Intact circadian rhythm despite cortisol hypersecretion in Alzheimer’s disease: A meta-analysis

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ABSTRACT
Hypersecretion of the glucocorticoid steroid hormone cortisol by individuals with Alzheimer’s disease (AD) has been suspected for several decades, during which time dozens of examinations of this phenomenon have been conducted and published. The goals of this investigation were to summarize this sizeable body of literature, test whether participant and methodological characteristics modify the magnitude of the AD-associated basal cortisol hypersecretion, and examine whether cortisol circadian rhythmicity is maintained among individuals with AD. To this end, the present meta-analysis and systematic review examined over 300 comparisons of indices of basal HPA-axis functioning between individuals with AD and cognitively normal older adults. AD was associated with basal cortisol elevations \( (\gamma = 0.45) \) but the magnitude of the effect was not systematically impacted by any of the participant characteristics considered or the time-of-day of the cortisol sampling. Further, there was no evidence of group differences among direct indices of circadian rhythmicity such as the cortisol awakening response or the diurnal cortisol slope. These results suggest that basal hypersecretion of cortisol, but not circadian dysrhythmia, is characteristic of individuals with AD. Mechanistically, the observed hypersecretion is consistent with the theorized AD-driven deterioration of the hippocampus and subsequent reduction in hypothalamic-pituitary-adrenal axis inhibition. Further investigation is warranted to elucidate the role and timing of cortisol elevations in the progression of AD.

1. Introduction

Alzheimer’s disease (AD) is a neurodegenerative disease characterized by the accumulation of amyloid-beta plaques and neurofibrillary tangle tangles in the brain, which are typically accompanied by neurodegeneration (Jack et al., 2018). Alterations in other biological systems have also been observed in individuals living with AD, however, the ubiquity and interpretation of these variations are less well established. One variation which has been the focus of a large body of research spanning nearly four decades is dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, manifesting as increased basal cortisol levels (Ahmad et al., 2019; Canet et al., 2018; Ouanes and Popp, 2019; Raskind et al., 1982; Spar and Gerner, 1982).

The HPA-axis is a biological system that controls both regulatory and stress-induced neuroendocrine function. Broadly, in response to a perceived stressor or circadian-based stimulation, the hypothalamic paraventricular nucleus signals the release of corticotropin-releasing hormone and arginine vasopressin, subsequently triggering the release of adrenocorticotropic hormone (ACTH) from the pituitary which serves to activate the synthesis and subsequent secretion of the glucocorticoid steroid hormone cortisol. A portion of this secreted cortisol is able to pass through the blood-brain barrier where it can bind to mineralocorticoid or glucocorticoid receptors. Binding in the hypothalamus and pituitary directly inhibit further cortisol secretion while binding in the hippocampus indirectly downregulates HPA axis activity (Herman et al., 2012; Spiga et al., 2014).

It is this indirect downregulation of the HPA-axis by the hippocampus that is often hypothesized to be hindered in AD. The hippocampus has been a central focus of AD research for decades due to the characteristic impairment of dependent functions such as episodic memory (Lopez et al., 2011; Moscovitch et al., 2016) and accelerated rates of atrophy (Jack et al., 1998; Zhao et al., 2019) among individuals with...
AD. It is hypothesized that this structural damage, which may itself be a consequence of basal cortisol elevations unrelated to or preceding the disease, contributes to impairments in episodic memory, and may limit the ability of the hippocampus to downregulate HPA axis activity, leading to chronically elevated cortisol levels among individuals with AD (Jacobson and Sapolsky, 1991).

Alternatively, researchers have pointed to the interactive roles of circadian dysrhythmia and stress, a driver of HPA-axis activity and thereby cortisol secretion, in AD (see Phan and Malkani, 2019). The hypothalamic suprachiasmatic nucleus (SCN, the central circadian pacemaker, controls a number of biological rhythms in humans. Similarly to observations of the hippocampus, AD appears to be associated with SCN degeneration (Harper et al., 2008; Stopa et al., 1999; Swaab et al., 1985) and this degeneration is associated with decreased circadian rhythm (Stopa et al., 1999; Uddin et al., 2020).

Under basal (i.e., non-stressed) conditions, signaling from the SCN to the HPA-axis produces a distinct circadian rhythm of cortisol, characterized by a sharp increase just after awakening and decline throughout the day (Henley et al., 2009; Spiga et al., 2014; Waite et al., 2012). Deviations from this rhythm, such the failure to mount a cortisol response to awakening (i.e., a blunted cortisol awakening response (CAR); Pruessner et al., 1997) or a decrease in the morning to evening drop in cortisol levels (i.e., a flattened diurnal cortisol slope (DCS)) are thought to reflect HPA-axis circadian dysregulation, and have been associated with a number of negative health outcomes including obesity and inflammation (Adam and Kumari, 2009; Adam et al., 2017; Stalder et al., 2016).

HPA-axis dysregulation has been an area of interest for AD researchers for several decades, resulting in a sizeable body of literature comparing basal cortisol levels and circadian patterns between individuals with AD and cognitively normal adults. These investigations have reported widely different findings ranging from mild, but not statistically significant, decreases in the cortisol levels of those with AD (Pomara et al., 1988) to cortisol levels that are more than 150% of those observed in cognitively normal older adults (Murialdo et al., 1993). A recent meta-analysis of a portion of this literature conducted by Zheng and colleagues (2020) reported that individuals with AD have moderately elevated morning cortisol levels compared to cognitively normal older adults (Zheng et al., 2020). While this article describes a comprehensive investigation of basal cortisol levels in AD (see also Lupien et al., 1996) and is an important step towards a more complete understanding of the biological processes involved in the progression of AD, the scope of the investigation was limited to morning cortisol levels, thereby precluding the examination of alterations in the rhythmicity of basal cortisol secretion.

The present meta-analysis and systematic review aimed to replicate and extend the findings of Zheng and colleagues (2020) by (a) confirming the presence of basal cortisol elevations among individuals with AD compared to cognitively normal older adults, (b) examining the influence of between-study factors such as the time-of-day of cortisol sampling and AD participants’ mental status on the magnitude of these elevations, and (c) systematically examining group differences in within-person markers of HPA-axis circadian rhythm.

2. Method

2.1. Literature search

The literature search, inclusion criteria evaluation and article coding were conducted by author UGS. The literature search used the PsycINFO and MEDLINE databases with the Boolean phrase (cortisol OR glucocorticoids) AND (Alzheimer’s OR dementia OR mild cognitive impairment OR MCI). The search was limited to articles published in peer-reviewed journals between 1/1/1982 and 11/1/2020 that (a) were published in English, (b) described empirical studies, and (c) included a human adult population. If manual review of the abstract (a) confirmed the literature search criteria, (b) indicated that at least one population with dementia (n > 2) of unspecified or AD-specific etiology was examined, and (c) indicated the use of a design including a cognitively normal control population, the full text of the article was read.

In order for an article to be considered methodologically eligible, the full text had to (a) confirm the earlier search and inclusion criteria, and (b) describe the in vivo collection and subsequent assay of cortisol from at least one biological sample using analogous protocols for both a cognitively normal and an AD population under basal (i.e., unstimulated) conditions (see Supplemental material Section 1.1). The information reported in the article text, tables, Supplementary materials or which was extractable from figures (via WebPlotDigitizer; Rohatgi, 2020) was considered. Methodologically eligible articles from which the (1) the sample size, (2) a conventional measure of central tendency, and (3) the corresponding spread for at least one measure of basal cortisol for both the AD and cognitively normal samples could be determined were included. Articles reporting duplicate data (e.g., identical samples or subsamples and basal cortisol measures) were excluded (see Supplemental material Section 2.1).

