Cortisol hypersecretion and the risk of Alzheimer's disease: a systematic review and meta-analysis

Bang Zheng [a]; Roy Tal [a]; Zhirong Yang [b]; Lefkos Middleton [a, c]; Chi Udeh-Momoh [a, d]*

Affiliations:

a. Ageing Epidemiology Research Unit, School of Public Health, Faculty of Medicine, Imperial College London, London W6 8RP, UK

b. Primary Care Unit, Department of Public Health and Primary Care, School of Clinical Medicine, University of Cambridge, Cambridge CB1 8RN, UK

c. Public Health Directorate, Imperial College NHS Healthcare Trust, London W6 8RP, UK

d. Translational Health Sciences, Faculty of Medicine, University of Bristol, Bristol BS1 3NY, UK

*Corresponding author address: Dr. Chi Udeh-Momoh, Ageing Epidemiology Research Unit, School of Public Health, Faculty of Medicine, Imperial College London, London W6 8RP, UK. E-mail: c.udeh@imperial.ac.uk; Tel: +44 20 3311 0320; Fax: +44 20 8846 7739.

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Abstract

Background: Morning cortisol levels have been reported to be elevated among patients with Alzheimer's disease (AD); yet no meta-analysis has been conducted to confirm the existence and magnitude of this association. It also remains unclear whether hypercortisolism is a risk factor for AD.

Methods: PubMed, EMBASE, and PsycINFO were systematically searched for eligible studies. Cross-sectional data were pooled using random effects meta-analyses; the differences in morning cortisol levels between patients and cognitively normal controls were quantified. Longitudinal studies were qualitatively synthesised due to methodological heterogeneity.

Results: 57 cross-sectional studies and 19 prospective cohort studies with 17,245 participants were included. Compared with cognitively normal controls, AD patients had moderately increased morning cortisol in blood (g=0.422, $P<0.001$; I^2=48.5%), saliva (g=0.540, $P<0.001$; I^2=13.6%), and cerebrospinal fluids (g=0.565, P=0.003; I^2=75.3%). A moderate elevation of morning cortisol was also detected in cerebrospinal fluids from patients with mild cognitive impairment (MCI) versus controls (g=0.309, P=0.001; I^2=0.0%). Cohort studies suggested that higher morning cortisol may accelerate cognitive decline in MCI or mild AD patients, but the results in cognitively healthy adults were inconsistent.

Conclusions: Morning cortisol was confirmed to be moderately elevated in AD patients and may have diagnostic and prognostic values for AD.

Keywords: cortisol; Alzheimer's disease; cognitive decline; biomarker; risk factor

1. Introduction

The healthcare and socio-economic burden of Alzheimer's disease (AD) has been increasing exponentially with the global trend of population ageing (Alzheimer's Association, 2018). Late-onset AD is posited to be a multi-factorial disorder caused by complex interactions between genetic, lifestyle, and environmental factors over time. However, the precise aetiology and pathogenesis, as well its nosological heterogeneity still remain unclear (Gauthier et al., 2016). Identification of potential biomarkers beyond the "β amyloid deposition, pathologic tau, and neurodegeneration" (ATN) biomarker framework is essential for elucidating the complex pathogenesis of AD and discovering new targets for drug development (Gauthier et al., 2016; Jack et al., 2018). Furthermore, a reliable set of predictive biomarkers that can detect pre-symptomatic elderly subjects at high risk of AD would add significant value for patient stratification in clinical trials and preventive interventions.

A growing body of evidence suggests that abnormal level of the glucocorticoid hormone is linked with key hallmarks of AD pathogenesis, specifically β-amyloid deposition, tau-hyperphosphorylation; alongside synaptic deficits (Green et al., 2006; Lante et al., 2015; Toledo et al., 2012; Wang et al., 2018) and cognitive impairment (Notarianni, 2013; Ouanes and Popp, 2019) in both animal models and human patients. Such observations lend credence to the "glucocorticoid-cascade" hypothesis which suggests that chronic glucocorticoid secretion, as seen after prolonged stress exposure, may contribute to the aetio-pathogenic pathway leading to cognitive decline and AD in the elderly (Sapolsky et al., 1986).

Activation of the hypothalamic-pituitary-adrenal (HPA) axis results in the production of glucocorticoids, secreted in the form of cortisol in humans, as a response to stress and for maintaining homeostasis (Russell and Lightman, 2019). Hormonal release follows a circadian rhythm that reaches peak levels after 30-45 minutes post morning awakening (referred to as

the cortisol awakening response; CAR (Stalder et al., 2016)) and declines throughout the rest of day to nadir levels (Russell and Lightman, 2019). AD-related HPA axis hyperactivity is most frequently observed as elevated peak cortisol levels in the morning (Gil-Bea et al., 2010; Giubilei et al., 2001; Laske et al., 2009; Notarianni, 2013). At the same time, several other studies reported no significant differences in morning cortisol levels between AD patients and cognitively normal controls (Paoletti et al., 2004; Wang et al., 2018). Despite these contradictory reports, no meta-analysis has yet been conducted to synthesise the available evidence and evaluate the existence or magnitude of the reported associations. Robust comparisons of AD-related cortisol elevation in peripheral samples (e.g., blood, saliva, urine, and hair) and in the cerebrospinal fluid (CSF) may also be important for informing future research and clinical applications. Furthermore, it remains unclear whether morning cortisol hypersecretion occurs in earlier disease stages (e.g., mild cognitive impairment; MCI). On the other hand, summarising evidence from longitudinal studies on baseline morning cortisol levels and subsequent decline in cognitive functions or incidence of AD/MCI will help elucidate the temporal relationships and establish an aetiological and potentially predictive role of morning hypercortisolism in AD development.

