**AV**), Veronica A. Witte (**VAW**), Julia Sacher (**JS**) (*JAMA Netw Open. 2019 Jun 5;2(6):e196126. doi: 10.1001/jamanetworkopen.2019.6126. PMID: 31225892; PMCID: PMC6593958.*)*

* Corresponding author

MH and RGZ contributed equally to this work; however their contributions reflect independent essential components of this paper: MH has performed the majority of visceral fat and subcutaneous adipose tissue segmentation and structural brain network covariance analysis, RGZ has conceptualized the sex specific risk trajectories of brain and cognitive aging and developed the perimenopausal transition as a prevention model for neurodegenerative disease development in women.

MH und RGZ teilen sich die Erstautorenschaft gemäß der Promotionsordnung der Medizinischen Fakultät Leipzig

Conception and design	MH, RGZ, GMS, SKM, KM, MLS, MS, AV, JS
Literature Review	MH, RGZ, SKM, VAW, JS
Data acquisition, analysis, or interpretation of data	MH, RGZ, FB, SKM, JK, MR, US, ML, MLS, MS, AV, VAW, JS
Statistical analysis	MH, RGZ, FB, SKM, ML, VAW
Obtained funding	ML, MLS, MS, AV, JS
Administrative, technical or material support	MR, US, MS, AV, JS
Figure & table creation	MH, RGZ, FB, SKM, VAW, JS
Drafting of the manuscript	MH, RGZ, GMS, JS
Critical revision of the manuscript for important intellectual content	all authors
Supervision	GMS, MR, MLS, MS, AV, VAW, JS

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *“Association of estradiol and visceral fat with structural brain networks and memory performance in adults“, in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den Rachel Zsido ……

Rachel Zsido

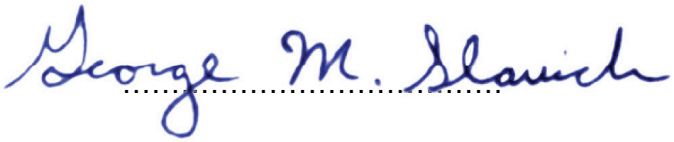
Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *"Association of estradiol and visceral fat with structural brain networks and memory performance in adults", in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den 03.01.2021

Matthias Heinrich

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *“Association of estradiol and visceral fat with structural brain networks and memory performance in adults“, in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Los Angeles, USA, 1/6/2021
Place, Date



Ph.D. George M. Slavich

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *"Association of estradiol and visceral fat with structural brain networks and memory performance in adults", in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den 23.12.25 Dr. Frauke Beyer

Dr. Frauke Beyer

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *"Association of estradiol and visceral fat with structural brain networks and memory performance in adults", in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den 09.01.2021 .....

Dr. Matthias Raschpichler

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *"Association of estradiol and visceral fat with structural brain networks and memory performance in adults", in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den 22.12.2020 .....

Ulrike Scharrer

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *"Association of estradiol and visceral fat with structural brain networks and memory performance in adults", in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den 05. JAN. 2021 .....

Prof. Dr. Markus Löffler

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *"Association of estradiol and visceral fat with structural brain networks and memory performance in adults", in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den 22.12.20 .....

Prof. Dr. med. Dr. phil. Matthias Schröter

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *"Association of estradiol and visceral fat with structural brain networks and memory performance in adults", in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den 30. 12. 2020 .....

Prof. Dr. Michael Stumvoll

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *“Association of estradiol and visceral fat with structural brain networks and memory performance in adults“, in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den ……

Prof. Dr. med. Arno Villringer

Hiermit bestätige ich, dass Matthias Heinrich bei der Entstehung und Veröffentlichung der wissenschaftlichen Publikation *“Association of estradiol and visceral fat with structural brain networks and memory performance in adults“, in* JAMA Network Open June 2019;2:6, an der Planung, Durchführung, Auswertung und Interpretation der Analysen, sowie dem Schreiben des Manuskriptes maßgeblich beteiligt war.

Leipzig, den 29.12.20

PD Dr. med. univ. et scient. med. habil. Julia Sacher

D. Conditional statement

Erklärung über die Vorbehaltlichkeit der Verfahrenseröffnung zur Verleihung des Titels Dr. med. / Dr. med. dent.

Der erfolgreiche Abschluss des letzten Staatsexamen (gemäß alter Approbationsordnung für Ärzte) bzw. der letzten Ärztlichen Prüfung (gemäß Approbationsordnung für Ärzte vom 27.06.2002) oder die Zahnärztliche Prüfung ist Voraussetzung für den Abschluss des Promotionsverfahrens und damit der Verleihung des akademischen Grades. Die Zulassung zum Promotionsverfahren ist insoweit nur vorläufig und steht unter der auflösenden Bedingung des Nichtbestehens des letzten Staatsexamens oder der Approbation zum Arzt/Zahnarzt. Dieser Abschluss ersetzt nach Regelung im § 12 der Promotionsordnung das Rigorosum. Das Rigorosum ist essentieller Bestandteil und notwendig zum erfolgreichen Abschluss des Promotionsverfahrens. Entsprechend den Reglungen in § 12 wird das eröffnete Promotionsverfahrens bei Nichtbeendigung des Studiums ohne Titelvergabe eingestellt.

Hiermit erkläre ich, dass mir dieser Sachverhalt im Rahmen der Eröffnung meines Promotionsverfahrens bekannt ist und ich im Falle des Fehlens der Voraussetzung des Abschlusses meines Promotionsverfahrens keine rechtlichen Ansprüche an eine Vergabe eines akademischen Grades oder Titels stelle.