If the relevant cortisol values could not be ascertained from the article, but the protocol described met the remaining criteria, the data were requested from the original authors via email. If these values could not be obtained, the articles were considered incomplete and were not included. Additionally, if the only reported cortisol measure(s) encompassed multiple samples from more than one time-of-day segment (i.e., Morning, Afternoon, and/or Evening; see Section 2.1.4.3 and Supplemental material Section 3.1), granular cortisol metrics were requested; however, the summary measures were used wherever possible if the more granular measures could not be obtained. Finally, in addition to the database search, articles known to the researchers meeting the remaining eligibility criteria were also included (see Fig. 1 and Supplemental material Section 4.1).

2.2. Recording and classification

In addition to the requisite cortisol values (i.e., the measure(s) of central tendency and associated spread) and the sample sizes of both the AD and cognitively normal groups, the average age, the proportion of female participants, and the average Mini Mental Status Examination (MMSE; Folstein et al., 1975) score were recorded or imputed whenever possible (see Supplemental material Section 5.1). For each cortisol measure, the biological fluid collected for cortisol assay, the clock-time (s) of the biological sample collection, and when applicable, the method of calculation for the reported measure, were recorded.

2.2.1. Cortisol indices

Cortisol measures were classified as concentration or circadian indices. Cortisol measures reflecting the concentration of cortisol in a biological sample at a single clock-time or the average of multiple clock-times were considered concentration indices. Higher values of concentration indices are indicative of higher cortisol levels. Conversely, cortisol measures reflecting the within-person difference or ratio in concentrations of cortisol at different clock-times were classified as circadian indices because these differences typically address aspects of the circadian rhythm such as the CAR or the DCS. Cosinor analyses metrics such as amplitude (Cornelissen, 2014) were also classified as circadian indices. All circadian indices were calculated at the within-person level by the primary authors.

2.2.2. Cognitive status grouping

All participant samples included in the analyses were designated as either AD or normal cognitive control (NCC). An AD designation was applied if the authors described the sample as having AD or dementia (without specifying etiology), regardless of the diagnostic or classification criteria used. Given that AD accounts for approximately 60–80% of all dementia cases (Alzheimer’s Association, 2020), it is reasonable to
assume that even in instances where no etiology was suggested, the majority of the sample would have been classified as having AD had traditional diagnostic criteria been applied. No delineations of dementia severity (e.g., mild versus moderate) were considered. However, samples composed exclusively of participants described as mildly cognitively impaired (MCI) or with Clinical Dementia Rating (CDR; Morris, 1993) scores of 0.5 were excluded. Similarly, the primary article authors’ designation of normal cognition was used without the requirement or consideration of cognitive test performance. For both the AD and NCC populations, samples selected to include a condition impacting cortisol levels (e.g., depression) were excluded. If multiple NCC groups were available, only the NCC group closest in age to the AD group was included.

2.3. Effect size estimation

In order to directly compare the magnitude of basal cortisol elevations across studies reporting different raw units, the standardized mean difference (SMD) of the AD and NCC means for each basal cortisol index was calculated in the form of Hedges’ $g$ (Hedges, 1981). Because this measure is calculated using the relevant means, SDs, and sample sizes, alternative measures of central tendency or spread (e.g., standard error of the mean, interquartile range etc.) were converted as accurately as possible (Higgins et al., 2019; Luo et al., 2018; Viechtbauer, 2020; Wan et al., 2014) prior to the calculation of $g$ (see Supplemental materials Section 6.1). All differences were calculated as AD minus NCC.

2.4. Analytic approach

Despite the initial intention to compare the magnitude of the concentration and circadian effect sizes, just twelve circadian effect sizes were reported, preventing meaningful meta-analytic investigation. Therefore, a series of meta-analytic models were fit to the effect sizes of the concentration indices while the consideration of the circadian indices was limited to a systematic review.

2.4.1. Meta-analytic modeling

Unless otherwise noted, analyses were conducted using functions from the metafor package (Viechtbauer, 2010) in R version 4.0 (R Core Team, 2020), beginning with the calculation of $g$ via the escalc function. All meta-analytic models were fit using the rma.mv function with restricted maximum likelihood estimation (Viechtbauer, 2005, 2010). A random-effects, rather than a fixed-effects, model approach was adopted due to the a priori assumption that differences in the methodological approaches or participant characteristics systematically impacted the observed effect size (Hedges and Vevea, 1998).

Due to the frequent report of multiple cortisol measures collected at different times-of-day from a single participant population, a three-level meta-analytic model was applied (Van den Noortgate et al., 2015). Like traditional meta-analytic models, this approach models between-study variance with the assumption that effect sizes reported in separate studies are independent of each other. However, unlike the standard random-effects method, the three-level approach simultaneously models the within-study variance accounting for the inherent dependence.
between multiple effect sizes calculated from the same participant sample. Modeling this dependence helps to prevent the underestimation of the effect size standard errors while maximizing the amount of independent information included in the meta-analysis (Van den Noortgate et al., 2015). In the present meta-analytic models, study (j) refers to the pair of AD and NCC samples whose mean cortisol levels were contrasted to calculate one or more effect sizes (k). Therefore, the inherent dependence between effect sizes reported in different articles, but derived from the same study, was modeled identically to that of the dependence among multiple effect sizes derived from a single study and reported in a single article.

As the name suggests, when using the three-level model, the variance in effect sizes is parsed into three levels: the variance resulting from the primary studies reflecting a random sample of effects from some hypothetical set of normal distributions (sampling variance or level one), the systematic variance between multiple effects calculated from the same sample of participants (within-study or level two), and the systematic variance between effects calculated from independent participant samples (between-study or level three; Van den Noortgate et al., 2015). As demonstrated by Assink and Wibbelink (2016) it is possible to estimate (a) the percentage of the total variance present at each of the three levels (i.e., sampling, within-study, and between-study) and (b) whether the variance in effect sizes at the each of the within- and between-study levels is significantly different from zero. This is done by contrasting the fit of the full, three-level model (wherein both the within-study and between-study variance are freely estimated) with the fit of a model wherein the relevant level-variance (i.e., either the within-study or between-study variance) is restricted to zero. If a likelihood ratio test indicates that the restricted model fit is significantly worse than the full model fit, then there is evidence of significant variation at the restricted level (Assink and Wibbelink, 2016).

2.4.3. Meta-analytic models of interest

The first model estimated the average weighted effect size over all studies, without the inclusion of any covariates. Next, a series of five models examined the influence of several methodological and participant characteristics on the magnitude of the observed effect size. Covariates of interest were selected on the basis of previous literature and availability in the primary studies.

2.4.4. Model two: time-of-day

The second model examined the influence of the time-of-day of the cortisol sampling on the observed average effect size. In accordance with previous meta-analytic investigations of basal cortisol levels (Murri et al., 2014; Steller and Miller, 2011), the concentration indices were calculated using the cortisol levels at the point of collection (07:00 h to 11:59 h), Afternoon (12:00 h to 18:59 h), or Evening (19:00 h to 06:59 h) based on the time of the biological sample collection. Effects derived from 24-h urinary cortisol collections or which included at least one sample from each of the three times-of-day classifications (i.e., Morning, Afternoon, and Evening), and for which more granular measures could not be derived, were classified as 24-h measures (see Supplemental Material Section 3.1). The influence of the time-of-day of cortisol sampling was investigated through the inclusion of a four-level (Morning, Afternoon, Evening, and 24-h) factor covariate in the model.

2.4.5. Model three: biological sampling fluid

The third model examined the influence of the biological sampling fluid used for the cortisol assay on the observed average effect size through the inclusion of a four-level (Blood, Saliva, Urine and CSF) factor covariate.