In this study, we conducted the first reported meta-analysis to-date, aiming at a systematic evaluation of available cross-sectional studies that assessed morning central or peripheral cortisol levels in AD/MCI patients as compared to cognitively normal individuals. We also synthesised longitudinal studies that examined morning hypercortisolism as a risk factor for AD development and cognitive decline among older populations.

2. Methods

2.1. Search strategy

Following the predefined study protocol, we systematically searched PubMed, EMBASE,

and PsycINFO databases to identify journal articles published by July 2019 that reported the association between morning cortisol level and AD or cognitive impairment in human adults. This study followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline (Moher et al., 2009). Studies were identified using search terms related to cortisol, Alzheimer's disease, and cognitive impairment. Medical Subject Headings (MeSH) terms and free text words were combined for the search. Detailed searching strategies are displayed in Supplementary Methods.

2.2. Inclusion/exclusion criteria

Studies were included if they used a cross-sectional, case-control, or cohort design and measured cortisol levels in blood (plasma/serum), saliva, or CSF samples. We only included studies with morning sampling (defined as measurement within the time period from morning awakening to 11:30 a.m.) which reflects circadian peak cortisol levels. Cross-sectional studies were included if they reported cortisol levels in clinically diagnosed AD or MCI patients versus a cognitively normal group in an appropriate and extractable form for pooled analysis (e.g., mean levels for each group with standard deviation (SD), standard error (SE), or confidence interval (CI); or mean differences with SE, CI or exact *P* value). For inclusion, case-control and cohort studies were required to have morning cortisol levels as exposure and subsequent AD/MCI onset or cognitive decline as outcomes. Effect estimates of longitudinal data (e.g., hazard ratios (HR), odds ratios (OR), regression or correlation coefficients) were also required for inclusion.

Studies were excluded if they were conducted in younger populations (<50 years old); did not report sampling time; only presented graphic data that were not extractable even with graphic editing software; did not report specific diagnostic criteria for AD or MCI; or focused on populations with comorbidities characterized by chronic HPA hyperactivity (e.g., major

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depression, schizophrenia and bipolar disorder, anorexia nervosa, chronic alcoholism, posttraumatic stress disorder, or burnout), or populations with disorders that directly affect cortisol biosynthesis (e.g., Cushing's disease and Addison's disease). In addition, experimental studies that measured HPA reactivity after challenges, such as the dexamethasone suppression test or other drug tests, were excluded unless they presented basal cortisol data prior to the intervention. Duplicated data, including separate studies that used an identical population or sub-population, were removed.

2.3. Data extraction and study quality assessment

We extracted the following information from eligible cross-sectional studies for a quantitative synthesis: (1) clinical diagnosis (AD or MCI) with the diagnostic criteria used and sample sizes of patient and control groups, (2) mean morning cortisol values for each group and corresponding SD, SE, or CI, (3) sample type, time of sampling, assay method and units, and (4) population characteristics (country, age, gender, comorbidities and medications known to confoundedly affect cortisol levels).

Data from eligible case-control and cohort studies were extracted for a qualitative synthesis due to substantial methodological heterogeneity. The data included: (1) population characteristics (country, sample size, age, gender, baseline cognitive status, follow-up years), (2) cortisol measures (sample type, time of sampling, assay method, exposure categories), (3) cognitive outcomes and assessments, (4) statistical methods and effect estimates (based on maximally-adjusted models when possible).

We used the Cochrane recommended software PlotDigitizer (www.plotdigitizer.sourceforge.net/) to extract graphic cortisol data in 19 papers where no precise numerical data was reported. We also contacted 23 authors by email to request summary data; three authors provided usable data (Johar et al., 2015; Peavy et al., 2009;

Venero et al., 2013).

The study quality of included papers was evaluated using the "National Institutes of Health (NIH) quality assessment tool for observational cohort and cross-sectional studies" (www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools). This tool consists of a checklist of 11 items, for which an overall quality rating of "good", "fair" or "poor" was assigned to each study after a qualitative evaluation.

Two authors (BZ and RT) independently screened the papers and performed data extraction and quality assessment. All discrepancies were settled after review of the corresponding papers by both and consensus discussions.

2.4. Data analysis

Meta-analyses were conducted to examine differences in mean morning cortisol levels between AD/MCI patients and healthy controls in cross-sectional studies, separated by sample-type (blood, saliva, and CSF) and diagnostic-group (AD and MCI) to minimize heterogeneity. Random-effects models with inverse-variance method were used to estimate the pooled effect size. The Hedges' g statistic was adopted as a measure of effect size (Durlak, 2009) based on standardized mean differences, as it is robust to small sample sizes of individual studies.