3.1.21
.....
Datum

.....
Unterschrift

E. Curriculum vitae

Persönliche Daten

Matthias Heinrich
Leipzig
Mobil: +4915225321308
mat-heinrich@gmx.de

Geburtsdatum	10.07.1993
Familienstand	ledig
Nationalität	Deutsch

Schulbildung

08/2004 – 07/2012	Luther Melanchthon Gymnasium, Luth. Wittenberg
08/2000 – 07/2004	Grundschule am Trajuhnschen Bach, Luth. Wittenberg

Hochschulbildung

23.12.2020	Erteilung der Approbation als Arzt durch die Landesdirektion Sachsen
12/2020	Drittes Staatsexamen Humanmedizin
10/2019	Zweites Staatsexamen Humanmedizin
seit 02/2016	Doktorand am Max Planck Institut für Kognitions- und Neurowissenschaften, Abteilung Neurologie in Kooperation mit der Tagesklinik für Kognitive Neurologie des Universitätsklinikums Leipzig (Prof. Dr. Arno Villringer, PD Dr. Julia Sacher)
09/2015	Erstes Staatsexamen Humanmedizin
10/2013 – 12/2020	Studium der Humanmedizin an der Universität Leipzig

Praktika & Fortbildungen

11/2019 – 10/2020	Praktisches Jahr im Rahmen des Humanmedizinstudiums
01.10.2019 – 31.12.2019	Tutor sowie Seminarleitung an der Erziehungswissenschaftlichen Fakultät der Universität Leipzig, Seminartitel: „Neuroanatomie und Neurophysiologie für PädagogInnen des Förderschwerpunktes körperlich-motorische Entwicklung“ (Prof. Dr. Markus Spreer)

18.02.2019 – 04.03.2019	stationäre Famulatur in der Soteria Klinik Leipzig GmbH im Bereich der Psychosomatik und Psychiatrie (Prof. Dr. med. Katarina Stengler)
01.10.2018 – 31.12.2018	Tutor sowie Seminarleitung an der Erziehungs-wissenschaftlichen Fakultät der Universität Leipzig, Seminartitel: „Neuroanatomie und Neurophysiologie für PädagogInnen des Förderschwerpunktes körperlich-motorische Entwicklung“ (Prof. Dr. Walter-Klose)
10.09.2018 – 24.09.2018	stationäre Famulatur im Klinikum St. Georg in Leipzig im Bereich der Gynäkologie und Geburtshilfe (Prof. Dr. med. habil. Uwe Köhler)
21.02.2018 – 22.03.2018	ambulante Famulatur bei Dipl.-Med. Andreas Gänsicke, Facharzt für Innere Medizin und Kardiologie
20.09.2017 – 23.09.2017	Teilnahme am 90. Kongress der Deutschen Gesellschaft für Neurologie mit Posterpräsentation und anschließender Diskussionsrunde vor Fachpublikum
13.11.2016 – 30.11.2016	15 - stündiges Repetitorium „Medizinische Biometrie mit Übungen am Computer“ am Institut für Medizinische Informatik, Statistik und Epidemiologie (IMISE)
01.10.2016 – 21.12.2016	30 – stündiger Kurs „Grundkenntnisse im Umgang mit SPSS (Version 24)“ am Universitätsrechenzentrum der Universität Leipzig (URZ)
01.10.2016 – 31.10.2017	Studentische Hilfskraft am Max Planck Institut für Kognitions- und Neurowissenschaften, Abteilung Neurologie (Prof. Dr. Arno Villringer) Forschungsgruppe: „Emotion and Neuroimaging“ (Dr. Julia Sacher)
12.07.2016 – 11.08.2016	Famulatur in der Schön Klinik Neustadt auf der Station für Innere Medizin mit den Schwerpunkten Kardiologie sowie Gastroenterologie (Prof. Dr. Boris Bätge, Prof. Dr. Peter Radke)
04.03.2013 – 15.03.2013	Praxishospitation bei Dipl.-Med. Andreas Gänsicke, Facharzt für Innere Medizin und Kardiologie
01.02.2013 – 15.02.2013	Klinikhospitation in der Inneren Medizin I der Paul Gerhardt Diakonie Krankenhaus und Pflege GmbH (Prof. Dr. Jehle)
01.10.2012 – 31.01.2013	Pflegepraktikum im Diakonissenkrankenhaus Dessau auf der Station für Innere Medizin im Rahmen eines FSJ (Träger: Diakonie Mitteldeutschland) mit dem Schwerpunkt Geriatrie

Leipzig, den 03.01.2020

F. List of publications

Zsido, R.G.; Heinrich M.; Slavich G.M.; Beyer F.; Kharabian Masouleh, S.; Kratzsch, J.; Raschpichler, M.; Mueller, K.; Scharrer, U.; Löffler, M.; Schroeter, M.L.; Stumvoll, M.; Villringer, A.; Witte, A.V.; Sacher, J.: Association of estradiol and visceral fat with structural brain networks and memory performance in adults, JAMA Network Open. (2019)

Beyer, F.; Garcia-Garcia, I.; Heinrich, M.; Schroeter, M. L.; Sacher, J.; Luck, T.; Riedel-Heller, S. G.; Stumvoll, M.; Villringer, A.; Witte, A. V.: Neuroanatomical correlates of food addiction symptoms and body mass index in the general population. Human Brain Mapping. (2019)

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