2.4.6. Model four: mmse performance

The fourth model examined the influence of the severity of cognitive impairment of the AD group, defined as the MMSE performance of the AD group (included as a continuous covariate centered at 24), on the observed effect size.

2.4.7. Model five: participant age

The fifth model examined the influence of participant age on the observed effect size. Two continuous measures of participant age were added to the model simultaneously: (a) the mean age of the AD group (centered at age 70) and (b) the difference in the mean age of the AD group and the NCC group, with positive values indicating an older AD compared to NCC sample.

2.4.8. Model six: participant sex

The sixth model examined the influence of the proportion of female participants on the observed effect size. Two continuous measures of participant sex were included in the model simultaneously: (a) the proportion of females included in the AD group, and (b) the difference in the proportion of females in the AD and NCC groups, such that a positive value indicated a greater proportion of female participants in the AD group compared to the NCC group.

3. Results

3.1. Literature search

As shown in Fig. 1, the database search criteria returned 626 unique articles. Of these, 111 were considered methodologically eligible for inclusion. After the identification of 22 duplicate participant samples the corresponding authors were contacted via email for clarification regarding sample procedures or demographics (N = 2), cortisol values meeting the criteria for inclusion (N = 9) or more granular cortisol metrics (N = 7). Three researchers generously provided the relevant information regarding four of the articles (Arsenault-Lapierre et al., 2010, 2012; Carlson et al., 1999; Ennis et al., 2017; see Supplemental Material Section 4.1). Six researchers no longer had access to the requested original data regarding seven articles and the remaining seven researchers could not be reached.

An additional five articles known to the authors which were not identified by the database search, largely due to those studies’ incidental measurement of cortisol with a focus on other hormones (e.g., the androgenization index) or the exclusive use of the abbreviation “AD” in place of the full search term “Alzheimer’s disease”, were also included (e.g., Christie et al., 1987; De Leo et al., 1988; de Leon et al., 1988; Paolletti et al., 2004; Peskind et al., 2001). As shown in Table 2, the approximate effect sizes from all five of these articles are in line with those of the database-identified articles, suggesting that the inclusion of these articles did not bias the present results.

3.2. Meta-analytic results

Sufficient information regarding basal cortisol levels could be obtained from a total of 87 articles (see Fig. 1), including 82 studies and contributing between 1 and 97 unique effect sizes (median = 1 per article) for a total of 313 comparisons between 2738 CE and 3738 NCC individuals. Participant samples were comprised of a median of 19 (range [6319]) AD and 19 (range [4754]) NCC individuals. Both AD and
| Authors                        | N (Total Female) | Age (Mean (SD)) | MMSE (Mean (SD)) | Effect (Mean (SD)) |
|-------------------------------|------------------|-----------------|------------------|-------------------|
| Armanini et al. (2003)        | 23(18)           | 67(8.8)         | 67(10.3)         | 20(2.3)           |
| Arnsen et al. (2010)          | 12(5)            | 70(8.0)         | 20(4.2)          |
| Arnsen et al. (2012)          | 28(7)            | 78(6.5)         | 23(18)           |
| Barca et al. (2019)           | 319(184)         | 79(9.2)         | 18(6.3)          |
| Bemelmans et al. (2007)       | 21(18)           | 85(5.1)         | -(-)             |
| Bernardi et al. (2000)        | 127(7)           | 78(6.1)         | -(-)             |
| Casalheira et al. (2019)      | 19(9)            | 76(16)          | -(-)             |
| Chang et al. (2018)           | 21(14)           | 78(5.7)         | 16(4.4)          |
| Christie et al. (1987)        | 17(12)           | 61(5.4)         | -(-)             |
| Craft et al. (1993)           | 10(1)            | 69(5.9)         | -(-)             |
| Cermansky et al. (2006)       | 10(6)            | 70(8.7)         | -(-)             |
| Cunningham et al. (2001)      | 50(2)            | 70(6.3)         | 21(3.2)          |
| Curto et al. (2017)           | 18(13)           | 74(14)          | 13(9.5)          |
| Czech et al. (2012)           | 79(44)           | 70(7.7)         | 24(2.0)          |
| Davis et al. (1986)           | 9(3)             | 67(7.7)         | 16(5.7)          |
| de Bruin et al. (2002)        | 29(16)           | 76(4.7)         | 17(3.2)          |
| de la Rubia Oritz et al. (2017)| 20(10)          | 74(2.1)         | 20(2.2)          |
| De Leo et al. (1988)          | 24(15)           | 73(7.6)         | -(-)             |
| de Leon et al. (1988)         | 9(-)             | 68(7.0)         | -(-)             |
| de Leon et al. (1997)         | 8(4)             | 69(9.2)         | 16(5.7)          |
| Dodi et al. (1991)            | 12(7)            | 75(7.3)         | 19(5.3)          |
| Doecke et al. (2012)          | 207(127)         | 70(7.0)         | 25(2.9)          |
| Enns et al. (1990)            | 10(147)          | 84(6.0)         | 25(3.1)          |
| Ferrera et al. (2000a)        | 20(20)           | 81(3.6)         | 9(1.0)           |
| Ferrier et al. (1988)         | 15(13)           | 80(8.0)         | -(-)             |
| Franceschi et al. (1991)      | 14(10)           | 73(5.9)         | -(-)             |
| Gómez et al. (1990a)          | 18(17)           | 67(6.2)         | 18(6.5)          |
| Gómez-Gallo et al. (2018)     | 49(12)           | 77(5.2)         | 19(4.9)          |
| Gómez-Gallego and Gómez-García (2018) | 80(56)       | 74(7.5)         | 19(4.9)          |
| Hartmann et al. (1997)        | 12(8)            | 63(8.4)         | 19(6.3)          |
| Hanfield et al. (2004)        | 27(12)           | 72(5.2)         | 18(3.4)          |
| Higuchi et al. (2000)         | 11(13)           | 73(5.2)         | 16(10)           |
| James et al. (2020)           | 65(43)           | 76(7.9)         | 21(2.4)          |
| Johar et al. (2015)           | 33(10)           | 78(6.8)         | 28(2.4)          |
| Kudoh et al. (1999)           | 7(4)             | 72(4.8)         | -(-)             |
| Lara et al. (2013)            | 59(36)           | 78(3.7)         | -(-)             |
| Lavelle et al. (2019)         | 60(23)           | 71(17.9)        | 21(8.3)          |
| Leake et al. (1990a)          | 12(7)            | 67(9.0)         | -(-)             |
| Leake et al. (1990)           | 11(9)            | 77(6.3)         | -(-)             |
| Leibhuber et al. (1993)       | 24(11)           | 77(5.7)         | 5(2.6)           |
| Lerner et al. (2000)          | 8(5)             | 68(5.8)         | 18(7.5)          |
| Lech et al. (2000)            | 10(7)            | 59(6.3)         | 15(6.6)          |
| Linder et al. (1993)          | 13(9)            | 75(5.5)         | -(-)             |
| Maeda et al. (1991)           | 10(8)            | 80(5.1)         | 14(11.3)         |
| Martignoni et al. (1990a)     | 10(2)            | 64(9.7)         | 16(5.2)          |
| Martignoni et al. (1990b)     | 8(1)             | 66(8.2)         | -(-)             |
| Masera et al. (2002)          | 17(7)            | 67(7.6)         | 18(14)           |
| Masugui et al. (1989)         | 10(1)            | 78(5.5)         | -(-)             |
| Miller et al. (1994)          | 18(7)            | 68(1.1)         | 1.49(0.47)       |
| O'Brien et al. (1994)         | 18(7)            | 68(1.1)         | 1.49(0.47)       |
| Paolotti et al. (2004)        | 96(64)           | 77(15.7)        | 18(3.7)          |
| Parnetti et al. (1990)        | 21(6)            | 72(10.0)        | 18(6.2)          |
| Parnetti et al. (1995)        | 12(4)            | 74(1.6)         | 13(1.9)          |
| Pacsuyla et al. (2000)        | 9(5)             | 76(6.0)         | 17(3.0)          |
| Peabody et al. (1986)         | 9(0)             | 63(6.4)         | 14(2.2)          |
| Penkis et al. (2016)          | 11(3)            | 72(6.6)         | 19(6.6)          |
| Penkis et al. (2001)          | 64(20)           | 67(8.0)         | 16(8.0)          |
| Pettig et al. (1999)          | 10(3)            | 70(7.9)         | 18(4.4)          |
| Pomara et al. (1984)          | 10(1)            | 71(8.8)         | 18(4.4)          |
| Pomara et al. (1988)          | 10(7)            | 70(8.5)         | 18(4.4)          |
| Pop et al. (2015)             | 105(63)          | 73(7.4)         | 23(3.1)          |
| Porter et al. (2002)          | 32(20)           | 75(5.7)         | 2(3.3)           |
| Raskind et al. (1982)         | 15(0)            | 64(2.3)         | -(-)             |
| Rasmussen et al. (1998)       | 13(8)            | 78(8.4)         | 18(6.4)          |