The heterogeneity of results across studies was assessed by the I^2 statistic and Q test in each analysis. A funnel plot was created to graphically identify publication bias in each meta-analysis with at least ten studies, followed by an Egger's test of asymmetry. Subgroup analyses and meta-regressions were conducted to investigate potential moderators on the cortisol-AD association and potential sources of heterogeneity, such as country (European countries or elsewhere), assay method (radioimmunoassay or else), sampling time (before or after 9 a.m. for blood samples, and upon awakening or after 30 minutes post awakening for

saliva samples), mean age and gender proportions. To examine the robustness of the main findings, sensitivity analyses were performed by excluding studies without age/gender matching for controls, studies involving comorbid or medicated participants that may cause confounding bias, or studies rated as poor quality. We also estimated the pooled unstandardized mean difference of cortisol levels (nmol/L) in plasma, serum, saliva, and CSF samples between patients and controls to facilitate clinical inference.

Statistical analyses were performed using Stata (version 14, StataCorp, College Station, TX). All statistical tests were two-sided and the significance level was defined as $P<0.05$.

3. Results

3.1. Search results and study characteristics

The initial literature search yielded a total of 3305 records. After screening, 57 cross-sectional studies and 19 prospective cohort studies reported in 72 papers remained (four papers presented both cross-sectional and cohort data); no eligible case-control studies were identified (Figure 1). Most cross-sectional studies measured blood cortisol (n=40; 20 with plasma and 20 with serum) or salivary cortisol (n=13), whilst only five studies investigated central cortisol in CSF samples (Table S1-S3 in Supplement and Supplementary References). The sampling time ranged from 7:00 a.m. to 11:30 a.m. The radioimmunoassay (RIA) was the most frequently used method for cortisol measurement (n=34); other methods included fluoroimmunoassay and the enzyme-linked immunosorbent assay (ELISA). Most studies compared AD patients versus cognitively normal controls (n=39), nine studies compared MCI patients versus controls, and seven studies investigated both. The majority of the studies used the National Institute of Neurological Disorders and Stroke-Alzheimer Disease and Related Disorders (NINCDS-ADRDA) criteria (McKhann et al., 1984) for AD diagnosis (n=31) and the International Working Group on Mild Cognitive Impairment criteria (Winblad

et al., 2004) for MCI diagnosis (n=10).

Most included prospective cohorts assessed cortisol levels in blood (n=13) or saliva samples (n=4) at baseline, and only two studies measured CSF cortisol levels (Table 1). Twelve studies were initiated in cognitively healthy older adults at baseline, six featured patients with AD or MCI at baseline, and one study included both populations. The follow-up length varied from one to ten years. Most studies used decline in cognitive test scores as outcome measure (n=14), four reported clinical conversion/progression as outcome, and one study used both outcome measures.

The study quality of most included studies was rated as "fair" or "good" using the NIH quality assessment tool, while 8 studies had poor overall quality. The commonest potential sources of bias among these studies are small sample sizes and lack of control for confounding factors.

3.2. Morning cortisol levels in AD/MCI patients versus cognitively normal individuals

3.2.1 Blood samples

The meta-analysis of 38 cross-sectional studies (2179 participants) using blood samples showed significantly increased morning cortisol levels in AD patients compared to cognitively normal controls (Figure 2). The effect size was moderate (Hedges' g=0.422, 95% CI: 0.289-0.556; $P<0.001$) and the level of heterogeneity across studies was not excessive (I^2=48.5%; P=0.001).

In contrast, the meta-analysis of five studies (1149 participants) on MCI patients did not identify any difference in blood cortisol levels between affected and cognitively normal individuals (Hedges' g=0.002, 95% CI: -0.162-0.167, P=0.978), with minor between-study heterogeneity (I^2=17.0%; P=0.306).

3.2.2 Saliva samples

The meta-analysis of five studies (284 participants) evaluating saliva cortisol also showed a moderately elevated level of morning cortisol in AD patients compared with controls (Hedges' g=0.540, 95% CI: 0.276-0.804, P<0.001; Figure 3). There was only minor heterogeneity across studies (I^2=13.6%; P=0.327).

The meta-analysis of ten studies (2212 participants) in MCI patients did not reveal a significant difference in salivary cortisol levels between patients and controls (Hedges' g=0.106, 95% CI: -0.047-0.258, P=0.174; Figure 3), with a modest degree of heterogeneity (I^2=36.4%; P=0.117). Both the funnel plots (Figure S1-S2 in Supplement) and Egger's tests (P>0.05) suggested there was no evidence of publication bias in the above analyses.

3.2.3 CSF samples

Only five studies reported morning CSF cortisol levels in AD versus cognitively normal controls (618 participants), of which four also examined MCI patients (506 participants). A moderate elevation in central cortisol was detected in AD patients compared to controls (Hedges' g=0.565, 95% CI: 0.198-0.931, P=0.003; Figure 4), with a substantial degree of heterogeneity (I^2=75.3%; P=0.003).

Central cortisol was also moderately elevated in MCI patients compared to controls (Hedges' g=0.309, 95% CI: 0.125-0.492, P=0.001; Figure 4), with minimal heterogeneity (I^2=0.0%; P=0.429).