(continued on next page)
NCC participants were predominantly older adults ($M = 72.89, SD = 6.04$ and $M = 70.53, SD = 7.91$ years, respectively), and predominantly female ($M = 57.96\%$, $SD = 22.80; M = 54.38\%, SD = 22.61$, respectively). MMSE performance was reported for 45 CE participant samples and indicated that the average MMSE score among the AD participants was 18.03, $SD = 3.70$, range [5.20, 25.30]). See Table 1 for detailed information on the included articles.

Of the 313 comparisons, no effects were highly influential (DFBE-TAS: range [−0.26, 0.20]), however five effects were outlying (studentized deleted residuals from 2.04 to 3.47) and were excluded from further analysis. There was significant evidence of asymmetry among the remaining 308 effects (Egger’s test: $t(306) = 6.38, p < 0.01$), indicating that the distribution of included effect sizes was not symmetrical around the average calculated effect size. Applying the trim and fill procedure to a two-level model excluding the five outlying effects demonstrated that an estimated 92 effects were missing from the left side, with an adjusted estimate of $g = 0.24$ ($p < 0.01$, $95\%$ CI [0.17, 0.30], $k = 400$; see Fig. 2). Although this effect size is significantly different from zero, it reflects a 0.23 unit decrease from the overall effect estimate from the two-level model ($g = 0.47$, $p < 0.01$, $95\%$ CI [0.42, 0.52], $k = 308$), and a 0.22 unit decrease from the three-level model with all effect sizes included used for the identification of outliers ($g = 0.46, p < 0.01$, $95\%$ CI [0.35, 0.57], $j = 82, k = 313$).

### 3.2.2. Model two: time-of-day

As evidenced in Fig. 3, the time-of-day of the cortisol collection did not significantly impact the magnitude of the effect size ($F(3, 289) = 1.05, p = 0.37$) and AD participants had significantly higher basal cortisol levels than NC participants during all three time-of-day blocks (i.e., Morning, Afternoon, and Evening) and when measured over a 24-h period ($g = 0.45, g = 0.43, g = 0.53$ and $g = 0.55$, respectively, all $p < 0.01$). Significant residual variation in the magnitude of the effect sizes ($g^2 = 0.24, Q(289) = 517.58, p < 0.01$) remained after including time-of-day of cortisol collection as a covariate in the model. The between-study variance remained significant ($\chi^2(1) = 172.86, p < 0.01$) and the within-study variance non-significant ($\chi^2(1) = 0.03, p = 0.43$), accounting for 56.32% and 0.15% of the total variance, respectively.

Given the significant residual variability, the dominance of the variability at the between-study level, and the prevalence of articles reporting effects determined from only one time of day, a post-hoc replication of Model Two was conducted, including only those studies reporting the results of at least two comparisons of cortisol samples obtained at different time-of-day blocks. This approach resulted in the inclusion of 202 effects from 20 independent studies and replicated the results of Model Two ($F(2, 199) = 1.47, p = 0.23$; see Model 2.1 in Table 1 (continued))

| Authors | N | Age | MMSE | Effect |
|---------|---|-----|------|--------|
| AD | NC | AD | NC | AD | NC |
| Rasmussen et al. (2001) | 10(10) | 7(7) | 80(5.8) | 73(6.2) | 17(-) | 30(-) | 1 | -0.78(0.51) |
| Rasmussen et al. (2002) | 38(21) | 22(12) | 76(7.8) | 75(7.5) | 17(5.1) | 30(0.70) | 1 | 0.69(0.28) |
| Rasmussen et al. (2011) | 7(0) | 7(0) | 76(5.5) | 73(5.8) | 21(5.6) | 30(0.50) | 10 | 0.49(0.54) |
| Rolandi et al. (1992) | 6(0) | 6(0) | 70(1.6) | 82(2.3) | -(-) | -(-) | 1 | 1.62(0.66) |
| Sousa-Talarico et al. (2008) | 40(27) | 40(35) | 80(6.0) | 72(6.3) | -(-) | -(-) | 1 | 0.31(0.22) |
| Spada et al. (2001) | 15(7) | 23(8) | 70(8.0) | 68(9.0) | -(-) | -(-) | 1 | 0.95(0.35) |
| Swannick et al. (1998) | 18(11) | 17(9) | 74(4.7) | 70(8.3) | 17(4.4) | 29(0.70) | 1 | 1.13(0.36) |
| Tollefson et al. (1999) | 25(20) | 25(20) | 72(9.9) | 72(7.9) | 19(2.9) | -(-) | 2 | 0.02(0.28) |
| Tsushima et al. (1992) | 26(10) | 19(4) | 67(7.4) | 66(6.0) | -(-) | -(-) | 2 | 0.35(0.30) |
| Umezaki et al. (2000) | 66(66) | 21(21) | 82(7.8) | 83(7.8) | 8(0.65) | 25(2.9) | 1 | 0.75(0.26) |
| Vanikova et al. (2016) | 16(16) | 22(22) | 75(11) | 67(8.3) | -(-) | -(-) | 1 | 0.38(0.33) |
| Wahbeh et al. (2008) | 19(11) | 15(10) | 75(7.0) | 75(5.0) | -(-) | -(-) | 5 | 1.24(0.38) |
| Wang et al. (2018) | 69(30) | 52(46) | 75(7.6) | 76(5.4) | -(-) | -(-) | 1 | 0.02(0.16) |
| Wirth et al. (2019) | 112(47) | 58(28) | 75(8.0) | 75(5.8) | 24(1.9) | 29(1.2) | 1 | 0.16(0.16) |
| Woolley et al. (2014) | 17(8) | 18(11) | 59(8.0) | 57(8.7) | 15(7.7) | -(-) | 8 | 0.43(0.34) |
| Zvereva et al. (2013) | 45(28) | 37(29) | 75(8.4) | 63(7.6) | 19(7.1) | 29(1.0) | 1 | 0.54(0.23) |

AD = Alzheimer’s disease; k = The number of effect sizes contributed by a given article; MMSE = Mini-Mental Status Examination score; NC = Normal cognitive control; SD = Standard deviation.