3.2.4 Subgroup and sensitivity analyses

Results of subgroup analyses and meta-regressions for blood and salivary cortisol showed no effect modifications by aforementioned factors (P>0.05, Table S4 in Supplement). Moreover, the pooled estimates did not change significantly in sensitivity analyses (Table S5 in Supplement). Insufficient number of studies with CSF samples limited our ability to conduct the above subgroup/sensitivity analyses for central cortisol.

The sensitivity analyses with unstandardized mean difference as effect size showed that compared with cognitively normal controls, AD patients had higher morning cortisol levels in plasma (d=51.0 nmol/L, 95% CI: 25.5-76.5, *P*<0.001), serum (d=64.7 nmol/L, 95% CI: 35.0-94.5, *P*<0.001), saliva (d=4.0 nmol/L, 95% CI: 0.5-7.4, *P*=0.024), and CSF samples (d=6.6 nmol/L, 95% CI: 2.9-10.4, *P*=0.001). MCI patients had 4.1 nmol/L (95% CI: 1.0-7.2, *P*=0.010) higher morning CSF cortisol levels on average than cognitively normal controls.

3.3. Longitudinal studies on associations between morning cortisol levels and risk of AD or cognitive decline

3.3.1 Baseline morning cortisol level and longitudinal changes of cognitive functioning

The characteristics of 19 cohort studies with 11,805 participants are summarised in Table 1. 15 of these studies used longitudinal cognitive test scores as outcome measure, the results of which are described separately according to baseline disease status (5, 9, and 1 studies included MCI/AD patients, cognitively healthy older adults, and both populations at baseline) and sample types. Among the six cohorts with MCI or AD patients at baseline, one and five cohorts measured CSF and blood cortisol at baseline. Popp et al. (2015) found that higher CSF cortisol level in MCI (but not AD) patients was associated with faster subsequent cognitive decline. Similarly, Csernansky et al. (2006) identified an association between higher blood cortisol and faster cognitive decline among MCI patients. In contrast, four small cohorts of AD patients showed no association between high blood cortisol and subsequent cognitive decline (Csernansky et al., 2006; Miller et al., 1998; Swanwick et al., 1996; Umegaki et al., 2000), of which two studies detected positive associations in subgroups of mild AD patients (Swanwick et al., 1996; Umegaki et al., 2000). One larger cohort of mild-to-moderate AD patients associated high blood cortisol with faster decline in cognitive test scores (Huang et al., 2009). Overall, the limited but consistent evidence from cohort studies

suggests that elevated morning cortisol in patients at early clinical AD stages may contribute to an accelerated subsequent cognitive decline in the clinical course of AD.

Inconsistent results were found in the 10 cohorts initiated in cognitively healthy adults at baseline (seven and three studies with blood and saliva samples). One study in postmenopausal women reported an association of high blood cortisol with larger decline in category fluency but not in other cognitive domains (Greendale et al., 2000). Another cohort with blood cortisol sample identified similar associations with changes in cognitive test scores only in participants with high levels of brain amyloid-β deposition according to positron emission tomography (PET) scan (Pietrzak et al., 2017). In contrast, one small cohort (Csernansky et al., 2006) and two larger cohorts (the Longitudinal Aging Study Amsterdam (Comijs et al., 2010) and the Rotterdam Study (Schrijvers et al., 2011; Kalmijn et al., 1998)) found no association between blood cortisol and rate of cognitive decline. As for studies with saliva samples, the Whitehall II study (Singh-Manoux et al., 2014) found no association between saliva cortisol and cognitive decline. The Longitudinal Aging Study Amsterdam (Gerritsen et al., 2011) showed that lower morning saliva cortisol was associated with increased verbal memory decline in *APOE*-ε4 carriers but not in non-carriers. Another cohort identified an association between high morning saliva cortisol and larger decline in visual memory among women but not men (Beluche et al., 2010).

3.3.2 Baseline morning cortisol level and the risk of AD or MCI incidence

Among the five cohort studies using AD or MCI incidence as outcome measure (one aforementioned study (Schrijvers et al., 2011) analysed both cognitive test scores and AD incidence as outcome measures), three, one, and one studies measured blood, saliva, and CSF cortisol, respectively. Lehallier et al. (2016) screened 224 variables (combination of clinical data and biomarkers) to establish prediction models for clinical conversion from MCI to AD, using data from Alzheimer's Disease Neuroimaging Initiative. They identified baseline

plasma cortisol as a necessary component along with other five fluid-based biomarkers (distinct from amyloid-β and tau) to achieve optimal prediction performance. The other four cohorts assessed clinical progression from cognitively normal to AD/MCI as main outcome. The Vienna Transdanube Aging Study (Hinterberger et al., 2013) reported an association between higher blood cortisol and increased risk of AD incidence (OR=1.10, 95% CI: 1.03-1.17), whereas the aforementioned Rotterdam Study (Schrijvers et al., 2011) found no such association (HR=0.99, 95% CI: 0.85-1.14). The ESA Study in Canada (Potvin et al., 2013) detected significant interactions between salivary cortisol and depression/anxiety on the risk of incident cognitive impairment. Finally, we have previously reported an increased risk of clinical progression to MCI/AD in preclinical adults with high CSF cortisol and pathological amyloid-β levels, and identified significant interactions between cortisol, amyloid-β, and cognitive reserve factors (Udeh-Momoh et al., 2019).

4. Discussion

This study provides the first quantitative and qualitative synthesis of available cross-sectional and longitudinal data on associations between peripheral and central morning cortisol levels and the risk of cognitive decline and clinical AD syndrome. Our meta-analyses confirmed a moderate elevation in blood, saliva, and CSF cortisol levels in AD patients versus cognitively normal individuals. Only CSF cortisol was significantly elevated in MCI patients versus controls, potentially reflecting a pathogenic effect from a direct exposure to cortisol within the blood-brain barrier.

Circadian-peak cortisol hypersecretion has been reported as an endocrine and metabolic feature of AD over the last three decades (Jacobson and Sapolsky, 1991; Ouanes and Popp, 2019). Our review of cross-sectional studies suggests this factor could be an important disease-state indicator for AD. Indeed, a population-based study that screened 125 plasma

analytes within a biomarker panel identified elevated morning cortisol as one of five predictors for AD diagnosis (Doecke et al., 2012). Since blood cortisol can be assayed routinely in clinical settings, this measure could be used in population-wide screening for AD. However, the diagnostic accuracy (e.g., sensitivity and specificity) of this single analyte, or in combination with a broader panel of biomarkers (Doecke et al., 2012), requires further validation.

Saliva cortisol, a measure of unbound and biologically active hormone, is also shown to be a reliable and non-invasive measure of comparable value for identifying AD cases (Gatti et al., 2009; Kirschbaum and Hellhammer, 1994). Indeed, saliva cortisol concentrations were found to correlate well with blood cortisol measures (Kirschbaum and Hellhammer, 1994; Poll et al., 2007). Moreover, saliva samples are easier to obtain by participants at home, thus having the advantage of avoiding the stress response that may occur in clinical settings and also stress induced by blood sampling. The saliva sampling time can be self-recorded by participants according to their awakening time, which can help address the influence of CAR effect on morning cortisol levels (Stalder et al., 2016). Although peripheral cortisol is more suitable as an easily obtainable clinical biomarker, central cortisol levels assessed in CSF samples offer the added value of reflecting brain exposure levels of cortisol. Our finding of the CSF cortisol elevation in MCI patients versus cognitively normal individuals suggests that hypercortisolism in the central nervous system may occur at early disease stages. For instance, Popp et al. (2015) analysed both blood and CSF samples from MCI patients and healthy controls; the authors reported significant differences in cortisol levels for the two investigated groups in CSF but not in blood samples. Future research on central cortisol levels in MCI patients is needed to confirm theses findings.

Our synthesis of prospective cohort studies supports the potentially prognostic role of morning hypercortisolism in MCI or mild AD patients but reveals conflicting results in

cognitively healthy subjects. These findings may indicate that HPA axis dysfunction is not involved at the pre-clinical stage of AD. Nevertheless, methodological limitations in some of the studies may explain the negative results reported. For instance, the outcome measurements of repeated cognitive tests (or clinical diagnosis that is partially based on cognitive tests) may suffer from practice effect, the magnitude of which has been shown to be larger in cognitively healthy adults than in AD patients (Calamia et al., 2012). Additionally, most of the studies did not use markers of AD pathology to characterise the pre-symptomatic AD phenotype (Jack et al., 2018). We and others have found that cortisol hypersecretion accelerates clinical progression or cognitive decline in preclinical individuals with pathological levels of $A\beta_{42}$ (Pietrzak et al., 2017; Udeh-Momoh et al., 2019). Similarly, not all MCI patients included in the cohorts are prodromal AD patients; future cohort studies with more precise phenotyping of participants with established AD biomarkers could reveal more robust association between cortisol and AD progression. Overall, the available evidence is not sufficient to draw firm conclusions regarding a putative aetiological or predictive role of cortisol in AD development. Future large-scale longitudinal studies in preclinical-AD participants with both clinical conversion and comprehensive cognitive tests as outcomes are increasingly important. In addition, pilot experimental studies of HPA or cortisol-targeted therapy (Soria et al., 2018) for AD prevention, particularly in individuals at the preclinical or prodromal stage of AD, may better establish the aetiological role of cortisol in AD development (e.g., the XanADu trial and the XanaHES trial for Xanamem™; ClinicalTrials.gov Identifier: NCT02727699 and NCT03830762).

The biological mechanisms underlying the association between cortisol hypersecretion and AD have not been fully elucidated. One proposed mechanism is that chronic hypercortisolism may trigger neuronal loss in the hippocampus (Lupien et al., 1998), which may in turn exacerbate cortisol hypersecretion via reduced inhibitory control of the HPA axis (Jacobson

and Sapolsky., 1991; Vitale et al., 2013). In addition, cortisol levels are known to be elevated in chronic stress paradigms that have been implicated as a potential risk factor for AD (Lucassen et al., 2014); thus hypercortisolism may mediate the effects of stress and allostatic load on AD risk (Aznar and Knudsen, 2011; Crook et al., 2018). In fact, the moderate increase of cortisol in AD patients as synthesised in our study was lower than that was induced by acute stress or immunosuppression (Smith et al., 2012), and more similar to the chronic response to mild stress (Pan et al., 2018; Wust et al., 2000). Furthermore, chronic cortisol hypersecretion might also contribute to AD pathology through systemic inflammation. For instance, chronic herpes simplex virus infection has been linked to increased AD risk (Itzhaki, 2014). Central cortisol might modulate the recurrent brain infection through its principal target in the brain, the mineralocorticoid receptor, which has been shown to be a cofactor for herpes simplex virus proliferation (Haas et al., 2018).