Authors provided information regarding mean(s) and/or SD(s); Mean(s) was estimated using the range; Mean(s) was estimated using the median and interquartile range (IQR); SD(s) was calculated using the median error of the mean (SEM); SD(s) was estimated using the 95% confidence interval (CI); SD(s) was estimated using the range; SD(s) was estimated using provided t-test or p-values (or thresholds); Means and/or SDs were estimated from figures. Note. The Effect size reported here reflects the mathematical average of all k Hedges’ g values contributed by a given article. This value does not necessarily reflect the exact effect size used by the models but is used here for illustrative purposes. Articles included in the meta-analysis are indicated by a * preceding the reference in the bibliography.
Table 2

| Variance          | # | k   | j  | $\sigma^2$ | Q  | %    | LRT | F   | b  | 95% CI   |
|-------------------|---|-----|----|-----------|----|------|-----|-----|----|----------|
| Residual          | 1 | 308 | 82 | 0.27      | 600*** | 0.04 | 0.003 | 62  | 201*** | 69.54*** | Overall 0.45*** (0.34-0.56) |
|                   | 2 | 293 | 73 | 0.24      | 518*** | 0.15 | 0.03  | 56  | 173*** | 1.05     | Morning 0.45*** (0.34-0.56) |
|                   |   | 135 |    |           |      |      |      |     |       |          | Afternoon 0.43*** (0.28-0.58) |
|                   |   | 58  |    |           |      |      |      |     |       |          | Evening 0.53*** (0.39-0.67) |
|                   |   | 94  |    |           |      |      |      |     |       |          | 24 h 0.55*** (0.14-0.96)    |
| Between           | 2.1 | 202 | 20 | 0.23      | 312*** | 0.70 | 0.29  | 48  | 121*** | 1.47     | Morning 0.52*** (0.34-0.71) |
|                   |   | 57  |    |           |      |      |      |     |       |          | Afternoon 0.48*** (0.29-0.67) |
|                   |   | 54  |    |           |      |      |      |     |       |          | Evening 0.59*** (0.41-0.77) |
|                   | 3  | 308 | 82 | 0.28      | 571*** | 0.29 | 0.17  | 63  | 186*** | 1.70     | Blood 0.46*** (0.33-0.59)   |
|                   |   | 257 |    |           |      |      |      |     |       |          | Saliva 0.36* (0.12-0.61)    |
|                   |   | 42  |    |           |      |      |      |     |       |          | Urine 0.68* (0.20-0.116)    |
|                   |   | 5   |    |           |      |      |      |     |       |          | CSF 0.73*** (0.44-1.03)     |
|                   |   | 4   |    |           |      |      |      |     |       |          | Plasma 0.43*** (0.25-0.61)  |
|                   | 3.1 | 256 | 63 | 0.32      | 399*** | –    | –    | 59  | 117*** | 0.14     | Serum 0.44*** (0.24-0.64)   |
|                   |   | 159 |    |           |      |      |      |     |       |          | Blood 0.58* (0.04-1.12)     |
|                   |   | 61  |    |           |      |      |      |     |       |          | MMSE -0.03 (-0.06 to 0.01)  |
|                   |   | 36  |    |           |      |      |      |     |       |          | Int. 0.27* (0.05-0.48)      |
|                   | 4  | 192 | 45 | 0.20      | 312*** | –    | –    | 54  | 74***  | 2.54     | Age(AD) -0.001 (-0.02 to 0.02) |
|                   |   | 54  |    |           |      |      |      |     |       |          | %F 0.50*** (0.37-0.63)       |
|                   |   | 193 |    |           |      |      |      |     |       |          | %F(AD) -0.001 (-0.02 to 0.02) |
|                   | 5  | 308 | 82 | 0.27      | 573*** | –    | –    | 62  | 193*** | 1.87     | %F(AD) -0.02 (-0.04 to 0.004) |
|                   |   | 77  |    | 0.26      | 559*** | 0.19 | 0.07  | 60  | 200*** | 0.43     | %F 0.51*** (0.23-0.80)       |
|                   |   | 30  |    | 0.26      | 599*** | 0.04 | 0.003 | 62  | 201*** | 0.30     | %F(AD) -0.08 (-0.54 to 0.39) |
|                   | 7  | 308 | 82 | 0.27      | 599*** | 0.04 | 0.003 | 62  | 201*** | 0.30     | %F -0.37 (-0.42 to 1.17)    |

- value is < 0.00000005; $\Delta$Age = The difference in age (in years) between the Alzheimer’s disease (AD) sample and the normal cognitive control (NCC) sample; $\Delta$%F = The difference in the proportion of female participants in each sample between the AD and NCC samples; # = The model number; % = The percentage of the total residual variance at each given level (within-study and between-study); %F(AD) = The proportion of the AD sample that was female; $\sigma^2$ = total residual variance (including the estimated sampling variance, and the variance at the within- and between-study levels); 95% CI = the 95% confidence interval for the corresponding b-value; Age(AD) = The mean age (in years) of the AD sample (moderator centered at 70); CSF = cerebrospinal fluid; F = the value of the F-test of all included moderators for each model; j = number studies contributing the k effect sizes included in each model; k = number of effect sizes included in each model (for factor models, the total k is reported, as well as the number of effect sizes at each factor level); LRT = likelihood ratio test statistic; MMSE = Mini-Mental Status Examination Score (covariate centered at 24); Non-standard: No standard diagnostic criteria for the classification of the AD group was reported; Q: = the value of the Q-test for residual heterogeneity; Standard: One or more standard diagnostic criteria were used to classify the AD group.

Note. For factor models (Models 2–3.1; 7) the intercept (i.e., the average Hedges’ g) for each factor level is shown separately, rather than in relation to a reference level. Associated significance-level markers therefore indicate whether the average effect size of each factor level is significantly different than zero, not that it significantly differs from the other relevant factor levels. As indicated by the associated F-values, none of the factor levels significantly differed from each other. p < 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001

Table 2).

3.2.3. Model three: sampling fluid

Prior to fitting Model Three, an exploratory analysis of the blood-based matrix subgroup of effects confirmed that the magnitude of the effect size did not systematically differ between cortisol assays from plasma, serum, or unspecified “blood” matrix ($F(2, 253) = 0.14, p = 0.87$; see Model 3.1 in Table 2). Therefore, all effect sizes from studies utilizing any blood-based matrix were grouped into the factor Blood for Model Three. The biological fluid used for cortisol sampling (Blood, Saliva, Urine, or CSF) did not significantly impact the magnitude of the average effect size ($F(3, 304) = 1.70, p = 0.17$). Significant residual effect size heterogeneity remained after controlling for cortisol sampling fluid, predominantly and significantly at the between-study level.

3.2.4. Models four, five, and six

None of the participant characteristics (MMSE performance of the AD sample, participant age, or the proportion of female participants) were significantly associated with the observed effect size ($p > 0.12$). For all three models, significant residual heterogeneity remained after controlling for each of the covariates (or set of covariates), with a significant majority of this variance at the between-study level and an insignificant portion at the within-study level (see Models Four, Five, and Six in Table 2).

Finally, as previously noted, the primary authors’ designation of AD was accepted regardless of whether or not standard diagnostic criteria was applied. In an effort to ensure that this decision did not systematically impact these results, a post-hoc sensitivity analysis was conducted that included a binary factor covariate indicating whether standard diagnostic criteria (i.e., one or more of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA), DSM-III, DSM-III-R, DSM-IV, DSM-IV-R or ICD-10 criteria (American Psychiatric Association, 1980, 1987, 1994, 2000; McKhann et al., 1984; World Health Organization, 1992)) was used to classify the AD participant sample. Of the 308 included effect sizes, 201 originated from 74 unique studies that used standard diagnostic criteria. There was no
Fig. 2. Funnel plot of effect sizes (Hedges’ g) by the log of the standard error of the effect size. Each point reflects a single effect size. Black, solid points reflect effect sizes included in Model One, and Models Two through Six when relevant covariate information was available. Red, crossed points reflect effect sizes that were shown to have associated studentized deleted residual greater than 1.96. These effects were excluded from all models of interest. Light blue, open points reflect the hypothetical effect sizes filled via the trim and fill procedure. The dashed vertical lines reflect the meta-analytic mean from Model One (shown in black) and the adjusted meta-analytic mean from the trim-and-fill procedure (shown in light blue). The dotted lines reflect the estimated 95% confidence intervals (calculated under the assumption of fixed-effects, shown for the Model One average effect (g = 0.45, p < 0.01) and trim-and-fill procedure adjusted average effect (g = 0.24, p < 0.01) in black and light blue respectively).