On the other hand, given the long preclinical phase of AD where the hallmark pathologies are developing, it is possible that cortisol hypersecretion may be triggered by these pre-existing AD pathologies (Toledo et al., 2012; Wang et al., 2018). The direction of this complex association (i.e., hypercortisolism as a cause or consequence of AD pathology) is yet unestablished and warrants further investigation (Ritchie and Ritchie, 2012). To elucidate the direction of relationship between cortisol and AD pathology, future cohort studies that measure both AD-related pathological/cognitive changes and cortisol concentrations longitudinally during follow-up, while controlling for other factors that influence HPA axis regulation (e.g., inflammation, metabolic factors, physiological stressors, comorbidities), are needed. The longitudinal measurement of cortisol could help clarify whether abnormal cortisol secretion is merely a side effect of AD or ageing process. Given the synthesised evidence on potential prognostic role of morning cortisol in MCI or mild AD patients in cohort studies, morning hypercortisolism in late-life could possibly serve as a marker of AD

progression, secondary to the existing AD pathologies, though biomarker validation research is still required.

Several limitations in this systematic review should be noted. Most included cross-sectional studies recruited patients from clinical settings, which may lead to selection bias. Nevertheless, clinical patients have the benefit of more precise AD diagnoses compared to community-based populations. The possibility of residual confounding bias cannot be ruled out in this study. Most included studies used RIA or other immunoassays to measure cortisol level, which have some methodological limitations (e.g., immunoassays for salivary cortisol may have poor specificity due to antibody cross-reactivity with cortisone in saliva (El-Farhan et al., 2017)). Future validation studies using Liquid chromatography – tandem mass spectrometry, which has showed improved sensitivity and specificity (El-Farhan et al., 2017), are needed. In addition, the methodological heterogeneity across the prospective cohort studies limited our ability to perform quantitative syntheses. Finally, given the circadian rhythm of cortisol secretion and the variation in cortisol levels measured at different times of the day, we focused our analyses on morning cortisol levels to minimise heterogeneity. Morning cortisol level has been proposed to reflect both basal hypercortisolemia and circadian cortisol hypersecretion (Notarianni, 2013), and the single measurement is more feasible and practical to be implemented in various clinical and research settings for AD diagnosis compared with other cortisol parameters that require multiple measurements (e.g., 24-hour mean cortisol level, CAR level, or diurnal cortisol slope). The dynamic nature of cortisol secretion gives rise to different patterns that can be evaluated experientially. For instance, we note the existence, albeit limited, of other studies reporting associations of diurnal cortisol change, CAR, circadian nadir cortisol, 24-hour urinary cortisol, or 24-hour mean cortisol level with AD risk (Dijckmans et al., 2017; Ennis et al., 2017; Gardner et al., 2019; Geerlings et al., 2015; Peavy et al., 2012; Tsui et al., 2019; Curto et al., 2017).

Abnormal cortisol levels in response to the dexamethasone suppression test have also been observed in AD patients and warrants further investigation (Linder et al., 1993). Future studies investigating these additional measures, including hourly ultradian hormone secretion (Kalafatakis et al., 2018), could provide a full picture of the influence of HPA axis and cortisol on AD pathogenesis and symptom development.

5. Conclusions

This systematic review and meta-analysis confirmed both peripheral and central morning cortisol levels to be moderately elevated in AD patients compared to cognitively normal individuals. Given inconsistencies in the available data, no firm conclusion could be drawn regarding the predictive role of hypercortisolism in AD symptom development among cognitively healthy adults. Nevertheless, limited but consistent evidence implies that higher morning cortisol may accelerate cognitive decline in MCI or mild AD patients.

and in the decision to submit the article for publication.

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Figure captions and Tables

Figure 1. PRISMA flowchart illustrating the stages involved in screening studies for the systematic review [a].

a. Four eligible studies analysed both cross-sectional data and longitudinal data; one eligible cross-sectional study collected both blood and CSF samples.

Figure 2. Forest plot of 40 studies comparing morning cortisol levels in blood samples between AD versus CN and MCI versus CN

Abbreviations: AD, Alzheimer's disease; CN, cognitive normal; MCI, mild cognitive impairment.

Figure 3. Forest plot of 13 studies comparing morning cortisol levels in saliva samples between AD versus CN and MCI versus CN

Abbreviations: AD, Alzheimer's disease; CN, cognitive normal; MCI, mild cognitive impairment.

Figure 4. Forest plot of five studies comparing morning cortisol levels in CSF samples between AD versus CN and MCI versus CN

Abbreviations: AD, Alzheimer's disease; CN, cognitive normal; MCI, mild cognitive impairment; CSF, cerebrospinal fluid.

Table 1. Characteristics of 19 prospective cohort studies on associations between morning cortisol level and cognitive outcomes.

Author, Publication year	Cohort, Country of study population	Baseline diagnosis, Sample size	Gender (no. of male)	Age at baseline(year)	Follow-up years	Sample type	Exposure categories	Cognitive outcomes	Statistical method	Effect sizes (HR, OR, β, r)	Adjusted variables
Swanwick et al., 1996	Ireland	AD: 25 (4 lost to follow-up)	9	60-80, mean 73.0 (5.8)	1	Blood	Rank of baseline cortisol	MMSE change	Kendall rank correlation	All: r=0.17, P>0.05; Subgroup of CDR=1 or 2: r=0, P>0.05; CDR=0.5: r=0.44, P<0.05	None; excluded depression patients or drug users
Kalmijn et al., 1998	Rotterdam Study, Netherlands	Healthy: 189	81	55-80	1.9	Fasting blood	1 SD increase	MMSE change	Logistic regression	OR=0.9 (95% CI: 0.6-1.4)	Age, sex, education, depressive symptoms
Miller et al., 1998	USA	AD: 16	--	71.4 (8.8)	1.7 (0.4)	Blood	Continuous cortisol	ADAS, MMSE change	Pearson correlation	MMSE: r= -0.17, P=0.53; ADAS-cog: r=0.49, P=0.07	None; excluded depression patients or drug users
Greendale et al., 2000	Rancho Bernardo Study, USA	Healthy: 136	0	~72	3-5	Fasting blood	Continuous cortisol	Changes in multiple cognitive tests	Linear regression	Category fluency: β= -0.027, P=0.030; Visual: β= -0.0, P>0.05; MMSE: β= -0.011, P=0.205; Trail-Making B: β= -0.214, P=0.310	Age, education, time between assessments, BMI, antihypertensive use, blood pressure and glucose, alcohol consumption
Umegaki et al., 2000	Japan	AD: 28	0	82.5 (5.0)	3.3	Fasting blood	Continuous cortisol	MMSE change	Linear regression	All: β=0.482, P=0.243; Subgroup of baseline MMSE≥14: β=0.763, P=0.031	None; excluded patients with major disease and smokers
Csernansky et al., 2006	USA	AD: 10 MCI: 23 Healthy: 21	26	~75	<4	Fasting blood	Continuous cortisol	Changes in multiple cognitive tests	Linear mixed-effects model	AD+MCI: β=0.150, P=0.007; MCI: P=0.017; AD or control: P>0.05	None
Kuningas et al., 2007	Leiden 85-plus Study, Netherlands	Total: 551; MMSE≥19: 460	186	85	4.2	Blood	1 SD increase	Multiple cognitive tests	Linear mixed-effects model	All: MMSE (β=-0.78, P=0.004); Subgroup of baseline MMSE≥19: MMSE (β=-0.37, P=0.003); Stroop Test (β=2.86, P=0.034), LDT (β=-0.74, P=0.013)	Sex, education, health related correlates

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Author, Publication year	Cohort, Country of study population	Baseline diagnosis, Sample size	Gender (no. of male)	Age at baseline (year)	Follow w-up years	Sampl e type	Exposure categories	Cognitive outcomes	Statistical method	Effect sizes (HR, OR, β, r)	Adjusted variables
Huang et al., 2009	Taiwan, China	AD: 172 (24 lost to follow-up)	71	73	2	Fastin g blood	Continuous cortisol	MMSE change	Linear regression	β=0.148, *P*<0.05	None
Beluche et al., 2010	France	Healthy: 197	111	65-90	2-4	Saliva	Tertiles	Changes in multiple cognitive tests	Logistic regression	Women: Benton Test (OR=5.1 (0.9-29), *P*=0.06), TMTB (OR=0.1 (0.007-0.8), *P*=0.03); Other analyses: *P*>0.05	Age, education; stratified by gender; excluded depression patients or drug users
Comijs et al., 2010	Longitudinal Aging Study Amsterdam, Netherlands	Healthy: 1154	561	65-88, mean 75.1 (6.5)	3-6	Blood	1 SD increase	Changes in multiple cognitive tests	Linear mixed-effects model	Cognitive decline (interaction of cortisol*time): *P*>0.05; cognitive function over time: verbal learning (β= -0.033, *P*=0.04)	Age, sex, education, cardiovascular diseases, hypertension, diabetes, BMI, smoking, alcohol consumption, depression
Gerritsen et al., 2011	Longitudinal Aging Study Amsterdam, Netherlands	Healthy: 911 (213 lost to follow-up)	423	74.5 (7.2)	4	Saliva	1 SD increase	Changes in multiple cognitive tests	Linear mixed-effects model	All: *P*>0.05; Verbal memory-immediate recall: *APOE*-4 carrier: β=0.14, *P*<0.05; non-carrier: β=-0.04, *P*>0.05.	Age, sex, education, diabetes, cardiovascular diseases, hypertension, BMI, sleep duration, smoking and alcohol, *APOE*-4, depressive symptoms
Schrijvers et al., 2011	Rotterdam Study, Netherlands	Healthy: 3341	1420	≥55, mean 72 (6.8)	7.1	Fastin g blood	1 SD increase and quartiles	Changes in multiple cognitive tests; incident dementia (DSM-III-	Linear regression, Cox regression	Annual change in test scores: *P*>0.05; Incident AD: HR=0.99 (95% CI: 0.85-1.14).	Age, sex, sampling time, education, depressive symptoms, waist circumference, blood pressure and glucose, cholesterol, smoking, baseline