Fig. 3. Scatterplot of the relationship between the time of day of cortisol sampling and the observed effect size. Each point or line reflects a single effect size. In order to maximize visual data inclusion, clock times were imputed for samples collected at waking were assigned to 07:00, 30 min after waking assigned to 07:30, morning samples (without any further clock-time description) were assigned to 09:30, before lunch samples were assigned to 11:30, 1 h after lunch samples were assigned to 13:00, and bedtime samples were assigned to 23:00. Dashed lines reflect effects calculated from cortisol sampled within the time window indicated by the width of the line, while dotted lines reflect effects calculated from the mean of multiple cortisol samples collected within the time window indicated by the width of the line. Solid lines indicate the average effect size and associated 95% confidence intervals for each of the four times of day (Morning, Afternoon, Evening, and 24-hour collections). As indicated by the results of Model 2, the time of cortisol sampling was not significantly associated with the observed effect size.

evidence that the use of standard diagnostic criteria, in comparison to an unspecified or non-standard classification criteria, significantly moderated the observed effect size (b = 0.10, p = 0.58; see Model 7 in Table 2).

3.3. Replication with e

Hedges’ g, while providing correction for small sample sizes, assumes homogeneity of variance among the samples. It is plausible that basal cortisol levels are more variable among the persons with AD, who may have increased disruption of the HPA-axis compared to NCC participants, which could lead to a violation of this assumption. Recently, Aoki et al. (2020) introduced ε (and corresponding package es.dif), a measure of the SMD without the assumption of homogeneity of variance. In order to examine whether the violation of the homogeneity of variance assumption significantly impacted the present findings, Models One through Seven were re-fit using ε in place of g (unless g was estimated via a reported t- or p-value, in which case g was retained). The model results were not interpretively different when using ε in place of g (see Supplemental Table 2).

3.4. Review of circadian metrics

Eight studies met the criteria for inclusion in the systematic review. Notably, concentration indices determined from all eight of the constituent samples were also included in the meta-analysis. Three of these studies were restricted to cortisol sampled across the daytime (e.g., waking to bedtime), while one study included cortisol sampled overnight (21:00 h to 12:00 h), and the remaining four sampled cortisol across a period of 24-hours with varying sampling frequency. Of the four studies considering the entire circadian rhythm, three applied cosinor or deconvolution techniques for the comparison of circadian metrics across groups. Although a number of measures describing various aspects of the circadian rhythm can be derived from these rhythm modeling approaches, this review was limited to metrics reflecting ratios or differences in observed or modeled cortisol levels. This restriction resulted in a total of 12 metrics for consideration, including measures of the DCS, diurnal variation, and a measure of the CAR. None of the calculated Hedges’ g values significantly differed from zero with the exception of a significantly elevated cosinor amplitude reported by Martignoni et al. (1990a) Table 3).

4. Discussion

The current meta-analysis confirms the presence of basal cortisol elevations among individuals with AD compared to cognitively normal older adults. Specifically, across more than 300 comparisons from 87 peer-reviewed articles published over a span of nearly 40 years, AD was associated with an increase in basal cortisol levels of approximately half of a standard deviation in magnitude. This effect is virtually identical to the meta-analytic findings of Zheng and colleagues (2020) who also reported moderate (g = [0.42, 0.57]) increases in cortisol levels among individuals with AD. However, Zheng and colleagues restricted their analyses to cortisol samples collected in the morning. The findings from Model Two of the present study demonstrate that the cortisol elevation in AD is present regardless of the time-of-day of cortisol sampling. In addition, our systematic review reveals no evidence for altered circadian rhythmicity of cortisol in AD, though this latter conclusion is tempered by the small number of studies quantifying circadian rhythmicity. These findings suggest that basal hypercortisolism is generally characteristic of AD and are consistent with the hypothesized reduction in HPA-axis inhibition due to decreased structural and functional integrity of the hippocampus in AD (Jack et al., 1998; Jacobson and Sapolsky, 1991; Lopez et al., 2011; Zhao et al., 2019).
with Alzheimer and disrupted circadian rhythmicity in AD (Uddin et al., 2020), the vast

4.1. Circadian rhythm maintenance

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4.1.1. Systematic review of circadian indices

The findings from the systematic review of circadian metrics provide the most direct evidence of the maintenance of the cortisol circadian rhythm in AD. With the exception of the significantly increased cosinor amplitude reported by Martignoni et al. (1990a), none of the studies examining markers of HPA-axis rhythmicity, such as the CAR, DCS, and the diurnal variation reported significant differences between AD and NCC older adults. Unfortunately, the low number and inconsistency of the reported circadian indices prohibited meaningful averaging or comparison of the circadian indices effect sizes with the meta-analytic effect sizes. It is notable, however, that both Ferrari et al. (2000b) and Hartmann et al. (1997) reported significantly greater 24-h mean cortisol concentrations among individuals with AD, but no significant differences in the cosine amplitude and nocturnal increase or the diurnal variation, respectively. Taken together, these are consistent with the general finding of maintenance of cortisol circadian rhythmicity in the presence an AD-associated increase in basal cortisol levels.

4.1.2. Other rhythmicity measures

In addition to the circadian rhythm examined in the systematic review, the cosinor and deconvolution analyses of the 24-h cortisol sampling provide additional evidence for the maintenance of the circadian rhythm among individuals with AD. Specifically, both cosinor analyses report an unaltered acrophase (roughly, the timing of the circadian maximum; Ferrari et al., 2000b; Martignoni et al., 1990a), thereby providing no evidence for a shift in the timing of the circadian rhythm in individuals with AD.

Finally, in an exceptionally thorough examination of basal cortisol levels and circadian rhythmicity in individuals with AD, Hartmann and colleagues (1997) collected plasma samples at 15-minute intervals across a 24-hour period, then applied a deconvolution analysis to the 97 samples for each individual. The authors reported that, compared to cognitively normal older adults, the cortisol circadian rhythm of individuals with AD did not systematically differ with respect to the cortisol half-life, the frequency of cortisol secretory bursts, the interpulse interval, the length of the quiescent period, or relative diurnal variation. It was further noted that while the mass of cortisol secreted per burst was numerically much greater (251 noml/l versus 155 nmol/l), this contrast did not reach statistical significance (g = 0.77, p = 0.092). To the authors’ knowledge, this study reflects the only comparison of the ultradian rhythm of cortisol across a full circadian period between individuals with AD and cognitively normal older adults. Therefore, although considered with caution, these results suggest that the ultradian rhythm of cortisol is largely unaffected with the potential exception of increased cortisol secretion per burst.

4.1.3. Time-of-day moderation

The invariance of the average effect size to the time-of-day of cortisol sampling observed in the meta-analytic Model Two further provides further evidence that HPA-axis rhythmicity is maintained in individuals with AD. Specifically, if a flattened DCS was characteristic of AD, one would expect a larger evening than morning effects, reflecting the failure to embrace the quiescent period. Alternatively, if individuals with AD failed to mount a cortisol response to awakening, one would anticipate smaller (or even negative) effects in the morning compared to the other times of day.