Author, Publication year	Cohort, Country of study population	Baseline diagnosis, Sample size	Gender (no. of male)	Age at baseline (year)	Follow w-up years	Sampl e type	Exposure categories	Cognitive outcomes	Statistical method	Effect sizes (HR, OR, β, r)	Adjusted variables
								R) and AD (NINCDS-ADRDA)			test score, *APOE*.
Hinterberg er et al., 2013	Vienna Transdanube Aging Study, Austria	Healthy: 492	--	75.7 (0.4)	2.5-7.5	Fasting blood	1-μg/dL increment	Probable AD (NINCDS-ADRDA)	Logistic regression	OR=1.10 (95% CI: 1.03-1.17), *P*=0.004	*APOE*, sex, education, depression, diabetes, hypertension, smoking, medication, serum folate
Potvin et al., 2013	ESA study. Canada	Healthy: 581	~243	~73.6 (6.0)	1	Saliva	1 SD increase	Incident cognitive impairmen t (MMSE)	Logistic regression	Interaction of cortisol with anxiety (*P*= 0.008), depressive episode (*P*=0.002), and number of chronic diseases (*P*=0.030)	Sampling time, age, education, sex, drug use, cardiovascular and chronic diseases, baseline MMSE score, anxiety symptoms, depressive episode
Singh-Manoux et al., 2014	Whitehall II study, UK	Healthy: 3229	2541	61	5	Saliva	Tertiles and 1 SD increase after log transformati on	Changes in multiple cognitive tests	Linear regression	Memory: β= -0.01, *P*>0.05; Reasoning: β=0.00, *P*>0.05; verbal fluency: β=0.00, *P*>0.05	Age, sex, ethnicity, education, time of waking, seasonality, depressive symptoms, stress, Framingham risk score, coronary heart disease, stroke, diabetes, medication
Popp et al., 2015	German Dementia Competence Network, Germany	MCI-AD: 37, AD: 46	45	≥50, mean 68.9	≤3.5, mean 2.15	CSF	Dichotomize d using median	Changes in multiple cognitive tests	Linear mixed-effects model	MCI: CERAD verbal immediate recall (β= -0.148, *P*=0.003), delayed recall (β= -0.055, *P*=0.033), ADAS-cog immediate recall (β=0.062, *P*=0.012), recognition (β=0.123, *P*=0.010); TMT A (β=1.777, *P*=0.004), TMT B (β=1.063, *P*=0.090), MMSE (β= -0.124, *P*=0.018), CDR-SOB (β=0.077, *P*=0.024);	Age, sex, education

Author, Publication year	Cohort, Country of study population	Baseline diagnosis, Sample size	Gender (no. of male)	Age at baseline (year)	Follow w-up years	Sample type	Exposure categories	Cognitive outcomes	Statistical method	Effect sizes (HR, OR, β, r)	Adjusted variables
										AD: *P*>0.05	
Lehallier et al., 2016	ADNI, USA/Canada	MCI: 94	66	55-90, mean 76	1-6	Blood	Continuous cortisol	Progression to AD	Elastic net algorithm for model selection	Cortisol level is a necessary component for the optimal prediction model	Sex and age
Pietrzak et al., 2017	AIBL, Australia	Healthy: 461	186	60-100, mean 69.3	<6	Fasting blood	Dichotomized using median	Changes in multiple cognitive tests	Linear mixed-effects model	Interaction of cortisol*Aβ*time on global cognition, episodic memory, executive function (*P*<0.05)	Age, sex, *APOE* genotype, anxiety symptoms, radiotracer type
Udeh-Momoh et al., 2019	ADNI, USA/Canada	Healthy: 91	46	55-90, mean 75.7	<10, median 7	CSF	Dichotomized using mean value	Progression to MCI or AD	Cox regression	Cortisol+/Aβ+ vs. Cortisol–/Aβ– group (HR=3.67, *P*=0.017); Interaction of cortisol*Aβ*cognitive reserve (*P*<0.001)	Age, sex, *APOE* genotype, GDS score

Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; HR, hazard ratio; OR, odds ratio; β, regression coefficient; r, correlation coefficient; MMSE, Mini-Mental State Examination; CDR, Clinical Dementia Rating; SD, standard deviation; BMI, body mass index; ADAS, Alzheimer's Disease Assessment Scale; LDT, Letter Digit Coding Test; TMT, Trail-Making Tests; DSM, Diagnostic and Statistical Manual; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; MCI-AD, MCI due to AD; CSF, cerebrospinal fluid; CERAD, Consortium to Establish a Registry for Alzheimer's Disease neuropsychological battery; ADNI, Alzheimer's Disease Neuroimaging Initiative; AIBL, Australian Imaging Biomarkers and Lifestyle Study of Ageing; Aβ, β amyloid; GDS, Geriatric Depression Scale.