Post-hoc Model 2.1 was conducted in an effort to better capture the potential moderating effect of time-of-day while reducing between-study variation by limiting the analyses to studies reporting effect sizes from at least two of the three time-of-day blocks (i.e., Morning, Afternoon, and Evening). Even in this restricted analysis, there was no evidence for a time-of-day effect, suggesting that it is not between-study variance in participant characteristics masking a true effect.

4.1.4. Within-study variance

The three-level meta-analytic approach was chosen to properly model the dependence between multiple effect sizes originating from individual studies. In this analysis, individual studies were considered to be nested within individuals with AD and cognitively normal older adults. Specifically, the within-study variance for each effect size is a function of the number of participants and sampling times. The between-study variance is a function of the between-study heterogeneity, which was estimated using a random-effects model. The within-study variance is a function of the number of participants and sampling times.

Table 3: Systematic review of articles comparing circadian indices between individuals with Alzheimer’s disease (AD) and normal cognitive controls (NCC).

| Article | Cortisol Sampling | Comparison (s) | Result |
|---------|-------------------|----------------|--------|
| Davis et al. (1985) (Same procedure as Davis et al., 1986) | Every 30 min (21:00 h – 08:00 h) | Diurnal difference | AD > NCC (g = 0.14, p = 0.08) |
| Martignoni et al. (1990a) | Every 4 h (08:00 h – 12:00 h) | Amplitude | AD > NCC (g = 1.57, p < 0.01) |
| Rolandi et al. (1992) | Every 4 h (08:00 h – 20:00 h) | Diurnal difference | AD > NCC (g = 0.13, p = 0.62) |
| Hartmann et al. (1997) | Every 15 min (08:00 h – 00:00 h) | Diurnal variation | AD > NCC (g = 0.03, p = 0.95) |
| Ferrari et al. (2000b) (Same procedure as Ferrari et al., 2000a) | Every 4 h (08:00 h – 20:00 h) | Nocturnal increase | AD > NCC (g = 0.18, p = 0.50) |
| Giubilei et al. (2001) | Every 15 min (08:00 h – 04:00 h) | Diurnal fluctuation % | AD > NCC (g = 0.53, p = 0.12) |
| Johar et al. (2015) | Waking + 30 Bedtime (Bed) | CAR Waking/Bed ratio | AD > NCC (g = 0.03, p = 0.79) |
| Barca et al. (2019) | Morning (AM) Evening (PM) | PM/AM ratio | AD > NCC (g = 0.13, p = 0.29) |

+30 = Cortisol level sampled 30 min after waking; CAR = +30 cortisol level minus Waking cortisol level; Diurnal COV = Diurnal coefficient of variation; Standard deviation of the cortisol values divided by the diurnal mean; Diurnal difference = Mathematical difference in the highest and lowest cortisol value for each individual; Diurnal fluctuation % = 08:00 cortisol level – 20:00 cortisol level/08:00 cortisol level x 100; Diurnal variation = Diurnal difference divided by diurnal mean (the mathematical mean of all cortisol measures taken from a single day); Nocturnal increase = 24:00 h cortisol level minus 08:00 h cortisol level. Note: For the circadian indices, the Hedges’ g values and associated p-values were calculated using the tsum.tes and tes functions from the rpsychi Okumura, Y., 2012. rpsychi: Statistics for psychiatric research, R package version 0.8 ed. and compute.es Del Re, A.C., 2011. compute.es: Compute Effect Sizes. packages respectively.

2 The Hedges’ g value and associated p-value reported here were calculated by the present authors based summary statistics from the primary study using the tsum.tes and tes functions from the rpsychi Okumura, Y., 2012. rpsychi: Statistics for psychiatric research, R package version 0.8 ed. and compute.es Del Re, A.C., 2011. compute.es: Compute Effect Sizes. packages respectively.
the same sample participant population in order to properly estimate the effect size standard deviations (see Van den Noortgate et al., 2015). An additional benefit of this model is that the percentage of variance at each level (i.e., between-study, within-study, and sampling) can be estimated (Assink and Wibbelink, 2016). Although this approach does not inherently attribute the variance at any level to a particular variable, due to the structure of data recording and inclusion, the only methodological factors of interest that vary across multiple effect sizes originating from the same study were (a) the time-of-day, and (b) the biological sampling fluid.

Of the 37 studies contributing multiple effect sizes, 31 contributed effects from multiple times-of-day. Notably, the differing times-of-day here do not necessarily correspond to the time-of-day blocks examined as a moderator. For example, effect sizes contributed by a single study calculated from cortisol sampled at 09:00 h and 10:00 h would be considered differing times-of-day in the context of within-study variation. Given the limited sources of variation, the finding that the within-study variance accounted for less than 1% of the total variance across all models suggests that the effects derived from a single participant sample at differing times are highly similar, which may be interpreted as further evidence that the circadian rhythm is maintained in AD.

4.1.5. Biological mechanisms

Although the biological mechanisms underpinning the observed pattern of basal cortisol elevations cannot be directly addressed here, the uniform basal cortisol elevations appear to be consistent with the hypothesized role of reduced hippocampal inhibition of the HPA-axis in the pathophysiology of AD (Ahmad et al., 2019; Jacobson and Sapolsky, 1991). Specifically, the dominant theory of pathological age-related hypercortisolism, the Glucocorticoid Cascade Hypothesis (Sapolsky et al., 1986), proposes that the exposure of hippocampal neurons to chronic cortisol elevations increases the vulnerability of these neurons to other insults, leading to neuronal damage or even overt neuron death. This damage reduces the hippocampal downregulation of the HPA-axis resulting in even greater cortisol elevations and subsequently even greater hippocampal damage (Sapolsky et al., 1986).

It follows from this hypothesis that greater damage to the hippocampus should be associated with greater cortisol elevations. Although there has been limited direct examination of this hypothesis in individuals with AD (c.f., de Leon et al., 1988), this relationship has been a focus among older adults with depression. Like AD, depression is associated with elevated basal cortisol levels (Murri et al., 2014; Stetler and Miller, 2011) which are often attributed to reduced hippocampal inhibition. Given these commonalities, it is notable that a recent meta-analysis of the relationship between cortisol levels and hippocampal volume among depressed older adults found a significant negative association between the two factors (Geerlings and Gerritsen, 2017), indicating that the severity of hippocampal structural damage is significantly associated with the severity of HPA-axis dysregulation.

It is important to highlight that while the hippocampus has been a major focus of AD research for decades, there is ample evidence that the prefrontal cortex also contributes to the down-regulation of HPA-axis activity (Herman et al., 2012) and that dysfunction of the prefrontal cortex contributes to AD symptomology (see Xu et al., 2006). Therefore, it is conceivable that the observed basal cortisol elevations in individuals with AD are attributable to structures outside of the hippocampus.

The finding of seemingly intact cortisol circadian rhythms among individuals with AD is not difficult to reconcile with earlier findings of SCN degeneration and the associated disruption of circadian systems among individuals with AD (Harper et al., 2008; Stopa et al., 1999; Swaab et al., 1985; Uddin et al., 2020) but there are several plausible explanations. First, there is evidence for peripheral control of cortisol circadian rhythms at the level of the adrenal gland (Focke and Iremonger, 2020). The secretion of cortisol from the adrenal gland is typically triggered by the release of ACTH from the pituitary which results from signaling from the SCN to the hypothalamus (Spiga et al., 2014). The pattern of circulating ACTH and cortisol are generally tightly associated, showing coincident secretory events separated by a period of approximately ten minutes (Henley et al., 2009). Given this coupling, the circadian variation in cortisol appears to be controlled upstream by the SCN via pulsatile ACTH secretion. However, rodent work has shown that hypophysectomized rodents with constant ACTH replacement maintain circadian variation of glucocorticoids (Meier, 1976), and more recent work suggests that this maintenance is the result of a clock system local to the adrenal gland (Focke and Iremonger, 2020; Son et al., 2008). It is unlikely that these mechanisms are entirely independent of the SCN, given that SCN lesions in rodents result in a loss of glucocorticoid circadian rhythmicity (Waite et al., 2012), however, the adrenal-level control may be sufficient to compensate for more minor cellular damage in the SCN (Focke and Iremonger, 2020; Harper et al., 2008; Stetler et al., 1999).

Second, absent adrenal-level contributions to the maintenance of the cortisol circadian rhythm, the neuronal damage to the SCN simply may not be severe enough to result in overt, group-level shifts in cortisol circadian variation. While several studies have reported significantly greater evidence of SCN degeneration among individuals with AD compared to cognitively normal older adults (Harper et al., 2008; Stopa et al., 1999; Swaab et al., 1985) this finding is not unanimous (Wang et al., 2015), and the degree of SCN degeneration is not significantly associated with changes in all circadian systems (Harper et al., 2008).

Finally, the possibility that the present analyses were underpowered to detect shifts in the circadian rhythm must also be considered. As evidenced by the systematic review, only a handful of studies explicitly aimed to compare cortisol circadian rhythmicity between individuals with AD and cognitively normal participants and these studies often included small sample sizes, sparse measurement schedules, or a combination of these factors. These limitations are particularly problematic for the comparison of cortisol given the ultradian pulsatile secretion and the inherent difficulties with comparing a waking-based rhythm using pre-determined clock-times. The grouping by clock-time presents a similar challenge in the context of the test of time-of-day as a moderator of the meta-analytic effect. Specifically, although the division of the time-of-day classifications matches previous investigations of basal cortisol (Murri et al., 2014; Stetler and Miller, 2011) and roughly correspond with the varying phases of the basal circadian rhythm, a great deal of noise is inevitably introduced by using a clock-time locked scale for a biological phenomenon.

4.2. Biological implications

In addition to providing insight into the relationship between the hippocampus and HPA-axis dysregulation, investigations involving individuals with depression also illustrate why the basal cortisol elevations observed here among individuals with AD cannot be unambiguously classified as a consequence of AD (i.e., due to AD-related hippocampal degeneration) and could in fact be a driver or precursor of AD pathology. Specifically, individuals with depression, who presumably experience elevated basal cortisol levels over a period of years to decades, are at increased risk to develop dementia later in life (Barnes et al., 2012; Cherbuin et al., 2015; Diniz et al., 2013; Ownby et al., 2006; Rasmussen et al., 2018). Given the association between hippocampal volume and basal cortisol levels in this group (Geerlings and Gerritsen, 2017), it is not unreasonable to hypothesize that these cortisol elevations could contribute to the deterioration of hippocampal-dependent cognitive functions typically used to diagnose AD.

Others have suggested that the increased risk of dementia among those with depression is driven by increased accumulation of amyloid-beta, the hallmark biomarker of AD (Canet et al., 2018; Sotiropoulos et al., 2008). There is evidence of increased amyloid-beta among individuals with depression (see Harrington et al., 2015 for review) and cellular and rodent work suggests there may be a causal link between cortisol elevations and amyloid-beta accumulation. Specifically, Green
et al. (2006) showed that the addition of glucocorticoids to cultured mouse neuronal cells increased the amount of amyloid-beta in a concentration and time-dependent manner. Similarly, glucocorticoid elevations, whether induced by exogenous administration or chronic stress, result in greater amyloid-beta presence in rodents (Dong et al., 2004; Green et al., 2006; Jeong et al., 2006; Kang et al., 2007).

Considering the current meta-analytic results in the context of these associations, it is appropriate to conclude that basal cortisol elevations are characteristic of individuals with AD, but we cannot conclude whether these elevations preceded or followed the onset of cognitive symptoms or speak to whether these elevations reflect a symptom of the disease or contribute to the onset or progression.

4.3. Invariance to participant traits

Despite previous reports of negative relationships between basal cortisol measures and MMSE performance in individuals with AD (Gil-Bea et al., 2010; Huang et al., 2009; Zverova et al., 2015), the MMSE score of the AD sample did not significantly moderate the difference in basal cortisol levels between the AD and NCC samples in this analysis. Although this should be considered with caution, especially given the cross-sectional nature of the research included here, the null association could be interpreted as evidence for the occurrence of a plateau in basal cortisol elevations early in the disease process.

In line with previous meta-analytic findings (Zheng et al., 2020), this analysis did not find evidence for a moderating effect of the age or the proportion of female participants of the AD sample on the magnitude of AD-related basal cortisol elevations. Further, there was no evidence that the difference in the mean age of the AD and NCC samples or the difference in the proportion of female participants systematically impacted the effect sizes. These results suggest that basal cortisol elevations are not an age or sex-dependent attribute of AD.

4.4. Limitations

The results from the present meta-analysis should be considered in the context of several limitations. First, although recent workgroups have advocated for the distinction between Alzheimer’s disease (a condition characterized by biological markers, primarily the accumulation of amyloid-beta plaques in the brain), and the Alzheimer’s clinical syndrome (a profile of cognitive symptoms assumed to result from the pathophysiology of AD; Jack et al., 2018), the vast majority of primary studies included here were published long before this distinction was even feasible in vivo. Therefore, the participants included in the AD groups could more accurately be described as exhibiting symptoms of the Alzheimer clinical syndrome because measures of biological markers of AD (i.e., measures of amyloid accumulation) was exceedingly rare and were not used for participant classification.

Second, the literature incorporated here spans a period of nearly forty years, during which time there have been numerous shifts in cortisol assay techniques, diagnostic and exclusionary criteria, reporting standards and participant recruitment. Although these shifts are expected and often indicate forward progress in scientific discovery, these changes severely limit the investigation of mediating variables at the meta-analytic level, including those of substantial interest in the AD literature such as APOE ɛ4 genotype. However, the strength of the overall effect, in spite of this significant heterogeneity, suggests that basal cortisol elevations are typical among individuals with AD.

In regards to the interpretation of the systematic review and meta-analytic findings, it is important to reiterate that a number of null effects have been ascribed to biological functions. That is, the absence of a significant moderating effect of time-of-day on the magnitude of the AD-associated basal cortisol increases has been interpreted here as evidence for the maintenance of SCN function. Similarly, the absence of a moderating effect of sex has been interpreted as evidence against sex-specific differences in the role of cortisol in AD. However, there remains the possibility that the null effects are the result of insufficient statistical power or the failure of our meta-analysis or the primary literature to include key covariates.

The present findings, while demonstrating that basal cortisol elevations are characteristic of AD, are not informative regarding the time course of cortisol changes. Future studies examining the timing of elevation onset, whether through longitudinal approaches or by comparison with different stages of clinical severity such as MCI (James et al., 2020; Johar et al., 2015; Pomara et al., 1984; Popp et al., 2009, 2015; Swanwick et al., 1998) may help to elucidate the role of these elevations in AD progression.

4.5. Summary and conclusions

This study reports that across nearly forty years of research, the average reported basal cortisol levels are approximately half of a SD higher among older adults with AD in comparison to cognitively normal older adults. These results support the hypothesis that HPA-axis dysregulation is characteristic of AD, although the typical circadian rhythm of cortisol secretion is maintained. It is still ambiguous when cortisol dysregulation begins in the disease process and how the associated basal cortisol elevations impact the disease and associated clinical syndrome.

Declaration of Competing Interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105367.

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